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Aims and Scope
Vox Sanguinis reports on important, novel developments in transfusion medicine. Original papers, reviews and international fora are published on all aspects of blood transfusion and tissue transplantation, comprising five main sections:

1 *Transfusion-Transmitted Disease and its Prevention* Identification and epidemiology of infectious agents transmissible by blood; Bacterial contamination of blood components; Donor recruitment and selection methods; Pathogen inactivation
2 *Blood-Component Collection and Production* Blood collection methods and devices (including apheresis); Plasma fractionation techniques and plasma derivatives; Preparation of labile blood components; Inventory management; Haematopoietic progenitor cell collection and storage; Collection and storage of tissues; Quality management and good manufacturing practice; Automation and information technology
3 *Transfusion Medicine and New Therapies* Transfusion thresholds and audits; Haemovigilance; Clinical trials regarding appropriate haemotherapy; Non-infectious adverse affects of transfusion; Therapeutic apheresis; Support of transplant patients; Immunotherapy
4 *Immunohaematology and Immunogenetics* Autoimmunity in haematology; Allo-immunity of blood; Pre-transfusion testing; Immunodiagnostics; Immunobiology; Complement in immunohaematology; Blood-typing reagents; Genetic markers of blood cells and serum proteins: polymorphisms and function; Genetic markers and disease; Parentage testing and forensic immunohaematology
5 *Cellular Therapy* (including clinical, quality and regulatory aspects) Cell-based therapies; Stem cell sources; Stem cell processing and storage; Stem cell products; Stem cell plasticity; Regenerative medicine with cells; Cellular immunotherapy; Molecular therapy; Gene therapy

This comprehensive coverage has made the journal essential reading for a wide range of specialists interested in the present state of transfusion research and practice.

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Vox Sanguinis ISBT International Society of Blood Transfusion

Plenary session - How viruses shape blood banking

PL-01-01 | Hepatitis C and blood banking the road to the Nobel Prize

H. Alter

No abstract available.

PL-01-02 | SARS-CoV2 surveillance by blood banks: Variants, re-infections and vaccine-breakthrough infections

E. Sabino

No abstract available.

PL-01-03 | Blood donor biobanks as a surveillance tool for future pandemics

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Early in the history of transfusion it was recognized that transfusion-transmitted infectious diseases constituted a serious risk for the recipient. More than 100 years ago, syphilis was reported to be transferable from donors to patients and transfusion has led to great advances in our understanding of several important infections such as hepatitis B, C and HIV. Blood donor studies have continued to contribute to our understanding of the epidemiology and pathogenesis of these and other infectious diseases. Recently, screening of blood donors have helped monitor epidemics of e.g., West Nile virus, *Coxiella burnetii*, Zika virus, and SARS-CoV-2.

The SARS-CoV-2 pandemic has highlighted the importance of flexible systems for effective surveillance of emerging infectious diseases. While authorities worldwide, early on reported daily updated data of the number of individuals testing positive for SARS-CoV-2 viral RNA true measures of incidence and prevalence of SARS-CoV-2 infection were difficult to establish.

Seroprevalence surveys assess the percentage of a population who has already been infected and hopefully is immune to re-infection. Such results can be used to estimate both attack and fatality rates for the infectious agent. However, these surveys are notoriously challenging to carry out simply by inviting individuals randomly selected in the general population. Not only do the surveys take time to plan and roll out, and are therefore expensive; response rates are typically low and varying over time, and the surveys are therefore prone to participation bias.

The blood donor population in contrast, is mostly static, and we and others have re-affirmed that donors are motivated to donate because it helps patients and not because of the possibility of being tested for antibodies to SARS-CoV-2.

Blood donors have to fulfill strict criteria and are deferred when sick or e.g., due to sexual risk behavior. Blood donors are therefore healthier than the background population and not suitable for the study of all types of infections.

However, as long as this "healthy donor effect" is appreciated, donor populations may still be used to monitor infections. This has been convincingly demonstrated during the last year when numerous blood services have used the blood bank infrastructure to swiftly carry out seroprevalence surveys.

Many blood services host large biobanks approved for research, often enriched by additional phenotypes and register data. Blood donor biobanks may be instrumental in the real-time monitoring of an epidemic as described above. Additionally, a unique feature of donor biobanks is the availability of samples collected before an epidemic is even identified. E.g., the US donor samples were used to assess whether SARS-CoV-2 was present prior to identification of the virus. As another example, in case-control studies, samples drawn before the donor experiences disease can be used to identify biomarkers of long-term effects of COVID-19 or adverse effects of vaccination.

The current pandemic has revealed serious shortcomings in our preparedness towards emerging infectious diseases. In the blood banks we have the infrastructure to maintain large-scale biobanks, collect additional phenotypes, and, in some areas, data may be merged with national health registers. We propose blood donor biobanks to be part of post-COVID-19 preparedness plans for the surveillance of emerging infectious diseases.

Immunohaematology - from routine to extreme

PL-02-01 | Genotyping & allele database

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The application of next generation sequencing (NGS) and high density DNA arrays are ushering in an era of precision medicine. However, for this potential to be fully realized in transfusion medicine and blood group genotyping there are several key steps that are required: (1) generation of array and NGS data with paired conventional serology or PCR based results; (2) development of a fully annotated and complete electronic databases of RBC antigen allele genotypes to phenotypes; (3) conversion of RBC genetic changes from their defined cDNA sequences to human reference genome coordinates; and (4) development of software capable of automatically interpreting the large amount of data. Over the last decade many groups have addressed these needs. This talk will cover this progress.

PL-02-02 | Challenges in the search for rare blood: Global networking saves the day

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Background: Appeals to blood donors have many focuses and recruitment efforts concentrate on different qualities, e.g.: O negative for trauma and neonates, CMV negative for various disease states, HLA types for platelets and leukopacks, African descendants for red cell antigen and HLA types for matching platelets. Donor recruitment for rare blood types is a challenge and may receive less resources than aforementioned. Because rare donors are just that, rare, at less than 1 in 1000 and some phenotypes likely as rare as 1 in 1 million, it is important that once identified, they are recruited appropriately, with correct inventory management. Successful management is paramount in the provision of rare blood and includes planned testing, purposeful registration of identified donors, ensuring maintenance and updating of current contact information, and progressive management of the donated units. One of the major disease states requiring chronic transfusion is Sickle Cell Disease and is prevalent in many parts of the world. In many countries, identification of donors to provide full phenotype matches and *RHCE* and *RHD* allele selected products as needed occupies much of the testing and resources of the laboratories. With genomic testing, patients once considered untransfusable, due to complex antigen negative requirements, have been successfully transfused using *RHCE* and to a lesser extent *RHD* allele selected product. The use of automated molecular testing platforms, has led to identification of not only high prevalence antigen negative types found primarily in African descent populations, like U–, Js(b–), and Hy– but also phenotypes such as Kp(b–), Di(b–) found in other populations. The lack of regulatory agency-approved automated testing for other antigens of high prevalence, has not improved the chances of finding phenotypes like Vel–, Lan–, or PP1P^{k}–. To find those, sibling testing or large-scale mass screening are often still the only options. Collection of autologous units from those patients who are eligible at the time of need (surgery or pregnancy) and allogeneic when the patients can be "regular" donors is to be desired. Whole exome sequencing may aid in identification of rare donors in the future when hundreds of thousands of donors can be efficiently tested.

Methods: Cases involving provision of blood products when no blood can be located domestically are most challenging, and some of these will be reviewed. Perspectives and data including donors registered, policies for importation of blood, use of ISBT International Rare Donor Panel and collaboration of the ISBT Working Party on Rare donors were reviewed for the cases. Process flows for both a domestic program and the ISBT International Rare Donor Panel were used.

Results: Four challenging cases from the USA's American Rare Donor Program involved providing En(a–) units for a complicated surgery, Vel negative for 2 patients slated for potential transplant that overlapped in needs involving over 150 units, O negative Jk(a–b–), and In(b–)E– that involved social media.

Conclusions: Through communicating experiences such as these that provided extreme challenges to the provision of rare blood products, and sharing the successes, more treatment centers will be aware of the process. In addition, sharing the important information for proper planning is essential to future success.

PL-02-03 | The art of designing red cell panels and screens

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Background: The detection and identification of antibodies to red cell antigens is one of the most important issues in transfusion medicine. There are 376 red cell antigens recognized by the ISBT Working Party on Red Cell Immunogenetics and Blood Group Terminology. Most of

them belong to one of the 43 blood group systems. Some antigens with unknown genetic background are part of collections (200 series), low incidence (700 series) or high incidence antigens (901 series). In the routine it is important to identify the clinically significant antibodies.

Aims: Antibody screen should contain red cells homozygous for the clinically important antigens of the RhDCcEe, Duffy, Kidd and MNS systems. As the antigen frequencies differ worldwide, additional requirements may be needed for some populations (e.g. Mia cells in Asia). The cells should not cross-react with HLA class I antibodies. The red cells used in antibody identification panels are usually typed for 20 to 30 antigens. Considering the high number of known antigens any additional information on the pheno- or genotype of the cells is useful. To improve the diagnostics we developed in-house antibody identification panels with red cells serologically typed for 26 antigens and genotyped for additional 30 alleles coding for blood group antigens. To optimize the identification of antibodies to high and to low prevalence antigens we also created a special identification panel containing 12 rare blood types.

Methods: The red cells used in the screens and panels were phenotyped for RhD, C, c, E, e, C^w, K, k, $Kp^{a/b}$, $Fy^{a/b}$, $Jk^{a/b}$, $Le^{a/b}$, P1, M, N, S, s, Xg^a, $Lu^{a/b}$. Following alleles were genotyped by using inhouse PCR-SSP methods: *DO*01/*02* (for $Do^{a/b}$), *LU*18/*19* ($Au^{a/b}$), *YT*01/*02* ($Yt^{a/b}$), *DI*01/*02* ($Di^{a/b}$), *IN*01/*02* ($In^{a/b}$), *KEL*06/*07* ($Js^{a/b}$), *LU*08/*14*, *LW*05/*07* ($LW^{a/b}$), *SC*01/*02*, *KN*01/*02* ($Kn^{a/b}$), *KN*03/*06* ($McC^{a/b}$), *KN*04/*07* (Sl^a/Vil), *KN*-05* (Yk^{a-}), *KN*09/*10* (KCAM/KDAS), *RHCE*02.09* (C^X), *VEL*-01* (Vel-). The special panels consisted of 12 rare blood types. They were produced from red cells frozen at -80°C containing following rare blood types positive for Wr^a, Di^a, Wu, Rb^a, C^x, E^w, JAHK, FPTT, BARC, PARG, Tar, Js^a, Mi^a, Vw, Mt^a, Lu14, Sc2, LW^b, Vil+, Kn^b, Yt^b and negative for Kp^b, k, MAR, Rh17, Lu8, P, Lu^b, Co^a, Vel, Yt^a, Jr^a, Fy3, Yk^a, Kn^a, KCAM, Sl^a, AnWj, Ch, Rg, Cs^a, Sd^a.

Results: 27,587 blood samples were tested in our reference laboratory from 2014 till 2020. In 352 samples (1.3%) following antibodies could be identified due to the information derived from the genotype of the test cells: 42 anti-Kn^a, 47 anti-Kn^b, 35 anti-Yk^a, 38 anti-KCAM, 3 anti-KDAS, 2 anti-Sl^a, 1 anti-McC^a, 45 anti-Yt^a, 24 anti-Yt^b, 10 anti-Lu8, 29 anti-Lu14, 5 anti-Au^b, 11 anti-Vel, 10 anti-Do^a, 5 anti-Do^b, 24 anti-Di^a, 4 anti-Wu, 3 anti-LW^a, 2 anti-LW^b, 1 anti-Sc1, 1 auto-anti-Sc1, 3 anti-Sc2, 1 anti-BARC, 1 anti-Tar, 2 anti-MAR, 2 auto-anti-MAR. Using the special panel following antibodies were identified: 602 anti-Wr^a, 306 anti-Rb^a, 100 anti-Vw, 15 anti-Mi^a, 6 anti-Mt^a, 5 anti-Kp^b, 6 anti-k, 41 anti-Lu^b, 7 anti-Co^a, 12 anti-Jr^a, 14 anti-AnWj, 1 anti-Fy3, 46 anti-Ch, 11 anti-Rg, 30 anti-Cs^a, 13 anti-Sd^a.

Conclusions: The use of red cell panels with extended pheno- and genotyping and the availability of panels with rare blood types extends the range of detectable antibody specificities. It accelerates the antibody identification and improves the pre-transfusion diagnostics.

Platelets & Granulocyte Immunobiology – On the trail of disease processes

PL-03-01 | Stored platelet extracellular vesicles and their role in trali induction

M. Mcvey[1,2,3]

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Transfusion-related acute lung injury (TRALI) is a respiratory transfusion reaction associated with considerable morbidity and mortality. TRALI is a multi-hit injury where pre-existing inflammation within transfusion recipients combined with injurious mediators within donated blood products culminate in increased lung barrier permeability and inflammatory damage. Donor blood products are thought to mediate their lung-injurious hit due to containing pathogenic antibodies or biological response mediators such as lipids. Transfer of long chain sphingolipid ceramide (CER) between cells in aqueous solutions such as blood is facilitated in part by lipophilic chaperones. Previously it has been shown that non-antibody TRALI is mediated at least in part by CER, which is able to injure lungs by eliciting endothelial barrier dysfunction. Red blood cells and platelet blood products accumulate CER during storage prior to transfusion. Transfused donor blood product extracellular vesicles (EVs) shuttle CER in the recipient's circulation during TRALI. As cells undergo stresses or activation (such as during storage under blood bank conditions) they release lipid-membrane bound EVs. Of interest during this cellular process sphingolipid metabolism increases CER production which has been shown to lead to increased release of EVs. EVs are small membrane bound structures which retain parent-cell features such as genetic material (e.g. DNA, RNAs), lipids (e.g. sphingolipids CER and spinngosine-1-phosphate (S1P)) which can then be transferred between cells within the circulation. Evidence is emerging as to the mechanistic contributions of EVs in TRALI. As CER accumulates and S1P is lost during the prolonged storage of blood products there appears to be a concomitant increase in EVs which share the pattern of containing elevated CER and decreased S1P. Loss of S1P is relevant to TRALI as under normal physiological conditions the presence of S1P counteracts the increased permeability elicited by CER. CER enriched and S1P deplete EVs appear to represent a biomarker of at-risk blood products but more so regulate TRALI by shuttling lipophilic CER to target cells within the lungs such as the pulmonary endothelium. CER enriched and S1P depleted EVs from prolonged storage (5 days) platelets have been shown to promote TRALI whereas S1P containing EVs (from 1 day stored platelets) with modest amounts of CER do not. This fascinating finding shows that EVs may represent a

biomarker for TRALI but also are themselves a pathogenic mediator of injury. Screening blood products throughout storage for changes in their EV signatures may be a way to predict and or prevent TRALI in high risk transfusion recipients. Further, considering the involvement of CER in not only mediating TRALI but also the genesis of EVs, manipulating sphingolipid metabolism (e.g. reducing CER formation or increasing S1P) or eliminating EVs via filtration are appealing strategies for preventing or treating TRALI.

PL-03-02 | A humanized animal model for understanding FNAIT

P. J. Newman[1]

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Fetal/neonatal alloimmune thrombocytopenia (FNAIT) is a life-threatening bleeding disorder caused by maternal antibodies directed against paternally inherited antigens present on the surface of fetal platelets. The human platelet alloantigen, HPA-1a is the most frequently implicated HPA for causing FNAIT in Caucasians. A single Leu33Pro amino acid polymorphism residing within the ~50 amino acid plexin/semaphorin/integrin (PSI) domain near the N-terminus of the integrin β3 subunit (platelet membrane glycoprotein (GP)IIIa), is responsible for generating the HPA-1a and HPA-1b epitopes in human GPIIIa, and serves as the central target for alloantibody-mediated platelet destruction. To simulate the etiology of human FNAIT, wild-type female mice were pre-immunized with platelets derived from transgenic mice engineered to express the human HPA-1a epitope on a murine GPIIIa backbone. These mice developed a strong alloimmune response specific for HPA-1a, and when bred with HPA-1a-positive males, gave birth to severely thrombocytopenic pups that exhibited an accompanying bleeding phenotype. Administration into pregnant female mice of either polyclonal intravenous immunoglobulin G (IVIG) or a human monoclonal blocking antibody specific for the HPA-1a epitope resulted in significant elevation of the neonatal platelet count, normalized hemostasis, and prevented bleeding. The establishment of an alloantigen-specific murine model that recapitulates many of the clinically important features of FNAIT should pave the way for the pre-clinical development and testing of novel therapeutic and prophylactic modalities to treat or prevent FNAIT in humans.

PL-03-03 | Serologic and molecular studies to identify neonatal alloimmune neutropenia in a cohort of 10,000 neonates

L. B. Lopes[1]

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Neonatal alloimmune neutropenia (NAIN) results from maternal alloimmunization to human neutrophil antigens (HNAs). The alloantibodies more frequently involved in NAN are against the HNA-1 and -2 systems; however, HNA-3, -4 and -5 system have also been associated. This study investigated maternal HNA/HLA alloantibodies involved with NAIN and identified the frequency of NAIN in Brazilian neonates.

Neonatal neutropenia, defined as neutrophil count < 1.5×10^9/L in cord blood, was investigated in samples from 10,000 unselected neonates, resulting in the selection of 88 neutropenic newborns and their 83 mothers (3 pairs of twins and 1 triplet). Genotyping was performed by PCR-SSP (HNA-1/-4) and PCR-RFLP (HNA-3/-5), to assess cases of maternal-fetal incompatibilities. Serologic studies for detecting maternal HNA/HLA alloantibodies were performed by GAT (granulocyte agglutination test), Flow-WIFT (white blood cells immunofluorescence test), and LABScreen-Multi-HNA-Kit (OneLambda®) (LSM).

Neonatal neutropenia was identified in 88/10,000 (0.9%) newborns. Genotyping studies revealed 60.2% maternal-fetal HNA incompatibilities, being 31.8% for HNA-1, 14.8% for HNA-3, 15.9% for HNA-4, and 21.6% for HNA-5; two maternal-fetal incompatibility cases occurred concomitantly for HNA-1 and HNA-3 systems. Serologic studies revealed 37.3% of mothers with positive results with at least one technique (GAT, Flow-WIFT and LSM). The detected anti-HNA specificities were confirmed in eight positive cases related to HNA-1/-3 systems. In cases with maternal-fetal HNA-4/-5 incompatibility, no specific neutrophil alloantibodies were found but anti-HLA I/II were present. Anti-HNA-2 was not identified.

This is a large Brazilian study which involved the investigation of antibodies against all five HNA systems in neutropenia cases and showed a frequency of neonatal neutropenia in Brazilians (0.9%) and a frequency of NAIN in 8/10,000 neonates. Among the HNA antibodies identified, we highlight the anti-HNA-1d and anti-HNA-3b, antibodies unusual in alloimmunised women, and rarely related to NAIN cases.

Blood products & Cellular Therapies – New questions on old problems

PL-04-01 | Are cold platelets here to stay?

A. Cap

No abstract available.

PL-04-02 | Plasticizers, their needs and risks

J. Acker

No abstract available.

PL-04-03 | Update on platelet lysates and clinical usage

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Increasing experimental and clinical data demonstrate that platelet concentrates (PC) produced by blood establishments can be used, even when outdated, as source material to generate human platelet lysate (HPL).

HPL is obtained by lysis of the platelets by several cycles of freeze-thaw but can also be prepared by activation and degranulation induced by $CaCl_2$ addition. The lysate is then subjected to centrifugation and/or filtration steps, including a sterile bacterial filtration, to obtain a cell-free solution rich in proteins. Depending upon the pool size, HPL should be virus-inactivated by treatments performed on the PC or after pooling.

HPL proteome is complex, made of cell growth- and repair-promoting biomolecules, including growth factors (PDGF; VEGF; BDNF; TGF-ß; EGF; HGF; etc.), cytokines, and chemokines (CXCL4 or PF4; CCL5 or RANTES), as well as anti-inflammatory and anti-oxidative factors. These biomolecules trigger cellular functions as well as protective and regenerative signaling pathways. HPL also contains extracellular vesicles (EVs) that may be responsible for some of its physiological activity.

HPL is emerging as a product with a therapeutic and clinical interest in cell therapy, tissue engineering, and regenerative medicine. One of the best-established use of HPL now is as a substitute for fetal bovine serum (FBS) as a xeno-free supplement of growth media for *ex vivo* clinical-grade isolation and propagation of human cells for transplantation. Convincing data demonstrating the consistent superiority of HPL over FBS for cell expansion have been obtained using mesenchymal stromal cells (MSCs) from various tissues (e.g., bone marrow, adipose tissues). Studies are ongoing to evaluate HPL benefit as a growth medium supplement for expanding differentiated human cells (e.g., chondrocytes, corneal endothelium and epithelium cells, tenocytes, etc.), and initial tests are published using chimeric antigen receptor-T cells (CAR-T cells).

Experimental and clinical evidences also concur to suggest the value of HPL (and other forms of platelet biomaterials) in tissue healing in orthopedic, maxillofacial, dermatologic, ophthalmologic fields (in particular dry eye treatment), sports medicine, etc. However, rigorous additional pre-clinical and clinical studies still need to be conducted for some indications. Work done in our laboratory show that a tailor-made HPL preparation designed for intranasal administration to the brain (to bypass the blood-brain barrier) is strongly neuroprotective in cellular and animal models of Parkinson's disease and traumatic brain injury.

In conclusion, there is a solid scientific rationale for developing standardized, well-characterized, and pathogen-reduced HPL products made from allogeneic PC collected by blood establishments. HPL can be of value for vital clinical applications, including safe propagation of human cells for transplantation and direct clinical use in various fields of regenerative medicine. There is, therefore, a strong potential for an increasing future demand for therapeutic-grade PC in cell therapy, tissue engineering, and regenerative medicine.

TTID – Never underestimate a virus!

PL-05-01 | Discovery and monitoring of undisclosed antiretroviral therapy use in HIV-positive South African blood donors

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Background: The South African National Blood Service (SANBS) excludes HIV-positives by pre-donation questioning and screens all blood donations for both HIV antibody and HIV RNA by nucleic acid testing (NAT), allowing the identification of prevalent HIV infections (antibody+/NAT+), incident HIV infections (antibody-/NAT+) and potential elite controllers (EC; antibody+/NAT-). During recruitment of EC's into a cohort study, there was anecdotal disclosure by potential ECs of prior knowledge of HIV infection and antiretroviral therapy (ARV) use at the time of donation. We aimed to i) determine the prevalence of undisclosed ARV use in all HIV-positive donors; and ii) to understand the extent of this "false EC" phenomenon; and iii) to determine changes in undisclosed ARV over time.

Methods: Blood donations were tested for HIV antibody (PRISM, Abbott) and HIV RNA using individual donation NAT (Procleix, Grifols). Stored plasma was tested for 4 or 5 antiretroviral (ARV) drugs depending on the year, using qualitative liquid chromatography–tandem mass spectrometry (sensitivity 0.02 μg/mL). We estimated the prevalence of undisclosed ARV use amongst all HIV-positive donors in 2017 and amongst antibody+/NAT- blood donors from 2010-2016; associations with socio-demographic and blood drive characteristics and calendar time were analysed using chi-square, trend tests and multivariable logistic regression.

Results: In 2017, 1250 of the 1462 donors who tested either HIV antibody+ or RNA+ had sufficient plasma for ARV testing of which 122 (9.8%) tested positive for the presence of ARVs and the latter was associated with age over 40 years (aOR=3.73, 95% CI1.98-7.04) and first time donor status (aOR=5.24 95%CI 2.48-11.11). Antibody +/NAT- donors had the highest prevalence (68/80; 85%) of undisclosed ARVs. From 2010-2016, of 226 HIV antibody+/RNA- donors, 150 (66.4%) had detectable ARV drugs and were considered "false EC". False EC status was associated with donation at mobile versus fixed donation sites (aOR=2.46, 95%CI 0.98-6.22) but not temporally with small blood donation incentives. In the multivariable analysis the odds of an antibody+/NAT- testing positive for ARVs increased significantly from 2010 to 2016 (aOR 7.57, 95%CI 1.96-32.17) in parallel with the number of South Africans receiving ARV therapy.

Conclusions: Almost 1 in 10 HIV RNA positive blood donors neglected to disclose their HIV status and ARV use when they

presented for blood donation. Of these, most had a "false EC" phenotype representing a large and growing proportion of all potential ECs discovered by SANBS. The phenomenon does not seem to be associated with small blood donation incentives but may be related to broader ARV coverage among HIV-positive South Africans. Behavioral reasons for concealing HIV status and ARV use remain to be elucidated.

PL-05-02 | Is SARSCoV2 a threat to the blood supply? Impact of variants

P. Cappy

No abstract available.

PL-05-03 | Blood donor testing for antibodies to COVID-19; impact of natural vs vaccine induced reactions

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The COVID-19 pandemic has had a profound impact upon transfusion medicine, particularly in the context of maintaining and managing an effective blood supply. In addition, the collection, qualification and distribution of COVID-19 convalescent plasma (CCP) have added complexities based on changing regulatory requirements and hospital demand based on unknown efficacy. Many blood collectors have implemented donor testing for antibodies to SARS-CoV-2, in part to attract donors and also to identify sources of CCP from qualified donors; however, identifying suitable donors has proven to be complex, involving additional donor questions and testing. The increase in antibody prevalence from natural infection based on the detection of spike (S1) antibodies has been profound: from ~1% in June, 2020 to ~35% to mid-April, 2021, with less than 10% of these lacking nucleocapsid antibodies. Current vaccination programs are having a major impact on the prevalence of antibodies with frequencies of vaccine-induced seropositivity soon to exceed those from natural infection. At this point, regulatory agencies have not considered antibodies from vaccination as adequate for plasma for therapeutic or prophylactic use.

At some point, the frequency of vaccination in the general population would be expected to ease the difficulties associated with collection of blood in the face of social distancing. However, it must now be recognized that variant strains of the virus may be expanding at increased rates and appear to be more infectious than the wild-type virus. This places added pressure on the need for rapid vaccination, that appear to also be efficacious to both the wild-type and variant strains, and continued effort to maintain personal safety. Whether or not newly emerging viral strains will impact the value of convalescent plasma remains to be seen. These issues will be discussed in the context of current information from SARS-CoV-2 antibody testing of donors in the USA.

Clinical/Adverse Events – You're never too old to be young

PL-06-01 | RBC transfusions in preterm neonates: Does the hemoglobin type matter?

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Anemia is a usual complication of preterm birth, with severity also depending on concomitant morbidities and the frequency of blood withdrawal for tests. Since prematurity anemia is scarcely responsive to medical therapies, neonates born at gestation age <28 weeks are usually heavily transfused during their hospitalization. The association between repeated transfusions and the poor outcome in this setting has been long acknowledged. Detrimental effects of transfusions can be ascribed to components released during the storage, eliciting the activation of the innate immune system and the inflammatory response. Indeed, the increase of many of these mediators as well as markers of oxidative stress has been documented in preterms. Various diseases complicating the clinical course after preterm birth rely on oxidative damage as the underlying pathogenic mechanism. Among them, retinopathy of prematurity (ROP) and bronchopulmonary dysplasia (BPD) particularly influence the neurodevelopmental outcomes of affected patients and lifelong affect their quality of life. A body of growing evidence from retrospective and prospective studies suggests a direct connection between the severity of ROP or BPD and low levels of fetal hemoglobin (HbF), especially when experienced during the early weeks of life. Nevertheless, RBC transfusions remain unavoidable in the majority of patients. HbF and adult Hb (HbA) have a similar three-dimensional structure, but different chemical and physical properties. The distinctive HbF characteristics, besides the higher oxygen affinity, include a higher efficiency in maintaining its tetrameric integrity, preventing the release of toxic free heme groups, and a critical redox effect due to its pseudo peroxidase activity. In addition, HbF exerts a greater ability than HbA in vessel tone regulation. Therefore, transfusing to preterm neonates RBC concentrated obtained from cord instead of adult blood, might be advantageous. For this purpose, we set up the fractionation of units solidary donated at our public cord blood bank, which could not be used for transplant due to the low total nucleated cell content. We first assessed the feasibility of using these allogeneic RBC concentrates to transfuse preterm newborns, reporting no adverse events and similar hematocrit increments as standard transfusions. In a subsequent proof-of-concept study, we also demonstrated that transfusing cord-RBC prevents the HbF depletion observed after standard transfusions. Overall this approach seems to be safe and feasible, but many questions remain to be answered before it may be widely adopted. The first relevant point is to prove that cord-RBC reduce ROP and/or BPD severity in a properly designed

randomized trial, which is conceivable only in face of extensive availability of cord blood units. It deserves to be mentioned that using cord blood for transfusion implies further additional concerns: to find rapid and dependable methods for bacterial testing, to assess the rate of hemolysis in stored units, to identify optimal additive solutions and acceptable storage duration. Moreover, there is a rather scarce availability of dedicated fractionation devices. However, despite all these drawbacks, the cord-RBC concentrates might represent a unique opportunity to preterm neonates for the high HbF content. Therefore, new researches in this area are warranted.

PL-06-02 | Tranexamic acid - Where are we now?

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Tranexamic acid has become an established agent in the prophylaxis and management of severe haemorrhage across a diverse range of clinical settings. It has been shown to reduce blood loss, blood product transfusion and improve bleeding associated patient outcomes. This has been demonstrated in patients undergoing both elective cardiac and non-cardiac surgical procedures, and also in emergency settings such as obstetric haemorrhage and major trauma. Although efficacious and safe, the current evidence stems from studies with significant heterogeneity in study design and clinical setting, in addition to variability in dose used and timing of administration. We explore and briefly review its current role, and the evidence for its use in cardiac surgery, obstetrics and trauma.

PL-06-03 | Red blood cell transfusion in older adults - What HB threshold is suitable

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Data from developed countries report that more than half of the red cells issued are transfused to older patients (>65 years). As many populations around the globe age this demand will persist and grow. This cohort is serviced by adult patient blood management guidelines as there are presently no blood management guidelines specific to this age group.

There has been much discussion in the literature on the use of restrictive versus liberal transfusion thresholds. However, the haemoglobin levels defined as restrictive or liberal often vary between studies making comparison challenging. Our systematic review and meta-analysis found that liberal rather than restrictive transfusion strategies might produce better outcomes in older patients (Simon et al. Lancet Haematology 2017).

It is well accepted that with age comes deterioration in the speed of response to changes in homeostasis and recovery to baseline function. In an attempt, to better understand the anaemia tolerance and consequently the transfusion triggers of older patients, we considered the pathophysiological changes associated with aging. This led us to create a model of oxygen delivery capacity in young, middle-aged and older adults at a range of haemoglobin levels. The model predicts that an older adult with a haemoglobin of 100 g/L has a similar peak oxygen delivery capacity to a young adult with a haemoglobin of 70 g/L, (Simon et al. Transfusion Medicine Review 2019). These findings reinforce the need to consider age-related physiological changes when assessing a patients transfusion requirements. However, it is important to recognise that there are other factors that also need to be considered such as the patients' comorbidities. Hence, there is a real need for more research into blood use by older patients, and we need to work toward creating patient blood management guidelines for the older adult. As that will support effective patient blood management in this high blood use age group, and also inform management of our scarce and precious blood supply.

Supporting Safe Transfusion – It's all about the brain

PL-07-01 | Artificial intelligence: Applications in healthcare and implications for transfusion medicine

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While there is considerable interest in artificial intelligence (AI) in healthcare, its application into clinical practice and healthcare decision-making can be challenging. In this talk, Dr. Mamdani will use examples of applied AI initiatives to highlight how data scientists can work more closely with end-users such as clinicians and healthcare decision-makers in improving the utility of their work to ultimately improve patient outcomes and healthcare system efficiency. Dr. Mamdani will also discuss the fundamental principles of data science in healthcare settings, and how healthcare participants can apply this new knowledge in the context of their own work. Implications for transfusion medicine will also be discussed.

PL-07-02 | How transfusion practitioners use data to effect change

L. Estcourt

No abstract available.

PL-07-03 | Strategies to eliminate errors in blood transfusions

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Background: Haemovigilance schemes were set up in many countries in the 1990s in order to track adverse reactions to blood transfusion, particularly transfusion-transmitted infection. However, from the first Serious Hazards of Transfusion annual report in 1998 errors were noted to be the most common problem, particularly transfusion of incorrect blood components related in part to the complexity of the 9-step transfusion process and the number of different staff groups involved.

Attempted solutions: Despite feedback, education and production of protocols, guidelines, and the establishment of transfusion networks, teams and employment of transfusion practitioners, the same errors continue. Correct patient identification is central (4 identifiers; date of birth, first name, family name and unique patient ID number) and must be confirmed at the time of blood sampling and again at transfusion using a checklist that includes positive identification from the patient where possible. With the introduction of improved laboratory standards, widespread introduction of laboratory information management systems together with competency assessments for staff participating in transfusion the number of ABO-incompatible transfusions and associated deaths has decreased in the UK. Laboratory IT systems are associated with fewer laboratory errors compared to manual methods. Transfusion training should remain mandatory for clinical staff and be assisted by simulation methods.

Where to go from here? Two important strategies will reduce errors, firstly a reduction in human interventions by the introduction of full vein-to-vein electronic identification systems, and secondly application of human factors science and a change in organisational culture away from blaming individuals to full review of the processes and environment where errors may occur. Full vein-to-vein electronic identification systems are associated with improved safety and fewer wrong blood in tube errors. We must move from the reactive 'safety-I' approach to include proactive 'safety-II' learning from what goes well and anticipating danger areas. Examination of work-as-done (adaptations made to procedures in practice) in comparison with work-as-imagined (policies and protocols) helps to understand where local and organisational modifications can improve safety and support the individual workers. Safe laboratory practice is improved with protocols but requires adequate numbers of trained staff. Resilient medical practice may require frequent adaptations for individual patients over time; much can be learned from application of human factors methods, particularly that clinical guidelines can be very useful but are not rules. However, the final bedside check must be a rule as this step when properly completed can prevent most ABO-incompatible transfusions.

Strategic Management - Rules & money

PL-08-01 | High level decision making on controversial issues

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Background: Ideally, decisions about blood donor criteria, policies, and infectious disease testing are based on clear scientific evidence. However, unlike clinical medicine, randomized controlled trials are not possible in determining optimal donor policies. On occasion, finding the balance between recipient safety, adequacy of supply, operational feasibility, and diversity and inclusiveness can be challenging. It may also be necessary to make decisions in the face of limited data.

Aims: This presentation will discuss approaches to aid decision-making in different situations, with a concrete example of each: making policy for an emerging pathogen that hypothetically may be transmissible by blood (Zika virus), considering a change in criteria related to donor safety (donor age limit), and deferral of a group at higher risk for HIV (men who have sex with men, MSM).

Methods: The emerging pathogen matrix provides a framework to determine if an emerging pathogen is likely to be transmissible by blood, and if risk can be reduced by donor criteria, testing, or manufacturing practices. The Risk Based Decision Making Framework for Blood Safety (RBDM), developed by the Alliance of Blood Operators, can be useful in evaluating all perspectives on an issue, including consultation of high interest groups most affected by the possible change in policy. Risk-modeling can be useful in evaluating extremely small risk increments that may be associated with policy changes. Useful data can be collected from public health sources, donor hemovigilance, post-implementation surveillance and compliance studies.

Results: Different approaches may be adopted by different blood suppliers, depending on epidemiologic data in each country, potential impact on blood availability and operational ability to implement various approaches.

Summary: Decision-making on controversial issues is challenging. Various decision-making tools can be useful, and there are several ways to obtain data to support decision making. Once a change is made, post-implementation surveillance can provide reassurance that a decision has not had adverse impacts. As data evolves, policies may need to reassessed.

PL-08-02 | Mind the gap – Sustainable funding for transfusion medicine in low and middle income countries

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Healthcare delivery and funding, both in low and middle income countries (LMIC) and in high income countries (HIC), is complex, involves

multitude of interdependencies, competing priorities and macroeconomic factors. The key role healthcare plays in promoting prosperity in general is reflected in the construct of the World Health Organisation (WHO) Sustainable Development Goals (SDG). Not only is "Health" central to the SDGs, it also relates to most of the other 16 goals. Significant effort is being expended on the attainment of these goals, in particular through the United Nations Global Action Plan (GAP), which involved 12 multilateral agencies engaged in health and development. In contrast to previous initiatives in global health such as PEPFAR, GAP is not funding-focused, but instead promotes a cultural shift within existing health architecture towards more purposeful and systematic collaboration.

It is within this complex environment that countries must also ensure a safe and sustainable blood supply. Transfusion medicine is but one of many components of a country's healthcare system. Focusing on funding transfusion services independent of the larger healthcare system is fraught with danger and high risk of failure; the near collapse of some African blood services after the end of PEPFAR funding being examples. The creation of an artificial gap between funding transfusion medicine and healthcare in general contributes to a lack of integration of transfusion medicine into the key components of a well-functioning health system. The latter comprising *strong leadership and governance* through the use of progressively robust *health information systems; health financing policies* which supports appropriate *human resourcing* and *service delivery systems* underpinned by affordable, universal-access to *essential medical products and technologies*.

While there is no doubt that external funding for transfusion medicine brought significant improvements in some areas, it also had multiple unintended negative consequences. In particular, the conditionality usually imposed by external funders broadened the gap between transfusion medicine and national healthcare systems, and also created costly, unsustainable administrative burdens which collapsed when funding ended.

Addressing the inherent and historical gaps in sustainable transfusion medicine funding will require a paradigm shift. The key commitments of the WHO GAP namely *engage*, *accelerate*, *align* and *account* may be a framework for such a shift. At the core is the need to *engage* with countries to contextualize their specific priorities, strengths and challenges; leveraging in-country strengths with the multilateral agencies' competence to *accelerate* progress; *align* both input and output efforts to ensure harmonization of operational and financial strategies, policies and approaches while building robust frameworks for measuring and *accounting* progress.

Transfusion medicine does not stand outside a country's healthcare realities; this is especially true for LMIC. Engagement and alignment with national health priority through advocacy and the use of appropriate vehicles such as the WHO SDG and GAP programs have the promise of sustainable improvement while narrowing the gap between aspirational goals and the reality of national resource constraints.

Donors & Donation - Let's hear it for donors!

PL-09-01 | Managing Donors & Blood supply and sufficiency in India during COVID

R. Sharma

No abstract available.

PL-09-02 | How can we redirectplasma?

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Plasma is a starting material for many life-saving medicines like immunoglobulins, coagulation factors and albumin, highly needed and many times the only treatment choice for rare chronic diseases or intensive care situations. Whether collected through whole blood donations (recovered plasma) or dedicated plasmapheresis (source plasma), it is continuously needed for manufacturing of these medicines. The global demand for plasma is growing steadily.

In 2016, the International Plasma and Fractionation Association launched the term plasma as a strategic resource (Strengers and Klein, 2016), categorising it as "an economically important raw material which is subject to a higher risk of supply interruption". At the same time, it was calling for a higher collection of plasma outside the United States, which was, and still is, the leading nation in plasma collection. Increased collection in also other parts of the world, including low- and middle-income countries, would enable better supply of plasma medicines and avoid local interruptions. Today, the awareness of the high dependence on US plasma is highly recognized. The COVID-19 pandemic has brought new attention to vulnerability of the collection system, but also taught a broader public the importance of the antibodies present in plasma and the link to plasma medicines. But despite raised awareness, there is still much that needs to be done to increase collection and to strategically redirect plasma to local or regional needs.

How can we then improve the situation, and redirect the plasma so that it better becomes available to make plasma medicines? One method is to do all effort to avoid wastage of plasma. In many countries, the collection of blood and plasma does not fulfill the quality requirement of plasma that is needed for production of plasma medicines, and the plasma is wasted. How a change can be approached in low-and middle- income countries is well described in the new WHO guideline. Another recent example is the change in the local requirements for plasma in the UK, where a long ban of the plasma collected in UK due to risk of vCJD was lifted, making the formerly wasted plasma available for manufacturing.

Sufficient plasma needs to be collected locally or regionally to enable the redirection of this plasma and the medicines to the same markets. The availability of plasma medicines needs to be considered an important part of a country´s public health policy, and the strategic plasma resource has to be taken into account into this. Many countries already have or are setting up national programs to collect plasma alongside whole blood collections.

In order to redirect plasma to its strategic use to make plasma medicines, all donors should be well informed and understand the impact of plasma resource on the public health. At the same time, it is in the public health interest to ensure that the health and safety of all donors remains protected.

PL-09-03 | Defending the rights of the donor

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What are the rights and expectations of donors? Why is it so important for all of us to protect them?

Firstly, as obvious as it may seem, the donor is - like the patient he or she is trying to help - a human being, whose dignity, health, and freedom of choice deserve to be equally protected throughout the donation process. Blood donation is indeed a matter human rights, where their protection is explicitly stated in several legislative text and official documents, especially regarding the necessary prohibition of all profit making from the human body or parts of it. From their point of view, donors expect to be welcomed, respected, taken care of and for their gesture of love to be recognised.

The answer to the question of how best to protect the rights and expectations of donors requires careful reflection on two fronts. On the one hand, it seems essential that, throughout the donation process, the donor is guaranteed the best possible reception and selection process, plus a flawless handling of the act of donation. In the long term, this also involves constantly checking that the donation activity and its frequency are genuinely compatible with the maximum protection of the donor's health and with ensuring the highest standards of quality and safety of donated blood and blood components. Fully respecting the gesture of solidarity made by the donor also means that transfusion facilities make good use of donated blood and blood components, avoiding any kind of waste. Moreover, it is the duty of all the actors involved in the system to help develop a community in which all donors can feel united and protected, and which can provide them with all the guidance they need to continue a long relationship of mutual contribution to our public health systems. From this point of view, as we shall see, the alliance between health personnel, professionals, authorities, and associations (which are able to make a substantial contribution to the cause, thanks to their proximity to donors) would represent a strategic asset for achieving these objectives.

On the other hand, the real guarantee of being able to reach the goals outlined above requires - more generally - a broader system of rules that shares and promotes the same values. In our view, the core principle that should be relied upon is the one of anonymous, voluntary, regular, and non-remunerated donation (VNRD). VNRD donors are indeed the "safest" allies for themselves and especially for the patients. In applying this principle, there should be no difference between whole blood donation and apheresis donation (especially plasma).

We shall see how this principle is inextricably linked with each of the above requirements concerning respect for the rights and expectations of the donor. More in general, VNRD principle should be fully embedded in every project aimed at reaching and maintaining self-sufficient and sustainable blood systems based on a comprehensive, coordinated, and long-term oriented approach, as well as on the idea of donated blood (and blood components) as a public, ethical, strategical and community good.

Finally, we will consider the contribution that donors themselves and their organisations can make throughout this process.

PL-09-04 | "Keep the heart of the world blood donor day beating: A challenge in times of COVID-19"

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Background: The first World Blood Donor Day (WBDD) was in 2004 and is now celebrated each year throughout the world on 14 June to recognize the contribution of voluntary unpaid blood donors in saving lives and improving health. The WBDD has the purpose to create awareness about the need for availability and appropriate use of safe blood and blood products. The date of 14 June is the birthday of Karl Landsteiner, who discovered the ABO blood groups. In 2020 Italy was designated as the host country of the celebration of the global event, however the COVID-19 outbreak called for its postponement. In 2021 the epidemiological contingency obliged to rethink the organization but it also gave the chance, despite all, to reach a wider audience than ever, through the use of virtual solutions and streaming technologies.

Aims: The aim of the talk is to present an overview of the last WBDDs and to focus on the forthcoming edition highlighting the new challenges and opportunities given by the COVID-19 burden, which urged to rediscuss the contents of the initiatives.

In particular, in view of this, the scientific symposium will propose to the audience, which will be mainly composed of stakeholders (institutional and scientific, blood donors associations etc.) coming from different economic and social contexts, topics that are nowadays more requested, especially with a training and benchmarking perspective (e.g. donor management, contingency planning, donor recruitment in emergency situations, horizon scanning etc.).

Methods: So to respond to the epidemiological situation, many organisational adjustments are being made. The participation in residential events will no longer be possible leaving the floor to a virtual scenario also for the main institutional celebrative event and for the donors village. The latters will be anticipated by a "road to WBDD", which consists of a Hackathon and webinars addressed to general public.

Results: The 2021 WBDD edition aims to meet the new specific information needs tailoring them according to the target audience and in response to the epidemiological contingency.

Summary/Conclusions: Due to the global outbreak, the new organisation of WBDD can be considered a strength in terms of reaching out a larger number of people worldwide.

Red Cell Immunohaematology – Serology

P-001 | Serological evidence demonstrating that RHCE*CEMI (RHCE*03N.01) is not a null allele

selected for main programme

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Background: While studying the weakened expression of a e antigen encoded by a *RHCE*ceMO* allele, Pirenne et al. described in 2001 (*Brit J Haematol*) a subject originating from Congo who was also carrying a second rare allele, on a *RHCE*cE* background, that was named *RHCE*cEMI* after the proband's name. *RHCE*cEMI* corresponds to a 9-nucleotide deletion in exon 3 (c.350_358del CCATGAGTG) of *RHCE*. As discussed in the original manuscript, "this deletion does not disrupt the reading frame, but is predicted to result in a shortened polypeptide of 414 residues, instead of 417…." Because there was no evidence of a E antigen expression (including by flow cytometry or Western blot analysis), and despite *RHCE*cEMI* transcripts were found, it was supposed that "the loss of residues from a transmembrane or a junction domain alters the protein conformation and may result in the complete absence of the mutant protein at the cell surface". Although a drastic reduction of expression of the RhcE mutant protein was not excluded by the authors, the *RHCE*cEMI* allele was classified as a null allele and registered as *RHCE*03N.01* or *RHCE*cEN.01* in the ISBT Blood Group Allele Tables.

Aims: Samples from 5 individuals originating from French Guyana (African ancestry) were referred to our laboratory from 2008 to 2020, for the suspicion of an exceptional D – – type. In our laboratory, those patients (4 pregnant women, 1 newborn) were confirmed with our routine reagents to be D+C–E–e–, but a very weak c antigen expression was observed. As all of them were found to be homozygous for the *RHCE*cEMI* allele, this prompted us to study their reactivity against potent anti-RH17 (-Hr_0) antisera produced by D – – individuals (confirmed to be RH:–17 at the molecular level).

Methods: Several sources of anti-RH17 previously identified in our laboratory were tittered in a micro-column anti-IgG antiglobulin (IAT) device against papain-treated standard RBCs. The strongest antisera (titer >256) were tested on the patients' RBCs in the same IAT technique, when ABO compatibility allowed. Data of previous serological studies (standard RHCE typing, specific studies with anti-E and anti-c monoclonal reagents, antibody screening) were also considered.

Results: All the 5 patients expressed a weak c antigen when tested with Ortho Biovue System but no reaction was found with anti-E and/or anti-c with other commercial reagents. Patients 2 and 3 were tested with a panel of monoclonal anti-E and anti-c reagents, but showed only a weakened reaction (2+) with one monoclonal anti-E reagent (Hiro18). For RH17 phenotyping, 2 patients could not be tested (no cryopreserved RBCs available). The 3 other patients (when the source of anti-RH17 was ABO compatible) gave positive reactions varying from 2+ to 4+, depending on each antiserum titer (256 to >2048). All pregnant women had a negative antibody screen, except one with a weak anti-Le^b.

Summary/Conclusions: The original subject who was found to carry a *RHCE*cEMI* allele could not be fully explored by serology because of the heterozygous status (*RHCE*ceMO/RHCE*cEMI*).

The serological study of 3 homozygous individuals clearly showed that the RhcE protein is unambiguously expressed, as potent anti-RH17 antisera were clearly reactive on such RBCs. Furthermore, the absence of Rh antibodies in the 4 pregnant women suggests that most epitopes of the standard RhcE protein are expressed. Nevertheless, it remains unclear whether the c and E antigens that are of weak expression could also be partial.

P-002 | Anti-GIL in an individual with a novel AQP3 inactivating mutation resulting in the rare GIL phenotype; A serological and molecular case study with clinical outcome data

selected for main programme

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Background: Aquaporin 3 (AQP3) is a glycerol and water channel protein present in a variety of cells, including erythrocytes. This multipass membrane aquaglyceroporin is the carrier of the GIL antigen, the single member of the Gill blood group system. The encoding gene, *AQP3*, consists of six exons located on chromosome 9p13. The GIL_{null} (GIL-) phenotype is very rare; of the six known GIL- individuals, five had a single mutation in *AQP3* intron 5 splice site, leading to the skipping of exon 5, a frameshift and premature termination of protein translation. The sixth, most recently

described example was encoded by a missense homozygous mutation in *AQP3* exon 3, which was predicted to dramatically affect the structure of the protein and in turn cause the lack of expression of GIL. Owing to the rarity, limited information is known about the clinical significance of anti-GIL.

Aims: We investigated samples from a pregnant patient presenting with an alloantibody to an unknown high frequency antigen, with the aim to determine the unknown specificity of the alloantibody and exclude other clinically significant antibodies. The patient's previous history of pregnancy and transfusion was unknown. We also aimed to provide clinical outcome data about this rare antibody.

Methods: Serological investigations were performed by standard LISS tube IAT with untreated and enzyme treated cells. High frequency antigen screening was carried out, as well as extended enzyme studies. Standard alloadsorption and elution methods were used. Genomic DNA was extracted from whole blood and all the exons of *AQP3* were amplified by PCR and Sanger sequenced.

Results: The patient's plasma was moderately reactive with all panel cells and fresh donor cells tested. Cells treated with enzymes (papain, trypsin, chymotrypsin, pronase) and DTT remained reactive with the patient's plasma. Alloadsorption studies revealed no underlying alloantibodies. The patient's cells were found to be negative with two examples of anti-GIL. An eluate prepared using the patient's plasma was compatible with the one and only example of GIL− cells available. Sequencing of *AQP3* revealed the patient to be homozygous for a novel duplication c.277_283dupCTGGCTC in exon 3. This duplication would introduce a reading frame shift, which in turn would encode a premature stop codon p.Arg95ProfsTer4, potentially resulting in a truncated AQP3.

After multidisciplinary planning, a live male infant was delivered by elective Caesarean section at 38+6 weeks gestation, weighing 3030g. Cell salvage was utilised during delivery, however weighed blood loss was only 300ml with no immediate postoperative complications. Cord bloods were taken for DAT which was negative. The baby did not require phototherapy for jaundice and he was discharged home on day 3 with routine postnatal care.

Summary/Conclusions: We have identified the latest novel molecular basis for the rare GIL- phenotype, c.277_283dupCTGGCTC in *AQP3* exon 3, resulting in a shortened AQP3 and no expression of GIL. In this case there were no signs of anti-GIL causing HDFN.

P-003 | Impact of prophylactic red blood cell (RBC) transfusion with extended antigen matching on alloimmunization in patients with sickle cell disease (SCD)

selected for main programme

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Background: RBC alloimmunization remains a significant problem for many patients with SCD. To reduce alloimmunization some strategies have been implemented to provide limited (C/c, E/e, K) or extended antigen matched (C/c, E/e, K, Fya/b, Jka/b, S/s) RBC transfusions to patients with SCD who need chronic transfusion support. There is no consensus if prophylactic extended antigen matching significantly reduces the rate of RBC alloimmunization in patients with SCD compared to limited antigen matching.

Aims: Based on this, the aim of this study was to evaluate the effects of prophylactic RBC transfusion with extended antigen matching on rate of alloimmunization in patients with SCD chronically transfused.

Methods: A retrospective study was conducted in the period from March 2000 to March 2020. Our study included 179 (73 male and 106 female) patients with SCD, homozygous for HbS, on chronic RBC transfusion therapy receiving prophylactic RBC transfusions with antigen matching. In this period, 91 patients received RBC units matched for ABO, Rh and K and 88 patients were matched with selected donors for ABO, Rh, K, Fya/Fy/b, Jka/Jkb and S/s antigens. Among the patients, 60 were alloimmunized against RBC antigens and 119 patients were not. Serological typing was performed in patients and donors by gel cards (Biorad). Molecular typing was performed in all patients who had recent transfusions or a positive direct antiglobulin test by Laboratory Developed Tests (LDT) and HEA BeadChip (Immucor) to predict their antigen profile. RH variant alleles were determined in patient's antigen-positive who developed Rh antibodies using *RHD* and RHCE BeadChips (Immucor). Adsorption onto autologous RBC was also performed to aid the differentiation of autoantibodies from alloantibodies.

Results: Alloimmunized patients received a range of 5-515 units while non-alloimmunized patients received a range of 5-289 units. The median age in the alloimmunized group is 38.4 and in non-alloimmunized is 41. Of the 60 alloimmunized patients, 27 (45%) were alloimmunized before they started receiving limited or extended antigen matched RBC transfusions. Of the 91 patients receiving Rh and K matched RBC units, 22 (24.2%) produced alloantibodies (3 Anti-Fy^a, 4 anti-Jk^b, 6 anti-S, 1 anti-Di^a, 1 anti-C^w, 4 anti-e, 1 anti-D, 2 anti-C) during these 20 years. Of the 88 patients receiving extended antigen matching, 11 (12.5%) were alloimmunized (1 anti-D, 1 anti-E, 3 anti-C, 4 anti-e, 1 anti-Js^a, 1 anti-Kp^a). Thirteen patients who developed Rh antibodies had RH variant alleles. Autoantibodies were found in 8 patients (6 receiving limited antigen matching and 2 receiving extended antigen matched RBC units).

Summary/Conclusions: RBC transfusions with extended antigen matching had significant effects on autoimmunization and alloimmunization rates in chronically transfused patients with SCD over 20 years. SCD patients may benefit from receiving RBC transfusions with extended antigen matching as demonstrated by the lack of antigens on FY, JK and MNS systems. However, this strategy does not avoid alloimmunization to antigens against Rh system.

P-004 | Automated extended antigen typing - Monoclonal reagents

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Background: Automated extended antigen typing is used as a qualitative method for donor/patient characterization to ensure compatible blood transfusions and organ transplantation. Currently, monoclonal antibody reagents for the extended antigen typing, such as S, s, Fya, Fyb and Cellano on the surface of human red blood cells are not widely available on automated platforms. Manual methods are both labor intensive and error prone, making it difficult to obtain highly reproducible results. In an attempt to make extended antigen typing using monoclonal blood grouping reagents more reproducible and reduce hands on technician time, Immucor has developed a set of fully automated monoclonal extended antigen typing assays for NEO Iris and NEO v2.0.

Aims: The aim of these studies was to evaluate the performance of the monoclonal extended antigen typing assays for the detection of S, s, Fya, Fyb and Cellano antigens on Immucor's automated platforms NEO Iris/NEO v2.0.

Methods: Immucor has developed four (4) fully automated assays for the detection of extended antigens S, s, Fya, Fyb and Cellano. While the S and s antigens will be detected simultaneously in an antithetical paired assay, the detection of Duffy antigens as well as the Cellano antigen will be performed individually in single assays. Anti-S, Anti-s and Anti-Fyb antibodies are of the IgM isotype and are therefore configured as direct agglutination assays. For the detection of the Fya and the Cellano antigen, Capture-R® Select based assays were created as the respective antibodies are IgG class antibodies.

Repeatability and reproducibility (R&R) of the assays was assessed following the 3 x 5 x 2 x 3 study design recommended by CLSI. Five (5) donor specimens per assay were tested in triplicate, morning and afternoon on five non-consecutive days within 10 days on three (3) automated instruments resulting in 450 results per assay. In a second study, the instrument's ability to reliably detect the antigen of interest was evaluated by running 961 (S/s paired assay), 650 (Cellano assay) and 907 (Fy^a and Fy^b single assays) patient and donor samples on the automated monoclonal blood grouping assays and comparing the data to the results obtained with the manual tube test method. The result consistency was also assessed by testing a certain amount of specimens with at least two (2) different lots of reagent. Additionally, turnaround times for processing the extended antigen typing assays on the NEO Iris/NEO v2.0 were determined.

Results: The automated monoclonal blood grouping assays demonstrate very high repeatability and reproducibility across different automated instruments and different time points. 450 results were analyzed per assay. No qualitative result changed from positive to negative or vice versa when tested over a time period of 10 days. Method comparison of the automated monoclonal blood grouping assay and manual tube test method demonstrated high result concordance. For all assays, high sensitivity and specificity on the 95% lower-bound confidence interval was achieved. Within the lot to lot comparison study not more than one (1) reaction grade difference was observed between lots. Turnaround times for the extended antigen typing assays on the automated platforms are considerably low compared to manual methods.

Summary/Conclusions: Verification of the automated monoclonal blood grouping assays demonstrate extremely high result consistency and thus provide a more accurate and efficient alternative to manual methods.

P-005 | 2M urea microplate method for JK:–3 screening

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Background: The Kidd blood group system consists of 3 antigens located on a multipass type 3 membrane glycoprotein that functions as the primary urea transporter on red blood cells (RBCs). JK:–3 is the null phenotype of Kidd and types serologically as Jk(a–b–). The current ISBT allele table for JK (v6.0) lists 21 null alleles on a *JK*01* background and 21 null alleles on a *JK*02* background, which makes screening for nulls difficult to perform by genotyping, as is the case for nulls in most blood group systems. JK:–3 is found in approximately 0.9% of Polynesians, however is rare in all other populations. Because anti-Jk^a and anti-Jk^b commercial antisera in the United States is expensive and polyclonal antisera from donors may be limited in supply, it is often not desirable to use antisera to test donors for Jk^a and Jk^b that do not have an R_1R_1, R_2R_2, R_0R_0, or rr phenotype. However, by not screening all donors in an antigen testing program, it is possible to miss a rare JK null donor.

Aims: It is well established that JK:–3 cells are resistant to lysis by 2M urea. Most methods are test tube based and use 1ml of 2M urea that is prepared with 0.4% NaCl. The aim of this study was to develop a method that would utilize 2M urea to screen for the JK:–3 phenotype, and in particular one that could be implemented in a moderate to large scale antigen testing program that uses standard 96 well U-bottom microplates. It should also not add significant hands on time to testing and be economical.

Methods: Suspensions of 1.5 – 2% RBCs are prepared in phosphate buffered saline (PBS) and are used for antigen testing via a microplate method for RH/K antigens by direct agglutination as well as additional antigens via an indirect antiglobulin test (IAT) as is routinely performed by our laboratory. After reading the reactions, the plates are centrifuged and the supernatant is decanted, leaving a dry cell button. To this is added 1 drop of 2M urea prepared in distilled water with no added NaCl (6.0 g of urea pellets

brought to 50 ml with H_2O). The microplate is mixed and hemolysis is read in 5 – 15 minutes. Wells with hemolysis are positive for JK:3. If no antigen testing is to be performed and only screening for JK:3, the 1.5 – 2% suspension is added to each well of the microplate, it is centrifuged and decanted, and subsequently tested per as above.

Results: To evaluate the method, standard RBC cells (positive control) were tested and compared to JK:–3 cells from our frozen rare cell library and fresh cells collected from two JK:–3 donors (negative control). It was found that cells testing as positive or negative by direct agglutination, by IAT, or with no antigen typing before screening all gave satisfactory results. It is important to read the test within 15 minutes because even JK:–3 cells will lyse if allowed to sit for a significant time longer than this. The method is extremely economical, with the 2M urea costing only approximately $0.50 USD (0.43 Euro) per 1000 donors tested.

Summary/Conclusions: Here we present an economical method that could be used on a moderate to large scale for screening donors for JK:–3 by 2M urea in microplates that can be easily tested on all donors as part of an antigen testing program.

P-006 | Unexpected passive anti-D in donors - Cause and effect

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Background: At Canadian Blood Services (CBS), red blood cell antibody screening is performed on all blood and plasma donors on each donation. This testing is Health Canada licensed and performed on the NEO automated platform (ImmucorGamma Norcross, GA). Historically, plasma containing clinically significant antibodies was released for further manufacturing of plasma protein products. In 2019, CBS' deferral process changed to align with fractionator requirements: plasma from donors with Anti-D (current or historical) is no longer used for fractionation. To prevent unnecessary and permanent plasma program deferrals, we began to investigate further when female donors of child-bearing age presented with a new Anti-D to determine if it may have been passively acquired.

Aims: Review the number and location of donors with passive Anti-D, clarify the typical donor and serological features of passive Anti-D, investigate possible causes for passive Anti-D identification in donors, and determine strategies to mitigate future occurrences.

Methods: Initial antibody screening is performed with Capture-R Ready Screen (Pooled Cells) reagent using a solid phase antibody detection method. The ID-Micro Typing System Gel test method (Ortho Clinical Diagnostics Raritan, New Jersey) and manual tube indirect antiglobulin test using PEG POTENTIATOR as an enhancement, are techniques in the antibody investigation process. Once an Anti-D is identified, the following are clues it may be passively acquired:

- Antibody strength is weaker than usual
- Female of child-bearing age
- First-time donor or previous negative antibody screen on recent donation
- Donor questionnaire indicating recent medical treatments or pregnancy

Donor with suspected passive Anti-D are contacted to determine history. If RhIG injection is confirmed, the antibody is resulted as passive Anti-D, thereby allowing the donor to participate in future plasma collections after a temporary deferral for receipt of a blood product.

Results: Over 10 months, passive Anti-D has been suspected in eight donors across Canada. Six of eight donors confirmed RhIG administration although all answered 'No' to the donor screening question concerning receipt of blood products. Six of the eight had donated previously, all with a prior negative antibody screen. All donors had an antibody investigation reactivity strength of less than or equal to 2, and six of eight had 1+ reactivity. It is expected that the antibody screen will be negative on next donation for donors with a passive Anti-D, but none have returned.

Summary/Conclusions: Distinguishing between an immune and passive Anti-D is now significant from a donor perspective - global demand for plasma protein products is high and supply is limited. Misidentifying passive Anti-D as immune results in permanent plasma program deferral and automatic discard of future plasma components. The donor screening process should identify donors who have received blood products and temporarily defer them for six months. To be able to answer the screening questions accurately, it is critical donors recognize RhIG as a blood product. It is evident from our investigations this understanding is lacking. Opportunities to improve education exist at two key process points: as a patient, when consenting to RhIG administration, and as a blood/plasma donor, at time of screening.

P-007 | Applicability of international guidelines for red cell specifications for use in low-resource settings

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Background: Red cell (RBC) transfusions are a life-sustaining therapy for patients with β-thalassemia and sickle cell disease (SCD), but having multiple transfusions is associated with a risk for alloimmunization. In 2018, the International Collaboration for Transfusion Medicine Guidelines (ICTMG) published a guideline (GL) with recommendations

P-007 Table 1. Laboratory testing capabilities for/on SCD patients

	Can perform 37C° IAT XM procedures on units*	Can perform an IAT RBC antibody screen*	Can perform IAT RBC antibody identifications*	Can perform RBC phenotyping beyond ABO, RhD
Yes/No	28/2	27/3	24/6	16/14
Performed for every SCD patient	22 (79%)	15 (56%)	NA	7 (44%)
Never performed	2 (7%)	8 (30%)	NA	7 (44%)
Performed for selected patients or rarely	4 (14%)	4 (14%)	NA	2 (12%)

*Or has easily-available access to a laboratory that can perform testing. IAT: indirect antiglobulin test, XM: crossmatch.

P-007 Table 2. Implementation of GL recommendations

Recommendations	Implemented for all SCD patients/ for some or rarely/for none (total)	Implemented for none of the SCD patients but very/partially/moderately/ not feasible to implement (total)
1. Patients with SCD who do not have alloantibodies and who are anticipated to have a transfusion (simple or exchange transfusion) should probably be transfused with CcEe and K matched RBCs to reduce the risk of alloimmunization	6/6/16 (28)	2/2/5/7 (16)
2. Patients with SCD who have one or more clinically significant alloantibodies should be transfused with antigen negative blood to alloantibody(ies), if feasible	12/5/8 (25)	0/0/5/3 (8)
3. Patients with SCD who have one or more alloantibodies should probably be transfused with CcEe K Fya Fyb Jka Jkb Ss matched RBCs to reduce the risk of alloimmunization, if feasible and if matching does not cause undue delays that adversely affect patient care	6/2/16 (24)	1/1/8/6 (16)

on RBC matching strategies to help standardize transfusion practices and optimize outcomes for these patients.

Aims: The ICTMG conducted a survey to understand the applicability of the GL in low-resource settings.

Methods: 225 ISBT members from low-or low-middle income countries were sent a link to an anonymous survey. Responses were included.

Results: 30 individuals (India 11, sub-Saharan Africa 9, and other/undisclosed 10) representing facilities supporting SCD patients responded. A minority of the respondents were aware of the GL before receiving the survey (11/28) and of ICTMG accompanying resources (10/21). Of the resources provided to supplement the GL, the Journal Club was considered the most supportive while the others were ranked equally (podcasts, presentation and algorithm). Most suggested that a companion set of recommendations adapted to low-resource settings would be helpful. Tables 1 and 2 show the results for laboratory testing capability and implementation of the recommendations.

Summary/Conclusions: Although only a small number of surveys were completed, our study suggests that few ISBT members were aware of the GL and resources. Furthermore, in countries where most SCD patients reside, the immunohematology testing recommended by the GL cannot always be performed and the recommendations have not always been implemented. Support for low-resource countries is required to assist with awareness and implementation of guidelines as these countries care for the majority of the SCD patients worldwide.

P-008 | Simultaneous blood group phenotyping of FYA, FYB, JKA, JKB, S (BIG) and S (SMALL), M and N using MDmulticard lateral flow technique

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Background: A lateral flow assay for simultaneous typing of Fy^a, Fy^b, Jk^a, Jk^b, S and s with stable end-point and without a centrifugation step is in routine use since several years (Caesar, Vox Sang, 2018).

Aims: The aim of this study was to evaluate the performance characteristics of simultaneous blood group typing in lateral flow technique of antigens M and N together with Fy^a, Fy^b, Jk^a, Jk^b, S and s.

Methods: In 229 fresh blood samples, comprising 170 samples of blood donors and 59 authentic clinical samples of hospitalized patients, Fy^a, Fy^b, Jk^a, Jk^b, S, s, M and N blood groups were tested. The lateral flow based assay containing these parameters (MDmulticard, Medion Grifols Diagnostics, Duedingen, Switzerland) was compared with established CE certified liquid antisera reagents (Anti-Fy^a Mono-Type, Anti-Fy^b Mono-Type, Anti-Jk^a for DG Gel, Anti-Jk^b for DG Gel, Anti-S Mono-Type, Anti-s Mono-Type, Anti-M Mono-Type Dual, Anti-N (LN3/MN879) Mono-Type, Medion Grifols Diagnostics) used in combination with gel technique (DG Gel Coombs Cards, DG Gel Neutral Card, Diagnostic Grifols, Parets del Valles, Spain).

The credit-card sized lateral flow test device consists of a membrane, which is equipped in a cassette housing. Two equidistant detection areas with parallel lines of antibody reagents against Fy^a, Fy^b, Jk^a, Jk^b, S, s, M and N are left and right of a central application zone. Both detection areas contain a process control spot (val) and an auto control spot (ctl). One cassette can be equipped for the simultaneous detection of up to 10 different blood group specificities.

For blood group typing, 100 ml of diluted whole blood or erythrocyte sediment are pipetted to the central application zone, followed by two consecutive rinsing steps with 300 ml each of a rinsing solution. Results may be interpreted after about 8 minutes. Positive results clearly impose as distinct red bands, whereas negative results lack the respective bands.

Results: For M and N antigens, all results of the blood samples tested were in full accordance with those of the CE certified comparative methods.

Summary/Conclusions: MDmulticard lateral flow technique was presented earlier with unique features, e. g. simultaneous multiparameter testing of ABD, Rh phenotype and K antigens without the need of centrifugation and results within 5 minutes. Later, we were able to reproduce these characteristics also in the detection of Fy^a, Fy^b, Jk^a, Jk^b, S and s. Here, we demonstrate that directly agglutinating Anti-N and Anti-M murine IgG class antibodies complement in one single, homogeneous assay an indirectly agglutinating murine/human IgG class antibody (Anti-Fy^a) and murine/human IgM class antibodies Anti-Fy^b, Anti-Jk^a, Anti-Jk^b, Anti-S and Anti-s), while blood group typing with IgG/IgM class antibodies require different phases and incubation times and temperatures in other techniques.

P-009 | Diagnostics and blood transfusion in patients with autoimmune haemolytic anaemia

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Background: Patients with autoimmune haemolytic anaemia (AIHA) frequently have severe anaemia that requires a blood transfusion. Autoantibody is usually present on patient's red blood cells (RBCs), but is also present in the patient's plasma, where it usually reacts with all RBCs, making it impossible to find a compatible blood. The survival of transfused RBCs is similar to patient's own RBCs. A broad reactive autoantibody may mask the presence of alloantibodies in compatibility tests, which may result in a haemolytic transfusion reaction (HTR).

Aims: Aim of this study was to analyse serological characteristics of autoantibodies and effectiveness of blood transfusion in patients with AIHA.

Methods: We analysed 72 adult patients with AIHA, who were treated at our hospital during period from 2014 to 2020. For diagnostics of AIHA, anaemia with features of haemolysis (elevated bilirubin and/or elevated lactate dehydrogenase and/or low haptoglobin level) and a positive direct antiglobulin test (DAT) were present, while other causes of haemolytic anaemia have been excluded. According to haemoglobin (Hgb) value at diagnosis, patients were divided into two groups: patients with severe (Hgb<80 g/L) and mild AIHA (Hgb≥80 g/L). Effective transfusion was defined as an increase in post-transfusion Hgb value ≥5 g/L. per units of blood.

Results: A total of 72 patients met the criteria for AIHA with a mean age of 61 years (range 21-90). Among them 51.4% were female. Primary AIHA was diagnosed in 41.7% and secondary AIHA in 58.3% of patients (haematological and lymphoproliferative diseases, immune diseases, solid tumours, infective diseases, after allogeneic haematopoietic blood stem cell transplantation and drugs). Mostly warm AIHA (73.6%), then cold agglutinin disease (CAD), and mixed AIHA (15.3% and 6.9%, respectively) were diagnosed. Other types of AIHA were paroxysmal cold haemoglobinuria (PCH), DAT negative AIHA and drug induced AIHA (each 1.4%). Mean Hgb value at the diagnosis was 63.6 g/L. A total of 77.8% of patients were transfused. Effective transfusion was noted in 63.1% transfusion events. Unknown data were noted in 12.3% transfusion events. Patients with warm AIHA and CAD had higher increase in mean Hgb value (9.0 g/L and 9.8 g/L, respectively) than in other types of AIHA (6.0 g/L). Increase in mean Hgb value was higher in patients with severe AIHA than in patients with mild AIHA (8.5 g/L and 4.7 g/L, respectively). In six 6 (8.3%) cases with AIHA, alloantibodies were detected. There were no HTRs reported that were caused by alloantibodies.

Summary/Conclusions: Effective transfusion was noted in more than 60% transfusion events. Patients with severe AIHA had higher increase in Hgb value compared to patients with mild AIHA. The highest increase was seen in patients with warm AIHA and CAD.

P-010 | Validation of the new DTT method adapted for gel testing to mitigate the panagglutination of anti-CD38 monoclonal antibody daratumumab in the indirect antiglobulin test (IAT)

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Background: Daratumumab (DARA) has been approved for the first time in 2015 for patients with multiple myeloma with relapsed/refractory disease. This anti-CD38 therapy leads to positive and panreactive agglutination reactions in indirect antiglobulin tests. Numerous alternate approaches have been suggested to overcome these limitations. Oporto Regional Blood Centre has adopted CD38 denaturation on test RBCs using DTT technique according to the AABB Technical Manual, which is time consuming, and damages several red blood groups antigens, including such clinically antigen as Kell. More recently, a new method was proposed employing low concentration DTT treatment of red blood cells (RBCs) adapted for column agglutination (Valencia method). Replacing pre-treatment of RBC by parallel incubation of plasma, cells, and DTT in the reaction chamber of the gel test cards, makes the method much faster with no need for

separate washing steps. Other advantage is the use of gel testing, which is the worldwised method most used in the blood transfusions services.

Aims: Our aim was to validate a new method (Valencia method) performed on a gel microcolumn, to overcome the interference caused by anti-CD38 monoclonal antibodies in pre-transfusion tests, using a lower concentration of DTT in order to preserve clinically RBC group antigens.

Methods: Plasma samples of two patients undergoing DARA were screened for the presence of irregular antibodies using commercial RBCs panels of 3 cells (ID-Diacell I-II-III, BioRad, Diamed GmbH) and Anti_Human Globulin (AHG) gel test cards (BioRad, Diamed GmbH). Low DTT concentration of 0,04M was prepared by diluting 0,2M stock solution with PBS at pH 7.0. The same samples were inoculated with alloantibodies from previously identified (Nekas sample: anti-K plus anti-Fy^a; Donor sample: anti-E plus anti-Fy^a), in the ratio of volume 2:1 respectively. Panreactivity was demonstrated in the panel of 11-RBC panel (ID-DiaPanel, BioRad, Diamed GmbH) treated with the new DTT method. The procedure for treating DTT was carried out in accordance with that described in article: "*Izaguirre EC, et al. New method for overcoming the interference produced by anti-CD38 monoclonal antibodies in compatibility testing. Blood Transfus. 2020 Jul;18(4):290-294*".

Results: The two samples of DARA patients were tested for the presence of irregular antibodies and showed panreactivity. After treatment with 0.04M DTT this interference was eliminated. In samples from DARA patients inoculated with clinically significant antibodies, panreactivity was noted prior to treatment with DTT. After treatment, the interference was negative, which permitted the identification of the expected alloantibodies.

Summary/Conclusions: The assays allowed to verify the elimination of the anti-CD38 interference with low DTT concentration (0.04 M) while maintaining the ability to identificate the clinically significant antibodies used in our study. The degree of reactivity of the antibodies tested has been shown to be maintained after treatment with DTT, with the new Valencia method. As described, the low concentration of DTT preserved K antigenicity. This method has revealed itself to be an asset to our service because it is easy to perform, fast and reliable.

P-011 | Serologic characteristics of an anti-RH17 from a homozygous RHCE*03N.02 individual with a novel *RHD**1035A allele

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Background: Routine molecular techniques to predict Rh antigens target common single nucleotide variations (SNV). *RHD* and *RHCE* are highly polymorphic, which in turn translate structural and expressional variations of the RhD and RhCE polypeptides. Altered phenotypes are due to SNVs/indels and hybrid alleles of *RHD* replacing sections of its *RHCE* homologue. The *RHCE*cE 907delC* is a rare deletion that silences the *RHCE*03* translation. Anti-Rh17 alloimmunization occurs in D−, DC^w-, and Dc- individuals as a broad polyclonal response to the RhCE epitopes.

Aims: Investigation of unusual cross-reactive anti-RH17 in an 85-year-old group O RhD+ Hispanic female who presented for a fractured hip repair. She previously received seven RBC units following open heart surgery.

Methods: PCR-hydrolysis probe LDT assays were in-house developed techniques. Bidirectional DNA sequence analysis utilized standard Sanger techniques (Grifols, San Marcos, TX) and Seqscape software (ABI). Serological RH1, RH2/RH4, and RH3/RH5 phenotyping was performed with monoclonal class IgM anti-D (MS201), anti-C (MS24), anti-c (MS33), anti-E (GAMA402), anti-e (MS16/MS21/MS63) (Immucor, Norcross, GA), anti-C (MS24), anti-E (MS260/MS12), and anti-e (MS16/MS21/MS63) (Bio-Rad, Dreieich, DE). Polyclonal anti-RH17 was derived from human source antisera. Polyspecific anti-IgG, -C3d was used in direct antiglobulin testing (DAT) (Immucor, Norcross, GA). Solid phase red cell adherence (SPRCA), monospecific anti-IgG, polyethylene glycol (PEG), ficin red cell enzyme treatment (Immucor, Norcross, GA) and low ionic strength solution (LISS) (Alba Bioscience Ltd, Penicuik, UK) were utilized in indirect antiglobulin testing (IAT) for antibody identification.

Results: The serum was nonreactive at immediate spin, LISS-37C, LISS-IAT, and PEG-IAT with autologous red cells. The polyspecific DAT was negative. Anti-c (RH4) reactivity was observed with SPRCA and LISS-IAT in which anti-C, -E, -e, and all other common minor specificities were excluded. Panagglutination was observed in IAT following ficin treatment of red blood cells and PEG-IAT of nontreated cells. The serum failed to react with three different RH:-17 (D−) donors. All antibody reactivity was removed in the R_1R_1 and R_2R_2 x1 alloadsorbed serum. An elution of the R_1R_1 and R_2R_2 adsorbing cells reacted at PEG-IAT with R_2R_2, R_zR_1, and rr cells, but failed to react with R_1R_1 cells.

The individual's RBCs phenotyped RH: 1, -2, -3, -4, -5 with multiple sources of antisera. The RBCs failed to react at PEG-IAT with two sources of anti-RH17. Initial SNV genotyping detected homozygous c.307C and c.676C predicting an R_2R_2 phenotype. *RHCE* sequencing detected a homozygous c.907delC SNV (*RHCE*03N.02*). *RHD* sequencing identified a novel homozygous c.1035A silent SNV. Genotyping also revealed a heterozygous *FY*02N.01* allele.

Summary/Conclusions: An *RHCE*03* deletional allele is exceptionally rare. The c.907delC creates a premature stop codon translating a nonfunctional RhCE polypeptide. This individual's immune response produced anti-RH17 with apparent cross-reactivity to RH3 and RH4 identified in the eluate of both the R_1R_1 and R_2R_2 adsorbing cells as well as RH4 specific cross-reactivity in the unadsorbed serum. To the best of our knowledge, the *RHD* c.1035A is a novel silent SNV. Though the *RHCE*03N.02* has previously been associated with Hispanic populations, the heterozygous *FY*02N.01* allele identified is an indication of both Hispanic and African ancestry.

P-012 | Red blood cell immunization in adult major B-thalassemia: Study on a tertiary care hospital in Tunis

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Background: Blood transfusion remains a key treatment in the management of major B-thalassemia. However, the development of anti-red blood cell antibodies remains a major problem.

Aims: This study was performed to evaluate the frequency of auto and allo-immunization among polytransfused major B-thalassemia patients.

Methods: This is a descriptive cross-sectional study carried out over 9 months at the hematology department of a tertiary care hospital in Tunis. Our study included adult polytransfused major B-thalassemia patients. These patients receive transfusions with compatible and phenotyped in the Rhesus system red blood cells units. The cross-match was realized using indirect antiglobuline test. Irregular red cell antibody screening as well as the direct antiglobulin test were realized every three months and whenever there was a poor transfusional efficiency. Alloantibody screening and identification was done using 3 cell and 11 cell panel (Diapanel, Bio-rad, Switzerland) respectively. The search for autoantibodies by the direct antiglobulin test was carried out using polyspecific gel card (Diamed card). Epidemiological, clinical and biological data were collected from medical records.

Results: Our study included 41 patients: 22 women (53.6%); 19 men (46.4%), sex ratio (M/W) = 0.86. The mean age was 26.21 years with extremes [19 _ 36 years]. 30 patients (73%) were splenectomized. The mean interval between transfusions was 2 weeks with extremes [2 days _ 4 weeks]. The mean number of red blood cells units per transfusion episode was 2 with extremes [1 _ 4]. Red blood cell immunization was documented in 30 patients (73%): 29 autoimmunization (71%) and 10 alloimmunization (24%) including 9 cases of association of auto and alloantibodies (22%).The autoantibodies were IgG in 75% of cases (22/29) and IgG + C3d in 25% of cases (7/29).These autoantibodies caused reticulocytosis in 52% of cases (15/29). Concerning the 10 cases of alloimmunization: we were able to identify 5 allo antibodies (one anti D, one anti C, one anti S and two anti Kpa). In our study, immunization was identified in 73% (22/30) of splenectomized patients (autoimmunization in 70% (21/30), alloimmunization in 27% (8/30) and association in 23% (7/30) and in 73% (8/11) of non-splenectomized patients (autoimmunization in 73% (8/11), alloimmunization in 18% (2/11) and association in 18% (2/11).

Summary/Conclusions: Allo immunisation was detected in 24% of multi transfused thalassemia patients and auto immunisation in 73% of cases. This study supports the interest of systematizing the transfusion of RH Kell compatible red blood cells units in polytransfused thalassemia patients in order to reduce the rate of alloimmunization to the most immunogenic red blood cell antigens.

P-013 | Difficulties in identifying antibodies in the Dombrock blood group

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Background: Antibodies in the Dombrock blood group system have been reported to cause acute and delayed hemolytic transfusion reactions. They can be difficult to be identified especially in patients with multiple alloantibodies.

Aims: Identify particularity of complex identification of antibody involving Dombrock blood group.

Methods: The clinical and biological findings of the patient are recorded, and a review of the literature was conducted to identify additional Case reports.

Results: A 68-year-old Tunisian man was followed for myelodysplastic syndrome. In the preceding six months, he had received four units of RBCs at our center and was known to have anti KEL1 due to non phenotyped transfusion received at another hospital. He was admitted for an acute anemia (Hb: 5g/dl) with clinical signs of intolerance. Two units were ordered but the cross match test was incompatible. Investigation revealed negative direct antiglobulin test (DAT) and a panagglutination in Indirect antigloblin test(IAT).At the central blood bank, no compatible blood was available within more than eighty blood product tested and there was no national database of rare blood donors in our country.
Therefore, we collected blood samples from siblings for testing it with the patient's serum. Compatible blood was found from family donors and the patient received three units without incident. Samples were sent to a reference laboratory "CNRGS in Paris to be investigated. The (IAT) revealed antibodies without common specificities, anti-DO1, anti-KEL1, anti-RH3. After adsorption on homologous red blood cells we isolated anti-DO1, anti-RH3.The direct elution showed an antibody directed against a high frequency antigen with a reactive pan agglutination in IAT on native red blood cells and negative on red blood cells treated with papain. The phenotype was DO:-1,2. The genotyping confirmed the presence of the DO*2 allele in the homozygous state. Therefore, the genotype was DO*2/*2 .

Summary/Conclusions: The cooperation among family members, hospital personnel, and reference laboratory staff made it possible to provide blood for the patient, identify antibodies of Dombrock blood group system and avoid transfusion accidents.

P-014 | Hemolytic disease of the newborn associated with anti-JKB

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Background: Kidd blood group system is clinically significant as the antibodies could cause acute and delayed transfusion reactions and hemolytic disease of newborn (HDN) as well. In general, the severity

of HDN due to anti-Jkb is rare and the clinical symptoms tend to be mild.

Aims: we would like to present a case of HDN due to anti-Jkb.

Methods: standard serological principles of AABB technical manual

Results: The 3-day-old female infant was quite well after birth, but yellowish skin developed since 2 days old. Her capillary bilirubin level was 16.2 mg/dL, the evidence favored neonatal hyperbilirubinemia and the clinical manifestation revealed hemolysis symptoms. Her laboratory findings showed elevated reticulocytes (4.8%), G6PD (11.9 U/gHb), total bilirubin 17.01(mg/dl), direct bilirubin 0.51(mg/dL); DAT (1+), IAT (+), mild anemia (Hb 16.6 g/dl, Hct 46.7%), and blood smear showed tear drop cell (2+), schistocyte (1+), and polychromatic RBC. Her mother's blood typed was A, D positive, and had a positive antibody screening test; antibody identification showed Anti-Jkb. The RBC phenotype of our female infant was B, Rh(D) positive, Jka(+), Jkb (+). After receiving intensive phototherapy for hyperbilirubinemia, her clinical condition improved significantly, bilirubin level was within a normal range (13.6 mg/dL), she was discharged then. During hospitalization, he was no need for transfusion.

Summary/Conclusions: The maternal allo- IgG antibody will pass through the placenta, attacking fetal red cells which carry the corresponding antigen causing hemolytic symptom. In our institution, the most common allo-immune hemolytic diseases of the fetus are caused by anti-E, and this is the first case that showed anti-Jkb. Most of the HDN caused by anti-Jkb are mild-to-moderate, and our case confirmed those findings. Therefore, keep good communication with pediatricians is important to make sure the patients have a correct clinical diagnosis and well medical care as early as possible.

P-015 | A comparison of column agglutination and solid phase red cell adherence technologies for red cell antibody detection

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Background: Clinically significant red cell allo-antibodies can mediate destruction of transfused red cells, causing minor to severe morbidity and potentially death. Methods for pre-transfusion testing, therefore, should be sensitive, accurate, have acceptable turnaround times and ease of set up and interpretation for the detection of antibodies. There are multiple platforms available for testing including column agglutination technology (CAT) and solid phase red cell adherence (SPRCA) produced by various companies. Existing data have compared up to 3 methods in parallel.

Aims: We aim to compare four platforms for sensitivity and specificity in the detection of clinically significant red cell allo-antibodies.

Methods: Plasma aliquots for the study were initially tested by 3-cell antibody screen from patients at a tertiary hospital by the Ortho Autovue Innova analyser which utilises glass-bead CAT. Fifty samples demonstrating negative red cell antibody screen results and forty-eight samples with positive red cell antibody screen results were selected for the comparison then frozen and stored at -30 degrees Celsius. Samples were thawed and tested in small batches using four different platforms; BioRad CAT, Grifols gel CAT, Immucor Capture-R Echo SPRCA analyser and repeat testing by Ortho CAT.

Results: The 3 CAT methods produced assay sensitivity of greater than 90% whereas the SPRCA method displayed a sensitivity of 85.1%. All platforms performed comparatively to the initial AutoVue Innova analyser results as demonstrated by a tetrachoric correlation co-efficient of greater than 95%. Low titre antibodies failed to be detected in 4 samples by BioRad CAT, 2 by Grifols CAT, 8 by Echo SPRCA and 4 by Ortho CAT. No false positives were produced by CAT however SPRCA produced 2 unexpected positive results that were later identified as Anti- E.

Summary/Conclusions: Results using the four testing platforms were comparable to the initial AutoVue Innova CAT results, with the Grifols method showing slightly higher sensitivity/ specificity compared with other methods. However, considering tetrachoric correlation exceeded 95% for all methods, these systems are considered reliable for detection of red cell antibodies for the prevention of adverse outcomes and morbidity related to red cell transfusion.

P-016 | Red blood cell alloimmunization and autoimmunization among transfusion-dependent patients in Saudi Arabia

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Background: Sickle cell disease (SCD) and thalassemia are prevalent inherited blood disorders in Saudi Arabia, particularly in Jazan area. Multiple red cell transfusion units are required for these patients, which may cause alloimmunization due to incompatible blood group antigens.

Aims: This study investigated the frequencies of alloimmunization and autoimmunization in patients with SCD and thalassemia in Jazan area of Saudi Arabia as well as the involved antibodies.

Methods: A cross sectional study was carried out among transfusion-dependent patients in Prince Mohammed bin Nasser Hospital, Jazan Province, Saudi Arabia. A total of 438 patients, (385 SCD, 52 β-thalassemia, and one α-thalassemia), who underwent to blood transfusions were enrolled to the present study.

Results: The frequencies of alloimmunization and autoimmunization in patients with SCD were 12.98 and 0.52%, respectively. Regarding patients with thalassemia, the alloimmunization and autoimmunization rates were 13.21 and 3.77%, respectively. The most prevalent antibodies among the entire study population were anti-E (17.19%) and anti-KEL1 (14.06%). Other observed alloantibodies included anti-S (1.56%), anti-s (1.56%), anti-D (7.81%), anti-C (3.13%), anti-c (6.25%),

anti-Lu^a (1.56%), anti-Kp^a (3.13%), anti-Fy^a (3.13%), anti-Jk^a(7.81%), and anti-Jk^b (4.96%).

Summary/Conclusions: The frequencies of alloimmunization and autoimmunization among SCD and thalassemia patients were determined in Jazan area. The outcome of this study emphasize the requirement of extended phenotyping for transfusion-dependent patients as well as blood donors for better transfusion practice. It is highly recommended to use blood group genotyping in parallel with serological phenotyping in Saudi Arabia for patients safety.

P-017 | False positive indirect coombs test results in a cancer patient at King Hussien Cancer Center due to a manufacturer's cells preservatives: A case study

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Background: In our Blood bank we rely on antibody screening and identification on two systems that are BIO-RAD and Grifols.Each of these systems had provided our blood bank with a comprehensive and a reliable gel heamaglutination systems which helped in the prevention of transfusion reactions.

Since we introduced these systems the advantages and the disadvantages of each system are clearly showing which complete each other in a way that helps us in troubleshooting with a vast experience we gain.

Aims: In our blood bank we investigated a patient that gave a positive antibody screening using Bio-Rad screening cells and a negative antibody screening on Grifols cells. Our aim was to rule out the presence of alloantibodies.

Methods:

- The 3 cell panel (screen-Cyte 0.8% I-II-III Grifols) and the 11 cell panel (Data-Cyte Plus 0.8%, Grifols) using Grifols poly specific anti human globulin cards (DG Gel Coombs)
- The 3 cell panel (ID-DiaCell I-II-III, Bio-Rad) and the 11 cell panel (ID-DiaPanel, Bio-Rad) using Biorad poly specific anti human globulin cards(ID – card LISS/Coombs)

Results: A case study: A66 years old female patient diagnosed with bladder cancer was admitted to KHCCs ER suffering from general fatigue and weakness due to low Hb level of 7.8g/dl with no history of previous blood transfusions or chemotherapy.

The physician ordered one unit of packed RBCs for her; after the request was received to Blood Bank the indirect coombs test showed a positive results with all three cells of Bio-Rad screening cells.

A panel of 11 cells was conducted using both Bio-Rad(ID-Dia Panel) and Grifols(Data cyte Plus 0.8%) systems and the results showed a negative reactivity with all Grifols cells and a positive reactivity with Bio-Rad cells. alongside we performed compatibility testing with 6 units of packed RBCs and all were compatible on Grifols antihuman globulin gel cards and recross matched two of the six units on BioRad antihuman globulin cards and they were also compatible. To confirm the previous results a second sample was ordered and the results were the same as the first sample.

After that we washed the screening cells of Bio-Rad 3 times with normal saline and resuspended them with LISS and then performed the indirect coombs test and the results showed a negative reactivity eliminating that the reaction was from irregular antibodies that reacts with BioRad cells .

To confirm that the preservative combined with the patients plasma was the cause of that positive reactivity we:

- Obtained results with Bio-Rads preservative material only.
- The preservative alone cross matched with Grifols cells.
- Mixed Bio-Rad preservative with patients plasma and crossed with Grifols cells.

The results were as the following: a negative reactivity with the preservatives alone whereas a positive reactivity with the plasma and the preservatives .

As a final step we used Bio-Rad screening cells on Grifols cards and they showed also a positive reactivity results excluding that the reaction was from the cards materials.

Summary/Conclusions: We concluded that the reaction caused by interactions from patients plasma constituents and buffers or antibiotics used in Bio-Radcells preservatives.

- Biorad cells are buffered in suspension medium at 0.8% (± 0.1%). Preservatives: the antibiotics trimethoprim and sulfamethoxazole.
- Grifols cells are added to preservatives (0.010% (w/v) neomycin and 0.017% (w/v) chloramphenicol).

For this patient in particular washed cells of Bio-Rad or Grifols cells was recommended to use.

P-018 | Variation of AIHA at central blood transfusion service – Indonesian Red Cross

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Background: Immune hemolytic anemia is characterized by clinical and laboratory features of hemolytic anemia with direct antiglobulin test (DAT) positivity. It could be autoimmune hemolytic anemia (AIHA), alloimmune, or drug-induced hemolysis based on the antigenic stimulus.

Aims: The study the occurrence of RBCs autoimmunization among multiple transfused AIHA patients and identify blood group antibodies potentially developed and induce antigen-antibody reaction affecting patients. Study was carried out in 133 multi-transfused patients recorded with AIHA registered at Central Blood Transfusion Service Indonesian Red Cross Jakarta. This study is useful to see the percentage of AIHA species found in referral patients in CBTS IRC. This result can be used for reference of the case of incompatibility in CBTS IRC

Methods: Study was carried out in 189 multi-transfused patients recorded with AIHA registered at CBTS IRC Jakarta. Immune hemolytic anemia is characterized by clinical and laboratory features of hemolytic anemia with direct antiglobulin test (DAT) positivity.
Results: In Central Blood Transfusion Service Indonesian Red Cross, we have 189 patients AIHA in 4 years (2016-2019). Furthermore, based on thermal amplitude of autoantibody, AIHA is classified as warm (12.32%), cold (12.43%), AIHA cold and Drug Induce (22%) and mixed (53,25%) type. Mixed AIHA is the most in referral patients and must be differentiated from warm AIHA with clinically insignificant cold agglutinins and cold hemagglutinin disease as their treatment is different. It may present as blood group discrepancy or cross-match incompatibility leading to delay in arranging suitable blood unit for transfusion. Therefore, a thorough immunohematology workup including monospecific DAT, indirect antiglobulin test at 4°C and 37°C, determination of thermal amplitude and titer is essential.
Summary/Conclusions: This study shows that in Indonesia most cases AIHA is AIHA Mix (cold and Warm) autoantibody. In the presence of this result, pre-transfusion management should be performed for AIHA patients. To see the success of therapy in AIHA patients can be done DAT examination before and after therapy.

P-019 | The effort to improve quality blood bank hospital 2019 in Jabodetabek, Indonesia

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Background: One of the efforts to improve the quality of Blood Transfusion services at Blood Bank Hospital is carried out through Quality Control including External Quality. External Quality Assessment Programs are quality assurance activities to held periodically by CBTS IRC to retrospectively assess the similarity various of results in Blood Bank Hospital in Jabodetabek (Jabodetabek means is firmed by the names of five cities: Jakarta, Bogor, Depok, Tangerang and Bekasi) that use various methods to detect irregularities.
Aims: to improve the quality of blood transfusion services at Blood Bank Hospital In Indonesia and be used as an indicator to improve the quality assessment in a sustainable manner.
Methods: Samples were sent according to the results of the questionnaire filled out by prospective EQAS participants. Samples were sent in the form of 1 set with a total number of 18 tubes consisting of a suspension of red blood cells and plasma of the patient and the donor which was then examined by the participants.
Results: By the total of Blood Bank Hospital in Jabodetabek Indonesia, only 37 participated in the EQAS Immunohematology in 2019. Of the total 37 EQAS Immunohematology participants in 2019, there were 14 (38%) Blood Bank Hospital that had appropriate results and 23 (62%) Blood Bank Hospital that had unsuitable results. The most discrepancies in the results of the EQAS Immunohematology Blood Bank Hospital 2019 were the blood group examination were 11 (48%), then cross-match examination 5 (22%) and finally the discrepancy of both was 7 (30%). From a total of 37 Hospital Blood Bank that participated in PME Immunohematology, the most blood type was examined using a tube test, amounting to 69.9% and cross-match using a gell test, which was 88.8%.
Summary/Conclusions: Based on the analysis of the results of the examination of the EQAS Immunohematology Blood Bank Hospital in 2019, we conclude that it is necessary to provide guidance to technical personnel in the laboratory, especially the result reading technique using the tube method and reading the crossmatch results on the gell test method.

P-020 | The incidence of the ABO hemolytic disease of the newborn and significance of various serological and laboratory investigations in its evaluation and diagnosis

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Background: The commonest IgG red cell antibodies in human serum are anti-A and anti-B. High concentrations of IgG antibodies are found only in group O subjects as compared to group A/B. There is a lack of information regarding the true incidence of ABO HDN and the significance of various laboratory investigations commonly employed in its evaluation.
Aims: Our study aimed to find out the true incidence of ABO HDN, among newborns delivered at Sriram Chandra Bhanja (SCB) Medical College, Cuttack, Odisha, India and to find out the significance of various serological and laboratory investigations commonly employed in its evaluation and diagnosis.
Methods: The study population consisted of 1260 full-term neonates delivered at our hospital; who met the inclusion and exclusion criteria of the study. The study subjects consisted of 468 ABO-incompatible mother and newborn pairs; Group AB mothers were excluded. Blood grouping and antibody screening were performed on the mother's sample. After delivery, umbilical cord blood were collected from the mothers and blood grouping of all newborns were done. Hemoglobin (Hb), reticulocyte count, peripheral smear, Direct agglutination test (DAT) and Heat elution of ABO-incompatible infants were done. The eluates were subsequently tested with A1 cell, B cell, and O cell. Cord blood of ABO-incompatible newborns was sent for serum bilirubin estimation. IgG anti-A and anti-B titer of the mothers (with newborns having ABO-HDN) were performed on the mother's serum sample after treating with Dithiothreitol (DTT) for analysis. Red cells of the Group A newborns with ABO-HDN were tested with anti-A1 lectins to know the major A subgroups. For the purpose of this study the criteria for abo HDN were- Newborns with ABO-incompatibility with mother with no other known cause of jaundice and hemolysis -Birth weight of 2.5 kg or greater -Jaundice and anemia observed within 48 hours of birth - And/or antibodies confirmed in the eluate-And/or Positive Direct agglutination test- Spherocytosis ± microcytosis.
Results: Out of 1260 newborns, 468 (37.14%) were ABO-incompatible and 792 (62.86%) were ABO compatible. The total

incidence of ABO-HDN in our study was 13.65% of the total newborns taken and 36.75% of the total ABO-incompatible newborns. The most common mother-newborn pair among the ABO HDN cases in our study was O-B pair 93 (54.07%) followed by O-A pair 77 (44.77%). There was a single (0.58%) case of ABO HDN in a Group B infant of a Group A mother. This A mother was typed to be of A2 subgroup. Affected male newborns were 104 (22.22%) and female newborns were 68 (14.53%). Among the ABO HDN cases; 92 (53.49%) were DAT positive and 80 (46.51%) were DAT negative. DAT showed 1+ strength of reaction in 48 (52.17%) cases. Heat elution was positive in 116 (67.44%) newborns and negative in 56 (32.56%) newborns. Out of the 77 Group A ABO-HDN cases, 73 were of A1 subgroup and only 4 cases were A2. Out of ABO-HDN cases, 121 (70.35%) cases were firstborn in the family. It was found that mothers of 86 (50%) out of 172 cases of ABO HDN had a titer of ≥1:2048. Only 24 (13.95%) infants had serious disease requiring exchange transfusion; Rest 52 (30.23%) ABO HDN cases were managed only by phototherapy and 96 (55.82%) didn't require any treatment.

Summary/Conclusions: It must remain a high priority for all pediatricians to achieve accurate diagnosis in all cases of HDN and emphasizes the point that persistent neonatal hemolysis should always be fully investigated and followed up appropriately.

P-021 | Comparing the platforms for ABO antibody titration

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Background: Manual method of titration by conventional tube technique (CTT) has certain limitations, which has led to exploration of alternative methods such as column agglutination technology (CAT) and hemagglutination (HA)/ solid phase red cell adherence (SPRCA). However, there is lack of standardization of these methods which has led to significant variability in reporting of titers. Also, actual concentration of clinically significant IgG antibodies is masked by presence of IgM antibodies, which leads to overestimation of titers. Hence, measurement of actual concentration of IgG requires treatment of serum with dithiothreitol (DTT) or heat to reduce interference of IgM antibodies.

Aims:

1. To compare the results obtained by CTT with CAT and HA/SPRCA
2. To study effect of DTT and heat on ABO isoagglutinin titers performed by CAT and CTT
3. To compare effect of DTT treatment and heat on ABO isoagglutinin titers performed by CAT and CTT
4. To compare results obtained by HA/SPRCA with those obtained by CTT and CAT with use of DTT and heat

Methods: This was a prospective, observational study conducted from October 2018 to March 2020. All consecutive O group donors were included in the study. Samples were tested by CTT, CAT and HA/SPRCA. Additionally, serum from each donor was treated with DTT and heat and tests were performed by CTT and CAT after treatment.

Results: A total of 2005 donors were included. While IgM and IgG measurement of anti-A and anti-B showed strong correlation between CTT (1+ strength) and CAT (1+, 2+ strengths), correlation between CTT (1+ strength) and HA/SPRCA (1+, 2+ strengths) was found to be weak. Reduction in IgG and IgM titers was observed with DTT and heat. CTT (post DTT) results showed one-fold higher decrease than CAT (post DTT). IgG titers showed strong correlation between CTT (1+ strength) and post DTT CAT (2+ strength). Concordance between CTT and CAT (post HI) was low. CTT results showed more decrease in median titers as compared to CAT after HI. CTT (post DTT) IgG results are similar or lower when compared to results obtained by SPRCA, while CAT (post DTT) IgG titers were higher with CAT (post DTT) when compared to HA/SPRCA. Results of titers obtained by CTT (post DTT) were lower as compared to CAT (post DTT) and HA/SPRCA, with majority giving results less than 64. CTT (post DTT) IgG results are similar or lower when compared to results obtained by SPRCA, while CAT (post DTT) IgG titers were higher with CAT (post DTT) when compared to HA/SPRCA. Results of titers obtained by CTT (post DTT and post HI) were lower as compared to CAT and HA/SPRCA, with majority giving results less than 64.

Summary/Conclusions: CAT shows higher ability in detecting ABO isoagglutinins than CTT. HA/SPRCA shows lower ability in detecting ABO isogglutinins than CTT. There is significant difference between antibody titers estimated using DTT treated and untreated serum. There is satisfactory effect of heat inactivation on titers, which is modest when performed by CAT as compared to CTT. DTT treatment was found to be superior to HI. However, for resource constraint settings, where DTT is not accessible, HI of serum offers a satisfactory alternative. Results obtained by HA/SPRCA were closer to post DTT CTT results. Results obtained by post DTT CAT were neither closer to HA/SPRCA nor post DTT CTT. Results obtained by HA/SPRCA were closer to post HI CTT results. Despite various drawbacks of manual method, authors use post DTT CTT for reporting titers.

P-022 | Effect of storage time for monoclonal antibody a product from CBTS Red Cross Indonesia

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Background: The use of antisera reagents has become a routine test in confirmation of ABO blood group in the field of blood transfusion services. To get quality reagents and correct examination results, the reagent has to be tested for stability. Antisera can be used immediately or stored for a certain time so that the antibody content in the antisera remains in an optimal state when used. Therefore, we conducted a study on the stability test of antisera A products from the PMI Central Blood Transfusion Unit.

Aims: This study aims to determine the effect of antisera storage time on the degree of agglutination.

Methods: The test method used for this study was two methods, namely the tube method and the slide method with examination criteria based on WHO standards, namely the potency test, avidity test and specificity test.

Results: The results of the stability test for Antisera A products from Central Blood Transfusion Services Red Cross Indonesia for the potency test on the LOT A1 code (A010118) showed that the highest titer on retesting in April and July 2018 was still stable at a titer 1: 1024 both on the cell A and AB cell tests. Whereas in October the cell A test showed a titer of 1: 1024 and the AB cell test showed a decrease in the titer at 1: 512. This result could be due to a decrease in the binding capacity of antibodies to the antisera over time and could be due to variations in the concentration of antigens on the surface of the red blood cells being examined. However, all the results of the Antisera A potency test, LOT A1 code (A010118) are still classified as good and stable because the value of each titer is still above the minimum feasibility value for product quality according to WHO with a minimum titer of 1: 128.

The results of the Antisera A specificity test on the LOT A1 code (A010118) showed that the degree of agglutination that occurred in the reexamination in April, July and October was 4+ in both the A cell and the AB cell test while the results on B cell tests and O cell tests were negative. The results of the Avidity test for Antisera A with the code LOT A1 (A010118) indicate that the time needed to form agglutination in January, April, July, in the A cell test is 3 seconds and 4 seconds in October. Meanwhile, the AB cell test is 3 seconds in January, 4 seconds in April and July, and 5 seconds in October. The degree of agglutination formed is 3+ after 2 minutes of the reaction. Although there was an increase in Avidity time, these results were still within the standard timeframe set by WHO, which was less than 60 seconds and the degree of agglutination formed was still in accordance with the passing standards of reagent quality, namely 3+ after 2 minutes of the reaction. Based on these results, Antisera A with the code LOT A1 (A010118) is still in a stable condition and suitable for use.

Summary/Conclusions: Based on the results of the research conducted, it can be concluded that the results of the stability test of antisera A products have an influence in the storage time of the antisera on agglutination power. The importance of paying attention to the storage time of antisera used before expired date.

P-023 | A case of severe hemolytic disease of the fetus and newborn (HDFN) caused by anti-Kp^a

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Background: The Kp^a antigen, or KEL3, was identified in 1957 and initially named Penny, but renamed when it was understood that the antigen belongs to the Kell blood group system. Approximately 2% in the Caucasian population are Kp^a positive and less than 0.01% in black population.

Anti-Kp^a is most often a benign IgG antibody that seldom causes hemolytic transfusion reactions (HTR). Very rarely, it may be the cause of HDFN: to our knowledge, two cases have earlier been described that led to intrauterine transfusions (IUTs) and four cases that led to treatment post-partum.

Anti-Kp^a is a common antibody in Sweden and during the last decade (2011-2020), we have identified 38 pregnancies at our center with maternal anti-Kp^a.

Aims: We describe a case of severe HDFN due to anti-Kp^a.

Methods: At pregnancy week 11, an anti-Kp^a was detected and identified in an AB RhD positive 1-para woman of Swedish origin, pregnant with the same partner as before. In the previous pregnancy, the antibody screening test was negative in the first trimester as well as at delivery. The partner is Kp(a+). In the current pregnancy titers were followed, and a monocyte monolayer assay (MMA) was performed in pregnancy week 29. Fetal ultrasonography of the middle cerebral artery peak systolic velocity (MCA PSV) was performed nine times from gestational week 29 to 35 and multiple of median (MoM) calculated. A MoM <1.5 correlates to fetal anemia.

Results: Titers performed in Ortho Vision varied between 256 and 512. The MMA was negative. The first MCA PSV showed, unexpectedly, signs of fetal anemia (MoM 1.57) and was confirmed three days later at pregnancy week 29+6 (MoM 1.70). An intrauterine transfusion (IUT) was performed; 70 mL of O RhD positive Kp(a-) red blood cells (RBCs) increased the hemoglobin from 3.9 g/dL and the hematocrit from 11.7% to 30.6%. In pregnancy week 31+1 a second IUT was performed; 45 mL of RBCs increased the hemoglobin from 10.5 g/dL to 13.4 g/dL and the hematocrit from 30.2% to 38.4%. The fetus was A RhD positive but no Kp(a) typing was performed, because of a weak positive direct antiglobulin test (DAT) and a very weak anti-Kp^a was eluted from the RBCs. The fetus was thereafter monitored by weekly ultrasound, with no further signs of anemia (MoM <1.5).

A boy was delivered by caesarian section (patient request) in pregnancy week 36+6 with Apgar 9+10+10. A few hours after birth the hemoglobin was 9.9 g/dL and the baby received one exchange transfusion and was treated with phototherapy for two days. Bilirubin had the highest peak on day 4 (242 μmol/L). He received three top-up transfusions, the first one 35 days after birth. We were then not able to detect any RBCs with blood group A. He received the other two top-up transfusions at the age of two months and three months.

Summary/Conclusions: Anti-Kp^a is most often a benign antibody that seldom causes HTR or HDFN, but in rare cases it may be a dangerous antibody for the fetuses and newborns. In our case, the fetal hemoglobin was 39.9 g/dL in gestational week 29+6. The mechanisms causing fetal anemia is not clear, but possibly similar to anti-K antibodies, by suppression of fetal erythropoiesis.

Red Cell Immunohaematology – Molecular

P-024 | Complete reference sequences for ABO blood group gene alleles by long-read sequencing

selected for main programme

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Background: Defining high-quality allele reference sequences for blood group genes has become increasingly important, in particular as next-generation sequencing (NGS) is being more widely used in molecular analysis of blood groups. Allele reference sequences need to (i) span the complete gene region including introns, (ii) have a fully resolved haplotype, (iii) offer confirmed serology, and (iv) be well-accessible in a public sequence repository. Generating such sequences is technically challenging. The main obstacle lies in resolving solid haplotypes, as both Sanger sequencing and short-read NGS are restricted by their read length (and therefore physical phase information). Hence, for many blood group genes, allele reference sequences remain rare. Even for ABO, the clinically most important and well-known system, only nine complete human *ABO* gene sequences have been deposited in the NCBI Nucleotide database (accessed 2021-03-30).

Aims: We aimed to establish high-quality reference sequences for common *ABO* alleles observed in Switzerland. To resolve allele haplotypes, we took advantage of the latest 3rd-generation long-read NGS technologies of Oxford Nanopore Technologies (ONT) and Pacific Biosciences (PacBio).

Methods: We selected samples from a large, well-characterized *ABO* genotype dataset (n=25,200) of serologically-typed blood donors from the greater Zurich area (Switzerland), which had been generated previously using MALDI-TOF MS. We aimed to sequence at least 20 alleles for each of the six main *ABO* groups, i.e. *ABO*A1*, *A2*, *B*, *O.01.01*, *O.01.02*, and *O.02*. In total, we selected 78 samples for sequencing. The entire *ABO* gene comprising ~24.2 kb was amplified in two overlapping long-range PCRs (13 kb and 17 kb). PCR amplicons were sequenced using a PCR-free barcoding protocol on a MinION device from ONT. To circumvent potential biases from standard reference-based read mapping, we performed for each sample a *de-novo* (i.e. reference-free) assembly. For cross-validating the ONT sequences, a subset of 12 samples (n=2 for each *ABO* group) was sequenced using PacBio (HiFi sequencing on a Sequel II system) and Illumina (MiSeq).

Results: We established generic long-range PCR reactions covering the entire *ABO* gene, including the large intron 1, which has been difficult to amplify so far. Amplicons from the 78 study samples were sequenced with ONT to a median sequence depth of 1400X. For all samples, both full-length *ABO* haplotype sequences could be resolved. Cross-validation with gold standard HiFi PacBio and Illumina data confirmed high quality of the ONT haplotype sequences. Considerable genetic variation was observed among the six *ABO* groups (*ABO*A1*, *A2*, *B*, *O.01.01*, *O.01.02*, and *O.02*). Within-group genetic diversity was highest for *ABO*O.01.01*, while genetic diversity was particularly low within the groups *ABO*A1* and *ABO*B*. Strikingly, our data revealed four SNVs being putatively specific for *ABO*A1*. Such diagnostic SNVs are currently lacking.

Summary/Conclusions: We have established a large dataset of 156 fully-phased *ABO* sequences for the most common *ABO* alleles in Switzerland. This collection will serve as a valuable reference resource for NGS-based *ABO* genotyping and sequencing. Our data uncovered four putatively *ABO*A1*-specific SNVs, which are currently being studied in detail to verify diagnostic specificity.

P-025 | Optimised droplet digital PCR approach for non-invasive foetal blood group typing in ALLO-immunised pregnant women

selected for main programme

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Background: Alloantibodies against red blood cells or platelets in women may cause destruction of foetal blood cells during pregnancy. This may lead to anemia or thrombocytopenia and in severe cases to foetal death. If observed in time, monitoring and treatment can prevent serious illness of the foetus. For many years, real-time PCR on cell-free DNA (cfDNA) isolated from maternal plasma has been used for prenatal genotyping to determine if a foetus is at risk. The amount of fetal cfDNA (cffDNA) in maternal plasma is extremely low and sensitivity of this approach is limited by non-specific amplification from an excess of maternal DNA. Recently O'Brien et al. (BJH, 2020) demonstrated that droplet digital PCR (ddPCR) is a good alternative to analyse low amounts of cffDNA. Droplets contain a single copy of either the maternal or the foetal gene, and presence of a foetal copy will therefore always result in a strongly positive signal.

Aims: Optimisation of droplet digital PCR assays for non-invasive foetal genotyping in women with alloantibodies against red blood cells and platelets.

Methods: The Quick-cfDNA Serum & Plasma kit (Zymo Research) was used to isolate cfDNA from 3 mL of maternal plasma, which is a larger volume then usually applied in other methods. Primers and probes for ddPCR assays were selected for screening of several foetal antigens (K,

k, D, C, c, E, HPA1a, HPA5b). In case of an antigen negative result, presence of isolated cffDNA has to be shown to reliably report the negative results. *ACTB* is used to show cfDNA and in case of a male foetus cffDNA is confirmed by the *SRY* and *TSPY1* genes. Methylated *RASSF1* (mRASSF1a) is used as a universal foetal marker, since the gene is hypermethylated in placenta and therefore in contrast to maternal cfDNA resistant to digestion with methylation-sensitive restriction enzymes.

Results: To increase ddPCR sensitivity, an additional heating step of 8 min at 90°C was applied to denature double-stranded cfDNA into two single-stranded molecules (ssDNA). If denaturation takes place prior to droplet generation, twice as many droplets will contain the target of interest. To further minimise the number of assays, multiplex ddPCRs were developed by combining primers and probes in different concentrations and using different fluorescent labels. Initially we digested cfDNA for mRASSF1a assays with BstUI, but also positive droplets were obtained with cfDNA from males. BstUI is reported to be less active on ssDNA and was replaced by HhaI, which is capable to digest ssDNA. Greater efficiency was achieved by double digestion with Bsh1236I. A fixed optimum ddPCR annealing temperature of 57°C was determined. We tested assays for C, c, E, D (exon 5/7), Y (*SRY/TSPY1)* and *RASSF1* combined with *ACTB* and assays for K/k, HPA1a/b and HPA5a/b on 8-25 positive and negative cffDNA samples. The median number of positive droplets in negative samples for each assay was 1. The limit of detection was determined as the minimal number of copies per mL plasma (mean+3SD), which was 2.1 for *SRY*, 3.6 for K, 6.3 for *RHD* and 9.5 for mRASSF1a. For each assay we could detect cffDNA in all samples with positive fetuses of at least 11 weeks of gestation. The *RHD* exon 5/7 ddPCR reliably detected D positive fetuses in mothers carrying RHD**pseudo/RHD*DVI* variant alleles. The ratio between *SRY* or mRASSF1a and blood groups served as extra confirmation of positive results.

Summary/Conclusions: The optimised droplet digital PCR assays has shown to be reliable methods to implement for fetal genotyping.

P-026 | A new low prevalence Diego blood group antigen found in a Caucasian blood donor

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Background: The serum of a 33 years old pregnant Caucasian woman with no transfusion history contained anti-Wr(a) and an additional antibody reacting in the indirect antiglobulin test with a single Wr(a) negative test cell in the antibody screening test. Antibody identification panels and multiple red cells positive for low prevalence antigens showed negative reactions.

Aims: The results suggested an antibody against an unknown or a possibly new low prevalence antigen. The blood donor of the positively reacting test cell was identified and a serological and molecular testing of him and his family members were performed.

Methods: The antibody screening and identification were performed in the indirect antiglobulin test using untreated, papain treated and DTT treated red cells in the gel technique.

The red cells of the blood donor, his mother, his brother, his two sisters and his two sons, who were all ABO compatible with the patient, were serologically tested with the patient's serum in the indirect antiglobulin test.

Massive parallel sequencing (MPS) of a blood group gene panel including all exons of 42 genes encoding the blood group systems ISBT001 to 038 was performed on the blood donor and his two sisters. A PCR-SSP method was established for genotyping of the rare mutation in *SLC4A1* and was used for genotyping the donor's family.

Results: Antibodies to following low prevalence antigens could be excluded: Wr^a, Di^a, Wu, Rb^a, Co^b, Yt^b, Lu14, Js^a, K17, K25, C^x, E^w, DAK, FPTT, Tar, Go^a, V, VS, Crawford, JAL, JAHK, PARG, LW^b, Ls^a, Ul^a, Tc^a, Sc2, Sc4, Vw, Mg, Mi^a, Hut, Mur, Hil, Miny, He, Dantu, Mt^a, St^a, Mit, Vr, Kn^b, Vil, Cs^b, Tc^b.

The serum of the patient reacted positive with the red cells of the donor, his brother and his older sister. It was negative with red cells of the donor's mother, his younger sister and his two sons. The MPS approach lead to the identification of a very rare missense mutation c.1447G>A (p.Gly483Ser; rs544557335) in exon 13 of the DI gene SLC4A1 of the donor and his older sister.

PCR-SSP confirmed the heterozygous genotype for the donor, his brother and his older sister.

All other family members were negative for the mutation. In the gnomAD database for the rs544557335 variant a very low frequency of 0.0002462 is given for Africans and 0.000008797 for Europeans. The mutation obviously defined a new low prevalence Diego antigen, which we named DIST.

Summary/Conclusions: We describe a novel low prevalence Diego antigen, provisionally named DIST, which is characterized by the *SLC4A1* c.1447G>A mutation leading to Gly483Ser exchange in the extra cellular part of the Diego protein. According to the gnomAD database the mutation has so far been found in one of 113,680 European individuals. The clinical significance of the antibody is not known. Further family studies of the pregnant woman are planned.

P-027 | An example of anti-CD99 in an antenatal patient with a novel homozygous mutation in CD99, resulting in the rare CD99- phenotype

selected for main programme

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Background: The Xg blood group system is comprised of two antigens, Xg^a (XG1) and CD99 (XG2) encoded by two closely linked

homologous genes *XG* (X chromosome) and *CD99* (X and Y chromosomes). CD99 is a high frequency antigen with a phenotypic relationship with Xg^a, where all Xg(a+) individuals have high expression of the CD99 antigen, whilst Xg(a−) females have low expression of CD99 and Xg(a−) males can either have low or high expression of CD99. To date, there have been six CD99- individuals reported, of which four individuals had the molecular bases characterised. All individuals were homozygous for different exon deletions in their *CD99* gene, resulting in a truncated CD99 glycoprotein.

Aims: To present findings of an antenatal case referred for serological investigation due to the presence of an unidentified antibody to a papain sensitive high frequency antigen.

Methods: Samples from a 27 year old pregnant patient were investigated. Serological investigation was performed by LISS tube IAT with untreated and papain treated cells. The patient's cells were tested with monoclonal anti-CD99 (12E7) and human anti-CD99. Alloadsorption/elution studies were carried out using standard techniques. Genomic DNA was isolated from whole blood of the patient, all 10 exons of the *CD99* gene were amplified by PCR and all PCR products were Sanger sequenced.

Results: The patient's cells were found to be CD99- with two examples of anti-CD99. Anti-CD99, -S and $-Fy^a$ were found to be present in the patient's plasma. The antibodies were determined by removing the anti-CD99 from the patient's plasma by alloadsorptions with cells matched for the patient's common antigen types and anti-S and $-Fy^a$ were identified in the patient's adsorbed plasma. The eluate prepared from the cells used in the first adsorption was shown to contain anti-CD99 only. The DNA sequencing showed the patient was homozygous for c.202C>T in *CD99* exon 5, encoding a premature stop codon p.Arg68Ter, which would potentially terminate the expression of the CD99 glycoprotein, resulting in the CD99- phenotype observed in this individual. Three additional homozygous mutations were observed in the patient's *CD99*: c.363C>T in exon 8, c.496A>G and c.518A>T in exon 9. These three mutations were located downstream of the c.202C>T that would terminate the CD99 translation and therefore the associated amino acids would not be expressed.

Summary/Conclusions: Here we have described a case of a pregnant patient with anti-CD99 and the rare CD99- phenotype. To date, all previously reported molecular bases of the CD99- phenotype were a result of *CD99* exon deletions; however, this patient was found to have a novel homozygous nonsense mutation in *CD99*, c.[202C>T, 363C>T, 496A>G, 518A>T], p.Arg68Ter.

P-028 | The DNA sequence variation around the intron 1 +5.8-KB site of ABO gene is associated with ABO blood type

selected for main programme

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Background: The ABO blood type is the most clinically important blood group system. Multiple polymorphisms in both coding and noncoding regions of *ABO* gene, except for intron 1, were analyzed and correlated with the associated ABO phenotypes. Intron 1 of *ABO* gene was about 1.3kb length and found to regulate ABO expression within the +5.8kb-site relative to the translation start site. Several variations within erythroid cell-specific regulatory element at +5.8kb-site were demonstrated to associate with ABO subtypes. Since +5.8kb-site was important for ABO expression and highly conserved, the polymorphisms nearby this region might be kept and linked to ABO blood group.

Aims: The aim of this study was to investigate the association between the sequence variations around intron 1 +5.8kb-site of *ABO* gene with ABO blood group, and to apply it on genotyping.

Methods: Thirty-five blood samples from Taiwanese blood donors were analyzed due to discrepancy in ABO group screen test. The ABO typing were examined by serological tests which followed AABB guideline and Judd's Methods in Immunohematology. For genotyping, all the samples were analyzed using traditional *ABO* genotyping methods, including polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) and sequencing of exons 6 and 7, while the further sequencing of exons 1 to 5 was performed if needed. PCR of intron 1 +5.8kb-site of *ABO* gene was carried out according to previous literature (Nakajima, Transfusion, 2013). The region of intron 1 +5.8kb-site analyzed in this study was between c.28+5770 ~ c.28+6530, and the reference sequence of *ABO* gene used was NG_006669.2. Haplotypes of the *ABO* allele were confirmed by nested PCR of *ABO* gene with gel elution procedure and DNA sequencing.

Results: *ABO* genotypes of all the samples enrolled were in accordance with the ABO serological typing. A total of 12 nt variations in the region of intron 1 +5.8kb-site were found to be associated with ABO phenotypes. The variations included c.28+5888C>A, +5951C>T, +5984C>A, +6043T>C, +6123T>C, +6150A>G, +6268C>T, +6269A>G, +6280T>C, +6293A>T, +6467T>C, and +6518T>A. After compared to the *ABO* genotype results, these variations could be discriminated and categorized into 4 different groups, including A, B, O^1, and O^{1v} groups. And each group displayed characteristic variation combinations. Based on the correlation between *ABO* genotype and the sequence variations nearby the +5.8kb-site, two cases with serological weak ABO phenotype were genotyped. Both of the two cases, A_x and AB_m phenotypes, had no nucleotide changes associated with weak phenotypes on all the 7 exons or exon-intron boundary. However, the sequence variations around +5.8kb-site indicated homologous *ABO*O.01.02* allele and homologous *ABO*A* allele for the two cases, which implied a deletion of this region in one allele. Haplotypes of the two alleles were further confirmed as *ABO*A1.02* (c.28+5443_11354del) and *ABO*B.01*(c.28+5110_10889del).

Summary/Conclusions: In the present study, sequence variations around the regulatory region at Intron 1 +5.8kb-site of *ABO* gene were analyzed. The polymorphisms here could be categorized into A, B, O^1, and O^{1v} groups in accordance with the *ABO* allelic groups of genotype as well as the associated ABO blood type. The investigation of this study provided a new viewpoint and strategy for *ABO* genotyping.

P-029 | A novel KEL C.1414-1G>T allele identified in a polish patient with anti-Ku antibody

selected for main programme

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Background: Mutations occurring in the *KEL* gene, encoding Kell antigens, may lead to the Kell$_{null}$ phenotype (K$_0$) showing the lack of Kell antigen expression on red blood cells. Identification of K$_0$ individuals is often based on the detection of anti-Ku in their plasma, produced after transfusion or pregnancy. The antibody is regarded as clinically significant.

Aims: To present a case report of a nine year old girl diagnosed with familial encephalopathy of unknown etiology (similar course of the disease in older brother) and congenital cerebellar hypoplasia. She was transfused with one unit of red blood cells, O RhD pos, during orthopedic surgery. Antibody screening before transfusion was negative. Several months later antibody screening was positive with all panel cells, except the patient's cells. The patient required second orthopedic surgery due to dislocation of both hip joints.

Methods: Antibody screening and identification were performed by IAT gel column (DiaMed/BioRad) with untreated, papain and DTT treated cells and also by saline test at room temperature and LISS tube IAT. In addition to routine tests the adsorption of serum using allogeneic RBCs of Jk(a-), S- phenotype (the same as the patient) was applied. Serum was tested with commercial panel of red blood cells and with samples obtained from SCARF (Serum Cells and Rare Fluids Exchange). Kell antigen phenotyping was determined with commercial anti-K, -k, -Kp^a, and with reference antisera, anti-Kp^b, -Js^b, -K11, -Ku and -Kx. The patient's genomic DNA was tested using RBC-FluoGene vERYfy and Rare kits (Inno-Train, Germany), whole exome sequencing (WES; SureSelectXT Human All Exon v5, Agilent) and confirmed with Sanger sequencing of *KEL* exons 12-13 of the family.

Results: The patient's RBCs were found to have the rare K$_0$ phenotype (K– k–, Kp(a–b–), Js(b–), K11–, Ku–, Kx+), with anti-Ku identified in her plasma. The antibody reacted 2+ by LISS IAT with untreated cells, marginally stronger with papain treated cells (4+) and also reacted moderate strength in saline direct agglutination tests at 18°C and 37°C. The patient's serum was compatible with 2 examples of K$_0$ cells and no additional antibodies were detected. Commercial PCR tests determined the patient's genotype as homozygous *KEL*02.03* (c.578C, c.841T) and c.1790T (*Js^b*). WES analysis focused on the *KEL* gene showed homozygous change in intron 12, c.1414-1G>T and no changes were found in the *XK* gene. Sanger sequencing confirmed the mutation located on *KEL*02.03* allele. *KEL* Sanger sequencing of parents and the brother revealed their heterozygous *KEL* c.1414-1G>T genotype. Since this *KEL* variant was not listed in any of the examined databases, it was deposited in the EMBL under accession number ERP124060.

Summary/Conclusions: A novel mutation *KEL* c.1414-1G>T found in a Polish family was observed on the *KEL*02.03* allele. The mutation probably leads to aberrant splicing of *KEL* and affects expression of Kell antigens similarly to known *KEL*02N.51* allele where at the same nucleotide position the other single nucleotide variation c.1414-1G>C (rs906814829) has been described in K$_0$ individuals.

P-030 | First Dutch individual with an Rh$_{null}$ phenotype

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Background: The Rh$_{null}$ phenotype is defined by complete absence of all antigens of the Rh blood group system. Based on the genetic mechanism, two types of Rh$_{null}$ are distinguished, the amorph and regulator type. The amorph type is caused by mutations at the *RH* locus on chromosome 1, while the regulator type is caused by inactivating mutations in the *RHAG* gene on chromosome 3. Homozygous or compound heterozygous *RHAG* null mutations will cause complete absence of the Rhag and Rh polypeptides on the membrane of red blood cells.

Aims: In the Netherlands, foetal RhD typing at 27 weeks of gestation is performed with cell-free DNA isolated from maternal plasma of RhD-negative pregnant women by real-time PCR targeting *RHD* exon 5 and 7, to guide the administration of anti-D immune prophylaxis in case of a D positive foetus.

Methods: In one of these samples, the test showed a high concentration of *RHD*-positive cell-free DNA indicating the presence of *RHD* gene sequences in the DNA fraction derived from the mother.

Results: Genetic analysis of the maternal genomic DNA isolated from leukocytes, revealed normal *RHD* and *RHCE* alleles (*RHD*01/01; RHCE*02/02*). Sequence analysis of the *RHAG* gene showed a homozygous or hemizygous variant in intron 1, c.157+1G>A (*RHAG*1N.03*). This variant does not allow expression of Rhag protein and thereby preventing also the expression of Rh proteins. Further Rh phenotyping revealed that the woman's erythrocytes were also serologically negative for other Rh antigens (CcEe and the high frequent antigen Rh29). Lack of Rhag protein was confirmed by absent binding of monoclonal anti-Rhag, establishing a regulator Rh null phenotype. Genetic investigation of genomic DNA from the parents of the pregnant woman showed that both are heterozygous for the *RHAG*01N.03* allele (*RHAG*01/01N.03*). Microscopic examination of the Rhnull blood smear showed stomatocytes. Rheological analysis showed that the erythrocytes were only slightly less deformable with a minor increase in

osmotic fragility. Also the expression level of outer membrane proteins was slightly lowered in the Rhnull erythrocytes, while her parent's erythrocytes had values at the lower limit of healthy controls. These mild aberrant results combined with the lack of obvious clinical symptoms suggests no direct indispensable role for the Rh/RHAG protein complex in erythrocytes. However, there is an established role for the Rh/RHAG protein complex in transporting ammonia, such transport defect is never described in relation to pathology.

Summary/Conclusions: Luckily, the Rhnull mother has had her second pregnancy without any alloantibody formation Ante- and postnatal RhIg was administered and a healthy RhD-positive child was born. After pregnancy, erythrocyte antibody screens remained negative. Fortunately, she consented to become a blood donor.

P-031 | 6-year ABO genotyping of ABO grouping discrepant cases

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Background: ABO is the most relevant blood group system in transfusion medicine. Routine ABO blood group typing may reveal serologic discrepancies concerning forward and reverse ABO determination or weak agglutination. *ABO* genotyping helps to explain the ambiguous reaction patterns through identification of molecular basis of the observed discrepancies.

Aims: 6-year summary of *ABO* genotyping of patients with ABO grouping discrepancies sent to IHTM (Poland) between 2015-2020.

Methods: 152 samples from Polish individuals with weak A (86), weak B (16) agglutination results or forward/reverse typing discrepancies in standard agglutination methods (50) were sent to IHTM for further serological testing: 1) gel cards DiaClon ID ABO/D with anti-A: clone A5, anti-B: clone G1/2, anti-A,B: clone ES131, ES15 + BIRMA 1 + ES4 and DiaClon ID ABD Confirmation for Donors with anti-A: clone M297/628=LA-2, anti-B: clone LM306/386=LB-2 (DiaMed/Bio-Rad); 2) tube technique with: anti-A (BIRMA 1; A-11H5, c.9113D10), anti-B (LB-2, B-6F9, c.9621A8); as well as with A,B,O cells. *ABO* genotyping was performed with RBC FluoGene ABO Basic Kit (Inno-Train); then by sequencing (exon 1 and +7.21kb site from the intron 1 to 3'UTR of *ABO*; additionally +5.8kb fragment of the intron 1 with GATA motif if necessary) using PCR/Sanger Sequencing Primers Pairs (ThermoFisher).

Results: In 131 cases *ABO* genotype using commercial PCR tests confirmed serological results. In 26 cases with weak A agglutination, with and without discrepancies between serological and genotyping results by commercial test, further sequencing analysis revealed novel *ABO*A c.dup543_563* allele (ENA accession number ERP119607) (4); weak *ABO*AW.04* (1), **AW.06* (1), **AW.13* (3), **AW.30.01* (5); microchimerism of *ABO*A1.01* (1), new *ABO*cisAB c.796C>A* (1) (#ERP119421), *ABO*A2.16* (1), lack of *ABO*A* allele (9). Among 10 individuals with weak B or discrepancies further sequencing revealed new *ABO*cisAB c.796C>A* (5); new *ABO*B c.28+5858T>C* allele in GATA motif of intron 1 (#ERP127550) (3); microchimerism of *ABO*B* (1); lack of *ABO*B* allele (1).

Summary/Conclusions: ABO genotyping using commercial PCR tests helped to explain the ambiguous results in 86% of cases sent for consultation to the IHTM. Further classical sequencing used for 24% cases, revealed molecular basis of the ABO grouping discrepancies in 73% individuals. In 13 cases novel *ABO* alleles affecting the activity of ABO transferases, were identified (8,5% of all samples sent to IHTM).

P-032 | A novel EMP3 null allele detected in a patient with the MAM- phenotype

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Background: The MAM blood group system has recently been discovered to be carried on epithelial membrane protein 3 (EMP3), encoded by *EMP3* encompassing five exons located on chromosome 19q13.33. Ten MAM− patients have been reported prior to this case, all with inactivating mutations in *EMP3* which have been shown to cause the rare MAM- phenotype. The mutations previously observed include; four patients with a nonsense c.123 C>G mutation in exon 3, three patients with complete deletions of *EMP3*, two patients with a 745bp deletion of exon 4 and one patient with a 822bp deletion of exon 5. Anti-MAM is known to be clinically significant and has caused severe HDFN.

Aims: We report a case study of a 27-year old female patient of Malaysian origin who had a spontaneous vaginal delivery at 34 weeks gestation. The baby presented with severe neonatal jaundice, while the patient presented with an unidentified alloantibody to a high frequency antigen in her plasma. Here we report serological analysis of the patient and her partner, and *EMP3* sequencing analysis of the patient's DNA, providing evidence for a novel genetic background of the MAM- phenotype.

Methods: Samples from the patient and her partner were investigated due to the presence of a strong unidentified antibody to a high frequency antigen present in the patient's plasma. Serological investigations were performed by standard LISS tube IAT and direct agglutination techniques. Genomic DNA was extracted from whole blood and PCR amplification and Sanger sequencing was carried out on coding exons of *EMP3*. Additional deletion breakpoint analysis was carried out using primers spanning the *EMP3* exon 5 region.

Results: The patient was found to have the rare MAM- phenotype, whilst the partner was found to have the MAM+ phenotype. Strong anti-MAM was found in the patient's plasma and the partner's cells were incompatible. The patient's cells were also shown to have weakened expression of In^b, which is carried on CD44, akin to other MAM- individuals. Sequencing of the patient's *EMP3* gene showed apparent

homozygosity for a 923bp deletion (c.341 to IVS5+688), starting 18 nucleotides into *EMP3* exon 5 and deleting the majority of the exon including the termination codon. These deletion breakpoints differ from those in the previously reported exon 5 deletion (IVS4-231 to IVS5+338) associated with the MAM- phenotype. This deletion therefore represents a novel inactivating mutation in *EMP3*, and a further cause of the MAM- phenotype.

Summary/Conclusions: We have identified a novel exon 5 deletion of *EMP3* in a patient with the rare MAM−phenotype. The patient presented post-delivery with the baby showing signs of HDFN. Although homozygosity for an exon 5 deletion has previously been reported in a MAM− individual, our analysis showed the breakpoints in this case to be different, identifying this as a novel *EMP3* null allele.

P-033 | A serologically undetected partial D (DIVA) mother and newborn with hyperbilirubinemia caused by anti-D: A case for prenatal *RHD* variant screening

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Background: *RhD* is polymorphic with many partial and weak partial RhD phenotypes having been identified. The *RHD* variant prevalence in which antisera reacts with the RhD antigen can reach 2.2% in certain multiethnic urban obstetric populations (Wang, Am J Clin Pathol, 2010). Consequently, subsequent prenatal care can be complicated by late recognition of partial RhD in seemingly RhD+ mothers and crucial for prevention of hemolytic disease of the fetus and newborn (HDFN). Cell-free fetal DNA (cffDNA) is a dependable source for detection of RhD at 11 to 13 weeks gestation. At risk partial RhD females are considered candidates for prophylactic RhIG prior to RhD alloimmunization. DIVa results from the regularly linked *RHD*DIVa* and *RHCE*ceTI* alleles that translate to partial D, partial c, and partial e antigens. Furthermore, Tippett and Sanger demonstrated alloanti-D from DIVa individuals fails to interact with phenotypically DIVa red cells. A 32-year-old G2P1001 group O, RH: 1, -2, -3, 4, 5 female of African descent with a positive antibody screen delivered by urgent C-section at 31 weeks gestation for preeclampsia with severe features.

Aims: Presentation of a pregnant DIVa female with anti-D and HDFN.

Methods: Monoclonal MS201 anti-D was utilized for tube (Immucor, Norcross, GA) and gel column agglutination testing (CAT) (Ortho, Pompano Beach, FL) RhD detection. DNA was sequenced and analyzed by manufacturer directions (Applied Biosystems, Carlsbad, CA). Low ionic strength solution (LISS) (Bio-Rad, Dreieich, DE) and polyethylene glycol (PEG) (ARC, Washington, D.C.) enhancements were utilized for indirect antiglobulin testing (IAT). Monospecific anti-IgG were used for direct antiglobulin testing (DAT) followed by acid elution (Immucor, Norcross, GA).

Results: The mother serologically phenotyped R_0r. Strong agglutination without mixed field was observed when red cells were tested against anti-D with gel CAT and immediate spin (IS) tube testing. The DAT was negative, and the individual's plasma failed to react with autologous cells at all phases. Alloanti-D reacted at IS, LISS-37C, LISS-IAT, and PEG-IAT. Anti-LW^a was excluded following testing of RhD-LWa+ red cells at LISS-IAT and PEG-IAT. Antibody-antigen reactivity was enhanced following DTT treatment of RhD+ red blood cells. The neonate was born with hyperbilirubinemia (peak total bilirubin 10.4 mg/dL) at birth [day of life (DOL) 1], requiring phototherapy until DOL 5. The neonatal red cells reacted when tested against anti-D with gel CAT and IS tube testing. The neonatal DAT was positive with anti-D identified in the plasma and eluate. Genetic testing of the mother and the neonate revealed an unopposed RHD**DIVa*, and *RHCE*ceTI / RHCE*ce* alleles confirming a partial D, C-E-c+e+ phenotype and *RHD*01* and *RHCE*ce48C, 733G* [*RHCE*01.20.02*] / *RHCE*ce*, concluding a D+C-E-c+e+ phenotype, respectively.

Summary/Conclusions: The neonate likely inherited the paternal *RHD*01* and *RHCE*01.20.02* alleles, as the *RHCE*ce* was maternal. HDFN would be unlikely when a fetus inherits an unopposed *RHD*DIVa* from a DIVa mother with anti-D. R_1r and R_0r females of childbearing age may be variant *RHD* screening candidates prior to 28 weeks gestation due to the association of variant *RHD* and *RHCE*ce* alleles. Serologic and genetic testing of the father or noninvasive techniques to collect cffDNA may be considered to determine the neonatal *RHD* alleles to assess the risk of HDFN following alloimmunization from late recognition of partial RhD females during pregnancy.

P-034 | RHCE nucleotide 48 polymorphism in Argentinean donors carrying *RHD*weak D type 1* alleles on R_0 haplotypes

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Background: *RHD*weak D type 1* variant is strongly associated with *RHCE*Ce* allele on R_1 haplotypes. However, previous research conducted in our laboratory showed that in approximately 17% of Argentineans with weak D type 1 phenotype, the *RHD*weak D type 1* allele is found on R_0 haplotypes. *RHCE*Ce* and *RHCE*CE* alleles carry a cytosine at nucleotide 48 (c.48C) in *RHCE* exon 1 while *RHCE*ce* and *RHCE*cE* are generally associated to a guanine at position 48 (c.48G). It has been observed that c.48C single nucleotide variation (SNV) is also found in some *RHCE*ce* variants (*RHCE*ce.01*) in the context of R_0 haplotypes.

Aims: The aim of this study was to analyse the *RHCE* polymorphism at nucleotide 48 in Argentinean donors carrying *RHD*weak D type 1* alleles on R_0 haplotypes.

Methods: DNA samples from 20 D$^{\text{weak type 1}}$ccee and 41 Dccee Argentinean donors were investigated. Two PCR procedures each containing

forward primers targeting c.48C or c.48G SNVs in *RHCE* exon 1, respectively, paired with an *RHCE* intron 1-specific reverse primer were used to investigate *RHCE* nucleotide 48 polymorphism. *RHD* zygosity was investigated by PCR-RFLP. The presence of a hybrid Rhesus box, that implies a *RHD* hemizygous status, was demonstrated by amplification of the downstream and hybrid Rhesus boxes followed by digestion of the PCR products with endonuclease *Pst*I.

Results: Molecular analysis allowed the detection of c.48C and c.48G polymorphisms in all samples carrying *RHD*weak D type 1* alleles on R_0 haplotypes (n=20, 100%). On the other hand, 19 of the 41 (46.3%) Dccee samples harboured c.48C and c.48G SNVs while 22 (53.7%) only showed guanine at position 48. All weak D type 1 samples on R_0 haplotypes were *RHD* hemizygous and only 1 *RHD* homozygous sample was found in the group of Dccee samples, which has both c.48C and c.48G SNVs.

Summary/Conclusions: These results suggest that *RHD*weak D type 1* alleles is linked to *RHCE*ce.01* variant when found on R_0 haplotypes. The c.48G>C transversion in *RHCE* exon 1 leads to p.Trp16Cys, usually present in conventional *RHCE*Ce* and *RHCE*CE* alleles, while Trp16 is associated with *RHCE*ce* and *RHCE*cE* alleles. The presence of Cys16 in *RHCE*ce* is associated with the R_0 haplotype in Africans, leading to a weak e antigen expression on red blood cells. The finding of 46.3% of Dccee samples carrying *RHCE*ce.01* alleles can be attributed to African ancestry in the Argentinean population. Genetic recombination events occurring in such admixed population could account for the strong association between a Caucasian allele (*RHD*weak D type 1*) and an African allele (*RHCE*ce.01*) found on R_0 haplotypes.

P-035 | Two novel Rhag alleles in a patient leading to an Rh_{mod} phenotype

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Background: The Rh polypeptide is dependent on the Rhag polypeptide for its expression. Therefore, absent or diminished expression of Rh antigens can be caused by variations in *RH* genes or by variations in the *RHAG* gene. Absence of the Rhag protein will lead to the so called Rh null phenotype (regulator type), while diminished expression of the Rhag protein will lead to the Rh_{mod} phenotype. Several *RHAG* alleles are known to cause a weakened membrane expression of both Rhag and Rh antigens. Some of these alleles are dominant and as such are responsible for a Rh_{mod} phenotype in a heterozygous state.

Aims: A patient needed surgery for treatment of pseudomyxoma peritonei. Pretransfusion testing showed a group O phenotype with very weak expression of the RhD and c antigen. Routine typing for C, E and e antigens produced negative results. However, with adsorption/elution techniques a weak expression of the E antigen was shown. Antibody investigation showed the presence of an antibody directed against Kp(a) and no other relevant antibodies. Follow-up investigation on the RhD typing with a panel of monoclonal antibodies showed no clues for loss of RhD epitopes. With a monoclonal anti-Rhag, weakened expression of Rhag was found.

Methods: RH genotyping by MLPA (Multiplex ligation-dependent probe amplification assay) showed two normal *RHD* and two normal *RHcE* alleles. This predicted a ccDDEE (R_2R_2) phenotype. Sanger sequence analysis of all 10 exons of the *RH* genes revealed no mutations, while in exon 2 of the *RHAG* gene two heterozygous variants were found. The two variants NM_000324:c.172C>T and NM_000324:c.242G>A (rs1446598944, MAF 0.000004) result in amino acid changes p.His58Tyr and p.Gly81Asp. By targeted next generation sequencing of *RHAG* it was found that both variants were located in different reads, confirming the presence of two different variant alleles. Both alleles were not described before.

Results: Based on the combined serological and molecular results we conclude that the patient has the rare Rh_{mod} phenotype and is positive for RhD, c and E antigens and negative for C and e antigens. Based on the Dutch transfusion guidelines, which includes preventive matching for Rh antigens and K compatible blood, when relevant alloantibodies are present, blood was selected negative for Kp(a) and negative for the C, e and K. A family study is planned to further study the effect of the two separate *RHAG* alleles on Rh expression levels.

Summary/Conclusions: We describe the first Dutch patient with the Rh_{mod} phenotype. The diminished expression of Rh and RHAG antigens is associated with two new mutations in the *RHAG* gene.

P-036 | 5-year non-invasive prenatal diagnostics of fetal RhD for targeted anti-D immunoprophylaxis in Poland

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Background: Alloimmunization against RhD antigen during pregnancy leads to hemolytic disease of fetus or newborn (HDFN). Antenatal anti-D immunoprophylaxis is recommended for all RhD-negative Polish women since 2015 but more than 30% carry RhD-negative compatible child and do not require anti-D immunoglobuline (RhIg). Noninvasive prenatal diagnostics of fetal *RHD* (*RHD* NIPD) predicts RhD status of a fetus and recommends antenatal immunoprophylaxis only for women carrying an RhD-positive child. *RHD* NIPD offered since 2016 only at IHTM is not covered by the national health care system.

Aims: Summary of 5 years of routine *RHD* NIPD.

Methods: cffDNA isolated using easyMag, Biomerieux, from plasma of 1229 RhD-negative pregnant women (in 8-38 week of gestation)

was tested for exons 5 and 7 of *RHD* and *CCR5* by real-time PCR using LC480II (Roche). Maternal DNA from whole blood was tested for identification of *RHD* variant using RBC FluoGene RBC-Dweak/variant (Inno-Train, Germany) or the *home-made* protocol.

Results: *RHD* gene was not detected in 409 pregnant women so the administration of RhIg was not recommended. In 25 women Ct-values for *RHD* and *CCR5* indicated the presence of D variant in maternal genome and further follow-up revealed *RHD*01W.1*, *2* and *3* in 15 cases. *RHD* NIPD predicted RhD positive fetuses in the remaining 795 cases and for them RhIg administration was recommended. In 1/795 cases RhD negative phenotype of the neonate was reported but re-analysis confirmed fetal *RHD*-positive result.

Summary/Conclusions: For 5 years *RHD* NIPT has predicted a compatible RhD-negative fetus in about 31% of all tested women and pointed out the pregnancies that required no anti-D immunoprophylaxis. In 2% women the test revealed maternal *RHD* variants further identified in 60% as the weak D type 1 and 2 - not prone to anti-D immunization.

P-037 | Fetal *RHD* genotyping with parallel estimation of fetal and total fraction using digital PCR

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Background: RhD-negative pregnant women may produce anti-D antibodies against fetal RhD antigen inducing hemolytic disease of fetus and newborn (HDFN). Non-invasive prenatal diagnostics predicts the fetal *RHD* genotype but the main limitation is lack of estimation of fetal fraction in case of a *RHD* negative result at the same workflow.

Aims: To establish a digital PCR protocol for the detection of fetal *RHD* together with estimation of fetal and total fraction of cell-free DNA.

Methods: DNA from plasma samples of 20 pregnant women (14-33 week of gestation) and 3 donors was extracted using easyMag (Biomerieux). The digital PCR protocol was tested using a triplex real-time PCR assay for *RHD exon 5* (a FAM-labelled probe), *SRY* (a Cy5-labelled probe) as fetal marker, *ABCB6* (a VIC-labelled probe) as a total marker using QIAcuity One 5plex (Qiagen). The results were compared to real-time PCR results.

Results: The dPCR results of *RHD/SRY/ABCB6* genotyping were concordant with the genotypes of donors, pregnant women and their children. In all cases 333-15333 *ABCB6* positive partitions were detected corresponding to 340-24543 copies/ml of plasma. In 10 cases of women carrying D-positive fetuses 11-88 *RHD* positive partitions were detected (12-92 copies/ml of plasma). In 10 cases carrying D-negative fetuses there was no *RHD* positive partitions except one with a false *RHD* positive partition, and in 7 of these cases 6-47 *SRY* positive partitions were detected (7-48 copies/ml of plasma).

Summary/Conclusions: The digital PCR triplex protocol detects fetal *RHD*, its marker and total fraction of cfDNA with high specificity making the confirmation the *RHD* negative results possible at the same workflow. Further validation is required on a large cohort and with other multiplex assays for female fetuses.

P-038 | A novel ABO*A2 variant allele identified in two patients with A_{weak} phenotype

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Background: Synthesis of A and B antigens are regulated by single nucleotide polymorphisms in the *ABO* gene encoding glycosyltransferase A (GTA) and B (GTB). The critical substitutions discriminating substrate specificity for GTA and GTB are located in exon 7. Thus, substitution(s) close to this catalytic site often lead to discrepancies in ABO blood group typing due to altered substrate binding site, which is often accompanied by reduced enzyme activity. To ensure patient safety, it is necessary to fully characterize these variants at the molecular level.

Aims: Samples from two patients of Caucasian origin were investigated in our laboratory due to discrepancy in ABO blood group typing.

Methods: ABO blood group phenotyping was done by standard gel column agglutination testing (BioRad; Grifols) and tube technique. Genomic DNA was isolated from whole blood and molecular investigation was initially performed by sequence specific primer (SSP)-PCR detecting common ABO variants (ABO-Type variant, BAG Diagnostics). The samples were further characterized by exon sequencing including flanking intronic regions of the *ABO* gene using published and in-house primers.

Results: Patient 1 showed strongly reduced expression of antigen A, detectable with Gel cards. Plasma from this patient contained strong anti-A1 also reacting in saline tube at 37 °C. A commercially available kit detected the characteristic single nucleotide variations (SNVs) known for alleles *ABO*A2.01* and *ABO*O.01.02*, usually associated with normal A antigen expression. The sample of patient 2 was referred to our laboratory due to strongly reduced expression of antigen A. Using Gel cards, the RBCs of patient 2 had normal B antigen and no detectable A antigen. Only in tube technique could a weak agglutination with an anti-A be detected. Reverse blood group typing revealed a strong positive reaction with A1 test cells in saline tube still reacting at 37 °C. By SSP-PCR the alleles *ABO*A2.01* and *ABO*B.01* confirmed the group AB. Sequencing of *ABO* revealed in both patient samples, in addition to the SNVs detected by SSP-PCR, a heterozygous substitution c.550G>A in exon 7. This mutation induces the amino acid substitution p.Val184Met within the catalytic domain of the glycosyltransferase. This substitution is known on a B background

as variant *ABO*BW.33*, but has not been reported previously on an *ABO*A2* allele to the best of our knowledge.

Summary/Conclusions: Here we report an ABO missense substitution c.550G>A, previously not associated with an A_{weak} phenotype. Based on serological data, this amino acid substitution p.Val184Met likely diminishes GTA activity. As plasma from both patients contained strong anti-A1, blood of group O is recommended in case of transfusion.

P-039 | A *RHD**C.1130C>T mutation induces differential reactivity with anti-D sera

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Background: In cases where serologic RhD blood group testing shows differential binding of two or more anti-D with the patient's red blood cells (RBC) commercial PCR methods can support blood group serology by identifying the most frequent weak D and partial D types. However, in rare cases extended molecular typing by DNA sequencing is required.

Aims: We describe a new *RHD* variant in a femal patient with weak reactivity of the patient's RBC with two monoclonal anti-D and negative results in different commercial PCR-SSP assays.

Methods: Routine RhD typing of the patent's RBC was performed by haemagglutination with two IgM monoclonal Anti-D (ABO/D+ DAT DiaClon (DiaMed, Cressier, Switzerland) with automatic reading (IH-500, BioRad, Munich, Germany). Commercial PCR-SSP assays were applied to determine the most frequent variations: RBC-Ready Gene D weak (Innotrain, Kronberg, Germany) and Partial D-Type (BAG Diagnostics, Lich, Germany). Genomic DNA sequencing of the ten *RHD* exons including short flanking intron sequences was performed with the BigDye Terminator v.3.1 chemistry (ABI, Weiterstadt, Germany) followed by electrophoretic separation in an ABI Prism 310 DNA analyzer. DNA sequences were aligned to published reference sequences.

Results: The patient's RBC showed weak reactivity (++) with both monoclonal anti-D. Both commercial PCR-SSP assays did not identify a weak or partial D type. However, gDNA sequencing showed a non-synomymous *RHD*c.1130C>T* substitution within exon 8 that induces an Ala377Val exchange. Based on the predicted molecular structure (Wagner FF et al. Blood 1999) the variation should be located within the 12th membrane passage of the RhD molecule and seems to induce a reduced RhD expression. The new mutation was submitted to GenBank under the accession number MT396943.

Summary/Conclusions: The *RHD*c.1130C>T* mutation affects anti-D binding and defines a new weak D type.

P-040 | Risk of alloimmunization and clinical relevance of partial little E Antigens in patients with sickle cell disease (SCD)

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Background: Rh antibodies in patients with SCD is associated with the lack of the corresponding conventional antigens on red blood cells (RBCs) or may be a result of inheritance of altered RH alleles. A plethora of molecular tools are being developed to detect Rh variants, but they do not distinguish those likely to prone immunization from those that are unlikely to prone immunization and delayed hemolytic transfusion reactions (DHTR).

Aims: Considering that e-partial antigens are the Rh variants most commonly found in patients with SCD, we investigated partial-e antigens with different molecular backgrounds, their alloimmunization risk and their clinical relevance in patients transfused with e-positive RBC units.

Methods: We selected 15 patients with partial e antigens who were previously exposed to e normal antigens. All patients were phenotyped for D, C, c, E, e by hemagglutination in gel cards and genotyped with RHCE BeadChip arrays (Bioarray, Immucor). Sanger sequencing was performed when necessary. Antibody screening and identification with autologous control were performed by gel test. Direct antiglobulin test, adsorption with autologous RBCs and crossmatching with allogeneic partial RBC antigens when possible were also performed. The clinical significance of the antibody was assessed by comparison of the hemoglobin levels recorded before and after transfusion at the time of antibody detection and by clinical suspicion of anemia and hemolysis.

Results: The mean number of e+ RBC units transfused per patient was 12. The variant *RHCE*ce* alleles encoding partial e antigens found in these patients were: 2 *RHCE*ceAR*, 1 *RHCE*ceMO*, *6 RHCE*ce733G*, 4 *RHCE*ce48C*, *733G*, and 2 *RHCE*ceS* in homozygous status. Anti-e was identified in 7/15 patients with variant *RHCE* alleles (2 *RHCE*ceAR*, 1 *RHCE*ceMO*, *2 RHCE*ce733G* and 2 *RHCE*ceS*). Serological features of the identified Rh antibodies were compatible with the presence of alloantibodies. Laboratory evidence and clinical symptoms of anemia and hemolysis compatible with a DHTR at time of antibody detection were identified in the patients homozygous for *RHCE*ceAR*, *RHCE*ceMO* and *RHCE*ceS*. Anti-hrB and anti-hrS clinically significant were also identified in 2 patients genotyped as *RHCE*ceS/RHCE*ceS* and in one patient with the *RHCE*ceAR/RHCE*ceAR* genotype. Four of six patients with the *RHCE*ce733G* allele and all patients with the *RHCE*ce48C*, *733G* allele did not develop anti-e. The 2 patients who developed anti-e with the *RHCE*ce733G* allele had a good survival of the transfused RBCs.

Summary/Conclusions: This study showed that of 15 patients with altered *RHCE*ce alleles* encoding partial e antigens, 7 became alloimmunized after being exposed to e normal antigens. Only the anti-e produced by patients with *RHCE*ce733G* allele was not related to DHTR. Our results demonstrate that the alloimmunization risk and

the clinical significance of Rh antibodies produced by SCD patients with partial e antigen due to variant *RHCE*ce* alleles may vary according to the specific variant inherited. Knowledge of the relevant e-partial antigens would facilitate the prevention of alloimmunization and hemolysis caused by the production of alloanti-e in patients with SCD.

P-041 | Evaluation of serology methods and molecular typing for ABO, RH and KEL blood group determination in Moscow City Blood Center

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Background: Proper blood type testing is one of major the aspects for safe blood transfusion. Generally, blood groups can be determined by routine serology tests, even in case of weak subgroups. However, there are some restrictions, which require genotyping.
Aims: To assess the practice of blood group determination for ABO, RH and KEL by serology methods and genotyping.
Methods: 78056 donor blood and 2315 patient blood specimens from various Moscow hospitals were analyzed. Serology tests were performed with gel cards ID-DiaClon kits, Coombs Anti-IgG, Rh-Subgroups +K, Anti-A1 Absorbed, DiaMed Basic Q.C. (Bio-Rad) on IH-1000 analyzer and immuClone on Galileo NEO analyzer (Immucor). Genotyping was used in case of discrepant results and challenges in blood group determination (chimerism, pannaglutination, etc.). DNA was isolated from the whole blood with "Qiagen" kit. Allele-specific PCR on commercial test kits (ABO-TYPE, ABO-TYPE Variant, RH-TYPE, D-Partial, D-Weak, KKD-TYPE BAG Diagnostics) was used for ABO, RH и KEL genotyping with following electrophoresis detection in 2% agarose gel. Sanger sequencing was chosen as a supporting genotyping method based on BigDyeTerminator v3.1 (Thermo Fisher Scientific) chemistry. Sequences were analyzed in software SeqA6 and Ensembl.
Results: Serology testing combined with modern molecular methods allowed to determine blood group antigens in 46 individuals who had challenges in ABO, RH and KEL determination. Among 10 individuals with A_{weak} serology results alleles *A2.01 (n=4), *AW.30.01 (n=1, c.646T>A) and *AW.06 (n=5, c.502C>G) were found. Interestingly, in 2 cases with B_{weak} primary testing by allele-specific PCR showed common allele *B.01. Further investigation using sequencing revealed rare allele *BW.15 (c.565A>G) that explained serology testing results. Among 10 persons with serologically weak D 3 persons had *RHD*weak D type 1* and 1 had *RHD*weak/partial 15 type* alleles, other 6 ones had normal *RHD*01* in homo- or hemizygous state. Other 11 individuals had D-negative, but C/E-positive phenotypes which needed to be checked for the presence of *RHD* positive alleles. 2 of them carried *RHD*01* in hemizygous state, whereas *RHD*-negative status was confirmed by genotyping in 9 persons. In these examples sequences, specific for *RHD* alleles, including *DEL*, *RHD Ψ*, *Cdes* и *RHD* (W16X), haven't been detected in resolving limits of allele-specific PCR commercial kit. Another case demonstrated troubles in KEL phenotyping when donor had been determined as K+ or K− by different analytical systems. Genotyping showed only *KEL*02* and the final result was interpreted as K−k+. Also we met a rare case of D-positive individual, who lacked CcEe antigens. The result of genotyping showed the absence of sequences, specific for *C*, c, *E*, *e* alleles. Besides, we have had 9 samples with posttransfusional chimerism and 2 ones with panagglutination. These factors caused difficulties for proper blood group typing. Following molecular based testing helped us to determine ABO, RH and KEL.
Summary/Conclusions: Our experience shows that combination of serology tests with genotyping methods seems to be advisable approach when challenges in blood group typing occur. Allele-specific PCR can be chosen as a genotyping method for resolving discrepancies providing modern and efficient serology tests are used. Sequencing technology might be performed in particular cases as an additional testing.

P-042 | The clinical significance of nondeletional ABO*O alleles

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Background: The ABO blood group system is the most clinically significant in transfusion practice. There are two principal types of O-alleles: deletional alleles with 261delG leading to nonfunctional truncated protein glycosyltransferase (GT) and nondeletional alleles, the major of which are O.02 with typical nucleotide substitution c.802G>A, encodes full-length GT with Gly268Arg without fermentative activity. Sometimes O.02 may encode weakly functional A-GT (GTA).
Aims: to describe the frequency of nondeletional O-alleles in Russia and rare hemolytic posttransfusion reaction after ABO-identical plasma transfusion in patient after renal transplantation with O.02.01 allele and weak A- glycosyltransferase activity.
Methods: Erythrocytes and serums 107 A2O donors, 9 polytransfused chimeras patients and patient's L. were typed by agglutination method using column gel ABO reverse grouping card (BioRad, Swiss) and by classic method on plate using anti-A, anti-A1, anti-H, anti-B monoclonal antibodies (Moabs) and Dolichus biflorus lectin (anti-A1) (Tsoliclons, Hematolog, Russia) and standard A1, B erythrocytes (BioRad, Swiss). Adsorption-elution with anti-A reagents, warm and cold elution from patient's red cells. DNA were isolated by kits (BAG, Germany). Molecular studies were performed by SSP-PCR and direct Sanger sequencing of ABO gene exons (preferably exons 6 and 7).
Results: In 116 examined individuals with 125 O-allele of the ABO system we identified 12 different variants: 9 variants with c.261delG in 118 samples - O.01.01-72.8%, O.01.02 - 5.6%, O.01.05 - 0, 8%, O.01.11 - 5.6%, O.01.13 - 0.8%, O.01.26 - 5.6%, O.01.44 - 1.6%, O.01.46 - 0.8%, O. 01.68 - 0.8%, and 3 variants of nondeletional

alleles with the substitution c.802G>A (p.Gly286Arg) in 7 samples: O.02.01 (4.0%), O.02.02 and O.02.03, 0.8% each.

Patient's L. erythrocytes investigation by gel column method showed no agglutination with anti-A and -B Moabs and patient's serum agglutinated standard A1 and B erythrocytes, so the conclusion was done, patient had O blood group with natural anti-A,-B antibodies. The result of the classic method on plate was another – O blood group with only natural anti-B antibodies. The strong unspecific agglutination between patient's serum and standard A/A1-erythrocytes disappeared after incubation with warm saline solution. By microscope the strong "money columns" were observed. Adsorption-elution methods and warm and cold elution did not reveal A antigen on patients erythrocytes. Immediate hemolysis were reported following transfusion of small volume of ABO identical plasma. Molecular methods revealed ABO*O.01.01 O.02.01 genotype: the first allele was deletional and the second one was nondeletional.

Summary/Conclusions: so we revealed the patient with nondeletional O allele with weak GTA activity manifested by small quantities of A antigen on red blood cells not detectable by adsorption-elution methods but by ABO-typing discrepancy in classic methods and hemolysis after ABO identical plasma transfusion. The ABO*O allele frequency were estimate in Russian population.

P-043 | Analysis of *RHD* alleles in patients in the USA

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Background: There are over 100 *RHD* alleles encoding partial D antigens and more than 145 alleles encoding weak D antigens. It is well accepted that patients that carry Weak D Types 1, 2 or 3 are not at risk for anti-D alloimmunization. In the US, hospitals are encouraged to resolve serologic weak D phenotypes by submitting specimen to molecular laboratory for RHD genotyping to more accurately assess this risk. In 2020, nearly 900 patient samples were submitted to a national molecular laboratory in the US.

Aims: The *RHD* alleles identified were analyzed to determine the frequency of alleles associated with allo-anti-D and compared to alleles that are not. Allele frequency were evaluated based on race/ethnicity.

Methods: Genomic DNA (gDNA) was isolated from peripheral blood mononuclear cells and genotyping was performed using the SNP panel *RHD* BeadChip (Immucor). PCR followed by RFLP was performed for c.1136 present in the *RHD*DAU* family of alleles. *RHD* exon sequencing was performed to resolve indeterminate calls as needed. Since the Weak D 4.0 allele is assigned a partial D phenotype by the ISBT (www.isbtweb.org/working-parties/red-cell-immunogenetics-and-blood-group-terminology/) with limited reports of alloimmunization, the data was parsed for this allele.

Results: From Jan-Dec 2020, 879 samples were submitted for *RHD* genotyping, with 41% from African Americans (AA), 41% from Caucasians (CAU), with the remainder from "other" (11.1%), Hispanic (3.2%), race not provided (1.1%), mixed race (0.7%), Asian (0.6%) and Native American (0.3%). In all, 63.5% were predicted to express a variant RhD phenotype (weak or partial) with 31.5% found to be homozygous or hemizygous for Weak D types 1 (16.7%), 2 (11.1%) or 3 (3.4%). In the CAU patients, 61.7% were one of these three types. In all, 22.1% of patients were predicted to be partial D+, or 31% if those with Weak D 4.0 were included. In the AA patients tested, 39.7% were predicted to be partial D+ with that increasing to 58.2% if those carrying Weak D 4.0 were included. The most common *RHD* variant allele in CAU was Weak D type 1 (N=121) with 82% of patients with Weak D type 1 being CAU. The most common *RHD* variant allele in AA was Weak D 4.0 (N=67) with 77% of patients with weak D 4.0 being AA. Sixteen different partial D types were found in this patient cohort with the most frequent being from the *RHD*DAR* (N=71, 8.1%) or *RHD*DAU* (N=56, 6.4%) families.

Summary/Conclusions: *RHD* genotyping of large cohort of patients submitted to a large national molecular laboratory in the US found that nearly two-thirds expressed variant RhD antigens. Whereas in Caucasians, the majority (61.7%) of these were Weak D Types 1, 2 and 3 with the most common being Weak D type 1, in AAs, only 3% expressed one of these three types. Interestingly, approximately one-third of those with race listed as "other" carried Weak D types 1, 2 or 3 while another third expressed a partial D phenotype. These findings highlight that information regarding patient race can be helpful when using *RHD* genotyping.

P-044 | Most JK*01W.01 donors display a normal JK phenotype

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Background: JK*01W.01 allele has been described as a frequent molecular basis of weak Jk^a expression in 2011 (1). We therefore included typing for this polymorphism in our blood donor screen to improve antigen prediction.

Aims: Using donor samples identified by Maldi-TOF, we aimed to confirm the observations by Olsson M et al. on JK*01W.01.

Methods: Blood donors were screened for blood group polymorphisms including rs1058396 and rs2298720 by Maldi-TOF. Distribution of the single nucleotide variants was compared. Routine serologic phenotype determination was done for identified donor samples with JK*01W.01/ JK*01W.01; JK*01W.01/JK*02; JK*01/ JK*01 and JK*01/JK*02 genotype. Antigen strength scores were calculated from titration with two commercial polyclonal anti-Jk^a in BioRad ID gel technique.

Results: Among 1604 donors with valid typing results, 8 were homozygous and 209 heterozygous for the E44K substitution. The frequency of the c.130G>A substitution was 0.07. All 8 homozygous donors were c.838G (JK*01 type allele). Of the c.130G/A heterozygous donors, 67 were c.838G, 72 c.838G/A and one c.838A (this sample is currently under investigation to confirm the constellation). Hence the c.130A polymorphism is firmly associated with the c.838G polymorphism. In routine

antigen testing, 4 JK*01W.01 homozygous and 2 JK*01W.01/JK*02 samples type das JK^a positive without obvious weakening. Titer scores of JK*01W.01 homozygous samples (19.5 to 22.5) were only slightly less than Jk*01 homozygous samples (22 to 24).

Summary/Conclusions: As expected, the c.130G>A substitution is frequent in our donor population. This substitution occurred exclusively on a JK*01 background resulting in JK*01W.01 alleles. Serologic differences between donors carrying Jk*01.1 and JK*01W.01 were minimal. This observation confirms a similar observation in the seminal manuscript (1) and is compatible with the hypothesis that samples with considerably weakened Jk^a antigen on a JK*01W.01 background detected among patients are likely to harbour additional polymorphisms reducing JK^a expression. For antigen prediction, the value of identifying c.130G>A is very limited.

P-045 | Experience in applying *RHD* genotyping strategy for accurate and rapid detection of *RHD* variants in serologic *RHD* negative Korean blood donors

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Background: Prevention of RhD alloimmunization is clinically important for D negative patients. Recently, it has been reported that alloanti-D were acquired by transfusion of serologic D negative red blood cells. By genotypic analysis of the blood components, all of them were confirmed as DEL variants. For that reason, the application of *RHD* genotyping for the D negative blood donor has been required in addition to serological assay. So, we established the genotyping algorithm for the detection of *RHD* variants and applied this algorithm to serologic D negative and blood donors who voluntarily agreed to the test.

Aims: The aims of this study were to analyze the distribution of DEL type variants among serologic D negative blood donors in Korea and were to attribute safety of blood supply related with D negative blood products.

Methods: From September 2016 to December 2020 we recruited 216 RhD negative blood donors who were C positive and/or E positive in previous serologic Rh typing. We got informed consents from the donors for this study. Genotypic screening was performed by PCR amplification of *RHD* promoter, exon 4, exon 7, and exon 10. Based on the results of PCR screening, we discriminate between true D-negative (*RHD* total deletion or *RHD-CE-D* hybrid) and *RHD* variants (including DEL type) among samples. When the results were *RHD* variants, we entered the data into BIMS (Blood Information Management System) and performed exon 9 sequencing to identify nucleotide changes including c.1227G>A(Asian DEL type). Full *RHD* sequencing was done if no mutations were detected at exon 9.

Results: 39 cases of C-E-c+e+ phenotypes were excluded from data analysis. Among the remaining 177 cases which were C positive and/or E positive, we found 68 cases (38.4%) of *RHD* total deletion, 35 cases (19.8%) of *RHD-CE-D* hybrid, and 74 cases (41.8%) of *RHD* variants. Among the cases of *RHD* variants, 73 cases (98.6%) have c.1227G>A substitution and were confirmed as Asian DEL type. 1 case showed no nucleotide change at exon 9, but full sequencing was not possible due to inappropriate sample quality.

Summary/Conclusions: 41.8% (74 cases) of serologic D negative blood donors with C positive and/ or E positive phenotypes were reclassified as *RHD* variants by genetic test. Because the transfusion of blood with complete or variant D antigen can cause RhD alloimmunization, *RHD* genotyping is required to all serologic D negative blood donors to discriminate *RHD* variants from true D negative subjects. The implementation of *RHD* genotyping algorithm for all D negative blood donors can improve blood transfusion safety. However, the rapid exclusion of DEL type blood donors from D negative donor pool can cause trouble in the supply of D negative blood. Identification of DEL type among recipients should be done at a similar rate as in the blood donors.

P-046 | HLA-DRB1 and cytokine polymorphisms in Brazilian patients with myelodysplastic syndromes and its association with red blood cell alloimmunization

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Background: Many patients with myelodysplastic syndrome (MDS) are at red blood cell (RBC) alloimmunization risk due to chronic RBC transfusion. However, differences in the immune response of transfused patients with MDS are not completely known.

Aims: This study aimed to investigate the association of HLA-DRB1 and cytokine polymorphisms with RBC alloimmunization in Brazilian MDS patients with prior exposure to RBC transfusion.

Methods: We evaluated a retrospective cohort of 87 polytransfused patients with MDS including 28 alloimmunized (PA) and 59 non-alloimmunized (PNA) treated in three Brazilian reference hospitals. HLA-DRB1 genotyping was performed by PCR-SSOP (Luminex platform) and Cytokine gene polymorphisms were analyzed by polymerase chain reaction (PCR) and TaqMan assays.

Results: Our results showed that PA patients were older and received a greater number of transfusions when compared to PNA patients (p=0.027 and 0.003, respectively). While the HLA-DRB1 allele frequencies did not differ between groups, *IL17A* –197G>A SNP showed a significant correlation with RBC alloimmunization. The allele A and AA genotype significantly more frequent in PA than PNA (allele A, 27.1% vs. 46.4%, p=0.012; OR= 2.3; 95% CI= 1.1-4.9; AA genotype, 6.8% vs. 25%, p=0.041; OR = 6.2; 95% CI 1.3–30.8). Moreover, a significant association of alloimmunization to Rh antigens with IL17A–197A allele and AA genotype was also identified in PA group (allele A, 27.1% vs. 45%, p = 0.036; OR= 2.5; 95% CI 1.1-5.7; AA genotype, 6.8% vs. 30%, p=0.042; OR = 7.9; 95% CI 1.5–42.3).

Summary/Conclusions: This study showed no association regarding HLA-DRB1 alleles for RBC alloimmunization risk or protection, however the IL17A–197G>A SNP was significantly associated with risk to RBC alloimmunization in this cohort of Brazilian patients with MDS.

P-047 | Transfusion-dependent patient's blood group genotyping

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Background: Transfusion management of patients with hematological diseases is often complicated. Presence of donor's erythrocytes in patient´s circulation after previous transfusions prevents accurate blood group phenotyping unless the phenotyping was not performed before the first transfusion.

Aims: The aim of this study was to evaluate the effectiveness of blood group genotyping and to compare the molecular typing of erythrocyte antigens with the established serological methods.

Methods: Blood samples from 10 blood donors and 95 multi-transfused patients including multiple myeloma (n=7), thalassemia (n=4), lymphomas (n=11), chronic myeloid leukemia (n=16), primary myelofibrosis (n=9), myelodysplastic syndrome (n=22), acute leukemia (n=22), aplastic anemia (n=5) were used for red blood cell genotyping and phenotyping. ABO blood group determination as well as typing of D, C, c, E, e and K antigens were performed using a gel agglutination test. The Rh-Type and KKD-Type (SSP-PCR RBC – FluoGene vERYfy (Inno-train Diagnostik) were used to determine the polymorphisms associated with antigen expression for RHD, RHCE and Kell blood group systems, respectively. Results of this determination were compared with results of RhD-, RhCcEe- and Kell-phenotyping.

Results: We observed a good correlation between serological and molecular methods for donors that were concordant for 100%. Patient´s results were concordant only for 54.7% and discordant for 45.3%. These discrepancies were confirmed by serological typing at subsequent hospitalizations. The results of 39 patients showed the presence of antigens C, c, E, e in the phenotype and the absence in the genotype. It means that these patients previously got some transfusions of blood components that were compatible but not identical in antigens of the Rh system. In 12 patients observed genotype-phenotype discrepancies could potentially cause alloimmunization. Ten patients who had been previously phenotyped as RhCc were genotyped as RHCE*CC, which necessitated the search for c-negative RBC units. Similarly, 2 patients who had been previously phenotyped as Rhee were genotyped as RHCE*EE and required transfusions of e-negative RBC units. In 2 patients antigens D and C were not detected in the phenotype, but were identified in the genotype. These patients suffered from myelodysplastic syndrome and chronic myeloid leukemia and absence of mentioned antigens in phenotype indicated the loss of expression of antigens of erythrocytes. In one patient, the lack of expression was of short duration and expression recovered after receiving treatment. In the second patient previously observed expression loss was associated with the development of the terminal recurrence with subsequent death. Discrepancies in antigen K were recorded in 2 patients; the antigen was absent in the phenotype, but was present in the genotype. The resulting discrepancies are associated with high transfusion activity in patients who received transfusions of erythrocyte components which did not contain the Kell antigen. All patients received transfusions of blood components taking into account the genotype of the Rh system. As a result of transfusion, no post-transfusion reactions and complications were recorded.

Summary/Conclusions: Blood group genotyping is important in transfusion management of chronically transfused patients, especially if these patients were not phenotyped before the first transfusion.

P-048 | Molecular versus serology testing in detecting *RHD* gene discrepancies

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Background: Rhesus (Rh) factor is an inherited erythrocyte membrane protein. There are two RHD phenotypes: RhD positive and RhD negative which are determined by the presence or absence of the highly immunogenic RhD protein. The Rh blood group system is the second most clinically significant of the blood groups, second to the ABO system. It is also the most polymorphic of the blood groups, with variations due to deletions, gene conversations and missense mutations.

The molecular laboratory performs RHD genotyping for screening, diagnostic and for emergency cases. Therefore, provide accurate pre-transfusion and compatibility testing between donors and patients. Hence, maintain the coordination and continuous supply of appropriate blood products for patients with blood group incompatible alogeneicity.

Aims: In here, we report two unrelated clinical cases with a discrepancy in the *RHD* gene between serological and molecular tests.

Methods: Two unrelated cases, a male (36 years old-patient 1) and a female (43 years old-patient 2) were typed by serological tests for RHD antigen detection (Immucor and Liss Gel Method, Biorad). Whole blood samples of those two cases (EDTA tubes) were referred to molecular lab to perform genotyping which was performed by ID RHD XTTM utilize Luminex xMAP® technology. Bi-Directional Sequencing was performed for the two genomic DNA samples.

Results: Serological findings: Patient-1 typed as D negative and patient 2 typed as partial/weak D.

Molecular findings: Patient -1 D positive due to *RHD**Pseudogene heterozygous genotype status.

Patient-2 D Negative.

Sequencing findings: Patient 1 Weak D with *RHD*weakD type 5*, *RHD*Psi* genotype. Patient 2 D Negative with *RHD* deletion, *RHD*del (1)* genotype.

Summary/Conclusions: Molecular lab results of the two samples with RH blood group showed discrepancies between serology and molecular/sequencing results. For patient-1 the RHD gene showed (*RHD**Pseudogene heterozygous) and was indicated to be RH negative in the serology result. However, due to the presence of the pseudogene in a heterozygous state, the molecular lab needed further investigation to confirm whether the trans- allele carries the functional copy of the *RH* gene. Sequencing result of this sample reported that the heterozygosity included one allele with the presence of the pseudogene and one allele with the presence of weak D type 5 variant and hence a weak D phenotype. This result is consistent with the molecular lab interpretation as the presence of the RH gene is due to the weak protein function of the weak D type 5 in one allele however due to the limitation of ID RHD XT test in predicting weak D type 5 this variant was not detected in the molecular test.

For patient-2 the *RHD* gene showed D Negative with RHD deletion, *RHD**del (1) genotype in both molecular and sequencing results due a deletion in the *RHD* gene (One allele is completely deleted and one allele with only exon 1 deleted) therefore was interpreted as D positive in the serology result due to the presence of weak protein function of the allele with exon 1 deletion. Those false positive results obtained by both ID RHD XT tests are considered discrepant results and then a malfunction.

The identification of these discrepancies provide an accurate approach to safely manage D negative units and avoid unnecessary use of Rh Immune Globulin (RhIG).

P-049 | Validation of blood group genotyping array on Ghanaian blood samples

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Background: In near future blood donor genotyping might replace serology. However, so far genotyping assays have mainly been validated in Europeans. The Ghanaian population is highly diverse and shows variation in blood group antigens, leading to increased risk of immunization in multitransfused patients, such as those with Sickle cell disease. Recently, a blood group array from which full RBC types could be extracted was developed and validated on European donors (Gleadall et al. Blood Adv 2020).

Aims: To determine the accuracy of this array in a cohort of 444 Ghanaian donors and to use the genotyping data to assess the frequency of different blood group antigens in the Ghanaian population.

Methods: Glycerolized RBCs, whole blood, and plasma were frozen at -80°C. DNA was extracted using automated Chemagen DNA extraction equipment, RBC genotyping was performed using the Axiom genotyping platform. Blood group antigens were inferred from the array data using the bloodTyper algorithm. RBC serology was performed using tube and microtiter technique. The phenotype frequencies of each antigen were compared with frequencies for European Americans (EA) and African Americans (AA) at bloodantigens.com

Results: A total of 232 RBC antigens belonging to 36 systems were tested. Variation in RH and MNS will be analyzed separately. The number of antigens tested per system ranged from 1 to 36 as listed in Table 1.

The genotype results were validated with serology on all 444 samples for ABO, RHDCcEe, JKa, Jkb and on 117 samples for MNS and FY. There was 100% concordance between the genotype and serology results for antigens c, e, s, Fy^a and Fy^b. The remaining antigens A, B, D, E, S, Jka, Jkb had ≥98% concordance.

In PIPK, LU, KEL, JK, DI, YT, XG, SC, LW, H, KX, IN, OK, RAPH, I, GLOB, GIL, RHAG, FORS, JR, LAN, VEL, CD59, KANNO and AUG systems, the frequencies of the antigens were comparable to reported frequencies among EA and AA. For ABO and LE, the frequencies of the antigens were as expected for Africans except Le^b (78%) which was slightly higher than expected In CO, all frequencies were comparable except that no Co(b) (0%) was observed. All but 1 person were homozygous for the GATA null mutation, resulting in absence of Fy antigens. In GE, the rare antigen An(a) was detected in 5 Ghanaians. In KN, antigen Vil was high (94%) in Ghanaian compared to EA (0.7%) and AA (80%) while the KCAM was low (15%) compared to 96% in EA and 43% in AA. In DO, all samples were negative for the HFA Jo(a) suggestive for failure of the probe.

P-049 Table 1. Number of antigens tested in each of the 36 blood group systems.

ISBT	001	002	003	004	005	006	007	008	009	010	011	012	013	014	015	016	018	019
N=	2	23	3	26	20	35	2	5	3	20	3	1	7	10	4	3	1	1
ISBT	020	021	022	023	024	025	026	027	028	029	030	031	032	033	034	035	036	038
N=	8	18	9	4	3	1	5	1	1	1	3	1	1	1	1	1	3	1

Summary/Conclusions: Although we observed high concordance between serological and array-based typing results for most of the major blood group systems, the accuracy was lower than in Europeans, indicating the need for adaptation of the probes or BloodTyper algorithm. Almost all antigen frequencies observed in our cohort were comparable as expected for AA showing the reliability of the array in typing of African samples. The antigens for which significant differences were observed could suggest that there is undocumented genetic variation at relevant loci in the Ghanaian population which requires further investigation The extended typing data that arrays provide, pending further validation, could improve the safety of blood transfusions for the Ghanaian people.

P-050 | Molecular blood group screening in Omani blood donors

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Background: It is well known that red blood cell allelic and phenotype frequencies vary between different populations. Most data in the literature include frequencies in the western countries. Blood group genotyping arrays have been widely used in Caucasian, African American, and Asian populations.

Aims: This study aims at evaluating the genotypes of common blood group antigens in the Omani blood donors and to assess the concordance rate with obtained phenotypes.

Methods: A total of 180 blood donors attending a blood bank at a tertiary care University Hospital were consented for enrolment. Samples were typed by serological methods for the six blood group systems; RhD/RHCE, Kell, Kidd, Duffy and MNS. Samples were processed for genotyping using RBC-FluoGene vERYfy eXtend kit (inno-train, Diagnostik, Inc). Phenotypic variants for 70 RBC antigens in the RHD/RHCE, Kell, Kidd, Duffy, MNS, Dombrock, Lutheran, Cartwright, Diego, VEL, Colton and Knops blood group systems were assessed.

Results: Concordance rate was > 95% in all blood group systems with exception of Fy^b (89%). Homozygous GATA-1 mutation predicting the

P-050 Table 1. Antigen genotype frequencies in the Diego, Dombrock, Kell, Cartwright, Vel and Knops-Helgeson blood group systems.

Allele	Antigen number	Number tested	Omanis	Whites	Blacks	Other populations
Diego						
Di(a+)	DI*1	175	0.57	0.01	0.02	Japanese 12%, Chinese 5%, Hispanics 1%
Di(b+)	DI*2	172	100			most populations 100%, Native Americans 96%
Wr(a+)	DI*2.03	172	0			<0.01% (all populations)
Wr(b+)	DI*2	175	99.43			100% (all populations)
Dombrock						
Do(a+)	DO*1	174	69.54	67	55	Japanese 24%, Thais 14%, *Arab/Iranian 43.8%
Do(b+)	DO*2	173	78.61	82	89	*Arab/Iranian 56.2%
Colton						
Co(a+)	CO*1	175	100	99.5	99.5	*Arab/Iranian 99.3%
Co(b+)	CO*2	165	1.21	90	90	*Arab/Iranian 0.7%
Kell						
Js(a+)	KEL*6	174	3.45	<0.01	20	*Arab/Iranian 0.4%
Js(b+)	KEL*7	166	98.8	100	99	*Arab/Iranian 99.6%
Cartwright						
YT(a+)	YT*1	173	98.84			Most populations > 99.8%, Israeli Jews 98.6%, Israeli Arabs 97.5%, Israeli Druse 97.4%, *Arab/Iranian 89.1%
YT(b+)	YT*2	175	7.43	8		Israeli Jews 21.3%, Israeli Arabs 23.5%, Israeli Druse 26%, *Arab/Iranian 10.9%
VEL						
Vel		174	100			*Arab/Iranian 99.1%
Knops-Helgeson						
KN(a+)	KN*1	175	98.86			
KN(b+)	KN*2	172	1.74	4.5	<0.01	

Fy(a-b-) null phenotype was detected in 81/112 (72%) of genotyped samples. The common Fy(b+) phenotype was predicted in 13/112 (11%) of samples genotyped, leading to discrepant Fy^b phenotype/genotype results. D variants and partial e (V+VS+; c.733 C>G) was found in 22/100 (22%) and 14/119 (11%) of the samples respectively. We report the allele frequencies in the Diego, Dombrock, Kell, Cartwright, Vel and Knops-Helgeson blood group systems (Table 1). Di(a-b+), Js(a-b+), YT(a+,b-) and Kn(a+,b-) frequencies were 99.4%, 95.8%, 91.9% and 97.7% respectively.

Summary/Conclusions: This is the first study reporting the detailed distribution of common and rare red cell genotypes in Omani donors. We report a high frequency of *FY*B^ES* allele due to homozygeous GATA-1 mutation. Further sequencing studies are required to validate our findings.

Red Cell Immunohaematology - Rare Donors

P-051 | Finnish JK(a-b-) donors supporting oncologic treatment of a US patient

selected for main programme

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Background: The Finnish Red Cross Blood Service (FRCBS) takes care of all blood service activities in Finland. Around 200 rare donors are listed in our database and a pool of frozen rare units is maintained. Rare blood groups vary a lot depend on the country and the ethnicity. Jk(a–b–) is more frequently found among the Finnish and Polynesian populations. Finnish rare donors are listed in the International Rare Donor Panel.

Aims: The American Rare Donor Program (ARDP) staff first contacted FRCBS in September 2020 with an urgent request for O RhD neg Jk(a-b-) units. The patient, an 85 year old woman, diagnosed with myelodysplastic syndrome has presented with a hemoglobin range of 6.3g – 7.8g and would require support over a period of time.

Methods: FRCBS has rather adequate pool of Jk(a-b-) donors, with a total of 23 active donors and 19 frozen units in September 2020. This is due to testing performed in 2009, we screened 99 000 donor samples using the Olympus PK 7300 urea test and found a total of 22 new Jk(a-b-) donors. New rare donors are also found using routine phenotyping and genotyping techniques and by testing patient and antenatal samples. Siblings of patients and donors who are found to be of a rare phenotype are contacted and tested for the same rarity.

The requirement for group O RhD negative units in addition to the rare blood group, presented an extra challenge. For the first shipment in September 2020, two donors donated on very short notice and the delivery went according to plan. The second two-unit request came in November 2020. Only one donor was available in the Northern part of Finland and luckily a mobile donation site happened to be in the area. Another unit was thawed, even with the 7 day shelf life using the ACP215 process, this presented a challenge for the delivery over the ocean. It was determined that if everything went like planned, there should be 3 days of shelf-life remaining on the thawed unit. Unfortunately, the units were lost during the shipment. Third shipment of two thawed units was sent to replace the lost RBC's. These units were delayed in US Customs for two days, but luckily were transfused before their expiration date. The fourth and the fifth shipments in the beginning of 2021 went successfully, although, the fifth delivery was completed by another courier company because our routine courier could not supply transport for 3 weeks. In the latest delivery, there was an issue with the icing of the shipment, but the units were able to be transfused.

Results: Although group O RhD neg Jk(a-b-) blood is very rare, even in Finland, 3 fresh and 5 thawed units were sent to the patient. In November 2020, Finland was the only country with O RhD neg Jk(a-b-) donors or units available in the world. Altogether five Jk(a-b-) donors helped this patient: one of them had been found in routine donor typing, two in the mass screening and two by sibling testing. These transfusions helped the patient survive and allowed treatment of her primary disease.

Summary/Conclusions: Shipping thawed blood units over the ocean, especially during the COVID-19 pandemic when the number of operating flights and courier staffing was reduced, was quite a challenge. The lost shipment was a very rare unfortunate incidence. This case highlights the importance of rare donor program strategies worldwide. Compatible blood is not always available for the patients with rare phenotypes, but international co-operation might help finding a suitable donor.

P-052 | A novel mutation in the Kidd (JK) blood group causing a JK*A null phenotype

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Background: The Kidd (JK) glycoprotein is the red blood cell urea transporter that rapidly transports urea into and out of RBCs to maintain both the osmotic stability and the shape of the RBC. There are three common Kidd phenotypes: JK (a+b-), JK (a-b+), JK(a+b+). The JK- null phenotype, JK (a-b-) is extremely rare. Kidd JK (a-b-) individuals can form clinically significant anti-JK3 antibodies, which can cause hemolytic transfusion reactions and hemolytic disease of the fetus or the newborn (HDFN).

Aims: In here, we report two clinical cases with a rare red blood cell JK- null phenotype and a new variant in the *JK*A* allele.

Methods: A-39-year old female patient at 37 weeks gestation presented for elective cesarean section and was admitted to the hospital with the need for transfusion support. Patient and relative RBCs samples were typed by standard serological tests for antigen detection (Ortho Liss IAT tube method, Immucor and Liss Gel Method, Biorad). Whole blood samples (EDTA tubes) for genotyping were received

from serology laboratory for molecular testing of blood groups which was performed by ID CORE XTTM utilize Luminex xMAP technology. DNA sequencing was requested and performed by Grifols Immunohematology Centre for Bi-Directional Sequencing.

Results: Serological findings: The patient's phenotype was negative for the following antigens: R_{Or}, K, Fyb, S, Lea, N and the JK-null phenotype: JK (a-b-). Direct antiglobulin test and auto control were also negative. Serological tests were performed for the patient and her relative showing a negative phenotype for O, K, Fyb, S, Lea, N, and Jk-null phenotype, JK(a-b-). However, antibody identification test showed an antibody against a high-frequency antigen, suggesting the presence of anti- Jk3 antibodies. Previously frozen JK (a-b-) RBCs confirmed the presence of anti-Jk3 antibodies.

Molecular findings: the molecular lab results for the patient and her relative showed that both have JKa(a+b+) phenotypes.

Sequencing findings: Both patient and her relative genotypes showed a JK*A SLC14A1: c.191G>A mutation in exon 4 in the JKA gene associated with a predicted phenotype JK(a-b-), matching with serological data.

Summary/Conclusions: Sequencing results of two DNA samples with Kidd blood group interrogated JK gene exons 3-10 and the genotypes SLC14A1: c.191G>A JK*A (191A) were found in a homozygous state. Those alleles are null alleles reported by ISBT, (JK*02N. 09). Due to this homozygosity the predicted phenotype in this gene was found not to be expressed. The reported discrepancies in the molecular lab result which predicted JKa (+) phenotype is due to a limitation of the ID CORE XT test in detecting all covered Kidd blood group variants which are (c.342-1G >A, SLC14A1: c.838 G >A and SLC14A1: c.871T>C). Those false positive results obtained by both ID CORE XT tests are considered discrepant results and then a malfunction. These limitations are covered by the general assay limitations described in the test package inserts. The current study identified a novel polymorphism variant in JK*A (191A) genotype. The new variant c.191G>A has not been reported on JK*A gene previously. Although serological status of JKa remains unclear, the phenotype of this sample is likely JKa negative. The identification of these discrepancies for clinically relevant antibodies allow appropriate monitoring in avoiding hemolytic transfusion reaction and HDFN hence early interventions and improved care.

P-053 | Compound heterozygosity induces the Gerbich-negative phenotype in a blood donor with a broadly reactive antibody: A family analysis

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Background: The Gerbich blood group system is encoded by the *GYPC* gene and includes homologous regions (Gourri E et al. Brit. J. Haematol. 2017). In rare cases, deletions of either exon 2 (YUS type) or exon 3 (Gerbich type) are responsible for the lack of the high frequency antigens Ge2 and/or Ge3. Individuals with the respective types can develop anti-Ge2 or anti-Ge3 with the capacity to induce transfusion reactions. A female blood donor attracted attention because of a broadly reactive antibody that exclusively did not react with Ge-2,-3 RBC. Thus an anti-Gerbich was assumed.

Aims: The molecular basis of the Gerbich-negative phenotype should be elucidated.

Methods: Antibody differentiation was performed in the indirect agglutinin test (IAT/ID card) on extended panels with native, DDT and enzyme treated RBC including cells lacking different high frequency antigens. Molecular typing included gDNA analysis of the donor, her parents and sister. DNA was amplified in two long range PCR reactions that are specific for either the *GYPC* exon 2 deletion or the *GYPC* exon 3 deletion and result in amplicons that are truncated by the respective deleted sequence. Sanger and NGS sequencing based on deletion specific amplicons, too.

Results: The donor's serum was positive at a titer of 1:16 with all native or DDT treated RBC tested so far except for papain treated RBC. Only one GE:-2,-3 RBC showed missing reactivity with the donor's serum in the IAT. The donor and her sister both typed as O RhD positive (CDe/cde), K negative. Genomic DNA analysis demonstrated that both siblings exhibited compound heterozygosity with a *GYPC* exon 2 deficiency on one chromosome and an exon 3 deficiency on the other chromosome. The father was heterozygous for the exon 2 deficiency and the wild type allele while the mother carried the exon 3 deficiency together with a wild type allele.

Summary/Conclusions: We present a rare case of a Gerbich-negative phenotype in an immunized female donor that is caused by compound heterozygosity for two different Gerbich deficiencies. Because both siblings have identical ABO, Rhesus, Kel and Gerbich blood types they could give blood for each other and for immunized patients with a Gerbich-negative phenotype.

P-054 | Managing requests for rare blood in a pandemic: American Rare Donor Program Data

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Background: The SARS-CoV-2 pandemic had a profound effect on the USA, reaching into every walk of life and work. American Rare Donor Program activity from 2019 (pre-pandemic) and 2020 were compared to evaluate the impact on the blood transfusion community.

Aims: Assess 2019 and 2020 requests for rare units and new rare donor submissions to the American Rare Donor Program (ARDP).

Methods: Interrogate 2019 and 2020 ARDP data and review by month. Data analysis included number of rare blood requests, fill rates of requests, liquid or frozen product sourced, and number of donors submitted to register into the program. An unfilled request indicates no rare units were sourced, meaning these patients did not receive the blood that was requested by their physicians, partially filled

requests are those where one or more units was sourced, but the request was not filled.

Results: Request for rare units were 849 in 2019 and 1000 in 2020, a 15% increase in 2020. The fill rate was the same for both years at 93%. The Unfilled Request rate was 4.8% in 2019 and 4.2% in 2020. The remainder of requests were partially filled at a similar rate, 2.2% in 2019 and 2.6% in 2020. Requests were filled with liquid units at similar rates, 55% in 2019, 56% in 2020. In a review of the months immediately following the USA lock-down for SARS-CoV-2 mitigation, April, May and June 2020, requests were 194 in 2019 compared to 237 in 2020, an 18% increase. The fill rates in these months were comparable - 93.3% in 2019 and 95.3% in 2020, a 2% increase in 2020. In the months that followed, July through December, ARDP received 446 requests in 2019 compared to 540 in 2020, a 17.5% increase. Submissions of new rare donors to be registered in the ARDP were 5949 in 2019 compared to 5035 in 2020, a 15.4% decrease. In April, May and June 2019, rare donor registrations were 1,474 compared to 427 in 2020, a decrease of 71%. In the months that followed, there were 3504 registrations in 2019 and 3422 in 2020, a 10% decrease.

Summary/Conclusions: In a comparison of 2019 to 2020, ARDP requests were increased 15% in 2020. The number of unfilled requests was similar in both years, 41 vs. 42. In 2020, the months of April, May and June were the most impacted, although the rest of 2020 also showed increases in requests and decreases in donor submissions. It is unclear if the decrease in submissions of new donors was due to fewer new donors presenting to donor centers, a decrease in screening activities at the centers, less submissions of new donors due to limited staff time or a combination of all.

P-055 | Yt(a-) rare blood phenotype patient: Real challenge to transfusion medicine

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Background: The Yt antigen system (also known as Cartwright) is present on the membrane of red blood cells and helps determine a person's blood type. Antibodies against the Yt system can lead to none to moderate haemolytic transfusion reaction. Yt(a-) is an extremely rare blood phenotype occurs 1/1000 population or less than that.

Aims: We present a case of 71 years old, Punjabi elderly man who was diagnosed with L2/L3 prolapse intervertebral disc for reconstructive operation. He has underlying Ischaemic Heart Disease (IHD), Chronic Kidney Disease (CKD) with history of Colon Cancer. During blood investigation, noted that he has rare blood group (anti-yta antibody) on the 22rd July 2020. He was planned for spine surgery on the 22rd September 2020. It was about 2 months duration to describe clinic characteristics in patient with rare blood and to determine adequate approaches to support patient with rare blood in need of transfusion.

Methods: It was multidisciplinary approach between Transfusion Medicine, Ortho-Spine team and Anesthesia team.

Results: Our plan of management were, Firstly, reassurance patient and family members regarding status of yta blood phenotype in Malaysia, the risk of transfuse incompatible blood if patient in need of blood transfusion during surgery, family screening if there are compatible blood in the family and plan for parenteral iron. Secondly, family screening was done on the 5 August 2020. Only 1 son tested in family screening. The blood send to National Blood Centre, Kuala Lumpur (NBC) on the same day. Thirdly, we linked with Rare Blood Donor Registry NBC, there was no fresh blood available in inventory for him for the time being. No frozen blood Yt(a-) in NBC. No donor match in Rare Blood Donor Registry in NBC. Fourthly, we tried to implement Patient Blood Management (PBM) by using autologous blood or cell saver. Unfortunately, He was not a suitable candidate to go through this PBM techniques as he is having IHD, CKD and history of Colon Cancer. Fifthly, we administered 2 doses of parenteral iron for him. Sixly, we served IV tranexamic acid during induction of anesthesia, support with crystalloid and colloid perioperatively, and to introduce IV FEIBA/ PCC (Prothrombin Complex Concentrate) 30 - 50iu/kg if he bled. He bled 400ml throughout the operation. There was no indication to transfuse blood. We gave him 1 dose of IV parenteral iron 24hours post operation. Day 3 of operation, he discharged well.

Summary/Conclusions: In conclusion, a close collaboration between the Transfusion Medicine personnel, surgeons and clinicians are essential for management of patients who need rare blood phenotype. The use of collaboration between regional, national and international Rare Blood Donor Registry are crucial to ensure patients requiring rare blood phenotype to receive the right blood to the right time.

P-056 | The rarest of the rare B subgroups (BX & B3) detected at a standalone life blood centre in Saurashtra Region, Gujarat, India

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Background: There are 43 blood group systems registered as per International Society of Blood Transfusion, including ABO & Rh. ABO blood group system still remains the most important one. These principal groups also have subgroups, but we come across them infrequently due to very low prevalence. Still most of the laboratories, especially in developing countries, are doing blood grouping only by cell grouping and the results are not counter checked by serum grouping. Serologically, the variants of "B" can be classified into B_3, B_x, B_m & B_{el}. B_3 shows a mixed field agglutination with Anti-B sera while in B_x weak agglutination with anti-B as well anti-B in serum can be seen. B_x & B_3 are very rare type of B group so most of the time it has been wrongly reported as O type due to very weak presence of antigen B on red cell membranes, which cannot be detected if low titre

P-056 Table 1. Case wise characteristics of B subgroups.

Case	Phenotype	Reagents					Antibodies in serum		Saliva status Substance present
		Anti-A	Anti-B	Anti-AB	Anti-H	Anti-D	A cells	B cells	
1	B_X	0	W+	1+	3+	4+	3+	W+	H
2	B_X	0	W+	1+	3+	0	4+	W+	H
3	B_X	0	W+	1+	3+	0	3+	W+	H
4	B_3	0	W+/MF	0	3+	4+	3+	0	*
5	B_3	0	W+/MF	0	3+	4+	3+	0	#

*Non-secretor.
#Saliva sample not received.

anti-B grouping sera is used in forward grouping and reverse grouping is not done. The current report five cases of subgroups of B (three cases of B_x and two cases of B_3).

Aims: To detect and resolve discrepancy of ABO grouping results so as to prevent ABO mis-match transfusion.

Methods: Blood group discrepancies were reported for cell and serum grouping in five cases (four normal persons & one patient) during routine blood grouping process performed on automated platform as well by CTT method. Clerical & Technical errors were also analysed to rule out the problems. Advanced immuno-haematology workup like increased serum-to cell proportion and incubation time period of cell-serum grouping at 22^0C & 4^0C were carried out but the discrepancies remained. Then cold adsorption and heat elution as per AABB guidelines were done. For the confirmation salivary testing (four cases) were performed. Blood group reports with all mandatory recommendation were issued to each persons and proper counselling was done. In one case the request for two units of red cells was received for a patient (B_3 group) in emergency; so "O" blood group Leuco-reduced RBC after CAT cross-match were issued while communication with treating clinician and patient's relative was done as well as their written consent was taken.

Results: In all three cases of B_X groups weak agglutination with anti-B and anti-AB antisera as well as weak clumps with B cells along with 4+ gradation with A cells were observed while in two cases of B_3 subtype weak agglutination with only B sera were seen and there was no agglutination with Anti-AB sera and with B cells. Results of all adsorption-elution technique with appropriate valid controls yielded only "Anti-B" on elution, which indicated the presence of weaker B variants of B group. Saliva testing could not be done only in one case as patient was unable to turn up at our blood centre for providing the sample. Out of four cases, presence of both B and H substances were detected in three cases, whereas the one was found to be non-secretor (Table-1- on next page). Out of five case in two cases Rh phenotype was negative (B_X Negative).

Summary/Conclusions: Cell and serum grouping of the recipient and donor must be done meticulously to identify such subgroups and avoid haemolytic transfusion reactions.

P-057 | Initiative for rare donor registry for A_2/A_2B subgroups with RH phenotyping: A first of its kind

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Background: Prevalence of ABO antigens in Indian population is 21.77% for A and 9.09% for AB, making chances of prevalence of A_2/A_2B subgroup rare. Studies have reported from various parts of India and across the globe that in general, prevalence of subgroup A_2B is more frequent than A_2. Anti- A_1 antibodies appear as cold agglutinins, present in 0.4% of A2 and 25% of A_2B individuals, making it clinically significant when reactive at 37°C, causing haemolysis of A_1 RBCs. Amongst the five RH antigens phenotyped serologically, highest prevalence in Indian population is of "e" antigen followed by "D", "C", "c" and "E" being the lowest. Alloimmunized patients with Anti- A_1 antibodies should receive AHG compatible A_2/A_2B PRBC. Cases are reported from all over the globe of A_2/A_2B patients having clinically significant Anti- A_1 antibody with implicated HTR and death; and no transfusion could be given to such patients with rare RH phenotypes due to non-availability of blood and database of A_2/A_2B donors with rare RH phenotypes for directed donation.

Aims: To create donor registry of A_2/A_2B blood donors with RH phenotype to provide transfusion to all A_2/A_2B patients to prevent alloimmunization of Anti- A_1 antibodies.

Methods: A retrospective data analysis was done in a tertiary care hospital based blood centre at capital city of India from January 2016 to December 2020. All donors were subjected to ABO RH grouping and RH phenotyping by Solid Phase method. Tube Technique was used to distinguish A_2/A_2B by agglutination reaction with Anti- A_1 Lectin. Data was analysed by appropriate statistical tools.

Results: Total of 40,051 donors were analysed during study period, of which, A and AB groups constituted 21.26% (n= 8517) and 9.02% (n= 3611) of donor population respectively, out of which only 0.46% (n= 187) belonged to A_2while A_2B was more frequent; 1.07% (n= 427). The RH phenotype in A_2/A_2B blood donors (n= 614) is as shown in Table. The rarest phenotypes found were R1Rz, r''r, r'r''. There was complete absence of RH phenotypes - R2Rz, RzRz, r'r' and r''r'' in A_2/A_2B blood donors.

P-057 Table 1. RH phenotype prevalence in A2/A2B blood donors.

RH Phenotype	A2 Donors; N (%)	A2B Donors; N (%)
No. of Donors	187	427
CCDee (R1R1)	76 (0.189%)	170 (0.424%)
CcDee (R1r)	64 (0.159%)	150 (0.374%)
CcDEe (R1R2)	25 (0.062%)	37 (0.092%)
ccddee (rr)	11 (0.027%)	28 (0.069%)
ccDEe (R2r)	6 (0.014%)	21 (0.052%)
ccDee (R0r)	2 (0.004%)	9 (0.03%)
ccDEE (R2R2)	3 (0.007%)	4 (0.009%)
Ccddee (r'r)	0	5 (0.012%)
CCDEe (R1Rz)	0	1 (0.0025%)
ccddEe(r''r)	0	1 (0.0025%)
CcddEe (r'r'')	0	1 (0.0025%)
CcDEE (R2Rz)	0	0
CCDEE (RzRz)	0	0
CCddee (r'r')	0	0
ccddEE (r''r'')	0	0

Summary/Conclusions: There is a need to create rare donor registry for A_2/A_2B subgroups and clinically significant blood group antigens and share at various regional, national and international levels. This is a first small step to create a rare donor registry of A_2/A_2B subgroups with RH phenotype, to provide transfusion to all A_2/A_2B patients, to prevent alloimmunization of Anti-A1 antibodies and to ensure improved quality of transfusion therapy.

TTID – Screening strategies for TTI

P-058 | An association of recent influenza or COVID-19 vaccination history with unconfirmed repeat reactive syphilis serology assay results in Canadian blood donors

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Background: False-positive syphilis serology results or syphilis serology results that do not confirm have occasionally been noted by some blood operators during influenza vaccination campaigns.

Aims: To understand the relationship between unconfirmed repeat reactive syphilis serology results and recent influenza or COVID-19 vaccination histories in Canadian Blood Services blood donors.

Methods: This study retrospectively analyzed repeat reactive syphilis serology results in Canadian Blood Services blood donors for the period September 1, 2017 and January 31, 2021. Canadian Blood Services collects blood donations (approx. 850,000 annually) in all Canadian provinces except Quebec. At time of donation all donors are asked if they recently had a vaccination within the prior three months. All donations are tested with the Beckman Coulter PK-TP System (Brea, California. USA), a microhemagglutination assay intended for the qualitative screening of blood donors for the detection of *Treponema pallidum* IgG and IgM antibodies in EDTA plasma and serum. Confirmatory testing was carried out on all PK-TP repeat reactive donations using both the *Treponema pallidum* particle agglutination assay (TPPA) and the rapid plasma reagin (RPR) assay (TPPA/RPR). Repeat reactive donations with confirmatory test results were matched to donor influenza and COVI-19 vaccine histories. Data was stored (Microsoft Excel; Redmond, WA) and statistically analyzed (GraphPad Prism 5; San Diego, CA).

Results: The distribution of repeat reactive and repeat reactive donations with a recent influenza or COVID-19 vaccination are indicated in Table 1.

In this time period, only three donors had received a COVID-19 vaccine; all were syphilis repeat reactive and did not confirm by TPPA/RPR. The low rate of COVID-19 vaccination in our donor population was likely due to a delayed vaccine distribution in Canada during January 2021. Rates of syphilis repeat reactive test results that did not confirm by TPPA/RPR were higher in influenza vaccinated than unvaccinated blood donors (Fisher's exact test $p=0.0161$, two-sided).

P-058 Table 1. Percentage of syphilis repeat reactive and true positive donations who received a recent influenza or COVID-19 vaccination.

		Influenza vaccine		COVID-19 vaccine	
Syphilis	Total n	n	%	n	%
Repeat reactive-only	748	132	17.65	3	0.40
Confirmed	154	15	9.74	0	0.00
Total	902	147	16.30	3	0.33

Summary/Conclusions: There is an association between syphilis repeat reactive test results that do not confirm by TPPA/RPR and a recent receipt of an influenza virus vaccination in Canadian blood donors. As expected, the numbers of donors with a recent COVID-19 vaccination were low in early 2021. The association between a recent influenza vaccination and syphilis repeat reactive test results that do not confirm by TPPA/RPR will need to be re-assessed. The mechanisms for this phenomenon are still not apparent.

P-059 | The impact of SARS-CoV-2 pandemic on the seroprevalence of TTI among blood donors

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Background: It is well known that the markers of transfusion transmissible infections (TTI) are more prevalent in family/replacement donors versus voluntary nonremunerated blood donors. The SARS-CoV-2 pandemic reduced the number of potential voluntary blood donors in different ways (people get infected, lock-down introducement, fear of infection, etc.). In 2020, a shortage of blood and an increased number of family/replacement donors was observed in our transfusion practice.

Aims: To compare the seroprevalence of TTI among blood donors observed in 2020 to the seroprevalence in the period from 2017 to 2019 in order to assess the potential impact of SARS-CoV-2 pandemic on blood safety.

Methods: In 2020, 21342 blood donors were screened for the presence of HBsAg, anti-HCV, HIV Ag/Ab anti-Treponema using Architect 2000 platform. Repeatedly reactive samples were subjected to confirmatory testing using HBsAg neutralization test, immunoblot for HCV and western blot for HIV. We analyzed data on the seroprevalence of TTI markers in first time and repeat donors using the donor information system (eDelphyn).

Results: In the period of 2017-2019 family donors were present with 1.5%, while in 2020 their number significantly increased to 40%. Seroprevalence of HBV, HCV and HIV in the period of 2017-2019 was 0.2%, 0.023% and 0.0065% respectively. In 2020, seroprevalence of HBV, HCV and HIV was 0.36%, 0.018% and 0.014% respectively. The overall seroprevalence of TTI in the period of 2017-2019 was 0.23% (out of 75763 donors, 174 were confirmed positive), while in 2020 it was 0.39% (out of 21342 donors, 84 were confirmed positive).

In 49711 first time donors who were tested in the period of 2017-2019, the seroprevalence of HBV, HCV and HIV was 0.3%, 0.03% and 0.004%, while in 26052 repeat donors the seroprevalence was 0.015%, 0.011% and also 0.011% respectively. In 12000 first time donors who were tested in 2020, the seroprevalence of HBV, HCV and HIV was 0.61%, 0.033% and 0.016%, while in 9342 repeat donors the seroprevalence was 0.032%, 0% and 0.01% respectively. Out of the total of 77 HBsAg confirmed positive donors, 74 (96%) were first time donors.

Summary/Conclusions: Due to the SARS-CoV-2 pandemic, the number of family/replacement donors dramatically increased. Consequently, the seropositivity for HBV and HIV was increased in 2020. The most significant increment was observed for HBsAg in first time donors.

P-060 | Validation of Elecsys ANTI-HBs II, CMV IgG, and Toxo IgG immunoassays for use with cadaveric plasma samples

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Background: Deceased donors are the primary source of organs and tissues for transplantation but the risk of infectious complications in the recipient is high and is the main cause of post-transplantation morbidity and mortality. Blood composition is modified (haemolysis, autolysis and/or bacterial growth) following circulatory death of a donor, which can alter the structure of markers for infectious diseases leading to false-positive or false-negative results in diagnostic assays. Therefore, screening tests for antibodies to infectious disease-causing agents such as hepatitis B virus, cytomegalovirus (CMV) and Toxoplasma gondii (Toxo) must be validated in cadaveric samples.

Aims: To evaluate the analytical performance of the Elecsys® Anti-HBs II, CMV IgG and Toxo IgG immunoassays on the cobas e 801 analyser (all Roche Diagnostics International Ltd, Rotkreuz, Switzerland, in pre- and post-mortem plasma samples. An exploratory analysis investigated whether additional centrifugation should be applied to samples prior to measurement with the aforementioned assays.

Methods: Accuracy/sensitivity and precision of the Elecsys Anti-HBs II, CMV IgG and Toxo IgG immunoassays for the quantitative detection of antibodies to hepatitis B surface antigen (HBsAg), CMV and Toxo, respectively, were evaluated at one site (Sanquin, Amsterdam, Netherlands; March–April 2019) using ethically approved, residual, anonymised, frozen, pre- and post-mortem plasma samples collected from December 2016–June 2017. For post-mortem samples, blood was collected ≤24 hours after last heartbeat. Pre- and post-mortem plasma samples spiked to a low positive (n=21) or medium/high positive (n=21) result were used for accuracy/sensitivity analysis, measured as the percentage deviation of the mean of post-mortem sample results to the mean of pre-mortem sample results. Precision was measured in six aliquots prepared from two pooled post-mortem blood samples and spiked to a low positive result, then measured within one run. Results were compared against predefined acceptance criteria as outlined in a guidance from the Paul-Ehrlich Institute (Langen, Germany): accuracy/sensitivity, ≤25% deviation of the mean between pre- and post-mortem samples; precision, coefficient of variation (CV) ≤15% between aliquots of one sample in one run. Thawed pre- and post-mortem plasma samples spiked to low positive (n=11) or medium/high positive (n=11) results were measured before and after centrifugation (10 minutes at 10,000 x g) and percentage recovery calculated.

Results: The acceptance criteria for the accuracy/sensitivity study were fulfilled across all three assays (Anti-HBs II: low positive, deviation 0.1% and medium/high positive, deviation 0.5%; CMV IgG: low positive, deviation 5.7% and medium/high positive, deviation 4.6%;

Toxo IgG: low positive, deviation 6.8% and medium/high positive, deviation 5.7%). High precision was observed across the three assays (Anti-HBs II: CV 2.3–2.5%; CMV IgG: CV 0.6–1.0%; Toxo IgG: CV 1.4–2.4%), satisfying the predefined acceptance criteria. Centrifugation had minimal effect on percentage recovery across all assays.

Summary/Conclusions: The Elecsys Anti-HBs II, CMV IgG and Toxo IgG immunoassays demonstrated acceptable accuracy/sensitivity and precision in pre- versus post-mortem samples. Thawed samples can be analysed without an extra centrifugation step.

P-061 | Effect of grey zone sample testing of transfusion transmissible infectious diseases on safety of blood- experience of National Blood Centre, Kuala Lumpur

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Background: One of the blood banks main goals is to collect and produce safe and sufficient human blood component intended for transfusion. However, the risk of misclassifying infected individuals is a crucial challenge. The situation becomes grave in developing countries, as more sophisticated testing technologies such as Nucleic Acid Testing (NAT) leads to increased cost of testing. Hence, the grey zone range is applied to enhance the current screening methodologies and to further ensure blood safety. Grey zone of a quantitative test is a range of values for which the test is deemed inconclusive. There is often an overlap between distributions of test results for subjects with and without the disease, where infected individuals could be false non-reactive, and non-infected individuals could be false reactive. In National Blood Centre, Kuala Lumpur, all samples were screened for transfusion transmitted infection (TTI) using serological assay (Abbott Architect i2000sr immunoassay analyser) and Nucleic Acid Testing (NAT). For serological testing, samples with signal to cut-off (S/CO) equals and above 1.00 is considered as reactive, and these blood products are discarded. To enhance the sensitivity of the test, grey zone range was applied. Grey zone is calculated 20% below the S/CO 1.00 for Anti-HCV (S/CO: 0.80-0.99) and 10% for HIV Ag/Ab and HBsAg (S/CO: 0.90-0.99). All samples with S/CO that fall within the grey zone range are consider as reactive sample, and are discarded.

Aims: An analysis was carried out to assess the role of grey zone range in enhancing the sensitivity of current serological testing. The utility of grey zone range and its role in enhancing the current screening methodologies was never been evaluated in our setting.

Methods: A retrospective study was conducted during the period from April 2018 to December 2020 in the Transfusion Microbiology Laboratory (TML). All reactive samples (including samples with grey zone reactivity) were calculated from donor reactive book. The confirmatory results for these grey zone samples were identified (Neutralisation test for HBsAg, and Line immunoassay (LIA) for HIV and HCV).

Results: Of the 683,233 healthy donors screened for mandatory TTI serology screening, HCV reactivity was found in 1928 (0.28%) samples, with HBV and HIV in 1324 (0.19%) and 567 (0.08%) samples respectively. Cumulative overall reactivity is 0.56% (3819 samples). Of the 3819 samples, about 608 (15.92%) samples were found to lie in a grey zone range, with 478 (12.52%) belonging to HCV, 95 (2.48%) belonging to HBV and 35 (0.91%) belonging to HIV. All 3819 samples with grey zone reactivity were Non-Reactive with NAT (Procleix Ultrio Elite Assay) test. All confirmatory test results of the HIV Ag/Ab, Anti-HCV and HBsAg reactive samples that were within the grey zone index value range were determined to be negative. However, 21 samples showed indeterminate results with 20 for HCV, and 1 for HBsAg. Nevertheless, upon follow up testing (up to 3 to 6 months period), all 21 samples did not seroconvert.

Summary/Conclusions: To summarize, the application of grey zone for serology screening leads to unnecessary donor loss, increase in costs for further testing and causing negative emotions to blood donors. Furthermore, with the used of NAT Procleix Ultrio Assay, the risk of TTI's has decreased considerably. In this case, it can be considered that they grey zone application is at least not required in HIV, HCV and HBV serological screening tests.

P-062 | Prevalence of hepatitis B virus serological markers in blood donors of South-Eastern Spain. Is it time to introduce anti-HBC analysis?

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Background: Spanish law establishes the obligation to test hepatitis B surface antigen (HBs-Ag) in donor's sera. To decrease the residual risk of HBV transfusion-transmission infection (TTI), HBV nucleic acid testing (NAT) was implemented. Nevertheless, HBV remains the most frequent TTI.

The prevalence of antibodies against core antigen of HBV (anti-HBVc), has decreased to 5.5% in the last 20 years. The addition of anti-HBc testing as a screening test could provide better level of blood safety.

Aims: To determine the reliability of the donor's interview to detect donors in higher risk of HBV infection.

To describe anti-HBc prevalence in donors selected by pre-donation risk assessment interview and to compare them to the Spanish general population.

To analyse HBV serological markers in NAT reactive and HBs-Ag negative donors.

Methods: Between January of 2017 and March of 2021 a total of 207432 voluntary non-remunerated donations were collected. All

samples were evaluated for HBs-Ag and HBV NAT and if any of these were reactive, they were also evaluated for anti-HBc. Donors were considered HBV positive when reactive for the 2 techniques. The cases with NAT repeatedly reactive and HBs-Ag negative, were considered occult hepatitis B (OBI) if anti-HBc was positive and window period infection when anti-HBc negative. The result of NAT was considered non-repeat-reactive (NRR NAT) if only initial NAT test was reactive and posterior repeated analysis non-reactive.

All serologic techniques used were chemiluminescent enzyme immune assays. NAT was tested by transcription mediated amplification assay.

Results: The determination of anti-HBc was performed in 302 donors: 237 from medical interview and 65 because of laboratory anomalies: HBs-Ag and/or NAT reactive (14 HBV positive, 9 OBI and 42 NRR NAT). Of the 42 cases with NRR NAT, 10 (23.8%) had anti-HBc. In the 19 cases with NAT reactive (OBI and NRR NAT with anti-HBc), 17 (90%) were regular donors, with a mean of 23 previous donations.

Regarding the 237 donors studied by medical interview, the majority, 180 (76%) were new donors and 18% came from countries with a higher incidence of HBV. Regarding the history, 156 (66%) reported having suffered from hepatitis and 81 (33%) had presented risk factors for suffering it. The prevalence of anti-HBc was 10.5% and increased to almost 30% in donors who reported a history of hepatitis. A clear increase in the prevalence of anti-HBc with age is observed, especially after 40 years, exceeding 12% in those over 50 years of age Table 1.

P-062 Table 1.

		Results, n (%)			
Age, years	n	Anti-HBc positive	HBs-Ag, NAT positive	Negative	Prevalence in Spain, %
18-29	42	1 (2.4%)	0	41	0.8%
30-39	38	3 (7.9%)	1	34	1.6%
40-49	78	8 (10.25%)	2	68	2.5%
50-70	79	10 (12.65%)	0	69	11.3%
Total	237	22 (9.3%)	3	212	4%

Summary/Conclusions: Medical interview is a key element to detect donors at risk of HBV infection, as the presence of anti-HBc in this group doubles general population's prevalence and it is even 6 times higher in those who refer any hepatitis history.

NRR NAT samples had a prevalence of anti-HBc 4 times greater than the general population. This may suggest that NRR NAT donors could be indeed OBI that we are not able to confirm with the available current tests.

The majority of the detected cases of anti-HBc (80%) were 40 years or older.

These data suggest that the implementation of anti-HBc screening could help us to detect sooner the donors with OBI or NRR NAT. However, the impact of losing a part of the oldest donors (>40 years old), should be evaluated.

P-063 | Comparison of performance of Abbott alinity s and prism for screening four mandatory infectious markers in Hong Kong blood donors

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Background: The Hong Kong Red Cross Blood Transfusion Service had been screening every blood donation for hepatitis B surface antigen (HBsAg), antibodies to hepatitis C virus (Anti-HCV), human immunodeficiency virus antigen & antibodies (HIV Ag/Ab Combo) and antibodies to human T-lymphotrophic virus Type I and Type II (HTLV I/II) serologically using PRISM (Abbott Diagnostics) since September 1999 and Abbott's Alinity s system from August 2019. Repeatedly reactive samples are subject to confirmation tests (HBsAg by neutralisation and for the other three markers by blotting assays). Blood components derived from donations with repeatedly reactive screening results are discarded.

Aims: To compare the performance of HBsAg, Anti-HCV, HIV Ag/Ab Combo and HTLV I/II assays on Alinity s and PRISM in terms of rates of initial reactive (IR), repeatedly reactive (RR), confirmed positive (CP) and non-specific reactive (repeatedly reactive with negative or indeterminate confirmation test results) (NSR).

Methods: A total of 316901 donations collected from 1 March 2018 to 5 August 2019 and 314250 donations from 6 August 2019 to 30 January 2021 were screened for the 4 mandatory markers by PRISM and Alinity s respectively. The derived specificities of the Alinity s assays were compared with the Abbott product requirements document (PRD) claim. Rates of IR, RR, CP and NSR of whole blood and apheresis donations tested by Alinity s and PRISM were compared statistically by two sample proportion Z-Test or Fisher Exact test. A p-value <0.05 was regarded as statistically significant.

Results: The specificities of the 4 assays on Alinity s were all consistent with Abbott's PRD claim (>99.50%). The IR, RR, CP and NSR rates of the 4 assays were listed in Table 1. IR rates of Alinity s were statistically lower than those of PRISM for HBsAg, Anti-HCV and HIV Ag/Ab Combo assays but was higher for HTLV I/II assay. RR and NSR rates of HBsAg assays were comparable between Alinity s and PRISM. RR and NSR rates of Alinity s were statistically higher for HIV Ag/Ab Combo and HTLV I/II assays than those of PRISM whereas they were lower for Anti-HCV assay. CP rates of Alinity s and PRISM were comparable with each other for all four infectious markers. There was no significant difference between the total number of blood donations discarded due to NSR results of all four mandatory infectious markers tested by Alinity s (612 donations, 0.19%) and PRISM (632 donations, 0.2%).

P-063 Table 1. IR, RR, CP and NSR rates of the 4 assays on Alinity s (A) and PRISM (P).

Assay	A /P	IR rate (%)	p-value	RR rate (%)	p-value	CP rate (%)	p-value	NSR rate (%)	p-value
HBsAg	A	0.112%	<0.01	0.103%	>0.1	0.087%	>0.1	0.017%	>0.5
	P	0.187%		0.128%		0.111%		0.017%	
Anti-HCV	A	0.062%	<0.01	0.061%	<0.01	0.012%	>0.1	0.050%	<0.01
	P	0.187%		0.127%		0.009%		0.118%	
HIV Ag/Ab Combo	A	0.050%	<0.01	0.042%	<0.05	0.002%	>0.5	0.040%	<0.05
	P	0.108%		0.037%		0.002%		0.035%	
HTLV I/II	A	0.097%	<0.01	0.088%	<0.01	0.00032%	>0.5	0.088%	<0.01
	P	0.082%		0.030%		0.00063%		0.030%	

Summary/Conclusions: The rates of CP of all the 4 infectious markers were not statistically different while screened by PRISM and Alinity s indicating stable prevalence of these infections in blood donors during the 2 testing periods. The IR and RR rates of Alinity s were statistically lower for Anti-HCV assay than those of PRISM whereas it was opposite for HTLV I/II assay. The lower NSR rate of Anti-HCV assay by Alinity s leading to less blood donations being unnecessarily discarded was nullified by the higher NSR rate of HTLV I/II assay.

P-064 | Impact of the introduction of malaria serological screening in a transfusion center, have we achieved any benefit?

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Background: Serological test for Malaria antibodies allows to shorten the times of exclusion from donation and improves transfusion safety.

Aims: We evaluated the impact of the implementation of serological screening for Malaria has had on blood donations and donors in our center.

Methods: In May 2018, the assessment of anti-Plasmodium antibodies was introduced routinely to donor samples from Malaria endemic countries and tourists returning from risk areas. The test used was EUROMMUN® ELISA (IgG). A "negative result" is considered with a <0.8 ratio, "indeterminate" between 0.8-1.1 and "positive" ≥1.1. As a complementary technique, the determination of Plasmodium spp. BinaxNOW® antigen was used.

All components of reactive (positive and indeterminate) donations were destroyed. Donors with reactive result were required for the drawing of a new blood sample to repeat serology and to analyze for the presence of the Plasmodium antigen. Donors were questioned again about previous diagnosis of Malaria or compatible symptoms. If serology was reactive again, the donor was excluded from blood donation for 3 years.

Results: Between May 1, 2018 and May 31, 2020, 99,755 donations were obtained and only 2,150 (2%) required serological screening for Malaria. Of the 2,150 donations analyzed, 373 (17%) were "reactive" (286 positive and 87 indeterminate). In positive cases, the mean of the ratio obtained was 2.13 ± 1.12 and the median was 1.7 (1.1-8.16). These products were obtained from 231 donors, who in 98 cases (42.5%) were new and in 133 cases (57.5%) had previous donations (average of 6 donations). These donors came from 24 different geographical areas: Latin America 82% (Ecuador 47.5%, Bolivia 16.5%, and Colombia 10.5%), Europe 13.5% (Spain 12% of the total) and 4.3% from other countries.

Of the 231 donors with reactive serology, 157 (68%) had positive results and 74 (32%) indeterminate. In 160 donors a new sample could be reanalyzed and the positive result was maintained in 111 cases (69.4%). In those cases, antigen tests (n=91) gave always negative results.

From the 231 donors with initially reactive serology, 135 ceased to further donate blood (58%), 111 were excluded due to repeatedly reactive serology, and 24 were pending to perform a new serology.

Summary/Conclusions: The introduction of serological tests for Malaria has resulted in the destruction of 373 donations and the loss of 135 donors in 2 years. This represents the loss of 0.37% of total whole blood donations in our facility during this period, and the exclusion of 6.3% of the donors analyzed for Malaria.

Most of the abnormal results were "indeterminate" or "positive" with a very low ratio. In the absence of a confirmatory antibody test, we have concerns about the validity of the interpretation of these serological results in blood donors.

The hypothetical advantage in the gain in donations due to the reduction of the exclusion period does not seem to justify the high loss of donations and donors due to the abnormal results of the detection of anti-Plasmodium antibodies.

P-065 | Blood viral metagenomics analysis in healthy donors using next-generation sequencing

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Background: Blood transfusion is an effective approach for clinical treatment of some patients. Now, the risk of transfusion transmitted related well-known viruses (such as HBV, HCV, HIV) can be considered as under control in most countries and showed very low residual risk of transfusion transmitted infection. However, other infectious viruses including not detected and/or unknown may be transmitted to recipients by blood-derived components from the donors. Currently, viral metagenomics analysis using the next-generation sequencing (NGS) should provide a possible opportunity to explore the entire pattern of viruses in blood donors, that could improve blood transfusion safety.

Aims: To explore the classification level and quantity of viruses in different blood components of blood donors in China, and propose preventive measures against potential viruses that may impair blood transfusion safety.

Methods: 32 buffy coat and plasma samples were collected from whole blood donors, each of buffy coat and plasma pair samples was from the same donor. 30 peripheral blood and platelets samples were from the same apheresis platelet donors. Viral nucleic acids were extracted using commercial kits. Then, a metagenomics library was constructed using a commercial kit according to the manufacturer's instructions. The library was sequenced on the illumina-MiSeq instrument. The raw sequences data and viral assignment were analyzed by CLC Genomics Workbench software.

Results: Human betaherpesvirus 5, Escherichia virus M13 and Macacamulatta polyomavirus 1 were found with high combined abundance among the whole blood donors, and the vast majority (over 90%) of them came from plasma. Anellovirus with multiple genera (alpha, beta, and gammatorquevirus) was also common seen in plasma. The abundance and quantity of viruses in buffy coat were significant differences with those in plasma ($p<0.01$) in whole blood donors. In addition, there are also significant differences of combined abundance in peripheral blood and platelets among the apheresis platelet donors ($P<0.01$). In whole blood donors, the abundance in plasma is higher than that in the buffy coat (over 3 times).while in apheresis platelet donors, the abundance in platelets is higher than that in the peripheral blood (over 3 times).

Summary/Conclusions: The abundance, quantity and classification level of the viruses were obviously differences in different blood components from the donors. This study demonstrates that NGS is a promising technology for detecting common or less frequent viruses in blood components.

P-066 | Performance evaluation study of Elecsys immunoassays in blood donor screening in Lima, Perú

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Background: Testing of blood donations for infectious disease markers plays an important role in maintaining the safety of blood transfusions. In Perú, it is mandatory to screen all blood donations for HIV, hepatitis B surface antigen (HBsAg), hepatitis C virus (HCV), *Treponema pallidum* (syphilis), *Trypanosoma cruzi* (Chagas disease), hepatitis B core antibodies (anti-HBc) and human T-lymphotropic virus (HTLV). Improvements in the development of blood screening assays reduces the risk of transmission of infectious diseases, providing high-quality blood products; thus, comparative evaluations assessing the diagnostic performance of alternative screening tests are highly recommended.

Aims: To evaluate the specificity of electrochemiluminescence immunoassay (ECLIA) technology of the Elecsys® immunoassays on the fully automated cobas e 601 analyser (Roche Diagnostics International Ltd) compared with the routine system based on chemiluminescence microparticle immunoassay (CMIA) technology of the ARCHITECT assays (Abbott Diagnostics), in blood donor specimens.

Methods: From January–June 2019, serum samples from 1100 unselected donors (repeat and first-time donors) at a blood screening centre in Lima, Perú, were routinely screened on the ARCHITECT i2000 analyser with ARCHITECT HIV Ag/Ab Combo, HBsAg Qualitative, Anti-HCV, Syphilis TP, Chagas, Anti-HBc II and rHTLV-I/II assays (all Abbott Diagnostics). Samples were re-tested within 48 hours on the cobas e 601 analyser with the corresponding Elecsys immunoassays: HIV combi PT, HBsAg II, Anti-HCV II, Syphilis, Chagas, Anti-HBc II and HTLV-I/II (all Roche Diagnostics). All tests were performed according to manufacturer instructions. Results were interpreted as reactive, borderline or non-reactive; borderline results were considered reactive for the purpose of this analysis. Initially reactive results were repeated in duplicate; all repeatedly reactive samples, either concordant or discordant between the two systems, were further investigated using confirmatory assays: HIV (INNO-LIA HIV I/II Score), HBsAg (Elecsys neutralisation, Anti-HBe, HBeAg, aHBc IgM), HCV (INNO-LIA HCV Score, Ortho HCV 3.0 ELISA SAVe, Mikrogen recomLine HCV IgG), Syphilis (INNO-LIA Syphilis Score, RPR, TPLA) and HTLV (INNO-LIA HTLV I/II Score).

Results: The specificity of the Elecsys and ARCHITECT immunoassays was comparable across all assays tested. Specificity values for the Elecsys immunoassays were: HIV combi PT, 99.36%; HBsAg II, 100%; Anti-HCV II, 99.82%; Syphilis, 99.91%; Chagas, 100%; Anti-HBc II, 100%; and HTLV, 100%. Specificity values for the ARCHITECT assays were: HIV Ag/Ab Combo, 99.82%; HBsAg Qualitative, 99.82%; Anti-

HCV, 99.45%; Syphilis TP, 99.36%; Chagas, 100%; Anti-HBc II, 100%; and rHTLV-I/II, 99.63%. All confirmed positive samples were detected by both systems (n=77).

Summary/Conclusions: The observed performance of the Elecsys immunoassays is comparable to that of the ARCHITECT assays, with specificities ranging from 99.36% (HIV) to 100% (HBsAg, Chagas, Anti-HBc and HTLV), demonstrating that Elecsys immunoassays are reliable tests for blood donor screening. The total number of false positives was 10 and 21 for the Elecsys and ARCHITECT immunoassays, respectively, suggesting that ECLIA technology may show an advantage over CMIA technology in minimising overall false-reactive cases, thus reducing the burden of confirmatory testing, risk of donor deferrals and loss of blood units.

P-067 | The epidemiology of hepatitis B and hepatitis C among β-thalassaemia major patients in Pakistan: A systematic review and meta-analysis

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Background: Beta thalassaemia major patients are leading consumers of the blood transfusions in Pakistan and therefore have a greater risk of acquiring transfusion-transmitted infections most notably hepatitis B and C. The present study includes a comprehensive review on the status of hepatitis B and C in beta thalassaemia major patients in Pakistan. For this purpose, we examined original articles assessing the epidemiology of hepatitis B and C in transfusion-dependent thalassaemia patients.

Aims: To assess the epidemiology of hepatitis B and C in transfusion-dependent thalassaemia patients.

Methods: We searched ten major subscription databases, i.e. Medline, PakMediNet, CINAHL, Scopus, PubMed, Web of Science, Embase, Science Direct, Google Scholar, and Directory of Open Access Journals (DOAJ). WHO resources were also explored for relevant reports. The search criteria included published articles up to December 31, 2019, with no language restrictions. Articles identified were introduced into the Endnote version X9 software and then screened for relevance and duplication. The study was based on the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) 2009 guidelines. To summarize the characteristics of included studies, numeric analysis was applied. The result was reported as pooled prevalence for the overall study and also for region-wise subgroups.

Results: A total of 33 studies conducted from 1995 to 2019, were included in the review. All 33 articles yielded information on HCV prevalence while 19 of them provided information on HBV prevalence. The overall sample size was 8,554 that tested the prevalence of HCV in thalassaemia patients. The sample size from the 19 studies that tested the prevalence of HBV was 6,184. The overall pooled prevalence of HBV was computed to be 4.13% while the pooled prevalence of HCV was 29.79%. The majority of the studies were obtained from Punjab province (33.33%), followed by Khyber Pakhtunkhwa province (24.24%).

Summary/Conclusions: maximum pooled prevalence of HBV and HCV among thalassaemia patients was observed in Punjab province (10.14% and 41.34% respectively). On the other hand, the least pooled prevalence for HBV was seen in Islamabad Capital Territory (3.05%) while for HCV it was in Khyber Pakhtunkhwa province (24.17%). The transfusion system in general and the screening system in specific could explain, at least partially, such findings. The total sample size of 33 studies was less than 10% of the total number of estimated thalassaemics, i.e. 100,000. Further studies or a national baseline survey are imperative to confirm the actual frequency of HBV and HCV in thalassaemia patients across the country.

P-068 | Epidemiology and trends of transfusion transmissible infections among voluntary and replacement blood donors at a regional blood centre in Pakistan

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Background: Blood transfusion is linked to a number of risks most notably the transmission of transfusion-transmitted infections (TTIs), including Hepatitis B virus (HBV), Hepatitis C virus (HCV), Human immunodeficiency virus (HIV), Syphilis, and Malaria. The risk posed by these blood-borne infectious agents is high in developing countries including Pakistan. This fact stresses the need for regular surveillance of TTIs.

Aims: The present study was undertaken to assess the seroprevalence of TTIs at a Regional Blood Centre.

Methods: This was a retrospective 4-year descriptive study undertaken at the Regional Blood Centre in Peshawar, Khyber Pakhtunkhwa Province of Pakistan, on the blood donor data from June 2016 to May 2020. A total of 41,817 donors donated blood during the study period and were screened for HBV, HCV, HIV, syphilis, and malaria. To ensure donor privacy, donors were identified via the use of codes and no personal information was available. The data were extracted from the ZAAVIA blood transfusion information system (BTIS) database.

Results: The study included a total of 41,817 donors, 41,493 (99.22%) males and 324 (0.78%) females. Most of them 22,343 (53.43%) were voluntary donors while 19,474 (46.57%) were replacement donors. An overall TTI prevalence rate of 4.61% was found. The TTI prevalence rate in voluntary donors was 3.90% while 5.42% in replacement

donors. The overall prevalence of HBV, HCV, HIV, syphilis and malaria was 1.95%, 1.38%, 0.23%, 0.91%, and 0.14%, respectively.

Summary/Conclusions: The current study documented a high prevalence (1,929 out of 41,817, 4.61%) of TTIs, especially in replacement donors (1,057 out of 19,474, 5.42%), and low participation of female donors. The recommendations include the promotion of voluntary blood donors, enrolment of female blood donors, and screening of donated blood through highly sensitive screening assay (i.e. nucleic acid testing).

TTID – Hepatitis B (HBV)

P-069 | Acute primary occult hepatitis B virus infection in Chinese repeat blood donors

selected for main programme

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Background: A residual risk of HBV transfusion-transmission remains associated with occult HBV infection (OBI) showing intermittently detectable low levels of viral DNA. Anti-HBc testing can reduced further this residual risk. However, rare cases of acute infection showing very low viremia without developing detectable HBsAg until anti-HBc/anti-HBs seroconversion have been reported, and were so-called acute primary OBIs.

Aims: Donors with HBV DNA as only marker of infection were followed-up to confirm early window phase of overt HBV infection or acute primary OBI. Viral genetic analysis was conducted to characterize the molecular mechanisms associated with acute primary OBI and to estimate the potential infectious risk.

Methods: A follow-up program of HBsAg-/HBV DNA+ donors is implemented in the Dalian blood center, China. Donors were called back and whole blood samples were collected. HBsAg, anti-HBc, and anti-HBs were tested with CLIAs. Viral load was measured with an in-house qPCR (LoQ: 20 IU/mL). HBV whole genome, PreCore/Core, Pre-S/S, and BCP regions were amplified and sequenced.

Results: Between 2010 and 2020, 58/450,549 (0.01%) donors tested positive for HBV DNA only, of which 27 were followed-up over a period of 17 to 2,202 days. Complementary serological and molecular testing identified 21 (77.8%) acute overt infections leading to 5 (18.5%) chronic (HBsAg+/DNA+/anti-HBc+) and 16 (59.2%) probable recovered (HBsAg+/DNA-/anti-HBc+/anti-HBs+ [n=14] and anti-HBs- [n=2]) infections. Three donors (11.1%) never showed HBsAg reactivity despite detectable viral DNA in at least two sequential samples over a period of 413 to 2,202 days, and seroconversion to anti-HBc at 91, 95, and 160 days post-index donation. They were classified as acute primary OBIs. Three donors anti-HBs only reactive in follow-up remained unclassified. Primary OBIs were repeat donors aged 34-45 years with no HBV marker in the index-1 donations collected 7-19 months earlier. One donor who has been vaccinated at the age of 20, had anti-HBs levels of 19 and 110 IU/L after 160 and 253 days of follow-up, respectively. Primary OBI strains were of genotype C and showed a higher aa diversity in the S protein compared to HBsAg+ genotype C controls (n=26): median 11% (2.2% >15.3%) vs 2.7% (0.0%>7.9%) (P<0.05). No significant difference was observed when compared to non-acute OBI strains (n=48). One primary OBI S sequence was identical to the HBsAg+ consensus sequence, while the other two showed 13 and 24 aa substitutions, including 9-10 unusual substitutions within the major hydrophilic region, particularly at positions s112-s120, that might negatively affect antigenicity. G1896A mutations associated with impaired HBeAg production was found in BCP sequences.

Summary/Conclusions: The existence of rare cases of acute primary OBI was confirmed. Acute primary OBI may participate to the HBV residual risk by challenging anti-HBc testing and requiring high-sensitive NAT assays. Preliminary genetic analysis suggested that mutations in S protein critical domains might be associated to primary acute OBI genesis. Extremely low viral replication throughout the acute phase of the infection as the only factor accounting for undetectable HBsAg cannot be ruled out. Functional analysis of viral replicative properties is needed to estimate infectivity of these variants.

TTID – Hepatitis C (HCV)

P-070 | Improved clinical specificity of the VITROS ® immunodiagnostic products anti-HCV assay*

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Background: This study was designed to assess the improved clinical performance of the VITROS Immunodiagnostic Products Anti-HCV assay (VITROS HCV) which is run on the VITROS ECi/ECiQ Immunodiagnostic Systems, the VITROS 3600 Immunodiagnostic System and the VITROS 5600/XT7600 Integrated Systems.

Aims: The VITROS HCV Assay Reagent was modified to reduce non-specific binding and improve the clinical specificity of the assay.

Methods: Specificity was assessed using 5259 blood donor samples and 200 clinical samples on both the modified VITROS HCV and the current VITROS HCV assays. Clinical Sensitivity was assessed using 403 anti-HCV positive samples and 20 HCV seroconversion panels tested with both the modified VITROS HCV and the current VITROS HCV assays. Assay precision was assessed consistent with CLSI EP05 with a 5-member panel tested over 29 days.

Results: The current assay had observed clinical specificity of 99.52% (5234/5259) for the blood donors and 100% (200/200) specificity for the clinical samples. The modified assay had observed clinical specificity of 99.85% (5251/5259) for the blood donors and 100% (200/200) specificity for the clinical samples. With-Lab precision assay ranged from 3.27 to 9.76% with the modified assay and from 4.00 to 9.98% with the current assay for the various reactivities tested. All 403 positive samples were reactive on both the current and modified VITROS HCV assays and for the 20 seroconversion panels the modified assay was within ± one bleed of the current assay.

Summary/Conclusions: The modified VITROS Immunodiagnostic Products Anti-HCV assay demonstrated improved clinical specificity compared to the current VITROS HCV assay with no impact on clinical sensitivity or assay precision.

*Under development.

TTID – Bacteria

P-071 | Bacterial proliferation in cold-stored platelet concentrates

selected for main programme

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Background: Cold storage of platelet concentrates (PC) has regained attention in recent years to treat patients with active bleeding. PC stored at 20-24°C have enhanced risk of bacterial growth, however few studies have addressed this concern with cold-stored PC (cPC) as it is assumed that refrigeration limits bacterial proliferation. As psychrotrophic bacteria (grow at 1-6°C) can proliferate in red blood cell concentrates, it is probable that these organisms are also able to multiply in cPC.

Aims: Evaluate bacterial growth in PC stored in refrigeration (1-6°C) compared to standard PC storage at 20-24°C.

Methods: This is a collaboratively study including 8 participant laboratories worldwide. Five transfusion relevant bacterial reference strains used in the study are described in Table 1. The study has a pool and split approach with options to start with two single (apheresis or buffy coat) PC units, followed by pooling and splitting, or with one double apheresis PC unit which will be split it into two. Each split PC is spiked at a target concentration of 25 CFU/PC bag. Inoculated PC units remain under standard storage (20-24°C) for 2 hours for bacteria acclimation. Then, one split PC unit remains at 20-24°C under agitation for seven days while the second split PC unit is stored at 1-6°C with no agitation for 21 days. Bacterial spiking day is Day 0 and PC stored at 20-24°C are sampled on Days 1, 2, 3, 4 and 7, while cPC are sampled on Days 7, 10, 14, 17, and 21 of storage, to determine bacterial concentration. The protocol is repeated ≥3 times for each bacterial species at each site.

Results: Study data collected from 3 sites are summarized in Table 1. While all bacteria can replicate in PC stored at 20-24°C, only psychrotrophic *S. liquefaciens*, *P. fluorescens* and *L. monocytogenes* grew in cold-stored PC. Notably, *S. liquefaciens* and *P. fluorescens* reached a concentration of ~10^8 CFU/ml by day 15 and day 10 of cold storage, respectively, while slow growing *L. monocytogenes* only reached a concentration of ~10^2 CFU/ml on day 21 of cold storage. Remaining 5 participant sites are expected to complete the study in 2021.

Summary/Conclusions: Proliferation of psychrotrophic bacteria was observed in cPC, with clinically relevant concentrations (>10^5 CFU/ml) observed after day 7 of storage for *P. fluorescens* and day 10 for *S. liquefaciens*. Published surveillance data from different blood centers show that psychrotrophic bacteria are not often isolated from PC. However, it is recommended to limit storage of cPC to minimize safety risk to patients receiving this PC product.

P-071 Table 1. Bacterial growth in cPC compared to standard stored PC (n≥6).

Bacteria	Characteristics[a]	Maximum concentration in PC stored at 20-24°C (storage day)	Maximum concentration in cPC (storage day)
Klebsiella pneumoniae PEI-B-P-08	Mesophilic	10^8 CFU/ml (day 2)	No growth
Staphylococcus aureus PEI-B-P-63	Mesophilic	10^8 CFU/ml (day 3)	No growth
Serratia liquefaciens PEI-A-184	Psychrotrophic – fast grower	10^8 CFU/ml (day 2)	10^8 CFU/ml (day 15)[b]
Pseudomonas fluorescens PEI-B-P-77	Psychrotrophic - fast grower	10^8 CFU/ml (day 2)	10^8 CFU/ml (day 10)[b]
Listeria monocytogenes PEI-A-199	Psychrotrophic - slow grower	10^7 CFU/ml (day 7)	10^2 CFU/ml (day 21)[b]

[a]Mesophilic: optimal growth at 20-40°C; Psychrotrophic: grow at 1-6°C

[b]On day 7 of storage, concentration was ~10^3 and ~10^5 CFU/ml for *S. liquefaciens* and *P. fluorescens*, respectively, and <10 CFU/ml for *L. monocytogenes*

P-072 | Expression of superantigen toxin genes produced by Staphylococcus aureus cultured in platelet concentrates: Genomic and transcriptomic analyses

selected for main programme

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Background: The Gram-positive bacterium *Staphylococcus aureus* is frequently isolated from contaminated platelet concentrates (PC). This organism occasionally evades detection during routine PC screening with culture methods causing false-negative septic transfusion events. Notably, *S. aureus* secretes a compendium of exotoxins including staphylococcal enterotoxins (SEs), responsible for septic shock symptoms in transfusion patients, as well as staphylococcal superantigen-like proteins (SSLs) that inhibit host immune cells thereby acting as immune evasion molecules.

Aims: Study the expression of superantigen toxin genes produced by *S. aureus* strains isolated from contaminated PC.

Methods: Next generation genome and transcriptome sequencing were employed to study the expression of superantigen (SAg) genes by four *S. aureus* isolated from PC (CBS2016-05, PS/BAC/169/17/W, CI/BAC/25/13/W, PS/BAC/31716/W, Table 1). The presence of SE genes was confirmed by PCR using gene specific primers. Comparative RNAseq analysis was performed on the strains grown to stationary phase in Trypticase Soya Broth (TSB) and PC. Verification of SE-type G (SEG) protein expression in both TSB and PC cultures was performed using Western blotting.

Results: Results are summarized in Table 1. Genome sequence analysis and PCR showed that the four *S. aureus* strains encode SAg genes. RNAseq data revealed expression of SE genes as well as SSLs in TSB and PC for all strains. Some SSLs genes were upregulated in PC in comparison to TSB. There were no significant differences in the expression of most SE genes in strains CBS2016-05 and PS/BAC/169/17/W, whereas *sea* (SE-type A) was slightly upregulated in PC compared to TSB, in CI/BAC/25/13/W and PS/BAC/31716/W. Remarkably, the repressor of toxins (*rot*), a negative regulator of most toxin genes, was expressed in PC at a similar level as in TBS, while the global regulator *agr* (accessory gene regulator) and its effector RNAIII:*hld* (δ-hemolysin) that positively enhance the expression of toxin genes were highly repressed in PC. SEG was detected in CBS2016-05 and PS/BAC/169/17/W by Western blotting.

Summary/Conclusions: *egc*: *enterotoxin gene cluster*, log2fold-change ≥ or ≤1, p<0.05.

The secretion of SAgs by *S. aureus* highlights the safety risk posed by this bacterium to PC recipients. Upregulation of some SSLs in PC is responsible for *S. aureus* resistance to immune clearance in this milieu. Furthermore, expression of SE genes in PC is biologically relevant as concentrations <0.1pg/mL are enough to cause septic shock symptoms in susceptible patients. Relevance of SE production in PC is highlighted in strain CBS2016-05, which was involved in a false negative septic transfusion reaction. SE-type U was detected in a remaining PC sample obtained after the transfusion event, which was likely responsible for the clinical symptoms displayed by the patient. This study shows the importance of assessing the presence of staphylococcal toxins during investigation of transfusion reactions caused by *S. aureus*.

P-072 Table 1. Expression of SAgs in *S. aureus* transcriptomes.

Strain	PC screening	SAg genes expression TSB and PC	Fold change (TSB vs PC)
CBS2016-05	False Negative	*egc* *ssls*	−0.00 to 0.49 −0.02 to **6.71**
PS/BAC/169/17W	Confirmed Positive	*egc* *ssls*	0.12 to 0.49 −0.09 to **4.10**
PS/BAC/317/16W	Confirmed Positive	*sea* *ssls*	**1.27** −0.07 to **1.54**
CI/BAC/25/13W	Near miss	*sea* *ssls*	0.28 −0.07 to **2.89**

egc: enterotoxin gene cluster; ssls: superantigen-like gene cluster; sea: enterotoxin type A gene log2fold-change ≥ or ≤ 1, p<0.05.

P-073 | Testing of expired platelet concentrates for residual bacterial contamination

selected for main programme

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Background: The Hong Kong Red Cross Blood Transfusion Service commenced screening all pre-released platelet concentrates (PCs) sampled at no sooner than 36 hours for bacterial surveillance test (BST) using aerobic BacT/ALERT 3D (bioMérieux, Durham, NC, USA) to minimize the risk of transfusion-associated sepsis in 1998 (Liu, Vox Sang, 1999). PCs with negative results after 24 hours of culture are released for transfusion while the culture bottles are monitored continuously until platelet expiry (5 days).

Aims: To determine the rate of detection failure during initial sampling in BST.

Methods: Initial sampling was taken at 36 to 48 hours on Day 2 post-collection. Expired platelets with negative BST results were retrieved for this study and were kept in platelet incubators with continuous agitation for 2 more days until secondary sampling. On day 8, 15-20 mL of platelet content was aliquoted into a transfer bag with sterile connectors; 5 mL sample was inoculated into each of an aerobic (BPA) and anaerobic (BPN) bottle for incubation up to another 7 days. Positive cultures were subject to Gram stain and confirmation by inoculating another 5 mL each from the original platelet bag into BPA and BPN bottles and incubating for 7 days or until positive signal identified, wherever came earlier. Initial and confirmed positive culture bottles were subject to bacterial identification. "False Positive" was defined as positive in initial culture bottles but negative in repeated bottles, no bacteria observed in initial positive bottles or no significant change in reflectance curve; whereas "Confirmed Positive" was defined as same bacteria ID identified in initial and repeated culture bottles.

Results: From March 2018 to December 2020, a total of 4001 outdated PCs were tested. Eight (0.2%) false-positive cultures were detected mainly due to instrument errors (Table 1). Most of the culture bottles giving no distinctive colour change in the bottle sensor and the reflectance curve were reloaded into the incubator and gave negative results at the end of incubation. No confirmed positive culture was observed.

Summary/Conclusions: There was no detection failure identified in the current sampling method of platelet concentrates on Day 2 for BST as no confirmed positive case was identified. The study provided evidence that our protocol has successfully prevented bacterially contaminated platelets from being escaped from detection. Based on the results of this study, extending the shelf life of PCs to 7 days can be considered.

P-073 Table 1. False positive results identified.

Case number	Time to positive (Day)	Gram stain on initial positive bottle	Repeat BPA and BPN cultures	Bacteria identified on positive bottle
P31*	1.24 (BPN)	No bacteria observed	Negative	No growth
P45#	0.56 (BPN)	Not tested	/	/
P134#	2.29 (BPA)	Not tested	/	/
P484#	0.17 (BPN)	Not tested	/	/
P627#	2.17 (BPN)	Not tested	/	/
P654@	6.87 (BPN)	Gram Positive Bacilli	Negative	Propionibacterium acnes
P937#	0.9 (BPN)	Not tested	/	/
P1324#	1.29 (BPN)	Not tested	/	/

#No distinctive color change in the bottle sensor and the reflectance curve. Bottles reloaded into incubator and negative at the end of incubation.
*No distinctive color change in the bottle sensor and the reflectance curve. Bottle reloaded into incubator and positive again at 2.25 days.
@Propionibacterium acnes identified in initial BPN bottles but not in repeated culture bottles.

P-074 | Enhancing growth and detection of cutibacterium acnes in media supplemented with Tween 80 as a source of oleic acid

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Background: *Cutibacterium acnes* is an anaerobic bacterium that thrives in the sebaceous niches of the skin. It is also the most frequently isolated anaerobic bacterial contaminant of platelet concentrates (PC) screened by the automated BACT/ALERT culture system. *C. acnes* exhibits slow growth in BACT/ALERT anaerobic (BPN) culture bottles, consequently approximately 35% of PC contaminated with *C. acnes* were issued to hospitals by Canadian Blood Services between 2017 and 2020 with a "negative to date" BACT/ALERT result. Though adverse transfusion reactions involving *C. acnes* are mild, scarce, and likely underreported, transfused *C. acnes* may contribute to chronic infections leading to endocarditis, and prosthetic valve failure. Enhancing the growth of *C. acnes* in BPN bottles is important to prevent the transfusion of *C. acnes* contaminated PCs. Oleic acid is a constituent of skin sebum which can be sourced from Tween 80 (T80) and has been shown to enhance the growth of *C. acnes* in basal media. We hypothesized that supplementing BPN media with T80 may enhance the growth of *C. acnes* leading to earlier detection in BACT/ALERT cultures.

Aims: Assess the impact of T80 supplementation of BPN culture media on the detection of *C. acnes* in the BACT/ALERT system.

Methods: Four *C. acnes* isolates obtained from contaminated PCs were used in this study and are listed in **Table 1**. Growth curves of the four strains were assessed in BPN media supplemented with T80 (0.1% v/v) under anaerobic conditions over 4 days. Additionally, BPN bottles were inoculated with bacterial suspensions prepared in supplemented BPN culture bottles with 4% T80 (v/v) to obtain a final bacterial concentration of 10 colony forming units/BPN bottle. Culture bottles were incubated in the BACT/ALERT system for 7 days or until spiked bottles flagged positive. The same protocol was used to assess the effect of T80 on the detection of other PC contaminants (*Staphylococcus saccharolyticus*, *Serratia marcescens*, and *Staphylococcus epidermidis*). Unsupplemented media served as a control for all growth experiments.

Results: T80 supplementation of BPN media enhanced the growth of all *C. acnes* strains and led to reduced detection times in the BACT/ALERT system (**Table 1**). In unsupplemented media, strains 19322 and 19422 were detected within 5 days, while strains 19153 and 19195 required longer incubation to be detected. Notably, the reduction in detection times ranged from 0.6 to 1.9 days, with strains displaying the slowest growth rates (19153 and 19195) exhibiting the highest reductions. Furthermore, T80 did not affect the detection of the other bacterial species tested.

Summary/Conclusions: Supplementation of BPN media with T80 enhanced the growth and detection of *C. acnes* in the BACT/ALERT system without affecting the detection of other transfusion relevant bacteria. Future work will focus on assessing the effect that supplementation of T80 will have on the detection of *C. acnes* in PC. This strategy could reduce false negative screening results and allow for earlier retrieval of contaminated products, thereby mitigating the transfusion risk for transfusion patients.

P-074 Table 1. Detection times of *C. acnes* isolates in the BACT/ALERT system.

Strain	BPN media (Days)	BPN media + T80 (Days)	Reduction in detection time (Days)
BPN-BT-19322	4.36	3.71	0.65
BPN-BT-19422	4.1	3.68	0.42
BPN-BT-19153	5.99	4.16	1.83
BPN-BT-19195	7.19	5.31	1.88

P-075 | The microbiome distribution characteristics of the whole blood and apheresis platelet from healthy donors based on 16S rDNA

selected for main programme

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Background: At present, it has been confirmed that 16S rDNA of bacteria is existed in the blood of healthy individuals. However, the characteristics of the microbiome in the blood of healthy donors are still not particularly clear, including the differences among the blood components and the blood donors in different population. Meanwhile, the role of these 16S rDNA of bacteria and the impact on blood transfusion-related risks is still under investigation.

Aims: This study aims to provide a comprehensive description of the 16S rDNA of bacterial flora distribution present in different blood components of blood donors in Chinese population.

Methods: 60 healthy blood donors were collected from blood Centre of Zhejiang province, in eastern of China. Among them, 30 individuals were whole blood and each of them was divided into 4 components: whole blood (WB), plasma(WP), buffy coat (WW), and red blood cells (WR). The other every 30 apheresis platelets donors were divided into platelets (P) and whole blood (PW). The bacterial 16S rDNA V3-V4 fragments were amplified using QIAseq® 16S/ITS Panel and sequenced by Next Generation Sequencing (NGS). The raw sequences data were analyzed by CLC Gennomics workbench software.

Results: The bacterial profiles of 16S DNA are significant differences for 6 kinds components ($p<0.01$) and spread over a relatively broad range among donors. The abundance of blood bacterial DNA located in WP and WR are significantly higher than that of WB and WW in the whole blood donors. The microbiome distribution characteristics of apheresis platelet showed that P is more abundant than PW. According to the 16S rDNA fragment analysis, a total of nine

phylums and dozens of genus are present in the different blood component group. At the phylum level, Proteobacteria is the most dominant one, which the abundance is over 75% in all 6 kinds components. Actinobacteria is the second dominant one existed in the WP, WW, WR and P component, and Firmicute is the next dominant one in the WB and PB components except Proteobacteria. At deeper taxonomic levels, there are significant differences of the bacteria species and dominant flora existed in different parts of the blood.

Summary/Conclusions: There are significant differences of the 16S rDNA of bacterial flora among each blood component both in whole blood and apheresis platelets. The abundance of 16S bacterial DNA or the microbiome profile could be monitored to improve the safety of the blood supply.

P-076 | Transcriptomic analyses reveal enhanced expression of virulence factors by a transfusion-transmitted Staphylococcus aureus strain grown in platelet concentrates

selected for main programme

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Background: Platelet concentrates (PCs) are prone to bacterial proliferation due to their pre-transfusion storage requirements at 20-24°C under agitation. *Staphylococcus aureus* is a PC contaminant that can be missed during PC screening with the automated BACT/ALERT culture system. This bacterium has been involved in septic transfusion reactions. We have therefore undertaken comparative transcriptomic analyses to unravel the mechanisms of *S. aureus* survival and evasion to immune clearance in PCs and missed detection during BACT/ALERT screening.

Aims:

1. Compare transcriptomes of a *S. aureus* strain involved in a septic transfusion reaction versus a *S. aureus* isolate captured during routine PC screening.
2. Compare gene expression of *S. aureus* grown in regular media and PCs.

Methods: PC isolates *S. aureus* CBS2016-05 and PS/BAC/317/16/W were used in this study. While CBS2016-05 was missed during routine PC screening and was involved in a septic transfusion reaction, PS/BAC/317/16W was captured during PC culture screening. Both strains were grown in Trypticase Soy Broth (TSB) and PCs up to stationary phase at 20-24°C under agitation. RNA was extracted and paired-end libraries were prepared using the Illumina Stranded Total RNA Prep kit and sequenced on the NextSeq 500 platform. Differential gene expression between the PC and TSB conditions for both bacterial strains was calculated using "lfcShrink()" function in DESeq2.

Results: Notable differences in the transcriptome of the two strains was observed, with virulence factors being prominently upregulated in *S. aureus* CBS2016-05 when grown in PCs in comparison to TSB (Table 1). Capsule biosynthesis genes (*capAB*) and its regulators (*arlR/S* and *spoVG/yabJ*) were highly upregulated in CBS2016-05. Moreover, the central regulator of *S. aureus* virulence RNAIII was strongly repressed in CBS2016-05. This along with upregulated expression of genes encoding for *S. aureus* surface proteins is essential for platelet activation and biofilm development. Additionally, CBS2016-05 had upregulated transcription of anaerobic pyruvate metabolism (*pflAB*) and arginine deiminase (ADI) pathway genes (*arcABDC*). Notably, *vraX*, a gene involved in inhibiting the classical complement pathway was upregulated in CBS2016-05. Overall, differential gene expression in PS/BAC/317/16/W was minimal or not detected compared to CBS2016-05 (**Table 1**).

P-076 Table 1. Gene transcription fold-change in PCs vs TSB.

	Fold-Change*	
Gene	**CBS2016-05**	**PS/BAC/317/16/W**
Capsular biosynthesis protein (*capA/B*)	5.9/5.9	-
Two-component signaling pathway ArlR/S	1/1.4	-
Two-component signaling pathway *spoVG*/yabJ	1.8/1.5	−1.7/−1.8
Virulence central regulator RNAIII	−7.4	−2.3
Surface proteins Clumping factors (*clfA/B*) and Protein A (*spa*)	1.7/2.7 and 5.7	−1.2/2.7 and −1.5
Arginine deiminase pathway (*arcABDC*)	1.5/4.7/2.6/4.7	−1.7(*arcA*)
Pyruvate formate lyase/ acetyltransferase (*pflAB*)	6.6/4.4	2.1/-
Complement inhibitor *vraX*	3.2	−1.2

*log2fold-change ≥ or ≤ 1, p<0.05.

Summary/Conclusions: *S. aureus* CBS2016-05 had increased expression of virulence factors involved in immune evasion and infection development. Also, this strain shifted towards anaerobic metabolism when grown in PCs, possibly lowering CO_2 production and impairing detection with BACT/ALERT cultures. Although further studies with more strains are needed, our data suggests that the PC storage environment modulates *S. aureus* gene expression in a strain-specific manner impacting detection during PC screening and safety of transfusion patients.

TTID – Newly emerging pathogens

P-077 | Further assurance of the safety of blood through the accurate detection of West Nile virus

selected for main programme

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Background: Since the advent of PCR, the safety of blood transfusion has benefitted from the assurance of early viral detection. However, with a multitude of assays all claiming equivalent sensitivity, it is hard to understand if all are equal. World Health Organization (WHO) International Standards (IS) are the highest order biological reference material, with an assigned arbitrary unit of measurement that provides an accurate means of calibration and comparison across different assay platforms.

In the 1990's the serological detection of Hepatitis C Virus (HCV) was replaced with molecular methods, in turn, improving blood safety. However, early HCV molecular assays showed as much as 3 Log_{10} variation when assessed against candidate WHO reference materials. A WHO IS for HCV was established and 25 years later we see assays detecting as little as 0.2 Log_{10} variation. Without a reference material this standardisation is not possible.

West Nile virus (WNV) is a blood-borne pathogen with the potential for transmission via transfusion, its global prevalence necessitates the requirement for standardisation of WNV Nucleic acid Amplification Techniques (NAT).

Aims: We conducted a study to evaluate two candidate materials for their suitability to become a WHO International Standard for the calibration of molecular assays for WNV to enable the standardisation of quantification across different assay platforms.

Methods: A coded panel of samples was distributed globally to participants using different assay methods. The panel included two candidate lyophilised heat inactivated whole virus preparations of laboratory cultured WNV-lineage 1 and 2, diluted in human plasma to ~7 Log_{10} copies/mL, as well as commonly used existing standards, a lentiviral vector construct and a related virus that was to be assessed for cross reactivity - Usutu virus. Participants were asked to test all samples in their routine assay on three separate occasions and return the results to NIBSC for analysis.

Results: Data was returned from 13 laboratories, providing a total of 17 data sets, where some laboratories returned data on more than one assay. Of these, 15 data sets were qualitative and 2 quantitative.

Most participants reported using commercial qualitative closed assays, typically these had been standardised using existing reference materials and showed good agreement.

Measurement agreement between different laboratories and assays improved further when data was expressed relative to the proposed candidate standards. E.g. the lineage 1 candidate demonstrated a 1.31 Log_{10} units/mL reduction in result range. This was the case regardless of lineage, demonstrating the suitability of either material to act as a calibrator.

All commercial WNV NAT assays showed cross reactivity to the Usutu virus, whereas the laboratory developed assays did not.

Summary/Conclusions: In October 2020 the WHO Expert Committee on Biological Standardization (ECBS) approved the proposal to establish the candidate for WNV lineage 1 (NIBSC code: 18/206) as the 1st WHO International Standard for WNV NAT with an assigned potency of 7.20 Log_{10} International Units/vial, the material is intended for the calibration of assays and secondary reference materials. In addition, the Lineage 2 material (NIBSC code: 18/208) was established as a WHO Reference Reagent.

The availability of these materials will improve the harmonisation of NAT assays for WNV, providing another level of assurance for the safety of global blood supply.

TTID – Pathogen inactivation

P-078 | Cost-effectiveness and budget impact of whole blood pathogen reduction in Ghana

selected for main programme

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Background: Despite the promise of pathogen reduction for reducing transfusion-associated adverse events in sub-Saharan Africa, no cost-effectiveness analysis is publicly available.

Aims: We modeled the impact of nationwide whole blood pathogen reduction (WBPR) in Ghana on transfusion-associated adverse event outcomes and conducted a cost-effectiveness analysis from a healthcare payer perspective.

Methods: Our mathematical risk reduction model estimated incidence of six infectious and one non-infectious transfusion-associated adverse events with and without WBPR. For each adverse event, we estimated the lifetime direct healthcare costs and disability-adjusted life years lost. For HIV, HCV, and HBV, we simulated disease progression using Markov models, accounting for the likelihood and timing of clinical detection and treatment. Local costs and clinical resource utilization patterns for adverse events were estimated by local clinicians, and other parameter values were estimated from the academic literature. We reported costs in 2019 US dollars and discounted future costs and outcomes at 3% annually. We performed probabilistic and univariate sensitivity analysis.

P-078 Table 1. Modeled impact of WBPR on six adverse events. M, million, K, thousand. DALY, disability-adjusted life year; FNHTR, febrile non-hemolytic transfusion reaction.

Outcome	Sepsis	Malaria	FNHTR	Syphilis	HBV	HCV	HIV
Cases reduced	9.7K (3.9K – 15.7K)	6.2K (2.2K – 9.9K)	1.9K (0.2K – 3.7K)	56 (17 – 99)	624 (233 – 1.1K)	779 (295 – 1.4K)	159 (55 – 300)
Net present cost per case, $	667 (526 – 845)	27 (20 – 35)	82 (57 – 111)	2.6 (1.3 – 4.6)	786 (584 – 1.0K)	1.5K (1.0K – 2.1K)	1.0K (931 – 1.3K)
Total costs reduced, $	6.5M (2.5M – 11.1M)	169K (56K – 285K)	158K (18K – 327K)	144 (38 – 328)	490K (171K – 915K)	1.2M (0.4M – 2.3M)	160K (58K – 323K)
DALYs averted	36K (13K – 63K)	759 (152 – 1.6K)	3.5 (0.4 – 8.4)	1.5 (0.4 – 3.1)	792 (296 – 1.5K)	884 (316 – 1.8K)	468 (157 – 961)

Results: We estimate that adding WBPR to Ghana's blood safety portfolio would avert 19,442 (95% credible interval 11,322 – 26,711) adverse events annually, primarily by averting bacterial sepsis (50%) and malaria (32%) infections, and would avert 38,490 (16,160 – 66,059) disability-adjusted life years each year. One year of WBPR would cost an estimated $8.0 million ($6.4 – $9.9 million) and eliminate $8.6 million ($4.4 – $13.4 million) in direct healthcare spending on transfusion-associated adverse events. In probabilistic sensitivity analysis, WBPR reduced overall healthcare spending in 57% of iterations and never cost more than $800 per disability-adjusted life year averted. This is well below the annual gross national income in Ghana, which was $2202 in 2019 (source: the World Bank). Findings were most sensitive to uncertainty in the probability that a bacterially contaminated donation leads to clinical sepsis.

Summary/Conclusions: WBPR would substantially reduce the burden of known transfusion-associated adverse events in Ghana and may reduce overall healthcare spending. Additional benefits not captured by this analysis may include averting secondary transmission of infectious diseases, reducing non-medical costs, and avoiding other adverse events, such as new or re-emerging transfusion-transmitted infections. Our findings suggest bacterial sepsis is an underappreciated blood safety threat in sub-Saharan Africa. More research on clinical outcomes following transfusion of a bacterially contaminated whole blood unit would reduce uncertainty.

P-079 | Preservation of neutralizing antibody function, in COVID-19 convalescent plasma, treated using methylene blue and visible light-based pathogen reduction technology

selected for main programme

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Background: Coronavirus disease 2019 (COVID-19) convalescent plasma (CCP) is considered a safe therapeutic option for SARS-CoV-2. However, a transfusion-transmitted disease is a risk that can be mitigated by pathogen reduction (PR).

Aims: The objective of this study was to evaluate the effect of methylene blue and visible light (THERAFLEX MB –TMB-) pathogen reduction technology on the functional properties of CCP (neutralizing antibodies –NtAb- targeting the SARS-CoV-2 S protein). We also wanted to check the correlation between SARS-CoV-2 IgGs recognizing the spike (S) protein receptor-binding domain (RBD), and NtAb.

Methods: CCP units (n = 35) from recovered COVID-19 male donors were treated with TMB. At the end of the PR procedure, MB was removed. All study participants were submitted to regular blood donor testing (ABO, RhD typing and infectious disease's screening) and were also screened for anti-SARS-Cov2 IgG antibodies. SARS-CoV-2 RBD S1 epitope IgGs were quantitated by ELISA and NtAb50 titers against the S protein were measured using a pseudotyped virus platform. Anti-SARS-CoV-2 RBD S1 epitope IgG was assayed with Euroimmun anti-SARS-CoV-2 IgG ELISA (Mountain Lakes, NJ). This is a semi-quantitative method where the S/CO ratio is considered as a titer. The cut-off was established at =/>1.1. Pre- and post-TMB treatment samples were tested for NtAb titer retention, and pre samples were also tested for IgG anti-SARS-Cov2.

Pre and post-PR treatment NtAb titer's differences were analysed using a paired sample t-test. Pearson's correlation coefficient was used to assess the relationship between continuous variables using the entire dataset. A $p < 0.05$ value was considered statistically significant. The analyses were performed using the SPSS v.20.0 software (SPSS, Chicago, IL.).

Results: Results are displayed in Table 1 and 2.

P-079 Table 1. Pre and post-PR NtAb50 titer's comparison.

	N	Mean	S.D.	Mean difference	p
Pre NtAb50 reciprocal titers	35	883.23	926.11	12.16	0.770
Post NtAb50 reciprocal titers	35	895.39	894.83		

NtAb titers did not differ between pre-and post-treatment samples ($p = 0.770$).

P-079 Table 2. Correlation between anti- Spike RBD IgG and NtAb50, pre- and post-PR.

	Pre NtAb50 reciprocal titers	Post NtAb50 reciprocal titers
Pearson's correlation	0.847	0.824
Sig. (bilateral)	0.000	0.000
N	35	35

Summary/Conclusions: SARS-CoV-2 NtAb function in COVID-19 convalescent plasma is well maintained following TMB. These results suggest that PR treatment does not impair the passive immunity provided by CCP. There is a strong correlation (Rho > 0.8) between anti-Spike RBD IgG and NtAb50 before and after PR. This fact allows blood centres to select CCP donors based on IgG ELISA titer's results.

P-080 | Efficient inactivation of SARS-CoV-2 in platelet concentrates in 100% plasma with amotosalen and ultraviolet a light treatment

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Background: Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) was identified in January 2020 as the responsible agent for COVID-19. First recognized in late 2019, the COVID-19 epidemic developed into a pandemic with, as of March 19th, 2021, more than 120 million cases and 2.6 million deaths reported globally. Low viral load SARS-CoV-2 RNA was detected in rare occasions in blood samples and blood components from asymptomatic blood donors, including platelet concentrates. In vitro studies showed SARS-CoV-2 particles on the surface of and inside platelets, the association of SARS-CoV-2 genomic RNA with platelets was reported in samples from symptomatic patients. This suggests that SARS-CoV-2 may be a potential risk for platelet transfusion in certain circumstances, and pathogen reduction may offer a potential to reduce the risk of transfusion transmission.

Aims: Assessment of the efficacy of amotosalen/UVA light pathogen reduction to inactivate SARS-CoV-2 in human platelets.

Methods: Five human apheresis platelet concentrates in 100% plasma (380 mL each) were inoculated with a 1:100 dilution of a viral stock obtained with a local SARS-CoV-2 clinical isolate (SARS-CoV-2/human/SAU/85791C/2020). The presence of anti-SARS-CoV-2 neutralizing antibodies in the platelet units was excluded by testing with an in-house neutralization assay prior to spiking. Spiked units were used to evaluate the efficacy of amotosalen/UVA treatment (INTERCEPT™ Blood System, Cerus Corporation, Concord, U.S.A.) to inactivate SARS-CoV-2 in platelet concentrates. Infectious and genomic viral titers were assessed by plaque assay on Vero E6 cells and quantitative RT-PCR (Altona Diagnostics, Hamburg, Germany), respectively, in spiked and treated samples in parallel with positive and negative controls.

Results: Treatment of spiked platelets (titer of the viral stock: 5.27 $\pm$ 0.2 $\log_{10}$ PFU/mL) with amotosalen/UVA light resulted in complete inactivation of infectious viral titer with a mean log reduction of >3.31 $\pm$ 0.23 $\log_{10}$ PFU/mL. No viral replication or cytopathic effect (CPE) was observed in permissive cells inoculated with inactivated samples even after 9 days of incubation and three successive passages. Evaluation of the genomic titer expressed in PFU equivalents (PEq/mL) in inactivated samples showed reduction to the limit of detection of >4.46 $\pm$ 0.51 $\log_{10}$ PEq/mL (median CT value in spiked samples pre-inactivation: 20.1 (18.0-22.2).

Summary/Conclusions: Complete and efficient inactivation of SARS-CoV-2 was observed with amotosalen/UVA light treatment of spiked human platelet units in 100% plasma suggesting that treatment of platelets with this pathogen reduction technology could reduce the risk of potential SARS-CoV-2 transfusion-transmission. These findings are consistent with prior results using the same technology for SARS-CoV-2 in plasma (reduction >3.32 $\pm$ 0.19 $\log_{10}$ PFU/mL) published recently by our group.

P-081 | High levels of contaminating lipids or red blood cells do not affect the inactivation capacity of the theraflex UV-platelets system

selected for main programme

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Background: The THERAFLEX UV-Platelets system (Macopharma) uses UVC-light for pathogen inactivation of platelet concentrates (PCs). Recently, the efficacy and safety of UVC-treated platelets was published in the phase III "CAPTURE" trial.

Aims: Contamination of PCs with lipids or red blood cells (RBCs) may reduce the transparency for UVC light, thus impairing the inactivation capacity of the THERAFLEX UV-Platelets procedure. In this study, the impact of lipids and residual RBCs in PCs on pathogen inactivation was investigated using different bacteria and virus species.

Methods: Plasma-reduced PCs were produced from pools of 4 buffy coats in 65% additive solution SSP+. Reddish PCs were prepared by spiking PCs with RBCs to a final concentration of 6×10^9 RBCs/bag. In order

to produce lipemic PCs, regular PCs were centrifuged and the supernatant was removed. Platelets were resuspended in a mixture of SSP+ (65%) and lipemic plasma (35%). The concentration of triglycerides in final PCs was 17 ± 10 mg/dL (control) and 83 ± 40 mg/dL (lipemic PCs).

In a pool and split design (control vs. reddish or lipemic), PCs (n=3 for each species) were spiked with bacteria or virus suspensions (≤10% v/v) and were then UVC-irradiated using the Macotronic UV illumination machine (Macopharma). Samples were taken after spiking and after illumination with different light doses (0.05, 0.1, 0.15 and 0.2 (standard) J/cm^2). The titer was determined by endpoint titration (viruses) or plating on agar plates (bacteria). Log_{10} reduction factors were calculated for the standard UVC dose.

Results: Klebsiella pneumoniae (PEI-B-P-08-01) and Escherichia coli (PEI-B-P-19) were efficiently inactivated, resulting in a log_{10} reduction of 6.9 – 7.0 in control and test PCs. VSV (VR-158) was inactivated by 6-7 log steps, and EMCV (VR-129B), a non-enveloped model virus for HAV, was inactivated by 3-4 log steps in control and test PCs. No differences in log reduction and inactivation kinetics were detected for lipemic or reddish PCs compared to control PCs.

P-081 Table 1.

	log_{10} reduction factor (mean±SD)	
	Control PC (n=3)	Reddish PC (n=3)
K. pneumoniae	6.9 ± 0.1	7.1 ± 0.3
E. coli	6.9 ± 0.1	7.0 ± 0.4
VSV	5.7 ± 0.1	5.8 ± 0.1
EMCV	4.0 ± 0.1	3.8 ± 0.1
	log_{10} reduction factor (mean±SD)	
	Control PC (n=3)	Lipemic PC (n=3)
K. pneumoniae	7.0 ± 0.2	6.9 ± 0.0
E coli	7.0 ± 0.1	6.9 ± 0.1
VSV	7.0 ± 0.1	7.2 ± 0.2
EMCV	3.2 ± 0.1	3.3 ± 0.2

Summary/Conclusions: Contaminations of PCs with RBCs or triglycerides did not affect the pathogen inactivation efficacy of the THERAFLEX UV-Platelets system. The results suggest that the UVC-based pathogen inactivation system is robust with respect to potentially contaminating blood components.

P-082 | Maintained quality of pathogen-inactivated platelets under various production and storage conditions demonstrate the robustness of the theraflex UV-platelets system

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Background: The THERAFLEX UV-Platelets system (Macopharma) uses UVC-light for pathogen inactivation of platelet concentrates (PCs). Recently, the efficacy and safety of UVC-treated platelets was published in the phase III "CAPTURE" trial.

Aims: Aim of the current study was to determine the influence of different production and storage conditions on the quality of UVC-treated platelets.

Methods: Plasma-reduced PCs (325 – 375 mL) in additive solution SSP + were produced from pools of 4-5 buffy coats (BCs) or by apheresis and were UVC-treated using the THERAFLEX UV-Platelets system. In a pool-and-split-design (n=4-6), PCs that were temporarily (about 1 hour) warmed up to 30°C before UVC treatment, UVC-treated with or without a resting time after preparation, or kept without agitation for 24 hours during storage, were compared to UVC-treated PCs handled under standard conditions. In vitro platelet quality (pH, glucose consumption and lactate generation, collagen-induced aggregation, CD62 expression) was assessed during storage for 7 days.

Results: In accordance to previous studies, UVC treatment was generally associated with a slight activation of the platelets and a slightly increased metabolic activity compared to untreated platelets. Aggregation response of platelets to collagen was improved after UVC treatment. There was no change in quality of UVC-treated platelets, if PCs were temporarily warmed up to 30°C before UVC treatment. The resting time after preparation did not have an impact on the quality of UVC-treated platelets. Interruption of agitation for 24 hours during storage resulted in increased glucose consumption and lactate production in both UVC-treated and control PCs. However, despite increased glycolysis UVC-treated and untreated platelets did not run out of glucose until day 7 and the pH was well maintained above 6.4, compliant with national and European guidelines.

Summary/Conclusions: The THERAFLEX UV-Platelets system is an effective technology for the inactivation of pathogens that does not use additional photochemicals. The in vitro quality of UVC treated is robust under various production conditions.

P-082 Table 1.

	Day 2			Day 5			Day 7		
		UVC-treated			UVC-treated			UVC-treated	
Parameter	Control	RT	30 °C	Control	RT	30 °C	Control	RT	30 °C
pH	7.16 ± 0.03	7.17 ± 0.03	7.17 ± 0.03	7.36 ± 0.05	7.31 ± 0.04	7.31 ± 0.05	7.41 ± 0.06	7.33 ± 0.04	7.34 ± 0.06
Lactate [mmol/L]	5.9 ± 0.6	5.8 ± 0.5	5.8 ± 0.5	8.5 ± 1.0	9.2 ± 0.9	9.0 ± 0.9	10.3 ± 1.1	11.8 ± 1.0	11.6 ± 1.0
Glucose [mmol/L]	7.2 ± 0.4	7.2 ± 0.5	7.3 ± 0.4	5.9 ± 0.5	5.6 ± 0.5	5.6 ± 0.4	5.2 ± 0.7	4.1 ± 0.5	4.2 ± 0.5
Collagen-induced aggregation [%] 10 µg/mL Collagen	85 ± 4	86 ± 2	85 ± 4	43 ± 15	57 ± 9	57 ± 9	21 ± 4	30 ± 6	34 ± 11
CD62P [%]	19.3 ± 8.6	25.7 ± 8.4	23.3 ± 6.4	23.2 ± 2.7	30.5 ± 3.4	29.8 ± 5.1	29.8 ± 2.9	35.0 ± 1.4	36.2 ± 2.6

P-083 | Usutu virus is efficiently inactivated in platelet concentrates by UVC light using the theraflex UV-platelets system

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Background: Usutu virus (USUV) belongs to the class of zoonotic mosquito-borne flaviviruses like West-Nile virus or Japanese Encephalitis virus. Having its origin in Africa, it was first detected in Europe in the 1990s. USUV infections mainly affect birds but infections in humans have also been described. These infections are predominantly asymptomatic, but some people experience neurological symptoms. Cross-reactivity of USUV with WNV in NAT testing showed that USUV infections are also common in blood donors.

Aims: This study aimed to investigate the efficacy of the THERAFLEX UV-Platelets system to inactivate USUV in platelet concentrates (PCs). The THERAFLEX UV-Platelets system (Macopharma) uses UVC light alone, without any additional photoactive substances.

Methods: Plasma reduced PCs from 4 buffy coats (35% plasma in additive solution SSP+) were spiked with virus suspension (10% v/v). PCs (n=3, 350 mL) were then UVC-irradiated on the Macotronic UV illumination machine (Macopharma) and samples were taken after spiking (load and hold sample) and after illumination with different light doses (0.05, 0.1, 0.15 and 0.2 (standard) J/cm^2). The titer of USUV (Genbank Accession # MT580899) was determined as tissue culture infective dose ($TCID_{50}$) by endpoint titration in microtiter plate assays on BHK-21 cells (Friedrich-Loeffler-Institute, Isle of Riems).

Results: The infectivity assays demonstrated that UVC-irradiation inactivates USUV in a dose-dependent manner. After spiking, an USUV titer of 8.3 ± 0.2 $\log_{10}$ $TCID_{50}$/mL was obtained in the PCs. At a UVC dose of 0.2 J/cm^2 the titer was reduced to 3.1 ± 0.9 $\log_{10}$ $TCID_{50}$/mL, resulting in a $\log_{10}$ reduction factor of 5.2 ± 0.7.

P-083 Table 1.

Sample (n=3)	$\log_{10}$ $TCID_{50}$ (mean±SD)	$\log_{10}$ reduction factor (mean±SD)
virus suspension	9.2 ± 0.1	
after spiking (load)	8.3 ± 0.2	0.0
0.05 J/cm^2	6.9 ± 0.2	1.3 ± 0.2
0.1 J/cm^2	5.9 ± 0.4	2.3 ± 0.3
0.15 J/cm^2	4.7 ± 0.5	3.5 ± 0.3
0.2 J/cm^2	3.1 ± 0.9	5.2 ± 0.7
hold	7.9 ± 0.4	0.4 ± 0.3

Summary/Conclusions: Our results demonstrate that the THERAFLEX UV-Platelets procedure is an effective technology to inactivate USUV in contaminated PCs.

P-084 | Pathogen inactivation (PI) of pool-PLTS using the intercept blood system in clinical practice

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Background: The use of pathogen inactivation (PI) enhances the safety associated with platelet (PLT) transfusion by preventing the pathogen (viruses and bacteria) transmission, reduces the risk of transfusion related adverse events and increases storage time from 5 to 7 days. This storage extension potential is very important in island regions with tourism. The difficulty of blood products transfer leads to reduced sufficiency or/and destructions because of expiration date. In addition, the epidemic of West Nile Virus during the summer months affects the PLTs offer in our country, while there is always the threat of an emerging pathogen. The main disadvantage of PI methods is the high cost, especially in their universal application and this demands the rational usage of them.

The aim of this study is to evaluate the 24-hour platelet recovery and 24-hour corrected count increments (24h CCIs) after the transfusion

of PI-treated pool-PLTs (PRPs) and the assessment of the post transfusion bleeding in patients with thrombocytopenia.

Aims: The aim of this study is to evaluate the 24-hour platelet recovery and 24-hour corrected count increments (24h CCIs) after the transfusion of PI-treated pool-PLTs (PRPs) and the assessment of the post transfusion bleeding in patients with thrombocytopenia.

Methods: Nineteen (19) adult patients were transfused with 88 pool-PLTs, which were treated with INTERCEPT (CERUS) Blood System. All patients were examined daily for bleeding according to the World Health Organization (WHO) Bleeding Scale The inclusion criteria were: age < 60 years, good survival rate with or without severe immunosuppression (under transplantation, induction chemotherapy of acute leukemia), without platelet refractoriness and WHO grade 0 or I. Thirty eight (38) transfusions were conducted in patients with platelet refractoriness or bleeding ≥ grade 2 and they were excluded from the study. In the rest 50 prophylactic transfusions of PRPs the following factors were evaluated: 24h CCI, platelet dosage (plt/m^2) and major donor-recipient ABO mismatch. The results were compared with 53 PI-untreated pool-PLTs (P-PCs) transfusions in 19 patients (historical controls). The statistical analysis was performed using t-test and X^2.

Results: The application of PI using INTERCEPT Blood System resulted in a mean platelet recovery of 92% (range 87-100%) with respect to the PLT concentration in the treated products. As it is shown in Table 1, the final PLT dose and/or the major donor-recipient ABO mismatch didn't differ significantly between PRPs and P-PCs while the 24hCCI was significantly lower in the group of PI-treated pool-PLTs (24hCCI: 6588.3 ± 5709.9 vs 14242.8 ± 8656.6, p<0.0001). However, the haemostatic efficacy of the transfusion appears to be maintained, as no severe bleeding was recorded in either group.

P-084 Table 1. Mean responses following platelet transfusions

	PRPs	P-PCs	p-value
Prior transfusion			
PLT count/µl	11900 ± 4858	12833 ± 3540	0.2
24h after transfusion			
PLT count/µl	24340 ± 8812	35041 ± 17800	
24h CCI	6588 ±5709	14242 ± 8656	<0.0001

Summary/Conclusions: Our results are in accordance with the bibliography. The pathogen inactivation technique is easy, enhances the transfusion safety without significant PLT loss and is efficient in preventing severe bleeding. The main drawback of this pathogen inactivation method remains the high cost. However, the application in low cost blood products, such as pool-PLTs, gives us the chance to use this method in the daily blood transfusion medicine for selected patients.

Systems Supporting Safe Transfusion – Organisational Issues

P-085 | Analyses of the variation of practices at blood centres during COVID-19 pandemic in India - A survey based cross-sectional study

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Background: India with more than 3,000 blood centres collect above 11 million units annually. Maintaining adequate blood supply during the COVID-19 pandemic was a huge challenge. The blood centres adopted unique ways of recruiting donors, adopting COVID appropriate behavior at collection centres, inventory management, manpower utilization, usage of PPE and modification of lab practices. In absence of very clear guidelines on above issues, we aim to study the variation in practices across the blood centres in India.

Aims: To assess the variation of practices across blood centres in India through an online survey.

Methods: This was a cross-sectional study targeting blood centres throughout the 28 states and 8 union territories in the country. The study was approved by the Institutional Ethics Committee. The doctor in charge of the blood centre was sent a Google form link with a detailed questionnaire. The participation in survey (consisting of 25 questions) was a voluntary exercise and anonymous to the responder as no blood centre specific questions were asked. The survey was conducted on one week from 4th to 10th May 2020.

The responses were automatically embedded in the MS office excel sheet. All the categorical variables were summarized as proportions and frequencies with percentages at 95% confidence intervals. Continuous variables were expressed as mean (SD) or median (range) and compared by Student's t-test or Mann-Whitney U test as appropriate.

Results: A total of 195 blood centers completely responded to the online survey. Most of the blood centres who responded were part of Government hospitals (60%), part of an academic institutes (55.6%) and were directly supporting a COVID hospital (67.5%). Almost 95,4% blood centers reported reduction of blood donation mainly due to lockdown (50%) and inability to conduct camps (17.3%). Scheduling blood donations was one of the most difficult to implement strategy for maintaining adequate blood donation (40.2%).

Blood center manpower management was also a challenge and up to 48% blood centers operated in two batches to ensure social distancing in blood banks and reduce the risk of exposure. Hemato-oncology (36.8%) and obstetrics (33.7%) were major utilizer of blood during the pandemic. There were marked variations in use of PPE by blood banks staff as face mask (37.2%) was the most common PPE used in donation area.

There was marked difference in strategies adopted while conducting immunohematology tests as 28.9% used a special plastic bag for sample transport, samples of COVID positive patients were tested using a biosafety cabinet (26.2%) by some centres and some even gave up tube testing (24%). Some blood centers also reported discard of blood units due to reduced demand (mainly platelets).

Summary/Conclusions: This pandemic has highlighted some of the major limitations of the health services, but blood services have risen to the challenge and strived to maintain the blood supply chain while ensuring blood donor and staff safety. The wide variations in the practices adopted highlights the need for uniform guidelines for blood services in future pandemics.

Systems Supporting Safe Transfusion – Information Technology

P-086 | Telemedicine in the institute for transfusion medicine in RNM – Our experience

selected for main programme

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Background: Telemedicine is a service that is rapidly evolving to provide increased access to high-quality healthcare that is efficient and cost-effective.

Aims: The shortage of specialist transfusion doctors as well as the age structure of the doctors employed at the Institute of Transfusion Medicine of Republic of North Macedonia has imposed the need to find a concept that will provide efficient operation of Transfusion Medicine Centers and Services over the years. At the same time, it was imperative to reduce the involvement of doctors in duties and regularities and to unify the way of working in all places.

Methods: After the creation of the VPN (Virtual Private Network) LogMeIn Hamachi was completed and the translation of the remote-validation software into Macedonian was also completed, the first telemedicine system was installed in the Transfusion Medicine Service - Specialty Hospital for Surgery Saint Naum Ohridski in March 2013. After completing installation in 3 Centers and 17 Transfusion Medicine Services, from 01 October 2018 telemedicine is in full use in our country. In July 2019, an installation was also made at the Resen Health Service. A regional separation was made of 4 regions according to which each of the doctors in the region validated the results elsewhere in the region where they belonged.

Results: Each of the Transfusion Medicine Centers and Services works the same way. The following tests are performed: 1. Blood group with reverse grouping; 2. Blood group for newborns; 3. Antibody screening with a commercial set of erythrocytes I-II; 4. Blood Test Profile consisting of: Donor Blood Type Confirmation, Patient Blood Type Confirmation, Patient Antibody Screening, Cross-match. Unification of work, unified report, archive of validated tests with images and information who has completed and validated the test, remote inspection and interpretation of pre-transfusion tests, rapid and efficient blood delivery, 24 hour doctor availability, especially in less available services with few doctors and cost savings are the major benefits of introducing telemedicine in our country. Simple analysis system for improving analysis' work processes, work organization and so on. From 01.01.2020 to 01.01.2021 are validated more than 110.000 results in the whole country through the telemedicine system, of which 2/3 are in working hours (from 8 to 15 h) and 1/3 after 15 h. Just over 35% of validated results are in the region that gravitates towards the Regional Transfusion Center Bitola.

Summary/Conclusions: The use of telemedicine has a strong impact on the improved and timely transfusion service for patients, improved organization and rationalization of work in the Institute for Transfusion Medicine of RNM and on substantial cost.

P-087 | Traceability impact of a standardized unique identifier for medical products of human origin

selected for main programme

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Background: Each medical product of human origin (MPHO) must be traceable from collection to disposition. Every MPHO labeled with the ISBT 128 standard is globally unique. However, MPHO traceability information commonly resides in laboratory information systems and is not consistently stored in a standard manner in electronic health records. A need to develop a single data item that includes critical traceability elements for use in electronic messaging and electronic health records was identified.

Aims: The purpose of the MPHO Unique Identifier is to provide a standardized format for a single unique identifier that incorporates the donation identification number (DIN), product description code, and division code.

Methods: A proposal detailing the critical traceability elements to be included in the MPHO Unique Identifier was developed. ISBT 128 labeling requirements, and the way information is encoded, purposefully vary due to the differing needs of various MPHO areas (*e.g.*, blood, cellular therapy, tissue, etc.). The new identifier was specifically designed with the intent of providing an effective and consistent way to store traceability information about an MPHO, regardless of MPHO product type, in an electronic health record. The ICCBBA Standards Committee approved the proposal. The MPHO Unique Identifier has been developed and is published in the

ISBT 128 Reference Table 42 (RT042) which provides mechanisms for identifying ISBT 128 information in electronic messages.

Results: The MPHO Unique Identifier is comprised of data elements sourced from multiple data structures already routinely used on MPHO labels. It is a twenty-nine character code that is a combination of the processing facility code (5 characters), product description code (5 characters), ISBT 128 DIN (13 characters), and division code (6 characters). Examples of MPHO Unique Identifiers are provided in Table 1.

P-087 Table 1.

Example 1: W0000E1234W0000181234560000Ab
- Processing facility is the same as the facility that assigned the DIN. - Blood Product Description Code - DIN - Division code from the 7th and 8th character of a Blood Product Code (4 padded leading zeros)
Example 2: W9999S1234W000018123456AA0000
- Processing facility code identifies a different facility than the one that assigned the DIN. - Cellular Therapy Product Description Code. - DIN - Division code when the Product Divisions Data Structure [032] is utilized.
Example 3: W9999T0123W000018123456000123
- Processing facility code identifies a different facility than the one that assigned the DIN. - Tissue Product Description code - DIN - Division code from the 6th, 7th, and 8th characters of a Tissue Product Code (3 padded leading zeros).

Summary/Conclusions: Health Authorities in many countries are promoting interoperability of electronic health records. Ever increasing global adoption of the ISBT 128 Standard for coding and labeling MPHO has provided the groundwork for inclusion of MPHO traceability information in electronic health records. Capturing the MPHO Unique Identifier as a single data item optimized for the electronic health record provides a harmonized format for traceability and electronic exchange of health information across the spectrum of MPHO. Additional benefits of the MPHO Unique identifier include providing a unique key for each MPHO which supports a more streamlined way of searching across MPHO product types and has significant potential to improve biovigilance.

P-088 | Interval information sharing among blood donors in Yangtze River Delta of China

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Background: The blood donation law of the people's Republic of China clearly stipulates that it is strictly forbidden for blood stations to collect excessive and frequent blood from blood donors in violation of regulations. In recent years, the trend of personnel flow in the Yangtze River Delta region of China continues to strengthen, the situation of cross provincial blood donation is more and more common, and there is the risk of re-donation in the interval of blood donation. Due to the lack of interconnection of blood management information system in the Yangtze River Delta region, the relevant information of blood donors has not been fully shared, which increases the difficulty of health consultation for cross provincial blood donors. In order to reduce the risk of re donation in the interval of blood donation and ensure the safety of blood donors and blood, we built the information sharing system of blood donors in the Yangtze River Delta region.

Aims: Continuously improve the blood donors and blood safety management level in Yangtze River Delta.

Methods: First of all, we determine the sharing requirements, including the sharing content, sharing frequency, and establish the sharing basic data set. Secondly, we determine the technical solutions, including networking mode and sharing mode, and formulate the interface specification. In terms of network and information security, we use the HTTPS protocol to ensure the security of information transmission, and carry out access control by verifying the identity of the sender of the request service. Finally, according to the data exchange interface specification, the blood stations in the Yangtze River Delta region have respectively organized and completed the development of interval blood donor information query service and interval blood donor information sharing database, and reformed the blood management information system of the province.

Results: The blood stations in Yangtze River Delta region of China have unified the information sharing standard of blood donors interval, developed the query service, and realized the information sharing of blood donors interval. Taking Zhejiang Province as an example, by the end of 2020, in the interval information sharing system of blood donors in the Yangtze River Delta region, 601953 blood donors registered in the provincial blood management information system were searched, among which 350 blood donors were found not meeting the requirements of blood donation interval, including 174 in Shanghai, 92 in Jiangsu and 84 in Anhui.

Summary/Conclusions: To avoid frequent blood donation of blood donors in the interval period can ensure the health of blood donors, that is, to ensure that the blood donors have enough time to return to the level before blood donation, so as to effectively ensure the safety of blood donors. In addition, the prevention of interval blood donation effectively ensures the quality of blood source and provides high-quality blood for clinic.

P-089 | Making blood safe through central deferral registry: SUKUSA

selected for main programme

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Background: Donor eligibility screening is one of the critical processes in blood bank quality system to ensure blood safety. The transfusion guideline (1) not only promulgates minimum testing requirement for transfusion-transmitted infection but also requires that certain questions

be asked of every donor and each prospective donor must be screened against the data base in the central registry such as SUKUSA. SUKUSA is a system that stored national deferral records from all 16 screening centres that can be use online access through a website or offline via SUKUSA Application. The intended purpose of the SUKUSA is to enhance and protect the safety of the blood supply by allowing blood collection facilities (BCFs) to identify donors whose blood has been previously designated as a potential health hazard and thus prevent blood from these previously deferred donors from entering the general blood inventory.

Aims: This study examined the frequency of blood donors who had been deferred from donation attempted to donate at similar or different blood collection centre to evaluate the important use of SUKUSA to ensure blood safety.

Methods: All confirmed positive donor information from 7 collection centres in Central Region were tabulated and analysed if any of them successfully donate for second time instead of being deferred. The data was gathered from 2007 to 2018 and presented in Table 1.

Results: A total of 445 confirmed positive donors have been found to attempt for next donation despite of being permanent deferred and recall for follow up. 285 of them are lapsed donor while 160 are regular donor. 237 donors or 53.3% try to donate at different collection facilities either in centre or mobile campaign.

P-089 Table 1.

Collection Site	Donordonated at same collection site		Donor donated at different collection site		Total
	Gap between twodonations with Confirmed Positive TTI				
	< 12 months (Regular)	> 12 months (Lapsed)	< 12 months (Regular)	> 12 months (Lapsed)	
P	20	20	45	105	190
K	49	66	15	43	173
S	4	8	3	22	37
T	15	8	2	6	31
B	3	3	2	1	9
R	1	0	0	1	2
Pi	1	0	0	2	3
Total	93	105	67	180	445

Summary/Conclusions: In summary this study showed that even the donor has been permanently deferred, there is possibility of them to travel and attempt to donate at similar blood collection sit or different. Based on these findings, SUKUSA play an important role at a local or regional level to help prevent deferred blood donors from deliberately donating elsewhere in the geographic area thus significantly improving the safety of the blood supply.

P-090 | A study on the application of intelligent cold chain system in blood cold chain monitoring

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Background: Blood cold chain is an important part that cannot be ignored in the whole process of blood collection and supply (from blood collection, processing, storage, transportation, to blood transfusion), and it is also a crucial aspect of blood safety. The intelligent blood temperature monitoring method can be used to monitor the temperature of blood throughout the whole process to ensure that blood products are always stored and transported in a complete, safe and eligible environment after they collected from the body. This can help establish a standard cold chain to ensure that blood products are effective.

Aims: Set up an intelligent blood cold chain monitoring system, to ensure that blood products are fully under the monitoring of system while they are stored and transported.

Methods: Build up a temperature monitoring system, which is composed of an open-case induction module, a temperature detection module, a geographic positioning module and a transmission control module. The open-case induction module, temperature detection module and geographic positioning module collect data respectively and send it to general control module. General control module communicates with monitoring terminal by transmission equipment. The monitoring terminal, including alarm module and storage module, will trigger an alarm to remind people to take interventions when the collected data falls within the preset temperature range.

Results: 391 bags of blood collected in the Wulin Institute of Blood Center of Zhejiang Province, labeled with RF donation code RFID tags and monitored by the intelligent blood cold chain monitoring system. In this system, each bag of blood was carefully monitored from blood collection, storage, to transfusion in clinical. Temperature of each bag was recorded and sent to system every one minute. In this study, we have encountered a temperature anomaly alarm and promptly intervened, all the rest were within normal temperature range. At the same time, we also carried out logistics cold chain monitoring on 376 bags of red blood cells and 359 bags of plasma sent to hospitals, the results showed that the temperature of blood products sent to hospitals met the requirements.

Summary/Conclusions: The intelligent cold chain monitoring system can carry out whole process and real-time temperature monitoring for each bag of blood. With this system, we can accurately and timely record blood temperature, give an alert when the temperature is abnormal, and perform a retrospective analysis of the historical temperature of blood. As a result, this system has been proved to be effective in the practice of blood logistics, which conforms to the standard of blood cold chain, and can be used as a helpful method of blood cold chain monitoring.

P-091 | Assessment of anomalies linked to the non-use of the donor management software for mobile blood collection: Review of 2 months of blood samples

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Background: Meeting the needs for blood products in Côte d'Ivoire remains a constant challenge. The number of annual samples is not sufficient for the needs of hospitals. In order to increase the number of blood donations, the National Blood Transfusion Center of Côte d'Ivoire has opted for the installation of several transfusion structures throughout the Ivorian territory, but also for the use of mobile teams. Several blood collection sites are thus visited either once a year or two to three times a year, thus becoming regular blood collection sites. However, many anomalies are often observed on these mobile sites.

Aims: The objective of this work is to take stock of the anomalies encountered on these mobile sites.

Methods: We carried out a descriptive and comparative prospective study between mobile and fixed collections for both regular and fixed donations. We took stock of two months of samples from January 01 to February 26, 2020. The data were extracted in the progesa medical-technical software.

Results: The most common anomalies were the high number of viral markers for new donors, both at fixed and mobile sites. For regular donors on a mobile site, we also noted a high rate of viral markers.

Summary/Conclusions: New donors are subject to positive viral markers for donated blood. However, the non-use of blood donor management software to highlight bans on donating blood for a positive viral marker constitutes a risk of always taking them; hence the need to edit banned donors before visiting a regular mobile site.

P-092 | Performance of HemoCue HB 301 between sysmex in blood transfusion medicine in Hospital Raja Permaisuri Bainun Perak, Malaysia

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Background: The HemoCue system utilizes the principle of oxidation of haemoglobin to hemiglobin by sodium nitrite and the subsequent conversion of hemiglobin to hemiglobinazide by sodium azide. This study is a correlation report between real performance of HemoCue and sysmex analyzer with samples tested at Hospital Raja Permaisuri Bainun (HRPB), Ipoh.

Aims: We aimed that HemoCue system correlates more than 95% with the sysmex analyzer in this hospital.

Methods: This study was performed from 27th April 2020 until 18th May 2020. The correlation study was carried out with eight (8) set HemoCue analyzer which is used in Transfusion Medicine Department, HRPB, Ipoh verses 5 part blood cell count Sysmex analyzer which is placed at Hematology Unit, Department of Pathology, HRPB. The eight (8) set of HemoCue are with serial no: 1306813280, 1415820318, 0737811115, 1211813194, 1306813262, 1543821001, 1211813227 & 1543821002. The test was carried out solely on testing the hemoglobin level of patient samples sent to HRPB, Ipoh. Total of 240 samples were run on this platform where each set HemoCue was run with 30 samples.

Results: Total of 240 blood samples were run concurrently with our existing HemoCue vs Sysmex analyzer. Results shows more than 98% of correlation between HemoCue and sysmex analyzer was able to achieve. HemoCue serial # 1306813262 (99.6%), 1543821001 (99.8%), 1211813227 (99.7%), 1543821002 (99.8%), 1415820318 (99.6%), 737811115 (99.5%), 1211813194 (98.9%) and 1306813280 (99.3%).

Summary/Conclusions: The performance of all eight (8) HemoCue analyzers was found satisfactory. Therefore, all eight (8) unit of HemoCue is proved to be able to produce a reliable and accurate results of Haemoglobin levels among donors in HRPB.

P-093 | Information sharing among HIV-positive blood donors in Eastern China

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Background: In 2009, Zhejiang Province began to share the information of HIV-positive blood donors in the province based on the provincial unified blood management information system, so as to jointly implement the permanent shielding of HIV-positive blood donors in the whole province. Since 2010, leading by Blood Center of Zhejiang Province and with the joint participation of Blood Center of Jiangsu and Shanghai, a joint shielding mechanism for HIV-positive blood donors has been gradually established, which is also the first cross-provincial joint shielding program in China. However, the three blood stations created the electronic form information of HIV positive blood donors separately ، and sended an encrypted E-mail for delivery, This way has the disadvantages of low automation degree and low timeliness, Therefore, it is difficult to further promote and carry out joint screening in a larger area. In 2017, The Eastern China Cooperation Group of Blood Collection and Supply Institutions ، consisting of Shanghai, Jiangsu, Zhejiang, Shandong, Anhui, Jiangxi and Fujian provinces or municipalities, made suggestion that Blood Center of Zhejiang Province leads to carry out the work for information sharing among HIV-positive blood donors in Eastern China.

Aims: To establish an information sharing system for HIV positive blood donors in Eastern China, Realizing the relevant information of the network direct report, automatic exchange.

Methods: Step 1: Establishing Information Standards. Basic data sets of HIV-positive blood donors in Eastern China have been developed, Including data element name, definition, data type, data length and precision, value range and other contents that need to be shared. In

addition to data standards, we also developed technical specifications for data exchange, including the data exchange formats (JSON, XML), data transfer protocol (HTTPS), messaging and feedback mechanism (SOAP Web services).Step 2: Confirming the information sharing pattern. According to the information construction of blood in various provinces and cities, the system adopts a hybrid sharing mode of centralized sharing and distributed polling. Among them, Zhejiang, Shandong, Anhui, Jiangxi and Fujian used the centralized sharing mode. The shared database is set on the Zhejiang Blood Cloud platform, and We also specially developed the data report web system and the related data report interface. The distributed polling mode is adopted between Jiangsu, Shanghai and Zhejiang blood cloud platform. Step 3: network and information security. In order to ensure the security of data transmission, the system adopts HTTPS security certificate, double factor authentication, authority management and other technical means.

Results: The information system began trial operation on January 1, 2018,by December 2020,1810 confirmed HIV-positive blood donors information has been shared in eastern China, Among them, 1034 cases were from Zhejiang Province, 286 from Jiangsu Province, 187 from Shanghai City, 161 from Fujian Province, 97 from Jiangxi Province, 31 from Anhui Province, and 14 from Shandong Province. According to the monitoring of blood cloud platform in Zhejiang Province, two confirmed HIV-positive blood donors from Shanghai and one from Jiangsu Province who donated blood in Zhejiang were successfully blocked.

Summary/Conclusions: Establishing an information sharing system for confirmed HIV-positive blood donors, can achieve joint screening of HIV-positive blood donors between regions in Eastern China.

Systems Supporting Safe Transfusion – Cost/Effectiveness

P-094 | Key drivers of cost-effectiveness for an amotosalen/ultraviolet a platelet pathogen inactivation technology

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Background: Globally, there has been a growing adoption of pathogen inactivation (PI) technologies to improve the safety of platelet transfusions. PI has been partially or universally implemented in 15 of the 31 countries in the European Union's single market; and in the United States around 28% of platelet products transfused are treated with PI. Routine use data, long-term haemovigilance reports, and post-market surveillance from Switzerland, France, Austria and Belgium, where the amotosalen and UVA light (A/UVA-PI) based technology (INTERCEPT Blood System™ Cerus Corporation, Concord, CA, USA) is being used, demonstrated equivalent efficacy for PI-treated versus non-PI treated platelets, with potential increased safety for PI-treated platelets as shown by the reduced risk of transfusion-transmitted infections.

An understanding of cost-effectiveness, based on a wide array of potential consequences, is required to help inform decision making for PI adoption.

Aims: The aim of the analysis was to quantify the key drivers of cost effectiveness for the A/UVA PI technology.

Methods: An economic model was constructed to assess the cost effectiveness of A/UVA-PI versus current safety measures for platelet transfusions from the perspective of the UK health care system. The model was constructed in line with methods guidance published by the National Institute for Health and Care Excellence (NICE) and input data were derived primarily from published sources relevant to the UK. The model simulated costs and health benefits for a cohort of platelet transfusion patients.

The model structure starts with a short-term decision tree which considers the period of transfusion in which patients may experience one of several adverse events, followed by a long-term Markov model which tracks outcomes over the patient's remaining lifetime.

To be comprehensive, the analysis captured a wide range of comparative benefits (elimination of donor deferrals and screening, product irradiation and testing requirements, decreased transfusion reactions, and protection against emerging threats), and comparative costs (additional equipment and maintenance needs, platelet demand and outdating).

Results: The results under base case assumptions suggested that adoption of A/UVA-PI provided greater health benefit at additional cost. While the total costs were greater, driven by the costs of the technology, savings were observed across other cost categories, including in donor deferral (-10%), screening and irradiation (-52%), bacterial culture and related recalls (-100%), transfusion-related adverse events (-76%), and resultant long-term healthcare costs (-100%). Combining the technology with new whole blood-derived buffy coat pooling methods resulted in a further cost-saving. Overall, the model was sensitive to assumptions, especially regarding the risk of emerging threats, the extent and scope of PI adoption, and the inclusion of multi-dose pooling methods.

Summary/Conclusions: PI systems offer a multitude of clinical and operational benefits. Results of this analysis demonstrate that, under specific assumptions, A/UVA-PI may also be a cost-effective use of healthcare resources. Multi-dose pooling methods hold potential for improved cost-effectiveness, while other key drivers were consistent with previous studies. Similar analysis can be performed for other health care systems by customizing inputs in the established model.

P-095 | Component wise financial implications of inappropriate blood transfusion on Indian healthcare system

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Background: For developing countries like India, maintaining a sufficient blood supply and ensuring its appropriate use has been a major challenge. In India the transfusion services are fragmented and there is no

uniformity in transfusion practices among clinicians. For their age, diagnosis, and comorbidities, a large number of patients are both unnecessarily transfused and over-transfused. These unnecessary transfusions increase the cost on both the patient and the healthcare system.

Aims: To extrapolate the cost of irrational blood component transfusion in India on healthcare system.

Methods: Study was funded by National Health Mission regarding implementation of patient blood management programme in Rajasthan State, India. Cross-sectional survey was performed from 9 different blood centres (7 government funded and 2 private operated) of Rajasthan state. Blood requisitions were reviewed for appropriateness and rationale use of blood based on the published guidelines. Diagnosis and indications for the transfusion, pretransfusion laboratory results, and blood components issued were collected during the study period from January 2021 to March 2021. All patients above 18 years of age who received at least one blood component were included. Cost analysis was done on inappropriate transfusions based on the guidelines issued by National Blood Transfusion Council (NBTC) and extrapolated to India as a whole country.

Results: A total of 8953 blood components were requested for 4391 patients and 6910 blood components were issued: 5377 red blood cell (RBC) units, 618 platelets [570 random donor platelets (RDP), 48 single donor apheresis platelets (SDAP)], 888 fresh frozen plasma (FFP) and 24 cryoprecipitate (CRYO) units. Major indications for RBC transfusion include anaemia (n=2981, 43%) and nearly one third of the total RBC transfusions (n=1125, 21%) were performed with hemoglobin levels of more than 7 g/dL including surgery and bleeding as indications. Similarly 29% (n=182, 29%) of the PLT transfusions (including RDP and SDAP) were prescribed with PLT count of more than 20 x 10^9/L for various indications like haematological/ non haematological malignancy, cardiovascular surgery and trauma etc. Cryo and FFP units were involved in 23% of all prescriptions. Among these, 33% (n=8) of the cryo and 5.2% (n=47) of the FFP were requested in the context of non-bleeding and INR< 2 as major indications. A major portion of blood components were issued (n=3277, 47%) without any indications mentioned on the requisition forms. Overall 4639, 67% of the transfusions were irrational. According to cost analysis, a total of 64,32,800 INR (approximately 88541 $) has been expended on irrational transfusions, equating to a total expenditure of 14,05,35,00,200 INR (approximately 19,34,32,377 $) for India and alone PRBC transfusions comprises of 96% of the total expenditure.

Summary/Conclusions: Except for the limitations on assessment of indications for prescribing each and every component, transfusion practices are not in agreement with the published guidelines. Our findings suggest that more than half transfusions are irrational which increase the cost and burden on blood transfusion services and healthcare systems. Implementing Patient Blood Management can effectively reduce the unnecessary transfusion and financial burden on blood transfusion services and will have a major impact on developing countries like India's healthcare system.

Systems Supporting Safe Transfusion – Training and Education

P-096 | Training of personnel involved in blood therapy – Polish experience

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Background: Training of medical staff is one of the crucial tasks on the path to effective and safe blood therapy.

Hospital and laboratory inspections revealed insufficient knowledge on the use of specific blood components, improper management of adverse events and reactions and insufficient understanding of possibilities of acquiring blood.

Aims: The aim was assessment of training formulas (on-site, on-line, e-learning) for members of transfusion committees, physicians responsible for blood management, diagnosticians and other hospital and blood establishment staff involved in blood therapy.

Methods: Material for analysis was data from training courses and anonymous questionnaires completed by participants and prepared separately for participants of each type of training.

In 2017-2020 a total of 659 persons participated in on-site courses; 248 diagnosticians, 196 physicians, 215 nurses. On-line courses organized in 2020 trained 260 persons including 74 diagnosticians, 94 physicians, 92 nurses. In 2019-2020, 4071 persons were trained in the e-learning formula; 1452 diagnosticians, 1308 physicians, 1311 nurses.

Lecture content was adjusted to the needs of each professional group.

The 3 training formulas rendered 1101 completed questionnaires which were subjected to analysis (659 – on-site, 260 - on-line, 182 - e-learning).

MS Excel program was used for analysis of questionnaire content. Microsoft Power Business Intelligence (Power BI) was used for data analysis and visualization of the outcome.

Results: 31% of participants who completed the questionnaires were members of transfusion committees (49% laboratory diagnosticians, 47% physicians, 4% nurses); 86% women and 14% men.

On a 5-point scale 4.3 points were ascribed to upgrading of professional qualification (4.37 – on-site, 4.59 - on-line and 4.31 e-learning).

Details of the analysis for each professional group to be demonstrated during study presentation.

Some subjects were found useless by 11% of participants of e-learning platform, 6.4% - of on-site and 6.2% of on-line courses. Other participants wanted the same subjects to be expanded.

37.4% participants of e-learning courses, 15.8% - of on-line and 2.4% - of on-site courses suggested topics that required expanding.

93.5% of e-learning participants rated the usefulness/effectivity of such training very high; 95.6% declared to recommend the formula due to:

- Free access to educational material.
- Opportunity of playing the recording whenever required.
- Time-saving.

The inconveniences were:

- Difficulties with focusing attention for long
- No direct contact with the lecturer.

Most trainees found no disadvantages.

Summary/Conclusions: In Poland no assessment studies were so far performed regarding national training of staff involved in transfusion medicine. Analysis of opinions/ commentaries of trainees demonstrated that some subjects required broader coverage.

The epidemiological situation induces a shift to the on-line training formula.

Unlimited access to the e-learning platform provides the opportunity of participation in training also to other medical staff not directly involved in activities related to transfusion of blood components but motivated to expand their knowledge in the area.

The outcome of the study demonstrates the training courses to be satisfactory and contributive to upgrading the awareness of participants. Undoubtedly, the training activity should be continued.

P-097 | Impact of various educational strategies on knowledge and practices of clinicians regarding bedside transfusion practices *selected for main programme*

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Background: As with the other countries, In India, the clinicians have limited knowledge about blood transfusion and good bedside transfusion practices as there is no formal training regarding this during their undergraduate and postgraduate training program.

Aims: To ascertain the knowledge and practices of clinical residents of PGIMER Chandigarh regarding bedside transfusion practices and to determine the impact of various educational strategies on improving these practices.

Methods: The study was a hospital based interventional study and different educational strategies were used as an interventional tool. The study duration was of 9 months done by Department of Transfusion Medicine in collaboration with Department of Hospital Administration and Department of Community Medicine and School of Public Health. The study was divided into three phases.

- **Pre-intervention phase**
- **Intervention Phase**
- **Intervention Phase**

Results: Assessment of knowledge of clinical residents regarding bedside transfusion practices

The overall score of residents for all 25 questions related to bedside transfusion practices was 61%. The results showed that majority of residents (97%) felt that a formal training program on clinical aspects of bedside transfusion practices should be a part of their teaching curriculum. This implies that though residents have a positive attitude towards learning good bedside transfusion practices yet majority (60%) have not read the institutes "Blood transfusion guidelines" and "Blood transfusion alerts" poster.

Assessment of bedside transfusion practices

Institutes blood transfusion guidelines were being followed in less than 30% observations and knowledge was not being translated to the bedside practices. A similar results were observed in study by Khetan et al from SGPGI Lucknow in which they observed Lack of awareness for institute guidelines (80% not aware), improper sampling practices (67%) lack of consent for blood transfusion (72%), improper warming of blood component (~80%). In a similar study by Sexsena et al from USA observed that compliance to good transfusion practices improved from 30% to 100% with sustained efforts of dedicated "Transfusion Nurses" over a period of four years.

Summary/Conclusions: The present study gave us the insight that there is a lot of scope of improvement in the bedside transfusion practices in our hospital which can only be achieved through continuous educational efforts followed by effective assessment and gap analysis.

Recommendations from the study

- All residents and staff nurses should have a formal training in all aspects of blood transfusion chain and it should be mandatory part of their residency program and on job training.
- Staff nurses should be involved in some parts of transfusion chain like monitoring and documentation of transfusion, obtaining consent for blood transfusion etc.
- All patients should have wrist band for patient identification check to avoid incorrect blood component.
- The department of Transfusion Medicine should have dedicated transfusion nurses to regularly do the assessment of blood transfusion practices and educate staff regularly regarding good bedside transfusion practices.

P-098 | Actions of improvement of the training activities with bite-sized teaching approach in blood components production unit

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Background: The formation and re-training of the personnel is an essential element for the safety and improvement of the quality in a Transfusion Service. During the COVID-19 pandemic the residential courses have slowed down to limit gatherings and secure interpersonal distances.

Aims: For some activities regarding the emocomponents transformations, like Washing the Erythrocyte Concentrates (W-EC), the evaluation and maintenance of the competences become necessary. We described a new organizational approach regarding the formation and practice of W-EC as a response to the complexity of the process in the times of a pandemic starting from the year 2021.

Methods: Approach inspired by PDCA integrated in Quality Management System (according to the ISO 9001:2015).

- PLAN: Literature revision (Evidence Base) compared to the type of teaching using keywords like: "bite-size" AND "education". Individuation and assignments of the responsibilities: Formators (FOR) and Tutors (TUT).
- DO: Execution of operative tries from the Tut (best practices); Recorded video footage.
- CHECK: Analysis and evaluation of the results; Risk and Opportunities Analysis with FMECA; Operative predisposition's checklists.
- ACT: Implementation of a new strategy with related improvement actions.

Results: Bibliographic research: executed in the time period between December 2020 and January 2021, has produced 5 articles limited to the last 5 years. Best practices results on EC of TUT compared between pre- and post- washing.
Responsibilities: 2 executives FOR; 2 technicians TUT.
Tests carried out on 12 units selected at random.
FMECA: highlights a medium-high risk of obtaining a non-conforming product. IPR: pre = 300; post attended = 100.
Operative checklist: 6 items regarding the essential and critical steps.
New Strategy: Double theoretical briefing (one with For, one with the Tut): max 10' concentrating on pros and cons of the characteristics regarding the type of processing that will be executed; Vision of the video footage of the Tut's activity; Practical execution of the activity for the person in training with contextual video recording following the operative checklist; Valuation of the obtained results based on the attributes indicated and following look-back of the manuality with the recording; Competence board upgrade in case of positive outcome (10% acceptable deviation if the indicators fall within the criteria of the Italian decree of 02-11-2015).

Summary/Conclusions: The brief and repetitive teaching can translate into a training strategy that right mitigate the risk of non-conforming worked product because of the operator-dependent variability. Also, though the direct involvement and confrontation between different professional profiles we can sensitise the team in the comprehension of the criticalities regarding the productive process maintaining the opportune competences and guaranteeing the quality attended performances.

P-098 Table 1.

Attribute	Pre-washing (min - MAX)	Post-washing (min - MAX)	Medium of Delta %
Hb gr/unit	59.49 (46.28 – 76.61)	55.89 (44.28 – 69.01)	5.8
Supernatant proteins residue	0.38 (0.215 – 1.03)	0.076 (0.0583 – 0.106)	76
% haemolysis	0.122 (0.089 – 0.164)	0.144 (0.098 – 0.205)	20

P-099 | Comparison analysis of EQAS blood bank hospital results in 2019 and 2020, Jabodetabek Indonesia: Immunohematology

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Background: One of the efforts to improve the quality of Blood Transfusion services at Blood Bank Hospital is carried out through Quality Control including External Quality. External Quality Assessment Programs are quality assurance activities to held periodically by CBTS IRC to retrospectively assess the similarity various of results in Blood Bank Hospital in Jabodetabek (Jabodetabek means is firmed by the names of fivecities: Jakarta, Bogor, Depok, Tangerang and Bekasi) that use various methods to detect irregularities. The analysis of the results on the quality improvement test was carried out by CTBC IRC to see the suitability of the EQAS inspection carried out by Blood Bank Hospital. The comparison of the data from the EQAS Blood Bank Hospital analysis in 2019 with the EQAS Blood Bank Hospital analysis data for 2020 was carried out in order to obtain information about the increase in the suitability of the results or the absence of an increase in the suitability of the results obtained in 2020 after efforts to increase the suitability were made in 2019.

Aims: to improve the quality of blood transfusion services at Blood Bank Hospital In Indonesia and be used as an indicator to improve the quality assessment in a sustainable manner.

Methods: Data comparisons were carried out after obtaining data from EQAS analysis results in 2019 and data from EQAS analysis results in 2020. The comparative analysis of the data was seen from the percentage of examination results that were in accordance with the results of the unsuitable examination.

Results: By the total of Blood Bank Hospital in Jabodetabek Indonesia that participated in EQAS Immunohematology in 2019 there were 37 blood bank hospital and in 2020 there were 31 blood banks hospital. Of the total EQAS Immunohematology participants in 2019, there were 14 (38%) Blood Banks hospital that had appropriate results and 23 (62%) Blood Banks hospital that had unsuitable results. The EQAS Immunohematology in 2020 had results that were far different from 2019, there were 22 (71%) Blood Banks Hospital with appropriate results, 7 (23%) Blood Banks Hospital that had unsuitable results and 2 (6%) Blood Bank Hospital that has not sent the results of the examination. The most discrepancies in the EQAS Immunohematology Blood Bank Hospital in 2019 were blood group examination by 48%, then crossmatch examination 22% and finally the inconsistency of both was 30% and at the Blood Bank Hospital EQAS Immunohematology in 2020, namely the blood group examination of 29 %, then cross-match checking was 43% and finally the incompatibility was both 29%.

Summary/Conclusions: Based on the analysis of the results from the examination of the EQAS immunohematology Blood Bank Hospital samples in 2019 and 2020, we conclude that there is a large change in the suitability of the results obtained in the analysis of EQAS results in 2020. It is necessary to conduct back coaching to technical staff in the laboratory so that it is expected that all Blood Bank Hospital can get results that match other blood banks at the next EQAS imunohematology.

Systems Supporting Safe Transfusion – Risk models, standards and regulation

P-100 | A who tool for risk-based decision making on blood safety interventions

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Background: Blood operators and blood regulators worldwide are periodically confronted with the challenge of assuring transfusion safety and a sufficient blood supply in the face of known and emerging bloodborne infections. This challenge is particularly acute in resource limited settings where the capacity to model risks and to evaluate alternative interventions may be constrained. To assist decision makers in this area, WHO developed the "Risk-Based Decision Support Tool for Blood Safety" as a software tool that can be applied in any country to obtain quantitative and qualitative information about risks, benefits and costs of candidate interventions in a decision-making framework. The tool is ready to use when addressing emerging infectious threats and new technology opportunities, and allows users to perform both a quantitative Multi-Criteria Decision Assessment (MCDA) and a novel step-by-step qualitative assessment.

Aims: The aim of this paper is to summarize the content and functionalities and illustrate the added value of using the new tool.

Methods: On basis of straightforward risk models for infectious disease transmission, parameters on the blood supply and characteristics of various test (like costs, effectiveness of detection, and false positivity rates), the tool generates an overview of costs and benefits (outcomes) for each of the optional safety interventions. Next, by applying a simple Multi-Criteria Decision Analysis the preferred intervention may be identified. However, to allow for a more deliberative selection of the optimal intervention, the tool also contains a guided qualitative step-by-step process for decision support. This process starts with having the user provide deliberative support for a preferred intervention when only considering the two most important outcomes (as pre-specified by the user). In each of the subsequent steps in the process one single outcome is added for consideration and the impact on the previously defined preference is explored and reported.

Results: A fictitious case study on the selection of a suitable safety intervention to reduce the risk of HIV transmission by transfusion in a particular setting was used to demonstrate the use and usefulness of the tool in practice. This example highlights strengths and weaknesses

of both the quantitative and qualitative approaches: the quantitative approach facilitates an assessment of the robustness of the decision but lacks nuances and interpretability, especially when multiple constraints are taken into consideration; conversely, while being unable to provide an assessment of the robustness of the preference, the qualitative step-by-step approach helps structuring the thought process and argumentation for a preferred intervention in a systematic manner. Next to the demonstration of the tool that is readily available from the website www.decisionsupportforbloodsafety.com, current limitations of the tool as well as potential extensions and options for modification of the outcomes presently defined in the tool will be discussed.

Summary/Conclusions: The relative strengths and weaknesses of the quantitative and step-by-step qualitative approach to risk-based decision making are complementary and mutually enhancing. A combination of the two approaches is therefore advisable to support the selection of appropriate blood safety interventions for any particular setting.

P-101 | A method for assessment of hygienic standard operating procedures (SOPS) in the blood transfusion centers during coronavirus disease 2019 (COVID-19) outbreak

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Background: The outbreak of coronavirus disease 2019 (COVID-19) has led to the alteration in hygienic conditions. During the outbreak of viral diseases with the potential of transmitted through blood components, polices for crisis management is critical to prevent the unexpected event in blood transfusion centers. In this situation, improvement of hygienic standard operating procedures (SOPs) in the blood donation department as the first step of blood collection practice is critical to the safety of the donors and blood supply. One of the reliable processes for the improvement of operating procedures in each organization is customer-centric evaluations as external audits.

Aims: The aim of this study was the assessment of hygienic SOPs in the blood donation centers by regular donors as external audits during the outbreak of COVID-19. We focused on regular donors for two reasons: 1) according to the principle of customer orientation, attention to the donor concern should be given priority and the conflict of interest of blood transfusion centers should be eliminated, 2) in a critical situation and increased need for specialized personnel in blood transfusion centers, the use of regular donors as the local inspector is reliable.

Methods: This study was performed from April 5th to April 20th, 2020. Regular donors were selected from 31 provinces of Iran with the following criteria: 1) The highest number of blood donations, 2) The sufficient knowledge of the blood donation process, and 3) The high social representation. The questionnaire containing 10 closed questions (yes/no questions) was provided to donors with the mentioned criteria to assess the SOPs of blood transfusion centers in the prevention of COVID-19 transmission. For positive answer of each question, an importance coefficient (IC) (0.75 - 1.5) was considered. Finally, the sum of the IC was considered as the total score for each province.

Results: Assessment of SOPs in blood donation department by regular blood donors in 31 provinces of Iran showed that 18 centers (58.1%) received IC scores >10(Strong performance), 7 centers (22.6%) received the range of IC scores between 7-10(acceptable performance), and 6 centers (19.4%) received IC scores <7(poor performance). The difference in IC scores between provinces was not statistically significant.

Summary/Conclusions: The result of this study confirms that the assessment of blood donation centers through the regular donor inspection is a reliable method to identify the strengths and weaknesses of blood transfusion center services and ultimately leads to correct intervention and improvement of hygienic SOPs to prevent COVID-19 transmission.

P-102 | Blood wastage in major haemorrhage incidents

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Background: Recognising major haemorrhage early is crucial for better patient outcomes. Hospitals are therefore required to have local Major Haemorrhage Protocols (MHP) in place to streamline the process of issuing emergency blood products. The Imperial College Healthcare Trust currently advises on issuing 4 units of red cells and 4 units of fresh frozen plasma (FFP) until the lab is notified to stand down. Furthermore, tranexamic acid (TXA), platelets and cryoprecipitate are also recommended to be considered at this stage on a clinical basis. While this protocol is being adhered to, the question of blood wastage remains a problem. According to NHS Blood & Transplant, 3% of red cells, 16% of FFP and 9% of platelets issued were wasted nationwide for MHP incidents in 2018. Since introducing this protocol, the amount of blood products transfused, returned, and wasted have not been properly investigated and the impact of anticoagulants and TXA usage on blood transfusion in this setting are not known.

Aims: The study was undertaken to assess the number of red cell, FFP and platelet units that were transfused, returned, or wasted due to MHP incidents at Charing Cross Hospital (CXH) over a 1-year period. Additionally, it aimed to understand the effect of anticoagulants and TXA usage on blood transfusion.

Methods: Retrospective review of MHP incidents from CXH were assessed over a 1-year period from March 2018 to March 2019. Patient information such as age, sex, indication for MHP, use of anticoagulants/tranexamic acid and blood group were obtained from

electronic patient records (CERNER). Transfusion history on blood products that were issued, returned, or wasted were acquired from the Laboratory Information Management System (TelePath).

Results: Over the 12 months, 57 MHP incidents occurred in this time frame, representing a cohort with an average age of 63.1(SD=17.5) and a greater proportion of males (71.4%) to females (28.6%). Majority of cases were due to gastrointestinal bleeds (56.1%) with other causes such as surgery, haematological disorders and haematuria being less common. For every MHP incident, 6.6 units of red cells, 5.4 units of FFP and 0.9 units of platelets were issued on average. Just over 50% of red cell units were transfused (51.2%), 44.8% were returned to the laboratory and 4% were wasted. Group O positive blood (57%) was the most issued group followed by group A positive blood (23%). However, group O negative blood was wasted the most as a proportion of units issued (14.3%). 54.4% of FFP and 67.9% of platelets were transfused, and a smaller proportion (2.6% and 1.9% respectively) were wasted with the rest being returned. Anticoagulants were utilised in 24 cases while TXA was given in 38 incidents. Anticoagulant and TXA usage made no significant difference (P>0.05) to blood products being issued, returned, or wasted in this cohort.

Summary/Conclusions: Blood wastage (4%) was found to be above the national average (3%). More concerning is the high proportion of O negative blood wasted (14.3%). FFP and platelets were wasted to a lesser degree, however for all products a large amount were returned to the laboratory. Furthermore, the use of TXA made no difference to the blood products that were transfused, returned, or wasted which could potentially be explained by the majority of cases being gastrointestinal bleeds as described in the HALT-IT study in 2020.

Systems Supporting Safe Transfusion – Quality management

P-103 | Shifting the paradigm to improve safety

selected for main programme

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Background: Haemovigilance is the systematic surveillance of serious adverse reactions (SAR) and events (SAE) related to the transfusion pathway from donor collection to recipient transfusion. The aim of haemovigilance is to collate and analyse SARs and SAEs, identify risks, and make recommendations for safer practice that can be shared with the wider community. The Serious Hazards of Transfusion (SHOT) scheme is responsible for haemovigilance for the United Kingdom and provides recommendations on avoiding harm based on the learning from errors. Root cause analysis following an incident may recognise aspects of good practice or praise individuals for their actions. This is the traditional Safety-I approach.

Aims: Safety-II is a proactive approach looking at safe episodes of care to inform improvement in healthcare systems. Understanding and appreciating how frontline staff handle dynamic situations throughout the day, constantly adapting, and getting so much right will help identify the factors and conditions that underpin the success.

Methods: Recognising the importance of combining Safety-I and Safety-II approaches, a new reporting category entitled 'SHOT-Acknowledging Continuing Excellence' has been introduced in 2020. Reporters are encouraged to submit examples of exceptional practice by individuals, teams or departments that was above and beyond routine practice and had widespread learning opportunities. Submissions that share innovative solutions to previous adverse events which have had a positive outcome on practice are also encouraged. This would help identify, appreciate, study, and learn from episodes of excellence in frontline healthcare.

Results: Reporting has just begun in this category. An example of a report submitted includes one that related to a full power outage which disconnected analysers, blood component storage devices, computer systems and telephone lines in a hospital transfusion laboratory. This report demonstrated the power of individuals working together to ensure that patient care was not adversely affected during this challenging time. SHOT was able to share the learning from this event, and the importance of robust contingency plans, via a national patient safety notice.

Summary/Conclusions: Learning from excellence has a valuable role to play in haemovigilance schemes. Learning from excellence and sharing good practice acts as a proactive safety measure in the absence of patient harm. As the number of SHOT-ACE reports increase, a repository of good practice will be developed and shared on the SHOT website. While this has been welcomed by reporters, it has not been implemented long enough for impact to be measured. It is also hoped that this encourages wider adoption of learning from excellence in the NHS. Combining Safety-I and Safety-II approaches is a concept that will help provide a more holistic understanding of the underlying reasons for errors and procedural violations and will help identify aspects of practice that could benefit from targeted interventions to help support staff in providing safe patient care. Reporting and studying success would augment learning, enhance patient outcomes and experience through quality improvement work and positively impact resilience and culture in the workplace.

P-104 | Curious incidents in the night-time: Laboratory 'out of hours' and lone working errors

selected for main programme

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Background: The Serious Hazards of Transfusion (SHOT) haemovigilance scheme collects and analyses data regarding serious adverse events (SAE) and reactions of transfusions in the United Kingdom (UK). Most transfusion laboratories provide blood components

24/7. As most tests and procedures are not performed overnight, staffing levels are usually reduced. UK Transfusion Laboratory Collaborative (Standard 3.6, 2014) state laboratory staff should always have access to specialist advice. Working alone is often listed as a contributory factor for SAE. This may be related to excessive workload, multitasking or lack of specialist support.

Aims: To determine the proportion of SAE which have occurred during non-routine working hours, identify which type of SAE is more likely and to recognise potential measures for mitigation.

Methods: Laboratory SAE accepted by SHOT from 2015-2020 were analysed to determine the time of day the incident occurred. Additional data in 2020 was analysed regarding lone working and occurrence during routine (RH) or outside of routine hours (OH) (excluding anti-D immunoglobulin errors). Incorrect blood component transfused-wrong component transfused (IBCT-WCT) SAE in 2020 provided weekend or weekday data.

Results: A total 8608 SAE were reported, of which 2706/8608 (31.4%) arose in the laboratory: 1121/2706 (41.4%) occurred 08:00-20:00, 193/2706 (7.1%) occurred 20:00-00:00, 274/2706 (10.1%) occurred 00:00-08:00 and information was not available in 1118/2706 (41.3%). This trend was seen in most categories. For handling and storage errors (HSE) 97/506 (19.2%) SAE occurred between 00:00-08:00. Major morbidity arose in 22 cases, death possibly related to transfusion in 14, and probably related in 2.

In 2020, 121/439 (27.6%) SAE occurred when lone working. For HSE 43/90 (47.8%) occurred when lone working, 35/43 (81.4%) involved a cold chain SAE due to equipment failure. A total 159/374 (42.5%) SAE arose during RH, 127/374 (34.0%) arose OH and no answer was given for 88/374 (23.5%). SAE occurred when lone working OH in 71/112 (63.4%) (excluding anti-D). For IBCT-WCT SAE in 2020, 29/44 (65.9%) arose on a weekday, 3/44 (6.8%) on a weekend and no answer was given in 12/44 (27.3%).

Summary/Conclusions: There was no disproportionate prevalence of SAE occurring in the laboratory outside of 08:00-20:00. Data may not be representative as many laboratories class after 17:00 as OH so will have decreased staffing provision after this time, and time of incident was not available in most reports. Data from 2020 shows near comparable rates of SAE during RH and OH. A higher volume of workload occurs during RH, therefore a disproportionally high rate of SAE occurred OH or when the individual is lone working.

Capacity plans must ensure provision of staff matched to workload and consider if any tasks can be allocated to RH. Staff must receive appropriate training and be competency assessed for all tasks undertaken before working alone. Specialist advice must always be available, and policies/procedures should include methods of escalation OH or when lone working. Mechanisms should be in place to inform staff who mainly work OH of policy changes in a timely manner, regularly review training/competency assessments and staff should be included in emergency scenario drills.

HSE are overrepresented OH and when lone working. Procedures to highlight and action SAE (e.g. cold chain breaches) should be reviewed to determine if sufficient instruction is given for OH and lone working.

P-105 | Use of failure modes and effect analysis as an effective tool to proactively reduce risks in blood bank practices of a newly established tertiary care hospital from Western India

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Background: The Blood Bank work involves multi-step linked processes and carries inherent risks affecting the quality of donations, testing, and selection of blood products for issue. Foundation of a newly established blood center is laid through establishment of quality systems which are capable of identifying and correcting these risks. These systems work on the principle of plan-do-check-act cycle leading to continuous quality improvement. We used Failure Mode and Effect Analysis (FMEA) as a risk management tool in a two year old blood bank attached to a 384 bedded tertiary care hospital. The intent of this study was to proactively identify and reduce risks in blood bank processes.

Aims:

- To identify the potential failure modes and their effects across the various sub-processes involved in blood bank work by using FMEA methodology.
- To apply interventions across prioritized failure modes to reduce risks in blood bank processes.

Methods: FMEA team was constituted and mapping was done for each blood bank process over 3 months (June-August 2019). The failure modes and their effects across blood bank processes were studied. Each failure mode was evaluated using severity of effect (S), likelihood of occurrence (O) and probability of detection (D). Risk priority numbers (RPN) were calculate by multiplying S, O and D scores. The RPN enabled FMEA team to prioritize failure modes for intervention. The FMEA team also defined evaluation indicators for these failure modes to enable monitoring over a longer period of time. Post intervention (September 2019); the failure modes were assessed again after 16 months in December 2020 to determine the reduction in RPN.

Results: The study identified 13 failure modes across 26 sub-processes in blood bank. The most important failures were regarding: 1. febrile non hemolytic adverse transfusion reactions (RPN 441). 2. Window period of testing methodology used for transfusion transmitted infection screening (RPN 300). 3. Blood unit of wrong blood group may be issued to the patient (RPN 270). 4. Blood donated by a first degree relative may be issued to the patient (RPN 243); Plasma/ Platelet product by a multiparous female donor may be issued to a patient (RPN 243). 5. Non exclusion of donors with high hemoglobin levels (RPN 240), 6. Microbial contamination of blood during blood collection (RPN 224). Top 6 failure modes (RPN>200) were prioritized for intervention. Among the selected failure modes, rank 1, 2, 5 and 6 were related to provision of resources and funding for equipment, raw material and testing technology. Rank 3 and 4 were related to policy modification, process improvement and staff training. After implementation of risk preventive measures and

reassessment, a reduction in RPN was detected for each risk. There was overall 89.3% reduction in RPN across top 6 failure modes. The evaluation indicators helped in monitoring the sustenance of interventions and efficacy of risk reduction measures.

Summary/Conclusions: This study enabled justification for policy change, funding and staff training needs. FMEA helped to identify high risk processes and prioritize risk mitigation in resource constrained settings. As a risk management tool, the FMEA played a vital role in improvement of blood bank processes at our center.

P-106 | Notification of adverse events that may affect the quality and safety of blood components during 2010-2019 in Greece

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Background: A major function of a national haemovigilance system is to notify adverse events (AE) associated with the collection, testing, processing, storage and distribution of blood and blood components which may affect the quality and safety of blood, whether or not it actually led to a recipient's adverse reaction. AE include: (1) serious events (SAE) leading to recipients' death or life-threatening, disabling or incapacitating conditions, or which could have resulted in prolonged hospitalization or morbidity - notification is obligatory under EU Directive 2002/98; (2) "near-miss" events - errors in the blood establishment, hospital blood bank or clinical sphere, that if undetected could result in failures that might put the recipient in danger; (3) uneventful transfusion errors that did not cause an AE - their recognition may reduce risk by identifying weaknesses in the clinical transfusion process.

Aims: We analysed all AE reported to the Coordinating Centre for Haemovigilance and Surveillance of Transfusion (SKAEM) in Greece in 2010-2019 as part of the national effort to improve the quality and safety of blood transfusion.

Methods: Annual aggregate reports of AE were notified to SKAEM following guidelines laid down in the relevant Directives of the European Commission. SAE, near-misses and uneventful errors which could affect the quality and safety of blood components due to a deviation in any activity (whole blood collection, apheresis collection, testing, processing, storage, distribution, materials and other) were reported and analysed by specification (product defect, equipment failure, human error, other). Incorrect blood component transfused resulting in ABO incompatibility with or without adverse reaction was examined.

Results: In 7,710,333 blood products that were processed in this period, 10,117 AEs were recorded (incidence 131/100,000). SAE were 318 (3.1% of all AE), near-misses 2705 (26.7%) and uneventful errors 7092 (70.1%). Excepting 2016, when 37% of records arose from equipment failure, near-misses and uneventful errors attributed to human error fell substantially from an average of 70% in 2010-2015 to 17% in 2016-2019, while equipment failures increased from 15% to 30%. This differs markedly from other European Union countries where 70% of AE are attributed to human error. Regarding transfusion of "the right product to the right patient" to avoid serious or even fatal AEs due to acute haemolytic reactions, chiefly from ABO incompatibility, 29 failure reports were made in the study period (incidence 0.4/100,000), including 22 serious, with one death. Two further SAE arose from apheresis platelet transfusion, due to laboratory error. All other incidents involving human errors were associated with failure to identify the patient.

Summary/Conclusions: Systematic comprehensive haemovigilance throughout the blood chain in Greece records all AE, near-misses and uneventful errors, regardless of severity and any harm actually caused, thus identifying issues that threaten the patient's safety as well as quality issues. Encouragingly, SAE are relatively rare. The timely detection of the large number of near-misses and uneventful errors, chiefly attributed to equipment and infrastructure, has not only contributed to avoiding serious problems for donors and recipients, but also underlines the need for improvements in the quality system of Blood Services as well as in the clinical departments of the country's hospitals.

P-107 | Quality control for pre-transfusion services at peaking time to inpatients

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Background: Quality indicator data in clinical laboratories plays an extremely important role. Most of clinical guidelines in clinical blood banking laboratoris attach importance to urgent pre-transfusion tests and emergency transfusion issue turnround times. Kaohsiung Veterans General Hospital in Taiwan has 1276 beds. According to our

Standard Operation Procedure which provides urgent pre-transfusion testing results within 30 minutes and non-urgent pre-transfusion testing results in 4 hours. This study is conducted to make the better of inpatient transfusion services, which improve effectively the completion time of non-urgent specimens to inpatients at peaking time in our hospital.

Aims: In 2019, Kaohsiung Veterans General Hospital Blood Bank Laboratory was performed Quality Control Circle (QCC) to reduce waiting time for supplying effectiveness of red blood cells to outpatient transfusion services at Cancer Centre. The outcomes on new strategies were performed the same to inpatients. The aim of this study was to investigate spending time of inpatient pre-transfusion testing at peaking time after QCC for improving transfusion services to inpatients.

Methods: Between 2019 to 2020, the overall 45161 routine pre-transfusion tests, which involved antibody screening and blood group test (ABO group and Rh type) were performed in our clinical blood bank laboratory. 29659 specimens were from inpatients, including 5318 were non-urgent specimens at peaking time (07:30 AM to 10:00 AM). In order to assess inpatients transfusion services, we designed two time intervals which were defined as (1) "the time of specimens to the clinical blood bank laboratory", and (2) 'the spending time of testing at laboratory to complete pre-transfusion examination". The data was collected from February to April (3 months before QC), August to October (3 months after QCC) in 2019, and the following every three months until May 2020. The new process-flow diagram such as new machine maintenance schedule, work follow exchange and specimen re-classification were developed by previous QCC program and the data utilization for 15 months was analysed. Firstly, comparison of each time interval of the process before and after QCC were performed to validate the result. Secondly, comparison of after QCC and the following data to two quarterly (Q1 to Q2). All statistical analyses were performed using SPSS 23.0.

Results: Adherence to our strategies, the mean (SD) of spending time to inpatient pre-transfusion at peaking time was 43.0 (18.0) minutes before the process and 38.09 (16.8) minutes after the process with significant improvement. Comparison the process before QCC, Q1 to Q2 quarterly (December 2019 to May 2020) were all significantly improvements which were 40.6 (16.9) and 39.2 (16.4) minutes, respectively.

Summary/Conclusions: We observed significantly improved the mean of spending time to inpatient pre-transfusion tests at peaking time of Q1 to Q2 quarterly after implementing our interventions. Those improvements may support effectively physicians to order blood components for better quality of transfusion services. This data quality improvement may provide information to our laboratory to continue to follow the completion time of non-urgent pre-transfusion tests and carry out a proper continuous training to new staff.

Platelet and Granulocyte Immunobiology – Platelet Immunology

P-108 | Enlarging thrombocyte donor pool by HLA imputation of biobank data

selected for main programme

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Background: The effective size of the HLA thrombocyte donor pool of the Finnish Red Cross Blood Service is only a few thousand individuals despite the recruitment of new donors annually. This causes difficulties in finding donors for highly immunized patients or patients with unusual HLA type.

Highly developed DNA techniques and improved computational methods enable a cost-effective and rapid way to screen large sample cohorts based on DNA variation. Several dedicated algorithms have been developed to predict (impute) HLA alleles and genotypes from single-nucleotide polymorphism (SNP) data generated e.g. in GWAS studies and collected by biobanks.

Aims: The aim of the project is to expand HLA-typed thrombocyte blood donor pool by defining HLA alleles of Blood Service Biobank participants genotyped for GWAS studies by using bioinformatics tools.

Methods: We imputed alleles of HLA-A and HLA-B genes at high resolution (i.e. unique protein-level) for 19543 Biobank donors using the method described in Ritari et al 2019. To assess the imputation accuracy, we compared the imputed HLA type with the pre-existing HLA type of 97 consecutive samples that had been typed with clinical and validated HLA typing methods: serology, PCR-SSO and Sanger sequencing.

Results:

P-108 Table 1.

HLA-allele	n	Serology Correct typings (%)	PCR-SSO, low resolution	PCR-SSO and Sanger sequencing, high resolution	Number of errors
HLA-A	194	36/36 (100.0)	36/36 (100.0)	121/122 (99.2)	1
HLA-B	194	35/36 (97.2)	30/30 (100.0)	127/128 (99.2)	2
All	**388**	**71/72 (98.6)**	**66/66 (100.0)**	**248/250 (99.2)**	**3**

Summary/Conclusions: The imputed and clinical HLA typing results were highly concordant varying between 97.2–100.0% depending on the HLA gene and the typing method. We observed three errors in altogether 388 typing results (the overall error rate was 0.77%). The highest concordance (100%) was achieved for both HLA-A and HLA-B genes at low resolution level which is also the required level for HLA matched thrombocytes.

Based on the results, we here suggest that imputed HLA type could be used as a preliminary screening method for donor selection prior to confirmatory HLA typing. Furthermore, imputed HLA data could be used in designing an optimal donor pool in a perspective of common and rare HLA types focusing on HLA homozygotes in order to avoid recipient HLA immunization.

P-109 | The alleles of HPA-1 to 6W, 15 and 21W systems were simultaneously detected using a real-time PCR method

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Background: Currently, thirty-five human platelet antigens (HPAs) systems have been nominated and the distributions of HPAs systems were various in the different populations. It was reported that the polymorphisms of the HPA-1 to 6w, 15 and 21w systems were found and the other HPAs systems commonly showed mono allelic in the Chinese population. Now many methods have been used for HPAs genotyping. However, the methods for HPA-1 to 6w, 15 and 21w systems simultaneously detection are rare.

Aims: This study aims to provide a method to detect the alleles of HPA-1 to 6w, 15 and 21w systems simultaneously, and also used the method for HPAs alleles detection in the apheresis platelet donors, China.

Methods: 569 healthy apheresis platelet donors were collected from blood Centre of Zhejiang province, in eastern of China after information contents. The genomic DNAs of the donors were extracted by commercial reagents. Two specific primers and two probes were designed for each of the HPA-1 to 6w, 15 and 21w systems. The primers and probes were amplified and hybridized in one well for each HPA systems for real-time PCR detection. All parameters for PCR is same and can simultaneously detect the alleles of the HPA-1 to 6w, 15 and 21w systems with eight wells reaction. The real-time PCR was preformed in a ABI QuantStudio Dx instrument and the professional test software was used to assign the HPA alleles. The PCR-SBT assay which has been published (Vox Sang. 2017;112(4):360-366) used as a control method.

Results: A real-time PCR method for simultaneously detection the alleles of HPA-1 to 6w, 15 and 21w systems was established, which uses the same PCR conditions and one well for each system. The results of two hundreds samples by real-time PCR were coincident with those of previously PCR-SBT assay. The numbers of HPA-1aa, ab, bb genotypes were 566, 3, 0 in 569 healthy apheresis platelets donors respectively; while 509, 59, 1 for HPA-2aa, ab, bb genotypes; 172, 286, 111 for HPA-3aa, ab, bb genotypes; 566, 3, 0 for HPA-4aa, ab, bb genotypes; 558, 11, 0 for HPA-5aa, ab, bb genotypes; 547, 22, 0 for HPA-6waa, ab, bb genotypes; 157, 297, 115 for HPA-15aa, ab, bb genotypes; 557, 12, 0 for HPA-21waa, ab, bb genotypes respectively. The genotype distributions of HPAs systems were fitted with Hardy-Weinberg equilibrium ($p>0.05$). The frequencies were 99.74% and 0.26% for HPA-1a and HPA-1b alleles, while 94.64% and 5.36% for HPA-2a and HPA-2b alleles, 55.36% and 44.64% for HPA-3a and HPA-3b alleles, 99.74% and 0.26% for HPA-4a and HPA-4b alleles, 99.03% and 0.97% for HPA-5a and HPA-5b alleles, 98.07% and 1.93% for HPA-6wa and HPA-6wb alleles, 53.69% and 46.31% for HPA-15a and HPA-15b alleles, 98.95% and 1.05% for HPA-21wa and HPA-21wb alleles.

Summary/Conclusions: A new real-time PCR method for HPA-1 to 6w, 15 and 21w systems simultaneously detection was successful established. This method is accuracy compared with the PCR-SBT and can provide a rapid HPA alleles analysis.

P-110 | Positive test to heparin-induced thrombocytopenia and COVID-19

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Background: Coronavirus disease 2019 (COVID-19), may predispose patients to thrombocytopenia and venous thromboembolic events (DVT and/or pulmonary embolism [PE]) including those receiving standard thromboprophylaxis with HBPM, due to hypoxia, excessive inflammation, platelet activation, endothelial dysfunction, and stasis.

Aims: We investigated whether this condition may have a prothrombotic disorder caused by platelet-activating antibodies directed against platelet factor 4 (PF4), as it has already been described with heparin and sometimes other environmental triggers.

Methods: We conducted a retrospective observational study with samples sent to our laboratory to test anti-PF4/heparin antibodies to rule out heparin-induced thrombocytopenia (HIT). From April 2020 to March 2021 a total of 56 samples have been studied. To measure direct antibody binding a PF4/heparin EIA (ELISA LIFECODES PF4 Enhanced) was performed, and antibody binding was measured by a secondary antihuman IgG, IgA and IgM reagent as described, each sample was duplicated. An absorbance read (OD) value of equal or greater than 0.400 was considered as positive; negative and positive control should reach OD minor or equal 0.300 and major or equal to 1.8 respectively. The patients were divided into two groups: those with the diagnosis of COVID-19 (28) and the rest (28). Statistical

analysis: Sample characteristics were described for the total sample using means and standard deviations for continuous variables and frequencies for categorical variables. Student's t-test for continuous variables and Chi-Square tests for categorical variables were used to examine mean and proportional differences of the select characteristics of the sample by group. P-values <0.05 were considered significant. SPSS v.20.0 software (SPSS, Chicago, IL.) was used.

Results: The sample consisted of 56 patients, 28 with COVID-19 and 28 without (selected from samples previous to December 2019). For the whole group, seventeen out of 56 patients were positive (30.4%), and taking into account those patients with COVID-19 there was an incidence of positivity of 42.86% while in the rest of the patients the positivity was 17.85% (p=0.042). Results are displayed in Table 1.

P-110 Table 1

		Heparin-associated PF4 antibodies		
		No	Yes	Total
COVID-19	No	23 (82.15%)	5 (17.85%)	28 (100%)
	Yes	16 (57.14%)	12 (42.86%)	28 (100%)
	Total	39 (69.6%)	17 (30.4%)	56 (100%)

Summary/Conclusions: COVID-19 is associated with development of a prothrombotic disorder that clinically may resemble heparin-induced thrombocytopenia. In our study COVID-19 patients showed a higher proportion of positivity to anti-PF4/heparin antibody test than the rest of the patients that may be explained by a common pathophysiologic path to HIT caused by platelet-activating antibodies that recognize multimolecular complexes between cationic PF4 and anionic heparin.

P-111 | Analysis of large scale platelet donor database with HLA-A,B and HPA 1-17W genotype in Zhejiang Province of China

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Background: Immune-mediated platelet transfusion refractory (PTR) is mostly caused by anti-HLA and/or anti-human platelet antigen systems (anti-HPAs). HLA and HPA compatible platelets for transfusion can be improved the efficiency.

Aims: To provide the compatible platelet products in time for clinical usage, we have analyzed the genotype and alleles frequencies of HLA-A,B loci and HPA 1-17w in the large scale platelet donor database. The age, gender and donation times of those donors were also investigated.

Methods: A total of 22097 unrelated healthy blood donors from Zhejiang province were recruited. HLA-A and -B genotyping was performed by polymerase chain reaction sequence specific oligonucleotide (PCR-SSO) or polymerase chain reaction sequence based typing (PCR-SBT). HPA 1-17w genotypes of 1888 platelet-donor specimens from 22097 were determined using PCR-SBT method. Allele and haplotype frequencies of were carried out using Arlequin3.5 software. (This work was supported by the Science Research Foundation of Zhejiang Province with 2019KY368 and 2017C33085).

Results: There are 13352 platelet-donors were male (60.4%) and 8745 were females (39.6%). The mean age was 38.4±7.5 years old for the male and 37.5±7.7 for the females. It is noteworthy that, the donation times was 32.8±37.6 for the males and 18.6±23.7 for the females. That means male platelet-donors are easier to recruit than female among the local people (p<0.01). The distribution of HLA and HPA frequency was consisted with the Hardy-Weinberg equilibrium. In total, the top three frequent alleles of HLA-A and HLA-B loci were A*11:01(23.3%), A*24:02 (16.6%), A*02:01(11.7%) and B*40:01(13.3%), B*46:01(12.0%), B*58:01 (8.1%). The A*02:07-B*46:01(7.4%), A*33:03-B*58:01(6.6%) and A*11:01-B*40:01(5.1%) were the most common two loci haplotypes. Among the 17 HPA systems, HPA-3 and HPA-15 were exhibited the high heterozygosity, with the genotype HPA-3a/a (0.290), HPA-3a/b (0.512), HPA-3b/b (0.198) and HPA-15a/a (0.296), HPA-15a/b (0.486), HPA-15b/b (0.218).The HPA-7w to -14w, HPA-16w and HPA-17w were showed aw monomorphic in all samples. The frequencies of the HPA-1b, HPA-2b, HPA-4b, HPA-5b and HPA-6bw alleles were low with 0.007, 0.054, 0.002, 0.011 and 0.017, respectively.

Summary/Conclusions: We have established a large scale platelet antigen genotyping database in our blood center, which provides useful information for platelet targeted recruitment. Meanwhile, it is helpful to provide HLA and HPA compatible apheresis platelets for immune-mediated platelet transfusion–refractory patient.

Platelet and Granulocyte Immunobiology – Granulocyte immunology

P-112 | Association between ABO blood groups and autoimmune neutropenia of infancy in Danish patients *selected for main programme*

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Background: Autoimmune neutropenia of infancy (AIN) is a frequent cause of neutropenia in children. The disease is caused by

antibodies against epitopes on the immunoglobulin G (IgG) Fc receptor type 3b (FcγIIIb). Several studies have shown associations of the ABO and RhD blood group systems with various autoimmune diseases.

Aims: Establish the association between ABO and RhD blood types, and AIN in Danish pediatric patients.

Methods: One hundred and thirteen AIN cases diagnosed before age five, were included. The control group consisted of 400 healthy and unrelated Danish blood donors. Molecular determination of ABO types was analyzed in all AIN cases with polymerase chain reaction (PCR) testing three SNPs (rs8176719, rs7853989 and rs8176743). Determination of RhD types in AIN cases was analyzed with RT-PCR for exon 5 and 7. ABO and RhD types was previously serological determined for the control group. Different methods have been used for ABO and RhD typing, this is in according to previously studies which have shown consistency between serologic and genomic typing for ABO and RhD. Statistic p-values were calculated as Fischer exact test and odds ratio with 95 % confidence interval.

Results: The distribution of ABO types, shows a higher frequency of blood type-A and -AB, and a lower frequency of blood type-O and -B in AIN cases compared to the control group (Table 1). The distribution for type-A and -O is statistically significant with odds ratios indicating that blood type-A results in a higher risk of AIN, while type-O has a lower risk. The distribution of type-B and -AB are not statistically significant. The ABO distribution in the control group was not significantly different from findings in other Danish ABO studies. There was no significant difference between RhD types between the AIN cases and the control group.

P-112 Table 1. Frequencies of ABO and RhD.

System	Allele	Cases n = 113 (%)	Controls n = 400 (%)	p-value	OR (95% CI)
ABO	A	60 (53.1)	164 (41.0)	**0.024**	1.6 (1.1-2.5)
	B	7 (6.2)	34 (8.5)	0.556	0.7 (0.3-1.6)
	AB	6 (5.3)	15 (3.7)	0.429	1.4 (0.5-3.8)
	O	40 (35.4)	187 (46.8)	**0.033**	0.6 (0.4-1.0)
Rh	RhD+	96 (85.0)	325 (81.2)	0.402	1.3 (0.7-2.3)
	RhD-	17 (15.0)	75 (18.8)		

Summary/Conclusions: The ABO A-antigen is associated with higher risk of AIN, while the O-type shows a protective effect. No association was found for RhD types. The mechanism underlying the association between autoimmunity and ABO blood types have not yet been elucidated.

Platelet and Granulocyte Immunobiology – Fetal-maternal immunology

P-113 | A retrospective cohort study on cases suspected of FNAIT to investigate the association between anti-HPA-5B antibodies and the occurrence of clinical FNAIT

selected for main programme

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Background: Fetal neonatal alloimmune thrombocytopenia (FNAIT) is a devastating disorder caused by maternal alloantibodies directed against fetal human platelet antigens (HPA) and characterised by thrombocytopenia and a risk of bleeding in fetuses and newborn infants. Most FNAIT cases in the Caucasian population are caused by anti-HPA-1a. It is questioned if anti-HPA-5b, the second most frequently encountered antibody in cases suspected of FNAIT, is a finding by chance in pregnancy, and if it can cause severe FNAIT. This latter question is raised since we and others observed anti-HPA-5b antibodies about 2% of pregnancies in prospective series.

Aims: To describe differences in clinical characteristics between anti-HPA-1a mediated FNAIT and anti-HPA-5b FNAIT. To compare the occurrence of severe bleeding between anti-HPA-1a and anti-HPA-5b mediated FNAIT. To investigate whether the presence of anti-HPA-5b antibodies is related to occurrence of clinical FNAIT.

Methods: Retrospective nationwide cohort study on cases which were sent to our laboratory for the screening of anti-HPA antibodies because of clinical suspicion of FNAIT. In case an anti-HPA antibody was found by MAIPA, the neonate and if available also the father was typed for the cognate antigen. Clinical outcome was collected by direct contact with the care giver with a structured questionnaire.

Results: In 1864 suspected FNAIT cases, anti-HPA-1a was found in 161 cases (8.6%) and anti-HPA-5b in 60 cases (3.2%) (cases with multiple HPA antibodies excluded). Besides HPA directed antibodies, another clinical condition possibly leading to thrombocytopenia was present in 21% and 45% of the anti-HPA-1a and anti-HPA-5b mediated cases, respectively. The proportion of cases with severe bleeding did not differ between the anti-HPA-1a and anti-HPA-5b mediated cases

(14/129; 11% and 4/40; 10% respectively). Severe thrombocytopenia (platelet count < 25 × 10^9/L) was not present in 2 out of 14 cases with severe bleeding with anti-HPA-1a and in 3 out of 4 in cases with anti-HPA-5b (of which 2 of the anti-HPA-5b cases were anticipated by maternal IVIg treatment). In multigravida pregnant women with a child suspected for FNAIT, all ($n = 81$) HPA-1a cases and 79% ($n = 38$) of the HPA-5b cases were HPA incompatible, whereas 86.2% and 52.2% was expected by chance. In 5 out of 6 cases with severe bleeding with anti-HPA-5b the neonate was HPA-5b positive.

Summary/Conclusions: In line with previous studies, our data points to an association of anti-HPA-5b with neonatal thrombocytopenia and bleeding. The frequency of anti-HPA5b antibodies among cases suspected for FNAIT is higher than we find in the general pregnant population. Also in pregnancies of multigravida women, we observed a higher frequency of HPA-5b incompatibility than would be expected by chance. To truly assess the natural history of anti-HPA-5b FNAIT a prospective screening study is needed.

P-114 | The conundrum of maternal HLA class I antibodies and neonatal thrombocytopenia – A case report

selected for main programme

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Background: The significance of maternal HLA class I alloantibodies for the induction of neonatal alloimmune thrombocytopenia (NAIT) has been discussed controversely in the past. Maternal HLA alloimmunisation by pregnancy is rather frequent and occasionally suspected NAIT has been brought into context with maternal HLA class I antibodies when no platelet specific (HPA) antibodies were detectable. Literature reviews tend to negate HLA antibodies as causative for NAIT.

Aims: We add further information by presenting a case of neonatal thrombocytopenia with extended laboratory investigation.

Methods: A full-term infant was born with 17x10^9 platelets/L and mild signs of petechiae so that NAIT was suspected. No further clinical signs or reasons for thrombocytopenia were reported. After application of random platelets the platelet count raised to 130x10^9/L but dropped again to 37×10^9/L within three days. After transfusion of one HLA class I compatible platelet unit platelet counts remained stable at about 127×10^9/L until day 11 and thereafter reached more than 205×10^9/L without further transfusion. Testing for HLA antibodies within maternal and neonatal blood was performed by Luminex LSA class I (Immucor Lifecodes, Norcross, GA, USA). HPA antibodies were detected by the MAIPA assay and Luminex PAK Lx (Immucor Lifecodes). Fetal and maternal HPA genotyping was performed by PCR-SSP (InnoTrain; Kronberg, Germany) and HLA class I genotyping was performed by PCR-SSO (Luminex, Immucor Lifecodes).

Results: In the maternal serum no HPA specific antibody reactivity was shown in the MAIPA, neither with donor nor with paternal platelets. However, maternal antibodies with specificities for HLA-A, B and C were detected at MFI (mean fluorescence intensity) values up to 8,000 by Luminex LSA (see Table 1). Interestingly, only maternal anti-HLA-A2 and -A69 were identified within the serum of the baby at MFI values up to 12,000. During a follow-up until day 36 neonatal MFI values slowly declined to 5,800. The baby itself carried the corresponding *HLA-A*02* allele. Even in the presence anti-HLA-A2 the platelet counts of the baby recovered beginning from day 11 without further transfusion.

P-114 Table 1.

Test	Mother	Child
HPA antibody (Day2)	Negative	Negative
HPA alleles	HPA-1aa, 2aa, 3aa, 4aa, 5aa, 6aa, 9aa, 15aa	HPA-1ab, 2aa, 3ab, 4aa, 5aa, 6aa, 9aa, 15aa
HLA class I antibody		
Day 2	HLA-A2, 69; B17; Cw2, 5, 6, 8 (PRA 16%)	
Day 4	HLA-A2, 69; B17; Cw2, 5, 6, 8 (PRA 16%)	HLA-A2, 69 (PRA 5%)
Day 14		HLA-A2, 69 (PRA 5%)
Day 20		HLA-A2, 69 (PRA 5%)
Day 30		HLA-A2, 69 (PRA 5%)
Day 36		HLA-A2, 69 (PRA 5%)
HLA class I alleles	HLA-A*30, *68; *B44	HLA-A*02, *68; B*44

Summary/Conclusions: In this case of suspected NAIT only HLA class I antibodies were detectable. The fact that the maternal HLA-A2 alloantibody present in the neonate's blood corresponds to the determined HLA-A*02 allele could support HLA antibody induced NAIT. However, antibody persistence over more than 7 weeks coming along with normalized platelet counts without further transfusions argues against this theory. Additionally, it remains unclear why selectively only HLA-A specific maternal antibodies were detected within the neonatal serum and why these antibodies persisted for such a long time. In our opinion the presented case negates HLA class I antibody induced NAIT rather than confirming the theory.

P-115 | *Abstract withdrawn*

P-116 | Neonatal alloimmune neutropenia laboratorial diagnosis: Ten years of experience in Portugal

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Background: Neonatal alloimmune neutropenia (NAIN) is an uncommon cause of neutropenia in newborns. It occurs due to the transplacental passage of maternal IgG antibodies directed against fetal neutrophil antigens inherited from the father. Alloantibodies against the Human Neutrophil Antigen (HNA) type 1 are the most frequently identified. The diagnosis of NAIN impacts in the follow-up and treatment provided to newborns. Currently, there is a paucity of data on NAIN in Portugal.

Aims: To describe the laboratory findings in infants suspected of having NAIN referred to our laboratory unit of cytometry and genetics, in Portugal.

Methods: An observational retrospective study was conducted. Newborns with NAIN referred to our Laboratory from 2010 to 2020 were identified and the laboratory results and clinical data were reviewed. Diagnosis of NAIN was done by flow cytometry (NAVIOS™, Beckman Coulter) by demonstrating the presence of IgG coated newborn's neutrophils and of anti-HNA alloantibodies in maternal serum with positive crossmatch between maternal sera and paternal neutrophils. HNA genotyping was performed in the newborns and their parents, using the HNA Genotyping Tray (OneLambda, ThermoFisher), which allows for detection of 9 HNA polymorphisms (1a, 1b, 1c, 3a, 3b, 4a, 4b, 5a and 5b). A descriptive statistical analysis of the laboratory findings was made.

Results: Twelve patients were referred to our Laboratory for investigation of NAIN during the study period. One did not have NAIN, a congenital neutropenia was diagnosed. Another was excluded because the clinical information was not available. Seven (70%) were female. Six (60%) were premature. The median gestation time was 33.5 weeks (IQR 6.3). The median absolute neutrophil count and age at the time of diagnosis was 230/mm3 (IQR 422.5) and 1 day (IQR 0.8). Five (50%) were positive for IgG coated newborn's neutrophils; 8 (80%) were positive for neutrophil crossmatch between maternal sera and paternal neutrophils and 3 were positive for both tests. Nine cases (90%) had incompatible HNA genotypes, with 2 cases having incompatibility for two polymorphisms: HNA-1b: 4 cases; HNA-1b + HNA-3a: 1 case; HNA-1b + HNA-5b: 1 case; HNA-1a: 3 cases.

Summary/Conclusions: The HNA maternal-fetal incompatibility most frequently found in Portugal is similar to that described in the international bibliography. We encourage pediatricians to investigate NAIN in newborns with neutropenia without any other established cause.

Platelet and Granulocyte Immunobiology – TRALI

P-117 | TRALI diagnosed in Poland between January 2014 - December 2020

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Background: Transfusion-Related Acute Lung Injury (TRALI) manifests as acute respiratory failure with non-cardiogenic pulmonary edema. Along with cross-group blood transfusion it is the most common cause of the fatal complication after blood transfusion. According to the 2004 definition, it is differentiated into TRALI and – depending on the pathomechanism – into immunological and non-immunological. TRALI diagnostics is an important element of ensuring transfusion safety. It also allows to decide how qualify the donors with detected antibodies and with blood components taken from them.

Aims: Analysis of anti-leukocyte antibody detection, their specificity and leukocyte antigen typing results in TRALI cases.

Methods: During the period between January 2014 and December 2020 anti-leukocyte antibodies were analyzed in sera from 155 patients with dyspnea suspected for transfusion-related lung injury and in 361 blood components transfused to these patients sent to the IHTM by Polish Regional Blood Transfusion Centers from all over the country.

Anti-HLA antibody detection was conducted with lymphocytotoxicity test and following solid-phase assays: ELISA and since 2016 assays prepared for Luminex (xMAP technology): LABScreen Mixed and LABScreen Multi (One Lambda). Anti-HLA specificity was confirmed in LABScreen Single Antigen Class I, LABScreen Single Antigen Class II (One Lambda) assays. Anti-HNA antibodies were detected in GIFT, GAT, MAIGA and LABScreen Multi tests.

Diagnostics of TRALI cases was widen by HLA and HNA antigen typing in blood donor and recipient with PCR Luminex SSO (One Lambda), PCR-SSP assay (HNA Ready Gene; Inno-Train) and Sanger sequencing of selected exons of genes encoding HNAs.

Even though in 2019 the new consensus was established, all the data was analyzed according to the 2004 TRALI definition due to the fact that most of our results had been obtained before 2019.

Results: Based on clinical symptoms and laboratory results TRALI was confirmed in 47/155 patients (30.3%), transfused with 147 blood components. Non-immune TRALI was diagnosed in 18/47 cases (38.3%). Immune TRALI was diagnosed in 29/47 cases (61.7%). Anti-leukocyte antibodies were detected in 20 patients with TRALI (42.6%) and in 30/147 blood components transfused to them (20.4%). 80% of immunized donors and recipients were women. In 12 out of 14 cases anti-HLA and anti-HNA specificity was complementary with HLA and HNA genotype of donors and recipients.

The remaining cases were diagnosed as: TACO in 62/155 patients (40%), post-transfusion dyspnea in 40/155 patients (25.8%) and allergic reaction in 6/155 patients (3.9%).

Summary/Conclusions: Correct diagnosis of TRALI requires multiple methods of detecting leukocyte antibodies. For TRALI prevention female blood donors and multiple blood recipients should be tested for the presence of anti-leukocyte antibody.

Cellular Therapy – Stem cell and tissue banking

P-118 | Establishment of a cell bank for cell therapy with HUC-MSCS: From the umbilical cord to the patients

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Background: During the last years, the umbilical cord (UC) has become an ideal source of human mesenchymal stem cells (hMSCs) due to its accessibility, painless procedures for donors, promising sources for cell therapy, and lower risk of viral contamination. UC collection is easy, without ethical concerns, and does not harm newborns or mothers. hUC-MSCs can be expanded, are remarkably stable, and do not elicit any strong immune responses. The plasticity, expandability and immunomodulatory action of these cells, even after prolonged cryopreservation, attribute them an immense potential for possible future promising applications in regenerative medicine.

Aims: To develop a mesenchymal stem cell bank from the UC to the final advanced therapy medicinal product that would ensure availability of high-quality, reliable-and-well characterized hUC-MSCs for clinical application after cryopreservation.

Methods: UC samples were collected with informed consent from mothers healthy and validated according tissue bank criteria. hUC-MSCs has been isolated and proliferated according to good manufacturing practices (GMPs) with human component derived medium, quality assurance, and quality control for their use in clinical applications. Master and Working Cell Banks (MCB and WCB, respectively) were expanded and cryopreserved for further preparation in the finished medicinal product.

Results: UC from 4 different donors were obtained from maternity hospitals after normal or cesarean deliveries for generation of hUC-MSCs. All maternal blood samples tested negative for infectious disease markers. MSCs were successfully isolated from all the four cords processed irrespective of the sex of the baby, gestation, and ethnicity. Data of MCB counts at passage 1 of all the four samples were about 15–45 million cells ($2\pm1 \times 10^6$ viable cells/vial were stored) from about 20 to 25 grams of the cord used for processing. After gradual cryopreservation, the expanded MCB vial contributed between 150-250 million cells ($8\text{-}12 \times 10^6$ viable cells/vial were stored) for the established WCB after no more than 4 passages. This cell bank allowed us to have good cryopreserved cell reservoirs available for clinical use in finished medical products for direct application to the patient, without having a loss of viability and cell identity during cryopreservation. Cells presented a typical immunophenotype from MSC being >80% for CD90, CD73, CD105, MHCI and <20% CD45, CD34, CD19, CD11b, MHCII, CD31. Sterility tests, mycoplasma detection, endotoxin test and adventitious agents test were realized as quality control in all MCB and WCB.

Summary/Conclusions: Our results show that appropriate validated protocols for the processing of UC with GMPs can aid in the development of bank of cryoperserved hUC-MSCs, having at all steps the cell potency, quality and characterization necessary for the therapeutic application in patients.

Cellular Therapy – Collection, processing, storage and release

P-119 | *Abstract withdrawn*

P-120 | Comprehensive characterization of the physiology of red blood cells following hypotonic dialysis-based drug encapsulation process

selected for main programme

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Background: Red blood cells (RBCs) can serve as super-carriers for therapeutic agents, given their biocompatibility and long lifespan in the circulation, and therefore can significantly improve the

pharmacokinetics and pharmacodynamics of many drugs. Using ERYCAPS® platform technology, drugs are encapsulated within RBCs by a proprietary loading method. Maintaining RBCs integrity is important for the utility of RBCs as drug carriers/bioreactors and can be a challenging aspect of drug encapsulation.

Aims: Thus, the goal of this study was to evaluate the impact of encapsulation process on RBCs physiology and integrity.

Methods: Several parameters were compared between eryaspase (asparaginase [ASNase] encapsulated in RBCs), processed RBCs without drug addition (proRBCs) and normal non-processed packed RBCs (pRBCs) to evaluate potential RBCs modifications: hematological parameters (hematological analyzer), morphological properties (ImageStream Flow Cytometry), RBCs deformability (Ektacytometry), mechanical retention (splenic microfiltration model), proteomic and metabolomic profiles (mass spectrometry) and lesion markers such as PS, CD47and the number of microparticles (MPs) (Flow Cytometry). Also, RBCs biodistribution was investigated in a mouse model.

Results: Encapsulation process did not irreversibly affect the morphology of RBCs. Indeed, an increase of 15% of echinocyte shape was observed in proRBCs and eryaspase compared to pRBCs, while the percentage of spherocytes was not affected. Overall, pRBCs, proRBCs and eryaspase showed similar proteomic profiles. The encapsulation process led to a reduction in metabolites into RBCs, such as amino acids, pyruvate, lactate and glutathione but glycolysis remained active with an increase of its intermediate metabolites and the pentose phosphate pathway was activated. MPs release was increased after the encapsulation process while no difference in CD47 level was observed, and the percentage of PS externalized remained in normal range values (<1.1%). Other changes included a decrease in the mean corpuscular volume and protein content, a slight increase of hemolysis and a decreased osmotic fragility. The maximum RBCs deformability in eryaspase and proRBCs occurred at lower osmolality and was slightly lower than in pRBCs (-10% to -16%, respectively). However, no mechanical retention was observed in pRBCs, proRBCs and eryaspase samples. Importantly, in the mouse model, the half-lives of each eryaspase (13.0 days) and proRBCs (15.1 days) were similar to that of pRBCs (20,8 days).

Summary/Conclusions: The encapsulation process led to moderate changes that do not appear to impact the use of RBCs as drug carriers. Our results suggest a loss of intracellular content and RBCs dehydration during the encapsulation process, which could explain the decreased maximum deformability. However, the absence of mechanical retention of processed RBCs in a spleen microfiltration model and the long eryaspase half-life *in vivo* in a mouse model suggest that this decrease does not have a significant impact on RBCs survival. The low-level of hemolysis and the decreased osmotic fragility suggest that RBCs were not made more fragile by the encapsulation process. Of interest, in clinical Phase 1 study of eryaspase, the ASNase terminal half-life was approximately 21 days; almost 15-fold increase compared to free ASNase forms. Eryaspase is currently being investigated in a Phase 3 trial in second-line pancreatic adenocarcinoma.

P-121 | Evaluation of Amicus Blue™ online ECP system in routine use for patients with Sézary syndrome

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Background: L'Hospital Universitari de Bellvitge has established a strong partnership between the Dermatology and Hematology departments to provide ECP as a first-line treatment in erythrodermic cutaneous T-cell lymphoma (CTCL) patients since 2016. We perform ca. 150 procedures/year using the offline ECP method. We use the Amicus Separator® since 2017 for TPE, RBCx, and MNC collection either for transplant or as part of offline ECP. In 2020, we began evaluation of the new online Amicus Blue™ ECP System (Fresenius Kabi, Germany).

Aims: We evaluated the system in routine use for treatment of Sézary syndrome, an aggressive form of CTCL that occurs in ca. 5% of patients. Our treatment plan is ECP 2 consecutive days every 15 days for 3 months, then 2 consecutive days every 4-8 weeks as disease progression allows.

Methods: 28 procedures were performed in 11 patients (7 female, 4 male) with a median weight of 65 (62-110)kg. Two patients received previous treatment, one PUVA, UVB and interferon [IFN]-α and the second PUVA, [IFN]-α and bexarotene. All others received ECP as a first-line treatment with no concomitant medications. All patients had previously received offline ECP[DJ1], and Amicus Blue was incorporated into their existing regimen. We always use peripheral[DJ2] venous access to avoid possible infections of central lines. Amicus v6.0, Phelix v2.0 and double-needle disposable kits were used. A 14:1 whole blood (WB) to ACD-A anticoagulant ratio was used in all but one procedure (10:1) for a patient with history of hypercoagulability. Citrate infusion rate was 1.35 mg/kg/min, and maximum WB draw rate 80 ml/min. Our offline ECP procedure targets 1 TBV to process, so we used the maximum WB to process of 4000 ml on Amicus Blue. Hematology counts were performed on the patient's WB and the treated MNCs.

Results: No adverse events were reported. WB flow rate was 57 (30-80) mL/min and total procedure time including collection, photoactivation and reinfusion was 114 (89-205) minutes. WB processed was 4007 (3810-4020) mL. MNC purity was 96 (94-99) %, and median collection efficiency (CE2) was 63%. The Hct of the collected cells was 2.1 (0.8-3.3) %. Median treated cell doses (x109) were: WBCs 3.9 (1.3-6.6), MNCs 3.7 (1.1-6.4), lymphocytes 1.5 (0.4-5.0), monocytes 1.6 (0.5-4.7). In 10 instances of consecutive procedures (n=20), there were no statistically significant differences in patient counts, treated cell counts/yields/CE between Day 1 and Day 2 procedures. Of note is that in 1 patient with very low platelet count, the intra-procedure drop was minimal on consecutive days (Day 1:

37 à 27k/μL, Day 2: 39 à 29k/μL). The platelet sparing characteristic of Amicus is well known, and we observed in ECP a median platelet CE of 4%.

Summary/Conclusions: Compared to our offline ECP procedure which takes on average 3 hours, the automated Amicus Blue procedure provides at least 30 minutes time savings. Our nurses can easily attend to more than one patient at a time because they do not need to leave the bedside to perform manual cell manipulation and photoactivation. Results for cell dose[DJ1] and control of Sézary syndrome symptoms in patients treated with the Amicus Blue did not change compared to previous offline ECP treatment. The short procedure times, easy handling and performance make the online Amicus Blue ECP procedure a good option for Sézary syndrome patients.

P-122 | Automated separation of cord blood units using the compomat G5

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Background: Cord blood Units (CBUs) has become a real alternative source of haematopoietic stem cells for bone marrow reconstitution in a variety of malignant and non-malignant disorders. Since the outcome of transplantation depends on the number of transplanted cells, there is a need to minimize the loss of cells during sample processing. Volume reduction is one of the most important objectives of banks and has advantages reducing the storage space and the dimethyl sulfoxide (DMSO) quantity in the final product. Volume reduction methodology must guarantee high cell recovery and red blood cell (RBC) depletion by reducing the CBUs to a standard volume.

Aims: The aim of this study was to develop a program for the volume reduction of CBUs in the "Compomat G5 device" to reduce the volume and increase TNC and CD34+ recovery of CBUs, crucial for successful engraftment of CB stem cells transplant.

Methods: Samples were collected after written and informed consent gained from both parents. CBUs were processed and cryopreserved within 48 hours of collection. All units were collected in triple-bag system, transferred in Separation Bag CompoFlex® P4208 200 ml T&B (Fresenius), centrifuged in oval buckets at 3200 x g for 12 min at 20°C, ensuring that the bags were well supported to prevent disruption of the buffy coat layer and separated with Compomaster NET G5 Plus program. All units were fractionated in about 30 min. Total nucleated cells (TNC) count was performed with Sysmex XN 1000™ (DASIT). $CD34^+$ cells and Viability were quantified by Flow cytometry FACSCANTO II (BD Biosciences). Clonogenic assays were performed using Short-term culture in semisolid medium Methocult® GF H 84444 - StemCell Tech.

Results: For this study, 42 CBUs were processed with Compomat G5. The volume of blood collected ranged from 88 to 194 ml (median: 130). Table 1 shows data for CBUs before and after volume reduction. For all units total TNC, $CD34^+$ cells content, viability and CFU after volume reduction were $15{,}62 \pm 3.74 \times 10^7$, $5{,}09 \pm 3.88 \times 10^6$; 94,76 ± 3.71 % and $223 \pm 125 \times 10^4$ respectively. We reached a RBC depletion of 64% ± 9.07.

Summary/Conclusions: To our knowledge there are no previous studies analyzing the volume reduction process of CBUs with the Compomat G5 device. In our study, BC volume was standardized to 31-40 ml (median 35), achieving TNC and CD34+ cells recoveries of more than 90% and RBC depletion of 64%.

The initial volume of the collected units does not affect the processing, and recovery of both TNC and $CD34^+$ progenitor cells was as efficient with smaller volumes as it was with larger units. In fact CBUs with an initial mean value of 2,45 $x10^6$ $CD34^+$ present the same recovery (90,97%) after volume reduction. In conclusion, the Compomat G5 device is a highly efficient method for TNC and $CD34^+$ cells recovery maintaining good cells viability; in the same time, it allows RBC depletion with a low final volume with the advantage of being an automatic and functionally closed system that shortens and standardizes the proceedings.

P-122 Table 1. Results are shown as mean ± SD. NA not applicable.

	Before Volume Reduction	After Volume Reduction	Recovery %	Reduction %
Volume (ml)	134.42 ± 24.16	35.15 ± 1.77	NA	71.33 ± 4.28
TNC × 10^7	17.24 ± 3.84	15.62 ± 3.74	90.56 ± 5.29	NA
$CD34^+ \times 10^6$	6.51 ± 6.17	5.09 ± 3.88	92.78 ± 7.23	NA
MNC %	42.5 ± 3.5	44.12 ± 6.08	89.45 ± 5.34	NA
Htc %	37.43 ± 3.86	46.38 ± 3.59	NA	NA
RBC ml	51.07 ± 12.23	17.46 ± 1.72	NA	64 ± 9.07
Viability %	92.71 ± 6.09	94,76 ±3,71	NA	NA
CFUC × 10^4	271 ± 230	223 ± 125	95.62 ± 16.73	NA

P-123 | *Abstract withdrawn*

P-124 | Mobilized hematopoietic stem cells from peripheral blood as a preferred source in autologous stem cell transplantation in adult patients with hematologic malignancies

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Background: Peripheral blood stem cell (PBSC) transplantation has been widely used for autologous and allogeneic transplantation therapy in various hematologic malignancies. Optimal donor and recipient outcomes require maximized stem cell collection efficiency and minimized non-target cell contamination. The aim of our study is to present our experience with collecting of mobilized PBSC in adult patients with hematologic malignancies.

Aims: The aim of our study is to present our experience with collecting of mobilized PBSC in adult patients with hematologic malignancies.

Methods: This is a retrospective study performed in the Institute for Transfusion Medicine of Republic of North Macedonia and University Hematology Hospital; the data was obtained from our database for period from January 2001 till January 2021. All patients were fully informed on the donation procedure and signed an informed consent for donation. Minimum dose required to ensure successful and sustained engraftment was 2×10^6 /kg CD34+ cells and 2×10^8 /kg mono-nucleated cells (MNC). PBSC harvesting was performed with continuous flow cell separator Baxter C53000, COBE Spectra and Terumo BCT Spectra Optia using conventional-volume apheresis processing the 2 - 2.5 total blood volumes per apheresis. A femoral catheter was used for harvesting. Acid Citrate Dextrose formula A (ACD-A) is used for anticoagulation during the harvesting. Mobilization regimens included granulocyte colony-stimulating factor (G-CSF) alone or combination of G-CSF and disease-specific chemotherapy.

Results: There were 771 apheresis procedures performed in 428 hematologic patients, aged 16-65. The single procedure usually took 180-270 minutes and the volume of collected stem cells was 50-380 ml. The needed number of MNC and CD34+ cells was successfully collected by 1.8 apheresis. Procedures for mobilization and collection of PBPC are generally well tolerated. The main indications for autologous stem cell transplantation in our patients were: multiple myeloma - 213 (50%), Hodgkin disease – 59 (1.6%), non-Hodgkin lymphoma – 58 (13%), acute lymphoblastic leukemia – 11 patients (3%), chronic lymphoblastic leukemia – 3 patients (0.8%) and 1 patient with Ewing Sarcoma (0.2%).

Summary/Conclusions: Properly mobilized and harvested PBSC at the appropriate time before PBSC transplantation is prerequisite for a successful transplantation.

P-125 | Experience of using extracorporeal photopheresis for the treatment of transplant against host reaction in Kazakhstan

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Background: Acute and chronic graft versus host disease (GvHD) remains the major cause of mortality or severe disability in allogenetic hematopoietic stem cell transplant (HSCT). The first-line therapy for GvHD are corticosteroids, but 25% of patients have hormone-resistant disease. In international practice, since the 90s, extracorporeal photopheresis (ECP) has been successfully used as a second-line therapy for hormone-resistant forms of GvHD in adults and children. In the Republic of Kazakhstan, HSCT has been performed since 2010 and year after year their number is constantly growing.

Aims: Implementation of ECP in Kazakhstan based on foreign protocols for the treatment of acute and chronic hormone-resistant forms of GvHD.

Methods: The mononuclear cells collection was carried out using an automatic blood cell separator device - Spectra Optia, photochemical treatment was carried out with the addition of 8-methoxypsoralen using a MacoGenic G2 device.

Results: Extracorporeal photopheresis in Kazakhstan has been used in the treatment of hormone-resistant forms of GvHD after allo-HSCT since 2018. In the given period, 162 ECP procedures were performed in 17 patients: 8 adults, 9 children.

The overall treatment response was observed in 12 patients. Complete response - in 3 (1 adult and 2 children) – regression of clinical manifestations, the dosage of immunosuppressants (methylprednisolone) was reduced down to withdrawal, a stable remission was achieved in the adult patient. Partial response – in 9 patients (5 adults and 4 children) - regression of clinical manifestations, the dosage of immunosuppressants reduced.

Summary/Conclusions: As a result of ECP, 70.6% of patients achieved positive results of therapy up to regression of GvHD symptoms, in the remaining 29.4%, treatment discontinued due to the underlying disease. The use of combined immunosuppressive therapy involving ECP allows to reduce the dose of immunosuppressants in patients with hormone-resistant forms of GvHD.

P-126 | Implementation of Amicus Blue™ system in routine use for autologous mononuclear cells collection in Abu Dhabi

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Background: Though cancer is the third-highest cause of death in UAE, Emirati citizens and residents have often sought treatment abroad for cell therapy and regenerative medicine. In 2020, the new Abu Dhabi Stem Cell Center (ADSCC) implemented the Amicus Blue™ System (Fresenius Kabi, Germany) for autologous mononuclear cells (MNC) collection so that cancer patients living in the UAE can be treated closer to home to remain with family. The Amicus Blue System was chosen for its ability to also perform online ECP and TPE, two therapeutic treatments useful for some transplant recipients.

Aims: For successful hematopoietic stem cell transplantation, an adequate number of stem cells must be mobilized and collected. We evaluated the system in routine use for collection of autologous MNCs in 8 patients with various diseases, targeting a CD34+ cell dose of 3.0 x 10^6/kg.

Methods: Patients were mobilized with G-CSF (20 mcg/kg) and Plerixafor. Peripheral venous access was used for all but 1 patient who had a central line, and calcium gluconate prophylaxis was given to all patients. Amicus v6.0 and double-needle disposable kits were used. A 12:1 whole blood (WB) to ACD-A anticoagulant ratio was used, with citrate infusion rate 1.25 mg/kg/min, and maximum WB draw rate 70 ml/min. WB processed was 3 TBV in the first apheresis session, and 2 TBV second session. The MNC offset was 1.5ml, RBC offset 6.8ml, MNC collection setpoint 0.60, and MNC sense level 0.45. Cycle volume was set 1000-1200 ml. The protocol was performed according to the manufacturer's instructions and followed the International Society of Hematotherapy and Graft Engineering (ISHAGE) guidelines for single platform. Patient CD34+ precount and product CD34+ content was measured using a 10-color Navios-EX flow cytometer (Beckman Coulter, USA) with Navios Acquisition software. The analysis protocol using Kaluza C software was done per ISAHAGE guidance. Two collection procedures were performed on consecutive days for most patients.

Results: Two mild adverse events occurred: one patient vomited, and another patient experienced minor muscle cramps. Patient CD34+ precounts were 39,5 (12-100)/μL. WB processed was 16.645 (6.295-23.876)mL. Product volume was 320 (183-431)mL. Two patients required 3 apheresis sessions, Patient 2 for the collection of a second dose to be frozen for future transplant, and Patient 4 due to lower than expected collection. Initial engraftment occurred at Day 10 for most patients, with 1 patient achieving engraftment at Day 9 and 1 patient at Day 11.

Summary/Conclusions: Adequate doses of stem cell products for transplant were collected with the Amicus Blue MNC procedure. All patients were reinfused and subsequently engrafted successfully within 9-11 days. Device parameters will be optimized to improve collection efficiency, and procedures established to adjust settings according to patient mobilization. ADSCC is proud to make this life-saving treatment available and accessible to those who need it in UAE.

P-126 Table 1.

Patient	Diagnosis	Sex	Weight (Kg)	Plerixafor20 mg/dose	Mobilization Days	Apheresis Sessions	Precount (cells/μL)	Dose C D34×10^9/kg	Days Until Engraftment
1	Multiple Myeloma	M	81	None	6	2	14, 12	3,67	10
2	Plasma Cell Leukemia	M	125	+3 doses	7	3	35, 48, 55	7,37	10
3	Diffuse Large B cell Lymphoma	M	78	None	6	2	100, 71	10,01	11
4	Multiple Myeloma	F	73	+ 2 doses	7	3	26, 82, 74	4,15	9
5	Hodgkin Lymphoma	F	20	+3 doses	5	2	40, 47	7,15	10
6	Multiple Myeloma	M	74	None	7	2	57, 14	3,94	10
7	T- Cell Lymphoma	M	65	None	6	2	39, 24	6,62	10
8	Plasma Cell Leukemia	M	79	+1 dose	6	2	30, 38	4,60	10

P-127 | Overwiew of apheresis collection of mobilized peripheral blood stem cells in healthy donors – Macedonian experience

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Background: Allogeneic hematopoietic stem cell transplantation is an established therapy for many hematologic disorders. Since the discoveries of the potential of Peripheral Blood Stem Cells (PBSC) in the hematopoietic reconstitution mid 1980s and early 1990s PBSC gradually replaced bone marrow as the preferred source of stem cells. The introduction of hematopoietic cytokines that can mobilize large number of progenitors into circulation accelerated PBSC usage.

Aims: The aim of our study is to present our experience with apheresis collecting of PBSC in healthy donors.

Methods: This is a retrospective study performed in the Institute for Transfusion Medicine of Republic of North Macedonia and University Hematology Hospital for period from 2000 till 2021. All donors were HLA typed and matched; they were fully informed on the donation procedure and signed an informed consent for donation. Minimum dose required to ensure successful and sustained engraftment was 2×10^6/kg CD34+ cells and $2x10^8$/kg mono-nucleated cells (MNC). PBSC harvesting was performed with continuous flow cell separator Baxter CS3000, COBE Spectra and Terumo BCT Spectra Optia using conventional-volume apheresis processing the 2 – 2.5 total blood volumes per apheresis. A femoral catheter was used for harvesting and Acid Citrate Dextrose formula A is used for anticoagulation. Recombinant human granulocyte colony-stimulating factor (G-CSF) is used to mobilize PBPC for collection. Harvesting of PBSC is usually performed after 4 to 5 days of G-CSF subcutaneous administration at a dose of 10 μg/kg body weight.

Results: Most of the donors were siblings of the patients treated at the University Hematology Hospital, including 3 unrelated voluntary donors who donated through BMDW. There were 182 apheresis procedures performed in 118 donors. There were 75 male and 43 females, aged 16-63. One to two apheresis procedures were required to collect adequate graft. The single procedure usually took 3-4,5 hours and the volume of collected stem cells was 50-400 ml. The needed number of MNC and CD34+ cells was successfully collected with 1,5 apheresis. There were 47 ABO incompatible donors. Procedures for mobilization and collection of PBPC from healthy donors are generally well tolerated. The only adverse effects of the apheresis procedure were bone pain as reaction of G-CSF and numbness of the extremities as reaction of ACD-A (hypocalcemia), which occur rarely and were very mild. The collected PBSC were used in allogeneic stem cell transplantation in patients with: acute myeloid leukemia - 65 patient (55%), acute lymphoblastic leukemia - 17 patients (14%), chronic myeloid leukemia – 9 patients (8%), severe aplastic anemia – 7 patient (6%), myeloproliferative disorders – 6 patients (5%), myelofibrosis – 5 patients (4.2%), non-Hodgkin lymphoma – 4 patient (3.5%), multiple myeloma – 3 patients (2.5%), and one patient with Hodgkin disease and one patient with chronic lymphoblastic leukemia in allogeneic SCT.

Summary/Conclusions: The apheresis collection of PBSC in healthy donors is an effective and safe procedure. We are developing our National Stem Cell Donors Registry as a part of Bone Marrow Donors Worldwide. In that way we hope we will help widen the world network of stem cell donors and enlarge the possibility for each patient to find the right match.

Cellular Therapy – Clinical applications

P-128 | Evaluation of Amicus Blue™ online ECP system in routine use for patients with GVHD

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Background: Our center performs ca. 400 extracorporeal photopheresis (ECP) procedures annually as a second-line treatment for chronic and acute GVHD, as well as first-line treatment of CTCL when patients present. In March 2020, we began use of the Amicus Blue™ ECP System (Fresenius Kabi, Germany) which includes a photoactivation device (Phelix), and functionally closed disposable kit for online ECP. This is a new protocol on the Amicus Separator®, which we have used in the past 7 years for MNC collection for offline ECP in conjunction with the Macogenic (Macopharma, France) for photoactivation.

Aims: We evaluated the system in routine use from March through October 2020 for collected cell yield, apoptosis and procedure time in adult patients prescribed ECP and eligible for our standard offline procedure.

Methods: 110 procedures were performed in 14 patients (9 male, 5 female) with a median (range) age of 45 (27-70) yrs and median weight of 64 (49-90) kg. Ten patients were under treatment for cGVHD (n=92) and 4 for aGVHD (n=16), with primary diagnoses that included AML, ALL, MDS, myelofibrosis, Hodgkin lymphoma, and aplasia. No changes were made to concomitant medications during this study. All patients had previously received offline ECP, and Amicus Blue was incorporated into their existing regimen. Nine patients used peripheral venous access, 3 patients had CVCs, and 2 patients had PICC lines 4 or 5 French. Amicus v6.0, Phelix v2.0 and double-needle disposable kits were used. A 12:1 whole blood (WB) to ACD-A anticoagulant ratio was used, 1.24 mg/kg/min citrate infusion rate and maximum WB draw rate 80 ml/min. The system processes 1 cycle of WB, which can be configured from

500 to 4000 ml. We used the default setting of 2000ml for most procedures (n=85) and took advantage of the program flexibility to process 3000ml for patients with low lymphocyte counts (n=25). Hematology counts were performed on the patient WB and the treated MNCs, and lymphocyte apoptosis was measured at 72 hour.

Results: No adverse events were reported. Three patients had mild citrate reactions resolved with calcium gluconate. WB flow rate was 40 (20-68) mL/min and total procedure time including collection, photoactivation and reinfusion was 97 (65-231) minutes. Fluid balance was 111% (103-116), as expected from the automatic addition of 170 ml saline for dilution of the collected MNCs. MNC purity was 96% (47-99), and median collection efficiency (CE2) was 54%.

Mean collected cell yields were: WBCs 1.9×10^9 or 29.1×10^6/kg, MNCs 1.7×10^9 or $27.6x10^6$/kg, lymphocytes 1.0×10^9 or 16.6×10^6/kg, monocytes $0.6x10^9$ or 10.5×10^6/kg. Platelets and granulocytes collected were low, with CE2 of 6% and 1%, respectively. Lymphocyte apoptosis was as expected.

Summary/Conclusions: Our study demonstrated that the Amicus Blue system offers a safe and convenient online ECP procedure, with results for cell yields and lymphocyte apoptosis comparable to published literature. Our patients and staff prefer the Amicus Blue ECP System for its shorter, predictable procedure time compared to offline ECP which takes on average 4 hours (2.5 hrs MNC collection + 1.5 hrs product processing and reinfusion). We observed no differences in clinical response compared to previous offline treatment. We have expanded our program to more patients due to the increased operational efficiencies compared to offline ECP.

P-129 | Miniphotopheresis is an extracorporeal photopheresis that does not require leukapheresis. Clinical experience

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Background: Extracorporeal photopheresis (ECP) has proven effective in the treatment of several diseases, including acute (aGVHD) and chronic graft-versus-host disease (cGVHD) after hematopoietic stem cell transplantation. In its standard form, ECP requires leukapheresis to obtain a mononuclear cell fraction. The possibility of using leukapheresis is limited by the requirements for vascular access and the somatic status of the patient. There is a relatively new method of performing ECP, called miniphotopheresis (miniECP), in which a fraction of mononuclear cells is isolated from a dose of whole blood obtained by the exfusion method.

Aims: We present preliminary results of using miniECP in patients with aGVHD and cGVHD.

Methods: The study included 11 patients with acute (7 patients) and chronic (4 patients) GVHD who received miniECP therapy for the period from June 2018 to January 2021. Mononuclear-rich leukocyte fractions were prepared from a dose of whole blood by manual method. The resulting fraction was diluted with 0.9% NaCl solution to a hematocrit of less than 3%. Then, 8-methoxypsoralen was injected into the cell product and programmed irradiation with ultraviolet A was performed. Autologous red blood cells and the finished cell product were administered to the patient after irradiation.

MiniECP mode: 2 times a week - 8 weeks, then 1 procedure per week - 8 weeks, then 1 procedure every 2 weeks - 8 weeks, then 1 procedure every 4 weeks - 3-4 months.

Response to therapy was evaluated at 4 and 8 weeks and at the end of therapy.

Results: Seven patients (63.6%, 5-aGVHD, 2-xGVHD) had a partial or complete response to miniECP by week 8 of therapy. One of the patients (stage 4 aGVHD: skin-4, gastrointestinal-4) died from infectious complications of HSCT; one patient (cGVHD, skin-moderate) had graft rejection and repeated HSCT; one patient (aGVHD, stage 2: skin-3) continues miniECP therapy. The remaining 4 patients (36.3%) had a complete response to treatment by the end of the miniECP course. Patients with a complete response to miniECP at the end of therapy had the following stages of GVHD: 1 patient stage 2 aGVHD (skin-3), 1 patient stage 3 aGVHD (skin-3, liver-2, gastrointestinal-3), 1 patient stage 4 aGVHD (skin-2, liver-3, gastrointestinal-4) and 1 patient with severe intestinal lesions cGVHD with hemocolitis and the need for bowel resection.

MiniECP was ineffective in 4 patients (36.3%): 2 patients with overlapping aGVHD and cGVHD (moderate skin lesions), 1 patient with moderate intestinal lesions cGVHD, 1 patient with stage 3 aGVHD (skin-3, liver- 2, gastrointestinal-2).

Among responders and non-responders for miniECP therapy no significant differences were found in the number of white blood cells in terms of the patients body weight in the finished cellular product. The correlation between the presence of response to miniECP therapy with the number of white blood cells in the finished cellular product was not determined. None of the patients had adverse reactions and complications associated with the miniECP therapy.

Summary/Conclusions: MiniECP is an attractive alternative for the treatment of patients with steroid-resistant or steroid-dependent GVHD who cannot undergo leukapheresis. Our results are preliminary but promising. We will continue to use this method as a second line therapy for patients with contraindications to leukapheresis.

Cellular Therapy – Histocompatibility in stem cell transplantation

P-130 | Study on the polymorphism of the untranslated regions of HLA-A locus by next-generation sequencing

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Background: HLA-A is one of the most polymorphic locus of the human leucocyte antigen (HLA) complex, which plays an important role in immune system. It is reported that HLA-A mRNA expression showed allelic difference. However, the mechanism of the phenomenon is not fully clear. Many studies showed there are significant nucleotide polymorphisms in the untranslated regions (UTRs) of HLA-A locus. UTRs contain most of regulatory elements such like the proximal promoter presenting cis-acting regulatory elements in 5′ UTR and noncoding RNA binding sites in 3′UTR. However, the function of these regulatory elements in the UTRs on the differential expression of HLA-A is not clear.

Aims: To determine the 5′UTR and 3′UTR polymorphisms of common alleles of HLA-A locus, which can further help to explore the relationship between allelic polymorphism and differential expression of HLA-A alleles.

Methods: The genomic DNA samples were collected from donors of the China Marrow Donor Program (CMDP) in our lab after information consents, and the HLA-A genotyping were determined using PCR-SBT in routine work. The fragment of 5.4 kb with the coverage from 5′UTR to 3′UTR of HLA-A loci was amplified by long fragment amplification technique. The library for the amplicon was prepared with TransNGS Tn5 DNA library Prep Kit and was sequenced with Illumina Miseq platform according to the manufacturer protocol. All the sequencing data in FASTQ format were analyzed by CLC Genomics workbench 20.0 (QIAGEN) with the default setting, mapping the reference of A*02:01:01:01(Gene ID:LC257683.1). The defined sequences of each HLA-A allele were aligned using Clustal Omega programs to define UTR polymorphisms between different alleles. Putative binding sites for transcription factors or miRNAs within the 5′UTR and 3′UTR of the HLA-A gene were determined using bioinformatics software.

Results: The sequences of 500 bp regions located upstream of the ATG start codon (5′UTR) and downstream of the stop codon (3′UTR) were obtained from homozygous individuals of some common alleles (A*01,*02,*03,*11,*24,*26,*30,*31,*32 and *33), respectively. There were 43 polymorphic positions within the 5′UTR and 3′UTR, including 42 single point mutations and 1 deletion. It is shown that HLA-A*11 is the most polymorphic in the 5′UTR, including four single nucleotide polymorphisms (SNPs) (c.-7G>C, c.-54G>C, c.-66G>T and c.-298G>A) only presented. However, HLA-A*31 is the most polymorphic allele in the 3′UTR with six SNPs (2951 G>T, 2983A>T, 3118A>G, 3174C>T, 3194T>C and 3203C>A). Moreover, two SNPs (-274C>A and -374G>C) were only found in the 5′UTR of HLA-A*31, and the position of -274 is predicted to contain a binding site for nuclear factor kappa by transcription factor search, the mutation of an adenine in this position could induce change in potential binding sites and affect the expression. Interestingly, HLA-A*32 and HLA-A*33 alleles shared the same 5′UTR and 3′UTR except in the position of 3029.

Summary/Conclusions: The untranslated regions are polymorphic and some specific polymorphic sites of different allele in the 5′UTR and 3′UTR were obtained, which will provide a basis for the further study of HLA-A expression regulation.

P-131 | High-resolution analysis identifies high polymorphism of KIR3DL3 in Zhejiang Han Chinese

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Background: Killer-cell immunoglobulin-like receptors (KIRs) are transmembrane glycoproteins expressed on the surface of natural killer (NK) cells and a subset of T cells. They interact with polymorphic human leukocyte antigen (HLA) class I molecules playing an important role in regulating NK cells against many immune-mediated diseases and leukemia. The KIR loci are highly diverse in gene content, copy number and allelic polymorphism within individuals and across geographical populations. Of the 15 KIR locus, KIR3DL3 is the only KIR gene present in every individual, and present one copy in all human KIR haplotypes. It's the most polymorphism gene, and various across geographically and ancestrally diverse populations, and was reported that KIR3DL3 polymorphism has a function critical for human survival. However, China, as the most populous country in the world, the polymorphism of KIR3DL3 allele remains elusive.

Aims: In this study, we analyzed the polymorphism of KIR3DL3 alleles in 176 Zhejiang Han individuals from Southeastern China, we described diversity of KIR3DL3 at high resolution, which will provide further insight into the role of KIR3DL3 in disease susceptibility of Zhejiang Chinese.

Methods: 176 unrelated individuals were collected, and informed consent was obtained from all participants. An exome capture based high-throughput sequencing method was used to capture and sequence KIR3DL3, then Pushing Immunogenetics to the Next Generation pipeline was performed to analyze alleles (Norman PJ et al. Am J Hum Genet 2016). Allele frequencies were calculated by direct counting, the number of observed alleles divided by total allele numbers. And the distribution of KIR3DL3 alleles was compared with other countries.

Results: A total of 23 KIR3DL3 alleles have been identified in Zhejiang Han Chinese, which encoded 17 allotypes. The top three frequent alleles are KIR3DL3*01002 (22.2%), KIR3DL3*01001(20.2%) and KIR3DL3*00902 (12.5%). KIR3DL3*010 was the most common allotype, with the frequent as high as 43.2%, then was KIR3DL3**009

(20.2%). The distribution was compared with Southern Africa, South America, Europe, and Oceania. The most frequent allotype detected was various among these populations. The most common one in Europeans is KIR3DL3*001, South American Yucpa is KIR3DL3*003, Papua New Guineans is KIR3DL3*014, and Khomani South Africans is KIR3DL3*038. Besides, allotypes KIR3DL3*009 and KIR3DL3*010 are common to all populations. But KIR3DL3*003, a relatively high frequent allotype in other pupations, was only 0.85% in Zhejiang Han Chinese. And KIR3DL3*001, the most frequent one in Europeans, was only 1.99% in Zhejiang Han Chinese. The results indicated that the distribution of KIR3DL3 was highly different among populations.

Summary/Conclusions: KIR3DL3 is the most polymorphism KIR gene, with 165 distinct alleles and encoding 93 distinct proteins have been identified. In Zhejiang Han Chinese, we found 23 alleles. Compared with other populations, the distribution of KIR3DL3 alleles in different populations have their own characteristics, which may have arisen due to selection pressure from infectious disease.

P-132 | The association between KIR-HLA gene polymorphism and acute lymphoblastic leukemia in Han population of Zhejiang Province, China

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Background: Acute lymphoblastic leukemia (ALL) is a malignant hematological disease that seriously endangers human health, with an increasing incidence in recent years, but the pathogenesis still remains unclear. The capacity of natural killer (NK) cells to destroy leukemic cells has been studied, while the regulation of NK cell function mainly depends on the interaction of the killer-cell immunoglobulin-like receptor (KIR) expressed on its cell surface with the corresponding human leukocyte antigens (HLA) ligands on the target cell.

Aims: To screen the KIR-HLA susceptibility or protective genes for ALL through analyzing the differences of the polymorphism in KIR-HLA genes between ALL patients and healthy people in Han population of Zhejiang province, China, which is of great value for the early detection of ALL and the search for new target genes for clinical immunotherapy.

Methods: We extracted genomic DNA from blood samples collected in 262 ALL patients and 300 random unrelated hematopoietic stem cell donors in Han population of Zhejiang province, China, and detected the KIR and HLA-A, -B and -C genes of the samples using PCR-SSO and PCR-SBT technology respectively. To explore the susceptibility and protective genes of KIR-HLA in ALL patients, the association between KIR-HLA gene and ALL is carried out at the three levels of KIR, HLA and KIR-HLA through statistical methods.

Results: According to cellular immunological classification, ALL patients are divided into B-ALL and T-ALL groups, and patients in B-ALL group are divided into Ph^+ B-ALL and Ph^- B-ALL groups followed by the results of Ph (BCR-ABL) test. The 5 patient groups were compared with the controls respectively, and the results are as follows: 1. KIR2DS4 and KIR3DL1 gene frequency in Ph^- B-ALL group is significantly higher than the controls ($P<0.0001$). The frequencies of genotype Bx6 in the T-ALL group ($P=0.0480$) and the Bx19 genotype in the Ph^- B-ALL group ($P=0.0483$) were significantly higher than those of the controls, while the frequency of AB/AB in the Ph^- B-ALL group was significantly lower than the controls ($P=0.0157$). In addition, we did a comparative analysis between patient groups and found the KIR2DS4 and KIR3DL1 gene frequencies in the Ph^- B-ALL group were significantly higher than the Ph^+ B-ALL group ($P<0.0001$), and the genotype AB/AB in the B-ALL group was Significantly lower than the T-ALL group ($P=0.009$). 2. There were 8 special HLA alleles found by preliminary statistics: the allele frequencies (AFs) of A*31:01, C*08:22, A*69:01, B*15:02, C*01:03 and B*40:01 in one or two patient groups were much higher than the controls ($P<0.05$), while A*02:03 and C*15:02 in the Ph^+ B-ALL group ($P=0.0133$) were lower than the controls. However, the differences for the 8 special HLA alleles were not significant ($Pc>0.05$) after the date was corrected by Bonferroni method. In addition, the frequency of A3/11 ligand in the Ph^+ B-ALL group was significantly higher than the controls ($P=0.046$). 3. The frequency of 3DL2-A3/11 gene combination in the Ph^+ B-ALL group ($P=0.0460$) is significantly higher than the controls.

Summary/Conclusions: In Han population of Zhejiang province, China, KIR2DS4 and KIR3DL1 genes and Bx19 genotype may be the susceptibility factors while AB/AB genotype may be the protective for Ph^- B-ALL patients, and Bx6 Genotypes may be the susceptibility genotype for T-ALL patients, and the A3/11 ligand and 3DL2-A3/11 gene combination may be the susceptible factors for Ph^+ B-ALL patients.

Clinical transfusion – Transfusion reactions

P-133 | Allergic reactions linked to IgA deficient patients: Fact or fiction?

selected for main programme

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Background: Congenital selective IgA deficiency (IgA <0.07g/L) is the most common immunodeficiency, affecting 0.1-1% of individuals, the majority of whom are asymptomatic. Antibodies (AB) against IgA can be found in a small proportion of patients with IgA deficiency and it has been reported that these may be associated with anaphylactic transfusion reactions.

UK guidelines (BCSH, 2012) recommend measuring IgA levels in all patients who have experienced moderate or severe allergic transfusion reactions but not in mild reactions (those associated with transient flushing, urticaria or rash only). However, a causal link between

acute transfusion reactions and anti-IgA AB has not been proven and the characteristics of reactions in IgA deficient patients have not been systematically reported. The UK Serious Hazards of Transfusion (SHOT) haemovigilance scheme collects and analyses anonymised reports of adverse events and reactions (of at least moderate severity) following blood transfusions.

Aims: This review was undertaken to characterise the types of reactions reported in patients subsequently identified as having severe IgA deficiency. We also calculated the incidence of IgA deficiency in patients experiencing severe allergic reactions (Grade 3 by ISBT classification: with bronchospasm, stridor, angio-oedema or circulatory problems which required urgent medical intervention or directly resulted in or prolonged hospital stay).

Methods: All febrile, allergic and hypotensive transfusion reactions reported to SHOT between 2010-2019 involving patients subsequently identified as having IgA deficiency were included in this review.

Results: A total of 25 transfusion reactions in 24 severely IgA deficient patients were reported to SHOT during this period. Eighteen were confirmed to have anti-IgA AB; the remaining 6 were not screened. Most reactions (24/25) involved red cells, with platelets implicated in one case. The reaction occurred within the first 15 minutes of transfusion in 15 patients (15/25, 60%). The most frequently reported features were rigors (16, 64%), shortness of breath without wheeze (12, 48%), fever (10, 40%), tachycardia (10, 40%), anxiety (10, 40%) and chest/abdominal pain (40, 38%). Classical allergic features were less common, with rash in 3 (12%), angio-oedema in 3 (12%) and wheeze in 4 (16%). Anaphylactic reactions with hypotension requiring treatment with adrenaline were seen in only 2 patients. Recovery was typically rapid and complete: within 4 hours in 10 of the 14 cases where this information was provided (71%). In total, 412 severe allergic reactions were reported to SHOT over this period. There was a record of IgA levels being measured in 132 (32%) and only 3 (3/132, 2%) were confirmed to have IgA deficiency. Two of these had detectable anti-IgA AB; the third was not screened.

Summary/Conclusions: Transfusion reactions reported to SHOT and associated with anti-IgA AB in patients with IgA deficiency are rare (less than 1 per million components transfused in the UK). Although a variety of symptoms are described, most are hyperacute (occurring within 15 minutes) with features of acute inflammation and marked systemic upset. Classic allergic or anaphylactic features are much less common. More systematic measurement of IgA levels in patients experiencing severe allergic reactions would be needed to determine whether the incidence of IgA deficiency in this group is significantly higher than in the general population.

P-134 | Transfusion reactions in elderly UK patients 2015–2019 *selected for main programme*

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Background: Serious Hazards of Transfusion (SHOT) is the UK independent haemovigilance scheme. Serious adverse reactions and errors related to transfusions of blood components are reported to SHOT via an electronic confidential reporting system.

Rates of red cell transfusion rise steeply with advancing age. Blood transfusions are important supportive treatment for cancer patients, in patients with chronic diseases such as heart failure and chronic kidney disease, most of whom are elderly and anaemic. Transfusion can be a lifesaving treatment but is not without risk. Adverse reactions to blood components can lead to major morbidity and mortality. This review focuses on serious adverse reactions reported in elderly patients in the UK.

Aims: To establish the types of transfusion reaction reported in patients aged 70 years or over.

Methods: A retrospective analysis of reports submitted to SHOT in the period January 2015 to December 2019 was carried out where the recipient of the blood component was aged 70 or over and had experienced an adverse reaction to the transfusion.

Results: A total of 2245 reports of clinical transfusion reactions were submitted during this period, 881/2245 (39.2 %) involved recipients aged 70 years or over, of these, 462/881 (52.5%) were female. The most common type of reaction was febrile allergic hypotensive reaction (FAHR) with 431/881 (48.9%) of reports. Transfusion associated circulatory overload (TACO) was seen in 324/881(36.8%). Other, less common reactions included haemolytic transfusion reactions 70/881 (7.9%), transfusion associated dyspnoea 33/881 (3.8%), transfusion transmitted infections 3/881 (0.3%), post transfusion purpura 2/881(0.2%) and transfusion related acute lung injury 1/881(0.1%).

Most reactions were in the FAHR category where there were 283/431 (65.7%) febrile reactions, 99/431 (22.9%) allergic, 36/431 (8.4%) mixed (allergic & febrile) and 13/431 (3.0%) hypotensive reactions. Recipients who developed TACO commonly had at least one co-morbidity, 122/324 (37.6%) had cardiac disease, malignancies were seen in 68/324 (20.9%), anaemia 59/324 (18.2%) and chronic renal disease in 53/324 (16.3%). Others included diabetes, sepsis, hypertension and liver disease. Where the patient received several components or were of low weight, they appeared more susceptible to TACO.

Red blood cells 717/881 (81.4%) were the most implicated component while platelet concentrates accounted for 120/881 cases (13.6%), fresh frozen plasma 16/881(1.8%), cryoprecipitate 6/881 (0.7%), the remaining 22 cases involved a combination of components. Nearly 30% of transfusions were urgent or given as an emergency.

Summary/Conclusions: FAHR and TACO accounted for nearly 75% of transfusion reactions in patients >70 years old. Multiple co-morbidities in these patients make recognition and management of transfusion reactions challenging. All patients should be assessed for TACO prior to transfusion to identify risk factors and appropriate mitigating actions need to be considered, this is particularly important for this elderly and vulnerable group of patients. The main limitation of this review is the lack of denominator data regarding number of transfusions in this age group during this period. All transfusions should be appropriate, patients monitored closely for any reaction and managed promptly, early recognition and management are vital to improve safety.

P-135 | *Abstract withdrawn*

P-136 | Haemoylsis in a premature newborn after treatment with intravenous immunoglobulin due to passively transmitted anti-D

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Background: Intravenous immunoglobulin (IVIG) is the most widely used plasma component in the world. It is approved for the treatment of increasing number of conditions associated with immunodeficiency. Commercial immune globulins, including IVIG, may contain measurable quantities of IgG class antibodies, mostly of anti-A or anti-B specificity, but also other non-ABO antibodies may be present. So far, few cases of haemolytic anaemias, most often due to anti-D or anti-A have been reported, as manufacturers tend to lower the titre of red blood cell antibodies.
Aims: The aim of this case report was to point out the potential severity of haemolysis in a D-positive newborn after IVIG treatment due to passively transmitted anti-D.
Methods: We report on a case of haemolysis in a premature newborn after treatment with IVIG due to passively transmitted anti-D.
Results: The patient was a premature born male, born at 34th weeks of gestation, with a diagnosis of combined immunodeficiency with multiple intestinal atresia. His blood type was determined to be A D-positive. Direct antiglobulin test was negative. Mother´s blood type was determined to be A D-positive. In her blood no alloantibodies were detected. At birth, haemoglobin, total bilirubin and direct bilirubin were within reference values. Immediately after birth, partial resection of the small intestine was performed, with the formation of gastroduodenal LL anastomosis and jejunostomy, after which the patient developed sepsis and thrombocytopenia.
On days 18 and 20 of patient's life, he was treated with IVIG. Shortly thereafter, haemoglobin decreased significantly. Other haemolytic markers, such as total bilirubin, direct bilirubin and lactate dehydrogenase, were increased. Under the suspicion of an immune haemolysis, the patient's sample was sent to our laboratory for the serological investigation. Patient had a positive direct antiglobulin test and the red blood cell antibody screening test was negative. In the eluate, anti-D was identified. Anti-D was also identified in the IVIG, that was used for the patient's treatment. The patient was additionally transfused with A D-negative red blood cell units, and he had a corresponding increase of haemoglobin value.
Summary/Conclusions: This case illustrates the potential severity of haemolysis in a D-positive newborn after IVIG treatment due to passively transmitted anti-D. Haemolytic anaemias due to passively transmitted red blood cell antibodies by IVIG treatment may be missed by the routine clinician's practice. Clinicians need to be alerted of the possible haemolysis after IVIG treatment. Until then, haemolysis remains an under-recognized complication of IVIG therapy, and hence the exact incidence of haemolytic anaemia.

P-137 | Is there room for an expert system in haemovigilance reporting?

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Background: +French haemovigilance requires reporting of all adverse reactions concerning transfused patients. ANSM, the French competent authority, is in charge of this reporting system, which has evolved a lot since 2000 with the dual objective of making it easier for a growing number of reporting persons (RP) while enhancing reliability of reports. A national software application, e-FIT, supports all the process. Fact sheets of adverse reactions are available and updated. ANSM regularly organizes education sessions for new RP and in-house training.
Aims: A workgroup is now thinking about implanting an expert system functionality in e-FIT, in order to help RP in adverse reactions diagnosis.
Methods: The first model uses the period 2017-2019, the three last complete available exercises, as reference database. The presence or absence of 30 clinical and biological signs during the adverse reactions is registered and the declarer selects the most probable diagnosis in a thesaurus of 26 items. It is thus easy to calculate in the reference database the prevalence of each sign in each diagnosis and its positive predictive value (PPV). After the registration of a new adverse reaction, a RP may confront his diagnosis with each diagnosis probability resulting from the arithmetical combination of each observed sign PPV. As PPV can be low in rare adverse reactions, even with pathognomonic signs, the model proposes also to have a look at diagnosis where the observed signs are the most prevalent. Beyond the only validation of the chosen diagnosis, this tool may help RP to complete their inquiry and look for missing clues, which can comfort or invalidate some diagnosis.
Results: This first model is already able to confirm lot of adverse reactions with a classical appearance.
Summary/Conclusions: It has to be complicated with non-sign items as the incriminated blood component or time of onset, among others. We also plan to use it to review some unclassified complications of transfusion in the database.

P-138 | Post-transfusion purpura- Insights from shot UK

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Background: Post-transfusion purpura (PTP), a rare complications of transfusion is defined as thrombocytopenia arising 5–12 days following transfusion of cellular blood components (red cells or

platelets) associated with the presence in the patient of antibodies directed against the HPA (human platelet antigen) systems. Anti-platelet antibodies destroy both the transfused and autologous platelets. The diagnosis is often delayed because of the interval between the transfusion and disease onset. Haemorrhage, occasionally major, can occur due to the low platelet counts. If untreated, the disease is self-limited with platelet count recovery usually within 21 days. The diagnosis is confirmed by the clinical presentation and the detection of platelet -specific alloantibodies. Most cases have HA-1a antibodies but antibodies against many other HPA antigens have been reported and occasionally multiple antibodies have been reported. PTP occurs primarily in women sensitised to platelet antigens by exposure during pregnancy or transfusion; the female-to-male ratio reported in literature to be approximately 26:1. The cases reported in the last decade to Serious Hazards of Transfusion (SHOT), the UK haemovigilance scheme have been reviewed here to identify common themes.

Aims: –

Methods: All PTP cases submitted to SHOT between 2010-2019 (10 years) were reviewed.

Results: A total of 11 PTP cases were reported with over 26million blood components issued in UK during this period. Since reporting began in 1996, a total of 57 PTP cases have been reported to SHOT, a reducing trend since the introduction of universal leucodepletion in UK in 1999. Most cases were seen in female patients (10/11, 91%). Severe thrombocytopenia, often.

Summary/Conclusions: PTP is extremely rare and the thrombocytopenia can be quite severe and life-threatening. Staff need to be able to recognise these delayed immunological transfusion reactions so that appropriate actions can be taken. PTP has a temporal relationship to a transfusion and clinicians must recognise this and investigate appropriately. Although the low platelet counts are transient, major haemorrhage resulting from the thrombocytopenia can lead to patient death and major morbidity. Avoiding unnecessary transfusions, monitoring patients for delayed reactions and educating patients about these potential risks are vital.

P-139 | Immune-mediated refractoriness to platelet transfusion (PLT): Which strategy? Description of a complex case of allo-immunization

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Background: Long exposure to PLT transfusions in hematological patients increases the risk of immunization against PLT antigens (Ag) (HLA and HPA). Diagnosis and treatment follow the algorithm applied and the complexity of the case: when the Cross-match (CM) number of compatible donors is less than 5 on 90, it is necessary to identify HLA match donors. HLA Ag typing on blood donors is not yet a routine practice making difficult to find HLA match donors, rapidly. Our case is herewith described: male, 73 years old with CLL, non-responder to treatments with IgG + cortisone, Rituximab and Eltrombopag, presence of allo-Ab both vs HLA and HPA.

Aims: The aim of this study is to show how our algorithm can be applied to the patient with ineffectiveness (CCI Correct Count Increment) evaluation, - Ab screening (TS), - Ab identification (TI), - CM, - HPA and HLA genotyping (TA) - search for the specificity of anti-HLA Ab and level, expressed in Mean Fluorescence Intensity (MFI) - HLA typing CM compatible donors. Platelets from Apheresis (PLTAF) are than collected and transfused.

Methods: TS with and without chloroquine (SPRCA Capture-P Ready Screen, Immucor). TI (Pak-Lx Luminex and Pak plus Immucor ELISA). HLA and HPA TA (PCR-SSO and HPA BeadChip, Immucor). MFI Abs (Luminex). CM (Capture-P, Immucor).

Results: TS was POSITIVE with and without chloroquine. The TI showed Ab anti-HLA I + Ab anti-HPA1b and 2b. The HPA TA showed homozygosity in all systems studied (HPA1-6, 9 and 15 a/a) while the HLA I typing: HLA A*31*68, B* 18*51, C*12*15. MFI was performed at T0 and T2 months: 59 different Abs were identified (19/59 towards HLA A, 35/59 HLA B and 5/59 HLA C). 37/59 specificities showed MFI x at 21799 (T0) and 19935 (T2), 21/59 MFI x at 8388 (T0) and 4189 (T2months); the 4/62 specificities with MFI at 5000 (T0) were undetectable at T2. From 17/12/2020 to 17/03/2021, 1878 random donors were CM'd with the patient: 28/1878 (1.5%) were compatible. 20/28 where typed for HLA I: 4/20 not matching, 15/20 mismatch and 1/20 mach. Two mismatch donors for Ags: A*1*30;C*5*14 and Ag A*32,B*40, C*02*07, after 30 days were respectively incompatible and compatible. The search for HLA I match donors among our Periodic Donors Group, registered also in the IBMDR, did not give any results. Transfusion support: from 12/10/2021 to 16/03/2021 60 PLT components were transfused: 32 pools and 1 PLTAF random with no PLT increase at 1h from transfusion (CCI X = 0), 15 pools with 0-1 Buffy-coat (BC) compatible with medium increment (MI) of 1000/µl (CCI X = 669), 9 pools with 2-3 BCs compatible with MI of 7000/µl (CCI X = 3080), 3 compatible PLTAFs (of which one match) with MI of 13400/µl (CCI X = 8679).

Summary/Conclusions: The problem of managing complex cases of immune-mediated PLT refractoriness is clear. As a first approach, the result of the CM with the largest possible number of donors (80/90) is decisive: if compatibles BC are less than 4 it is necessary to assign partially compatible pools and submit compatible donors to HLA I TA. Matching donors can be enrolled for PLTAFs, mismatching ones can be enrolled according to the patient's antibody/MFI specificity. Therefore, in these particular cases, the search for Ab specificities over time is of considerable help, in order to monitor the modulation of refractoriness. HPA typing is not considered since refractory nature is primarily attributable to HLA I.

Clinical transfusion – Neonatal and Pediatric transfusion

P-140 | Oxidative stress gene expression profile in endothelial cells exposed to adult or fetal hemoglobin

selected for main programme

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Background: Repeated Red Blood Cells (RBC) transfusions in extremely low gestational age neonates (ELGAN) predict a poor outcome, with a higher risk for mortality and morbidity. A close association between low levels of fetal hemoglobin (HbF) and diseases driven by the oxidative damage, as retinopathy of prematurity and bronchopulmonary dysplasia, has been recently reported. In comparison with adult Hb (HbA), HbF is endowed with higher oxygen affinity, greater redox potential and higher tetrameric stability, all functions potentially protective in oxidative challenge. Cord blood RBC transfusions effectively prevent the HbF loss consequent to standard transfusions.

Aims: To investigate molecular mechanisms underlying the potential protective role of HbF, we assessed the expression profile of several genes involved in the oxidative stress pathways in endothelial cells exposed to either HbA or HbF.

Methods: Endothelial cells (EC) were obtained from umbilical cord blood and confluent layers at passages II were used. Packed RBC were obtained by fractionating whole blood units from adult donors or cord blood units from full term infants. Both packed RBC types with equal proportion of SAG-M were stored at +4°C for 7 days. Then, cord and adult packed RBCs were centrifuged; supernatant and RBCs were collected stored at -20°C. To obtain free hemoglobin, RBC aliquots were thawed at 37°C. Free Hb concentration was measured and samples were diluted in culture medium at a final concentration of 4g/L (corresponding to the average Hb concentration of a packed RBC unit with 0.8% of hemolysis, the maximum hemolysis rate allowed at term of storage).EC were then exposed for 6 hours to 7-day supernatants from cord or adult RBCs units, or to the medium containing free HbF or HbA. Total RNA was extracted and reverted. Gene expression profile was evaluated using the "Human Oxidative Stress Plus" (PAHS-065Y, SABiociences, Qiagen).Linear model fitting and differentially expressed genes (DEGs) estimation were done using the R package limma. DEGs were called at a significance level of $P < 0.05$, after Benjamini-Hochberg correction. DEGs enrichment analysis over Gene Ontology (biological process) were done using the online tool enrichR.

Results: Adult and fetal free hemoglobin and cord RBC supernatant induced a similar expression pattern of the most significantly regulated genes (NOX4←, APOE↓ and AKR1C2↓). This pattern of expression was opposite to that observed after the exposure to adult RBC supernatant, suggesting an eventual role for residual leukocytes contained in packed RBCs from adult donors. DEGs enrichment analysis showed that fetal and adult Hb act on similar but not identical pathways (see Best Gene Ontology in Table 1). Even more pronounced differences were observed in cells are exposed to day-7 supernatants of cord or adult packed RBC units (see Broader and Best Gene Ontology in Table 1).

Summary/Conclusions: These preliminary data suggest that fetal and adult Hb may have a different impact on the oxidative stress. This finding may be relevant to the transfusion management of ELGAN patients with diseases driven by the oxidative damage.

P-140 Table 1. Results of DEGs enrichment analysis

Condition	N° of gene	Broader Gene Ontology	Best Gene Ontology
Free HbF	17	response to stimulus	Superoxide metabolic process
Free HbA	26	response to stimulus	Removal of superoxide radicals
Cord supernatant	12	regulation of cellular process	Superoxide metabolic process
Adult supernatant	11	biological regulation	Sterol catabolic process

P-141 | Survey on neonatal transfusion practices in 18 European countries

selected for main programme

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Background: Infants born preterm are frequently transfused, but high quality evidence supporting transfusion guidelines remains limited. Recent studies suggest that restrictive red blood cell (RBC) transfusion strategies are non-inferior to liberal strategies and have reported an increased risk of bleeding and mortality as a result of a liberal (50×10^9/L) platelet transfusion threshold compared to a restrictive (25×10^9/L) threshold. It is unclear to what extent the results of existing studies have been incorporated into clinical practice. Data on variation in clinical transfusion practices in Europe are lacking, but are crucial for the development of quality improvement projects, guideline revisions and the design of new clinical trials.

Aims: To describe transfusion practices for preterm infants in European Neonatal Intensive Care Units (NICUs).

Methods: We distributed an online survey among neonatologists working in NICUs caring for neonates born at less than 32 weeks' gestational age from October 2020 until December 2020 in 18 European countries: Austria, Belgium, Finland, Germany, Hungary, Italy, Malta, The Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, Switzerland and the United Kingdom. One response per NICU was requested. The survey consisted of 32 questions, including questions about transfusion thresholds, indications, dose and duration for RBCs, platelets and Fresh Frozen Plasma (FFP). Clinicians were presented with sets of clinical scenarios for RBC and platelet transfusions and asked to indicate the hemoglobin, hematocrit or platelet count thresholds used in their center.

Results: The analysis included 354 NICUs, with a median response rate of 57%. There was substantial variation in practice for transfusion thresholds, indications, duration and dose for RBC, platelet and FFP transfusions. The interquartile ranges (IQR) for hemoglobin thresholds in different clinical scenarios varied between 6-20 g/L, with higher ranges indicating more variation in practice. The IQR for platelet count thresholds in different clinical scenarios varied between 0-50×10^9 platelets/L. In 53% of NICU's, clinically stable neonates without signs of bleeding received platelet transfusions for platelet count thresholds higher than 25×10^9/L. In 38% of NICUs, non-bleeding neonates with coagulopathy received FFP transfusions. Transfusion rate was highly variable, with an IQR for RBCs of 6 ml/kg/hr (2-8) and an IQR for platelets of 35 ml/kg/hr (5-40).

Summary/Conclusions: There is substantial variation in clinical transfusion practices in European NICUs. This survey is a crucial first step towards improving neonatal transfusion practices and provides valuable starting points for quality improvement projects, guideline revisions and the design of future clinical trials.

P-142 | RBC transfusions in ELGAN: Effect of fetal and adult hemoglobin of severe ROP

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Background: Extremely low gestational age neonates (ELGAN, i.e., born before 28 gestation weeks) are among the most heavily transfused pediatric patients. In this clinical setting, repeated RBC transfusions independently predict a poor outcome, with a higher risk for mortality and morbidity. A close association between low levels of fetal hemoglobin (HbF) and severity of retinopathy of prematurity (ROP) has been reported. This observation prompted in our hospital a previous study on the feasibility of transfusing allogeneic cord blood (CB) RBCs instead of adult RBCs. In a subsequent proof-of-concept study in a small number of patients, we demonstrated that CB-RBCs prevent and/or reduce the HbF loss. However, no data are available so far on the efficacy of this transfusion strategy. In both studies patients received adult or CB RBCs, depending whether an ABO/Rh D matched CB RBC unit was available at the time of the transfusion request.

Aims: This analysis is focused on ROP severity in patients at highest risk for ROP. We investigated ROP severity in a series of patients grouped according whether they were transfused exclusively with adult -RBCs or received also CB-RBCs.

Methods: Patients were selected from databases relative to our previous studies on CB transfusions (carried out in 2014 and 2019) and from a database prospectively collected (2019 and 2020) at our

neonatal intensive care unit (NICU). Study inclusion criteria were gestational age (GA) of 28 weeks or less, and survival until 42 weeks of postmenstrual age. Standard of care protocols for oxygen therapy, respiratory and nutritional support, as well as transfusion thresholds and criteria for diagnosis and treatment of intraventricular hemorrhage (IVH), bronchopulmonary dysplasia (BPD) and ROP remained unchanged during the entire study duration (2014-2020).

Results: Overall, 32 patients were included in the analysis. Among them, 8 patients received CB transfusions and 24 adult RBCs. The cord and adult RBC groups had comparable GA at birth, birth weight, and Apgar indexes. The rate of documented infections, IVH, and BPD, as well as the number of days in oxygen therapy were similar in both groups. Moreover, the CB and adult RBC patients received a comparable number of transfusions. Severe ROP occurred in 3 out of 8 patients in the CB group (37.5%) and in 11 out of 24 receiving adult RBCs (45.8%; p=0.504). Among the 11 patients receiving adult RBCs, 8 required treatment for ROP, in comparison with none in the 8 patients in the CB RBC group (p=0.070). In all cases, treatment consisted of anti-vascular endothelial growth factor administration.

Summary/Conclusions: The main limitation of this retrospective analysis is that a great proportion of patients in the CB group (6 out of 8) did not receive exclusively cord RBC transfusions but also adult blood. Several retrospective and prospective studies reported that repeated transfusions increase the risk for ROP. Considering that patients in CB and adult groups received similar number of transfusions, our data confirm the recent observations on the protective role of fetal hemoglobin. The use of CB transfusions to lower ROP severity and treatment requirement in ELGAN deserves to be further investigated in randomized studies.

P-143 | Intrauterine fetal transfusion in red blood cell alloimmunization: The blood bank perspective

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Background: The main cause of foetal anaemia is maternal red blood cell alloimmunization. When the maternal immune system is sensitized to foetal foreign erythrocyte antigens, it stimulates the production of immunoglobulin G antibodies, which can cross the placenta. Maternal antibodies cause haemolysis if the foetus is positive for the specific erythrocyte antigens of paternal origin, resulting in haemolytic disease of the foetus and newborn (HDFN). Presently, the more accepted treatment of foetal anaemia is intravascular intrauterine transfusion (IUT). The goal is to prevent or treat foetal hydrops and to allow the pregnancy to a gestational age with higher neonatal survival rate. The red blood cells for IUT should be 0 Rh D negative and/or negative for the antigen corresponding to the mother antibodies; indirect antiglobulin technique crossmatch compatible with maternal serum; less than 5 days old; CMV negative and/or leukocyte depleted; irradiated ≤24h; and with a haematocrit of 75-80%.

Aims: The aim of our study is to evaluate IUT prepared in the Immunohematology and Transfusional Medicine Department of Maternidade Dr. Alfredo da Costa, and its main immune causes.

Methods: We conducted a retrospective study between 2000 and 2020, with special emphasis in the last 5 years. The data were collected from the patient's clinical files.

Results: Between 2000 and 2020, were prepared 112 IUT for a total of 56 foetus. On average, each foetus received 2 IUT (min 1; max 5). The leading cause of severe foetal anaemia requiring IUT was HDFN secondary to maternal red blood cell alloimmunization (38, 67.9%), other causes were non-immune (18). The most prevalent antibody was anti-D (35, 95.1%), alone (13) or in association with other antibodies. Less prevalent were antibodies anti-K (1) and anti-c (1).

In the last 5 years of the study period (2015-2020), were prepared a total of 46 IUT for 13 foetuses with haemolytic anaemia associated to maternal red blood cell alloimmunization. The mean haemoglobin pre-IUT was 7,6 g/dL (min 1,2; max 12,3) and the mean haematocrit was 22,2% (min 3,7; max 36,5). Among the 13 foetuses, 2 were born in other maternity hospitals, 1 was a stillborn and 10 (76.9%) were live births; 6 neonates received red cell small volume transfusion, 3 received exchange-transfusion and 1 was not transfused.

Summary/Conclusions: Fetal anemia is a serious complication in pregnancy and is associated with significant perinatal mortality and morbidity. IUT is considered the most successful treatment for fetal anemia due to alloimmunization. The leading cause of HDFN is red blood cell alloimmunization (mainly by anti-D antibodies), being the most common indication for IUT. Overall, the evidence indicates that we should continue to promote Rh D immune globulin prophylaxis after any obstetric event, at 28 weeks and postpartum.

P-144 | Determinants and transfusion practices in pediatric critical care unit: A prospective study in thrombocytopenic children

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Background: Prevalence of thrombocytopenia varies in various Intensive Care Units, ranging from 13% to 58% depending on disease profile of patients. Platelet count is now considered a predictor of outcome. Restrictive transfusion policies save transfusion recipients from inherent risks of transfusion adverse reactions.

Aims: To evaluate the incidence of thrombocytopenia, determinants, transfusion practices, outcome and prognostic value of platelet count.

Methods: A prospective study was conducted in Tertiary care hospital from October 2018 to December 2019. Total of 450 children of age >1 month and <18 years admitted in level III Paediatric Intensive Care Unit (PICU) and level II High Dependency Unit (HDU) were included in the study. Patients' demography, laboratory data, length of stay,

severity score, transfusion requirement and outcome were noted and analyzed for various determinants.

Results: Patients' age varied from 4 months to 15 years with male preponderance. Incidence of thrombocytopenia was 24%; 20.22% patients presented at admission and 3.78% patients developed thrombocytopenia during hospital stay. Fever, shock and bleeding manifestations were found in 70.44%, 5.77% and 9.11% cases respectively; more in thrombocytopenia than in non-thrombocytopenia patients. Circulatory failure and shock were found in 17.59% thrombocytopenic and 2.04% non-thrombocytopenia patients. Coagulopathy was found in 27.78% of thrombocytopenia patients. Kidneys were affected in 61.11% patients in thrombocytopenia. Single & multiorgan failure were more in thrombocytopenia patients (34.25% & 12.96% respectively) than non-thrombocytopenia patients. Severity score, using PRISM III score, was high (>8) in 14% thrombocytopenia patients. Length of stay of >10 days was more in thrombocytopenia cases (4.62 %vs. 2.33%). Outcome was favourable; 99.89% patients discharged and 1.11% had unfavourable prognosis. Mortality rate of 3.7% and 0.29% in thrombocytopenia and non-thrombocytopenia patients respectively support that thrombocytopenia is a good prognostic indicator for outcome. 4.44% patients required transfusion; 2.78% in non-thrombocytopenia and 10.98% in thrombocytopenia category. 2.77% thrombocytopenia patients required multiple component transfusion and 0.92% cases required only platelet transfusion.

Summary/Conclusions: Severe or life threatening bleeding was not present in thrombocytopenia patients in this study. There was less number of Thrombocytopenia Associated Multiple Organ Failure and very less transfusion requirements reinforcing that various clinical practice international guidelines for restrictive strategy of platelet transfusions should be adhered and patients be treated for underlying causes and monitored closely.

P-145 | Transfusion reaction in paediatric population: Lessons from four years' experience

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Background: Haemovigilance is a key concept in transfusion. One of its aspects is collecting information on unexpected adverse outcomes resulting from blood products' use to prevent their occurrence. Given the specificity of paediatric transfusions, it should have a distinct haemovigilance schemes. In Tunisia, Haemovigilance is mandatory, using a paper report, and covers all grades of adverse events.

Aims: The aim of this study was to identify different elements of haemovigilance concerning this specific group of patients in northern region Tunisian centers, for a better follow-up and therapeutic management.

Methods: All transfusion adverse reactions were reported to the central unit of blood transfusion; covering 14 teaching and two regional hospitals in the capital city Tunis. Data were collected from transfusion incident report, retrospectively over a four-year period and involved all cases below the age of 18 years.

Results: The study included 161 patients; Mean age 6,48 year [1 month – 17 years] with a gender ratio 1,14 (males: 86; females: 75). 68.3% of investigations adverse events were carried on to term, from which 34.5% were considered allergic reactions, 25.4% were classified as febrile non haemolytic transfusion reaction (FNHTR) and 18.1% immunisation. We reported 7 cases of hyperkalemia, one case of ABO-incompatible transfusion and one case of TRALI. Transfusion reactions of grade 1 severity were the most frequent (N = 139), followed by grade 3 severity (N = 17). Only one case of grade 4 severity was reported, classified undetermined.

Summary/Conclusions: This study shows that allergic reactions and FNHTR were the most common in pediatric population. A better knowledge and use of qualified blood products could be helpful to decrease adverse transfusion events. Therefore, specific training dealing with qualified blood products' use, is needed to limit these events.

P-146 | *Abstract withdrawn*

P-147 | Investigation of patient factors associated with the number of transfusions required during chemotherapy for high-risk neuroblastoma

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Background: Blood transfusion is an important supportive care for high-risk neuroblastoma. When the number of transfusions increases, transfusion-associated adverse reactions may be more problematic. However, the factors determining the degree of myelosuppression and the number of transfusions during chemotherapy for high-risk neuroblastoma remain unclear.

Aims: We aimed to determine the number of blood transfusions required during almost similar chemotherapy for high-risk neuroblastoma and clarified patient factors, particularly those related to the number of blood transfusions required.

Methods: We investigated patient factors determining the number of required transfusions in 15 high-risk neuroblastoma patients who received five courses of chemotherapy. Clinical data, cytokine profile, and colony-forming assay with bone marrow samples at diagnosis were analyzed.

Results: The required number of transfusions of both platelets and erythrocytes decreased once in the second course and then increased

as the course progressed. The variability among cases increased as the chemotherapy course progressed. In cases of low peripheral blood platelet count and lower fibrinogen level at diagnosis, the number of platelet transfusions was higher during chemotherapy. In contrast, there was a negative correlation between the forming ability of granulocyte-macrophage or erythroid colonies and the number of erythrocyte transfusions in the latter period.

Summary/Conclusions: In the early stages of chemotherapy, bone marrow infiltration in neuroblastoma and/or coagulopathy complication may cause thrombocytopenia and requirement of platelet transfusion; conversely, in the later stages, the number of erythrocyte transfusions may be defined by the patient's inherent hematopoietic ability. These factors may be useful in predicting the required number of transfusions.

Clinical transfusion – Therapeutic apheresis

P-148 | Comparison of different volumes of calcium gluconate added in replacement fluid to maintain ionised calcium levels during therapeutic plasma exchange procedures

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Background: Apheresis procedures requires anticoagulation of blood in extracorporeal circuit. ACD-A is commonly used anticoagulant in therapeutic plasma exchange procedures (TPE). Ionised calcium levels fall in the plasma of patients undergoing TPE as a result of citrate anticoagulation, removal of calcium in patient's plasma and the intrinsic calcium binding properties of albumin, used as replacement fluid. So, we planned to add different volumes of calcium gluconate in replacement fluid to observe the change in ionised calcium levels during and post TPE procedure.

Aims: To compare the effect of different volumes of calcium added to 5% Human Serum Albumin (HSA) on intraprocedural and post-procedural ionised calcium levels in neurological patients undergoing TPE.

Methods: This is a prospective observational study in which we followed ionised calcium levels during 35 therapeutic plasma exchange procedures done in neurological indications where 5% human serum albumin was used as replacement fluid. In first 20 procedures (group-1), we added 5 ml of 10% calcium gluconate in 500 ml of 5% HSA (1ml in 100 ml) whereas in other 15 procedures (group-2), 10 ml of 10% calcium gluconate was added (2 ml/100 ml). The pre-procedural ionised calcium, intra procedural (at 45-50 minutes) and post procedural values were noted and compared in two groups. The inlet to AC ratio was kept similar (1:12) for all the procedures and no additional supplementation of calcium was done in normal saline or as separate infusion. Patients were monitored closely for any signs/symptoms of hypocalcaemia (citrate effect or toxicity).

Results: We observed that mean baseline ionised calcium in group-1 was 0.990±0.10 mmol/L and in group-2, it was 0.970±0.12 mmol/L, which was comparable (p=0.63). In group -1, the mean intraprocedural (0.924±0.08 mmol/L) and mean post procedural (0.873±0.08 mmol/L) levels significantly decreased as compared to baseline levels (P=0.000, P=0.000) and also when compared among themselves. whereas, in group 2, there was no change in intraprocedural (0.869±0.23 mmol/L, P=0.58) and post procedural levels (0.938±0.23 mmol/L; P=1.00) as compared to baseline levels. Also, difference between intraprocedural and post procedural levels was not statistically significant (P=0.467). Citrate effect was observed in 1 out of 20 procedures (5%) in group-1 whereas in another group no adverse event was observed.

Summary/Conclusions: The addition of 2ml of 10% calcium gluconate in 100ml of 5% HSA is safe and a better option than 1 ml in 100 ml of 5% HSA to maintain levels of ionised calcium throughout the procedure with lower chances of adverse events related to hypocalcaemia.

P-149 | Therapeutic plasma exchange - A boon therapy for Moran syndrome (case series and review of literature)

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Background: Morvan syndrome (MVS)-a rare autoimmune disorder with peripheral nerve hyperexcitability, central nervous systems (CNS) symptoms and autonomic dysfunction has male predominance with prevalence of 1 in 1000000. Apart from other therapeutic options, therapeutic Plasma Exchange (TPE) for MVS (VGKCs) is category II indication (as per American Society for Apheresis 2019 recommendation) due to paucity of currently available literature.

Aims: We aim to present our experience with the role of TPE in MVS patients, to evaluate their treatment response with special attention to any adverse effects of treatment and disease, in relation with their antibodies status.

Methods: The cross sectional study included data of 10 patients diagnosed with Morvan's syndrome and to whom TPE was instituted as adjuvant therapy from 2015 to Feb 2021 (6 years). Approximately 30-40 ml /kg of plasma was removed during each procedure with the help of MCS plus 9000 (Hemonetics, Unites States) apheresis platform alternatively over a period of five days. Crystalloid (0.9 % Normal saline) and fresh frozen plasma were used as replacement fluid. Another peripheral venous access was established for infusion of calcium.

Results: Total 10 patients enrolled in this study. Male to female ratio was 4:1 and median age at presentation was 35.5 years. All patients were treated initially with anti-epileptics and methylprednisolone along with plasma exchanges further as shown in Table1. Significant improvements were seen after plasma exchanges in terms of reduced symptoms including insomnia, hyperhidrosis and myokymia. All patients are on continued follow up.

P-149 Table 1. Summary of patient characteristics, including symptoms and details of TPE.

Patient	Age	Sex	Caspr2 Abs	LGI Abs	Paraneoplastic Neuronal antibodypanel	Symptoms	TPE/ Cycles	Improved or not	Other Rx
1	35	M	Pos	Neg	Neg	Fasciculations in both UL and LL, Insomnia, Dysautonomia	5	Yes	Anti-epileptics, MP
2	37	M	Pos	Pos	Neg, Thymoma +	Fasciculations, severe LL pains, insomnia, weight loss,loose stools	5	Yes	Anti-epileptics, MP
3	45	M	Pos	Neg	Neg	Fasciculations, severe LL pains, insomnia	5	Yes	Anti-epileptics, MP
4	17	M	Neg	Neg	Neg	Insomnia, NMT, pains, Hyperhidrosis, wt. loss	5	Yes	Anti-epileptics, MP
5	37	F	Pos	Neg	Neg	Parasthesias, Fasciculations	5	Yes	Anti-epileptics, MP
6	36	M	Pos	Neg	Neg	Fasciculations in both LL, Seizures, Absent DTR, Hyperhidrosis,insomnia, Myokymia	8	Yes	Anti-epileptics
7	21	F	Pos	Neg	Neg	Fasciculations in both LL, pain and paraesthesia in both the legsinsomnia, Myokymia	5	Yes	Anti-epileptics, MP
	20	M	Pos	Neg	Neg	Pain in both LL, Fasciculation, Hyperhidrosis, NMT, Insomnia	5	Yes	MP
9	53	M	Neg	Neg	Neg	Loose stools, Hyperhydrosis, Fasciculations all over the body, hyperphasiadrooling, insomnia	5	Yes	Anti-Epileptics
10	20	M	Pos	Neg	Neg	Pain in both LL, insomnia, Hyperhidrosis, Fasciculations	7	Yes	Anti-epileptics, MP

Summary/Conclusions: We report a case series of MVS with central and peripheral nervous system hyperexcitability with detectable serum VGKC antibodies by radioimmunoassay and remarkable response to plasma exchange. We provide the first review of responses of TPE in MVS cases from India. Approximately 60 cases are published in French literature and others, but only 27 cases were reported till date in English literature cases. In Indian subcontinent only scattered cases were reported.

Conclusion: TPE proved to be instrumental in the management and providing optimum results in MVS along with other adjuvant treatment.

P-150 | Monitoring RBC protoporphyrin levels with real-time live confocal fluorescence microscopy in a child undergoing therapeutic apheresis for erythropoietic protoporphyria associated liver failure

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Background: Erythropoietic protoporphyria (EPP) is a rare inherited disorder characterized by a reduced activity of ferrochelatase which catalyzes the final step in heme biosynthesis. This results in accumulation of protoporphyrin (PP) in RBCs, skin, and liver. While acute painful photosensitivity is pathognomonic, severe liver dysfunction occurs in only 1-4% of patients. The latter leads to a marked increase of hepatotoxic PP in plasma and RBCs which in turn can accelerate liver damage. Optimum therapy to break this vicious circle remains to be established, but benefits of therapeutic plasma and RBC exchange transfusion have anecdotally been reported at least to bridge patients prior to liver transplantation or hematopoietic stem cell transplantation (HSCT). HSCT is curative because it prevents overproduction of protoporphyrin.

Aims: Levels of PP in plasma and RBCs are determined by specialist porphyria laboratories, only. Therefore we aimed at implementing a method suitable for monitoring PP levels in order to optimize therapeutic plasma and RBC exchange transfusion therapy. Because of the specific fluorescence spectrum of PP the detection of PP carrying red cells ("fluorocytes") was done by real-time live confocal fluorescence microscopy.

Methods: A 7 year old 20 kg female (TBV 1600 ml) with EPP and endstage liver failure was transferred to the pediatric ICU at Innsbruck University Hospital for liver transplantation. During the perioperative period (46 days), she underwent therapeutic plasma (n=16) and RBC exchange transfusion procedures (n= 6). Living donor split liver transplantation (LDSLT) from the patient´s father was performed at day 24. Before each exchange procedure a custom prime was done with 1 matched and irradiated RBC unit. The 1.5fold of the patient´s plasma or

erythrocyte volume were exchanged per procedure. The PP carrying RBCs ("fluorocytes") were detected as autofluorescence (excitation: 410 nm) with a microlens-enhanced Nipkow spinning disk-based system allowing live confocal imaging in blood samples before and after 6 exchange procedures (5 RBC, 1 plasma exchange) from day 10.

Results: A rather weak, but distinct fluorescence on about 10% of the patient´s erythrocytes was detected before the RBC exchange transfusion on day 14. A pronounced photobleaching of the fluorocytes occurred during microscopy. In the further course, a continuous decrease of the proportion of fluorocytes was observed. In parallel, the difference between the percentage of fluorocytes before and after each RBC exchange procedure increased. After LDSLT, the fluorocytes sharply declined until they were no more detectable on day 46 (22 days after LDSLT). The patient improved as her liver function recovered. However, the total porphyrin concentration in the blood remained unchanged between 10 and 22.8 μM (limit <1.3 μM).

Summary/Conclusions: Therapeutic apheresis as a bridging therapy for EPP associated liver failure is a challenge, especially in low weight children. Hence, a method to assess the efficacy of every single procedure is highly desirable. Our preliminary work demonstrates that real-time live confocal fluorescence microscopy is a useful tool to map this complex situation. The discrepancy of our results with the concentration of total porphyrins remains to be elucidated. Thus, a method for the quantitative determination of fluorocytes is being developed.

Clinical transfusion – Evidence based transfusion medicine practice

P-151 | Transfusion support in first 100 days of allogeneic HSCT

selected for main programme

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Background: Patients receiving hematopoietic stem cell transplantation (HSCT) require extensive transfusion support until red blood cell and platelet engraftment occurs. Different kinds of ABO-incompatible (ABOi) HSCT have their unique complications, including hemolysis, passenger lymphocyte syndrome, delayed red blood cell engraftment and pure red cell aplasia. Transfusion support varies with this spectrum and forms an integral part of the management of ABOi HSCT recipients.

Aims: To study the trend of blood component transfusion in first 100 days of ABO compatible (ABOc) and ABOi allogeneic matched, related HSCT.

Methods: This was a prospective study conducted in the department of Transfusion Medicine at a large tertiary healthcare center between 2016 and 2019. All ABOc and ABOi allogeneic matched, related peripheral blood stem cell transplant recipients who were under regular follow-up for at least 100 days after transplant were included. Data of component wise transfusion from day of transplant to 100 days post-transplant was actively collected. A restrictive transfusion strategy was used during this study with red cell transfusion trigger being 7g/dL in a stable patient unless the patient was symptomatic and platelet transfusion trigger being 10,000/cumm in the absence of bleeding. The blood group of all components was decided on a case-to-case basis after a discussion between physicians from Transfusion Medicine and Hemato-Oncology and Bone Marrow Transplant unit.

Results: During the study duration there were a total of 62 matched, related HPC transplants including 38 ABOc and 24 ABOi transplants. Out of these, 42.9% were female HSCT recipients and 57.1% were male HSCT recipients. Out of the 112 ABOi transplants, 66.7% were minor incompatible, 8.3% was major incompatible and 25% were bidirectional incompatible transplants. Two patients developed passenger lymphocyte syndrome and one developed pure red cell aplasia. Mean number of days to platelet engraftment (20,000/μL after 3 consecutive days without platelet transfusion) were 18.4 and 16.9 in ABOi and ABOc categories respectively. Mean number of days to neutrophil engraftment (absolute neutrophil count more than 500/μL for 3 consecutive days) were 15.1 and 12.1 in ABOi and ABOc categories respectively. Mean CD34 cell dose harvested by apheresis was 7.7 and 6.2 million per kg for ABOc and ABOi categories respectively. Mean number of red cells, random donor platelet concentrates and single donor platelet concentrates issued for transfusion in ABOc category were 7.1, 8.7 and 5.5 respectively while it was 6.7, 9.6 and 3.4 in ABOi category respectively. The component-wise difference in number of units transfused in the first 100 days was not found to be statistically significant between the two categories.

Summary/Conclusions: Contrary to the usual belief that ABO incompatible HSCT recipients require more number of transfusions due to various complications, the transfusion practices at the present center did not show a significant difference in the component-wise transfusions between these two categories. A restrictive transfusion strategy for HSCT recipients is an important measure in reducing the overall number of transfusions. Careful consideration of the blood group of components to be transfused by both the department of Transfusion Medicine as well as the Hemato-Oncology and Bone Marrow Transplant unit helped in improving the outcome of ABO incompatible transplants.

P-152 | Factors affecting red cell consumption in transfusion-dependent beta-thalassemia patients

selected for main programme

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Background: Patients with homozygous beta-thalassemia are among the most heavily transfused subjects and represent a model of red cell

transfusion dependency. The factors which influence red cell consumption in those patients are only partially known and there are very few studies addressing this topic.

Aims: Data collected within a prospective clinical trial (ClinicalTrials.gov: NCT03992001) were further analyzed to verify which factors influence red cell consumption. The factors studied include patient characteristics: age, sex, height, weight, body mass index (BMI), blood volume (BV), pre-transfusion hemoglobin concentration, splenectomy; blood component and transfusion characteristics: length of the interval between transfusions, storage age.

Methods: Patients were adults with transfusion-dependent beta-thalassemia major, free from clinical conditions which could modify red cell consumption acutely. For each transfusion, patient characteristics, including pre-transfusion hemoglobin concentration, and storage age of the red cell concentrates (RCC) were recorded. RCC (pre-storage leukodepleted, suspended in SAGM) were sampled before transfusion to measure hemoglobin concentration and hematocrit. RCC units were weighed together with the infusion set before and after transfusion to measure the amount of hemoglobin actually transfused (Hb_{trx}). BV was calculated from sex, height and weight. Pre-transfusion total body hemoglobin content ($Hb_{pre\text{-}trx}$) was estimated from BV and the pre-transfusion hemoglobin concentration. $Hb_{post\text{-}trx}$ was the sum of $Hb_{pre\text{-}trx}$ and Hb_{trx}. The difference (Hb_{delta}) between $Hb_{post\text{-}trx}$ and $Hb_{pre\text{-}trx}$ calculated from the pre-transfusion hemoglobin concentration of the next transfusion gave the amount of hemoglobin consumed during the interval between two successive transfusions. Finally, Hb_{delta} was divided by the interval (days) to obtain an estimate of the average daily hemoglobin consumption (Hb_{cons}).

Results: Data from 51 patients (24 females) were available. Their age was 47±6 years, the average pre-transfusion hemoglobin concentration 9.4±0.5g/dL, BV 3.93±0.63L. Thirty-two patients were splenectomized. Fourteen of the other patients had a moderately enlarged spleen (≤16.5cm). Of all the transfusions available for analysis, we chose those (N=1046) where only one RCC unit had been transfused or all units had the same storage age. A total of 1567 RCC were transfused. Their storage age was 5.8±3.0 days, with a minimum of 2 and a maximum of 16 days. The transfusion interval was 14.8±3.4 days. The average Hb_{cons} was 5.7±1.8 gHb/day. Hb_{cons} was 20% greater in non-splenectomized patients (6.4±1.9 vs 5.3±1.7 gHb/day, Mann-Whitney test, $p<0.00001$). Among non-splenectomized patients, spleen diameter was positively correlated with Hb_{cons} (Spearman rho=0.258, $p<0.00001$). In the univariate analysis, height, weight, BMI, and BV were positively correlated with Hb_{cons}, whilst age and female sex were negatively correlated. However, in the multivariate analysis, only splenectomy and BV remained significantly associated. Pre-transfusion hemoglobin concentration was also positively correlated with Hb_{cons} in the multivariate analysis, but interval and storage age were not.

Summary/Conclusions: BV and splenectomy greatly influence red cell consumption. Our patients received relatively fresh RCC only, as appropriate for thalassemic patients. Within the first two weeks of storage, the storage length does not impact on red cell consumption.

P-153 | *Abstract withdrawn*

P-154 | Development of the consultant clinical scientist role in the UK - Haematology and transfusion medicine

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Background: The Higher Specialist Scientific Training (HSST) program in the UK prepares healthcare scientists for the challenging role of Consultant Clinical Scientist within the National Health Service (NHS).

Aims: This 5-year work based program, underpinned by a part time doctorate, is managed and delivered by the National School of Healthcare Science (NSHCS) and funded by Health Education England (HEE).

Methods: The HSST training programs for pathology specialities and life sciences are implemented in conjunction with the Royal College of Pathologists with many available curricula including Haematology and Transfusion Science. Other allied curricula include clinical immunology, histocompatibility & immunogenetics, microbiology and virology together with an innovative new course in bio-informatics These programs entail a blend of training for essential skills required in senior scientific roles within the NHS either in hospitals or blood services, including leadership, innovation, research and higher specialist scientific and clinical knowledge. Trainees are required to gain Fellowship of the Royal college of Pathologists through specialist FRCPath examinations. The funded academic element entails a Professional Doctorate (DClinSci) and a Postgraduate Diploma (PgDIP) in Leadership and Management. In addition, trainees benefit from a 5-year training budget to support costs of attending conferences, professional examinations and research costs.

Results: There are 58 candidates enrolled on the Scientist Training Programme (**STP**) for Haematology and Transfusion Science that provides eligibility for entry to HSST training either for the Haematology or for Transfusion Science program. This number will hopefully increase further with release of an updated STP curriculum in 2022. The entry criteria have also been widened to increase eligibility for biomedical scientists to also apply for these HSST training posts. There are currently 11 trainees on the HSST Haematology program and a further 11 on the Transfusion Science program with training for many of the latter strongly supported by the UK Blood Services. The majority of HSST trainees are in service candidates with key NHS service delivery roles in their departments either within hospitals or the UK Blood services complementing the aims of the program with practical experience and opportunities to implement skills learnt whilst on the course.

Summary/Conclusions: The trainees on the initial HSST cohorts are now beginning to complete the program and move into Consultant Scientist roles taking on significant clinical and scientific responsibilities with scope for easing the burden in areas where significant

workforce gaps have been previously identified. During the COVID19 pandemic, HSST trainees have taken on further roles and responsibilities highlighting their adaptability and resilience which reflects the quality of their training and experience.

HSSTs in key pathology specialties are bringing high-level scientific expertise, research skills and leadership training relevant to the practice of Haematology and Transfusion Medicine with benefit to patients across the whole of the NHS. The relatively low number of posts at present suggest this is a highly under-recognised and under-utilised resource with potential for a blended medical and scientific consultant workforce as providing a flexible solution to staffing and recruitment issues.

P-155 | Transfusion indication completion for red cell requests in chronic transfusion recipients - Impact of CPOE

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Background: Restrictive transfusion protocols with a hemoglobin thresholds of 70 g/L aim to reduce risks associated with transfusions, conserve blood products and reduce costs. The use of Computerized Provider Order Entry (CPOE), Clinician Decision Support, and education have been shown to help providers adopt these strategies. However, these studies focused on acute rather than chronic transfusions with limited analysis on transfusion indications. As CTRs often lack a defined hemoglobin trigger value for transfusions, transfusion indication is important in determining transfusion need.

Aims: The aim of this study was to determine providers' compliance in completion of transfusion indications in ordering RBCs for CTRs as part of a quality improvement project. CTRs are defined as those receiving transfusion receiving a transfusion at least once every two months for six months or longer. Additional aims were to evaluate patient demographics, hemoglobin (Hb) trigger and education opportunities.

Methods: As part of a quality improvement exercise, extracts of all transfusions in 2020 following implementation of CPOE in November of 2019 were obtained from the electronic medical record. Patients who meet the definition of being CTRs were included in a chronic transfusion database, consisting of CTRs from 2011-2020. Transfusion indications are presented as a cascade in the CPOE. There were 2 types of blood orders: therapy plans allowing providers to select transfusion indications once and carried through subsequent transfusion events, while standard orders require providers to input transfusion indications each time. Hb trigger and demographics were evaluated and were compared to that of 2011, 2015 and 2019.

Results: 310 CTRs were identified in 2020, with 8866 RBC units transfused. More CTRs were identified in 2020 compared to 2011, 2015, and 2019 with 219, 212 and 221 respectively. The overall transfusion indication completion rate was 94%; therapy plans had a higher completion rate than standard orders (96% vs 88%, p<0.001). 14.8% (n=556) of total orders did not include transfusion indications. The average Hb trigger in 2020 was 77.2 g/L, compared to the average Hb trigger of 78.7 g/L in 2019, 81.4 g/L in 2015, and 81.7 g/L in 2011. The Hb trigger was higher in therapy plan orders compared to non-therapy plans (81.1 g/L and 75.0 g/L, respectively, p<0.001). The Hb trigger was 73.6 g/L for therapy plans excluding hemoglobinopathy versus 95.4 g/L in therapy plans for only hemoglobinopathy (both exchange and simple transfusion).

Summary/Conclusions: As CTRs lack a defined hemoglobin trigger value for transfusions, transfusion indication is important in determination of need of transfusion. Although 94% of CTRs had transfusion indication completed for RBC orders, therapy plans had a higher completion compliance compared to non-therapy plans. 14.8% orders did not have transfusion indications but clear rationale for the lack of indication was not clear in this order set. The Hb trigger following implementation of CPOE was lower than historical but still higher than restrictive thresholds for acute transfusion but that may be appropriate for chronically transfused outpatients. A need for ongoing training and education regarding appropriate ordering and thresholds were identified.

P-156 | Patient blood management in coronary artery bypass graft surgery by optimising timing of surgical intervention in patients taking antiplatelet medication-A study on platelet function based algorithm

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Background: Irreversible inhibition of platelet function associated with dual anti-platelet therapy (DAPT) carries a substantial risk of bleeding in patients undergoing CABG. Platelet function test like thromboelastography platelet mapping (TEG-PM) could be used to predict blood loss in CABG patients.

Aims: The objective was to validate a TEG-PM based algorithm in CABG cases and to evaluate whether TEG-PM assay could be used as a preoperative tool to reduce the transfusion requirement by determining timing of surgery in patients who were on DAPT.

Methods: It was a single center observational pilot study where prospective data were collected on 15 adult patients who were admitted for elective CABG and had PCI done within last one year. Each patient was receiving aspirin and clopidogrel in combination and implanted with a drug-eluting coronary stent. Blood samples were drawn after 72 hours stoppage of DAPT in each patient and TEG-PM was performed as a preoperative test. Citrated samples were used in presence of Kaolin+$CaCl_2$ and heparinized samples were used with Activator F (reptilase and factor XIIIa) for platelet mapping. The contribution of P2Y12 receptor or cyclooxygenase pathways was measured by the addition of the agonists, ADP or arachidonic acid (AA). The % of inhibition was calculated to each agonist by the formula:100- [(MA $_{ADP/AA}$ – MA $_{Fibrin}$) / (MA $_{Thrombin}$ – MA $_{Fibrin}$) X 100]. Eligible patients (n=15) were categorized into three groups depending on the % of inhibition. Patients who had <50% inhibition with

agonists (n=4) were operated within 24 hours of assay. Patients with >70% inhibition (n=8) with AA or ADP, surgery was deterred till 48 hours, and patients who had >50% but <70% inhibition (n=3) to any of the agonists were operated within 24 hours after preoperative prophylactic platelet transfusion. Perioperative and 24 hours postoperative transfusion requirement was recorded as end-point for each group and compared with the previously published data on CABG operated without TEG by "Datta, Indian J Hematol Blood Transfus, 2021". Continuous variables were expressed as mean ± SD. Student's *t*-test was applied for comparison of means and significance was established at $p < 0.05$.

Results: Patients operated on TEG-PM algorithm [Table 1] showed a significant reduction in utilization of blood components such as PRBC (1.73 versus 3.5 U; $p < 0.05$) and FFP (1.6 versus 3.8 U; $p < 0.05$) when compared with the data published by "Datta, Indian J Hematol Blood Transfus, 2021".

P-156 Table 1. Summary of all parameters and transfusion details.

	Mean ± SD	Median	IQR (Q1-Q3)
Age	61.2 ± 9.82	64	60-66.5
Baseline R (min)	5.17 ± 1.96	4.8	3.8-6.6
Baseline K (min)	1.72 ± 0.99	1.3	1.05-1.95
Baseline Alpha (degrees)	63.47 ± 11.98	63.6	52.2-74.6
Baseline MA (MA_{CK} in mm)	71.9 ± 7.71	73.9	67.05-78
AA (MA_{AA} in mm)	36.17 ± 18.73	39.5	18.85-53.2
% of inhibition AA	60.69 ± 30.14	53.4	34.1-91.7
ADP (MA_{ADP} in mm)	32.2 ± 19.53	24.5	17.55-47.9
% of inhibition ADP	67.54 ± 30.03	86.2	41.6-93.2
PRBC transfusion	1.73 ± 0.46	2	1.5-2
FFP transfusion	1.6 ± 1.18	2	0.5-2.5

Summary/Conclusions: Distribution of ADP inhibition was 67.54±30.03% and AA inhibition were 60.69±30.14%, showing a variable offset of the effect of both medications starting after 72 hours of cessation. Overall transfusion requirement was reduced by using TEG-PM algorithm. We conclude that preoperative platelet function testing to determine the timing of surgery by TEG-PM assay in DAPT treated patients may be a useful targeted therapeutic strategy to reduce transfusion requirement in CABG cases.

P-157 | Successful pregnancy in women with major thalassemia

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Background: Very few pregnancies are reported among patients with beta thalassemia major because they generally have a poor sexual development and may be at high obstetrical risk. The biggest challenge identified in a low-resource country is the coordination of multidisciplinary centers, and the management of regular transfusions. There is very few data in literature on the obstetric and gynecological problems in women affected with major clinical form Of thalassemia.

Aims: This case demonstrates that women with beta thalassemia major who are otherwise healthy should not be discouraged from trying to conceive, and could conclude a normal full-term pregnancy with the birth of a healthy child.

Methods: the clinical and biological findings of the patient are recorded, and a review of the literature was conducted to identify additional Case reports.

Results: We present the case of 30 year old Tunisian woman with thalassemia major, she was referred from thalassemia department at 13 week's gestation, she was receiving regular transfusions every 21 days, with matched, and leukoreduced PRBC, and chelation therapy consisted of FERRIPROX, although she suffered from hemochromatosis with hepatic iron overload.

During the pregnancy she required twice a month a blood transfusion, she received a total of 24 PRBC to maintain her hemoglobin above 9.5 g/dl, in addition, glycemic control and urine analysis revealed no abnormal deviation. Serological spot tests for (HIV) antibody, hepatitis B antigen (HBsAg), and hepatitis C virus (HCV) antibody were negative and Antiglobulin testing was done, and no antibody was detected. Ultrasonic assessments showed normal fetal growth of the fetus and the triple screening was correct. Besides she has never been hospitalized. She was delivered at term by cesarean section of female infant with normal growth and development, without necessitation of blood transfusion.

Summary/Conclusions: A close and interdisciplinary care by obstetricians and hematologists is very important, in fact, the preconceptionally consultation and the monitoring during a pregnancy, can be optimized especially in a country with limited resources.

P-158 | Intraoperative blood loss and blood transfusion requirement among liver transplant recipients. A national single center experience 2020

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Background: Liver Transplantation (LT) is a complicated surgical procedure with high risk for massive intraoperative blood loss due to pre-existing coagulopathy, portosystemic shunts with collateral circulations and splenomegaly. The transfusion service will direct most of their resources towards LT programmes with great impact on cost. The purpose of this study was to evaluate single center transfusion strategies and to identify the risk factors associated with the intraoperative blood loss and blood transfusion.

Aims: The purpose of this study was to evaluate single center transfusion strategies and to identify the risk factors associated with the intraoperative blood loss and blood transfusion.

Methods: The study includes 18 patients who underwent LT at Hospital Selayang between January 2020 and December 2020. Retrospective analysis of data included preoperative assessment of coagulopathy, intraoperative blood loss, blood component transfusion

Results: The mean age in the study group was 36.4 ± 12.68 years. The mean intraoperative blood loss was 4450 ± 1646 ml requiring 4.17 ± 3.3 Packed Red Blood Cell (PRBC) Units, 7.56 ± 5.5 platelet units, and 9.50 ± 6.0 fresh frozen plasma (FFP) units. The independent risk factor for High Blood Loss (HBL) group was lower preoperative platelet count and it is statistically significant (p = 0.024). The HBL group is associated with higher usage of PRBC (p = 0.024) and Platelet Units (p = 0.031) and it is statistically significant. The length of stay (LOS) in ICU averaging 8.6 ± 4.95 days and there is no significant differences comparing the HBL and LBL group (p = 0.552). The mortality < 90 days for all recipients was 22.2%.

Summary/Conclusions: The pre-operative platelet count for is the most important factor associated with HBL in LT procedure. Usage of PRBC and Platelet units were statistically higher in the HBL group. Comparing HBL and LBL patients, there is no difference in terms of the LOS in ICU post-operatively. A larger sample size would be needed in view of relatively small sample size.

P-159 | Platelets transfusion has no benefit in dengue fever-related thrombocytopenia

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Background: Thrombocytopenia is a common biological sign in dengue fever. In some severe cases, bleeding can occurs, leading sometimes to platelet transfusion. However, the value of platelet transfusion is controversial.

Aims: Our study aimed to assess thrombocytopenia, bleeding and platelets transfusion in dengue virus infection during an outbreak in Burkina Faso.

Methods: We included cases of dengue fever (clinical signs associated to a positive test to AgNS1 and/or anti-DENV antibody IgM) admitted in seven health facilities in a cross-sectional study. We consider thrombocytopenia, bleeding and platelets transfusion as dependant variables. All statistic tests were performed at a significance level of 5%.

Results: A total of 296 patients were included, comprising 154 males (52%). The frequency of bleeding was 25%, with no gender difference (p=0.347). Elderly patients (over 55 years) were less likely to bleed (OR = 0.10, CI 95% [0.02-0.63]; p = 0.014), but were more transfused (OR = 8.96, CI 95% [1.07-74.90]; p = 0.043) than younger ones. There was more bleeding patients with moderate thrombocytopenia (OR = 6.84, CI 95% [2.72-17.25]; p = 0.001) and severe thrombocytopenia (OR = 6.66, CI 95% [2.36-18.86]; p = 0.008). The median in-hospital length of stay was significantly long (median = 5 days [IQR: 4 – 6.5]) in transfused patients compared to non-transfused ones (median = 4 [IQR: 2 – 5]) days (p = 0.0001). At exit, the median increase in platelet count in transfused patients was 27,000/mm^3 versus 0/mm^3 in non-transfused patients (p = 0.220) and the mortality rate the two groups was 0% versus 1.2%; p = 0.42.

Summary/Conclusions: There was no significant increase in platelet count in transfused and not transfused patients, meaning that platelet transfusion had no longer benefit in reduction of clinical or severe bleeding or improvement in platelet count recovery. Further studies are expected to better explore this issue.

Clinical transfusion – Haemorrhage and massive transfusion

P-160 | Regression analysis to predict the factor VIII activity of hemophilia A patients without inhibitor who received emicizumab therapy

selected for main programme

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Background: Hemophilia A (HA) is an X-linked hereditary bleeding disorder caused by deficiency of coagulation factor (F) VIII activity. Emicizumab, a novel, bispecific humanized monoclonal antibody, has been approved as a new treatment option for HA patients, providing an equivalent FVIII activity of at most 15%. It is necessary to supplement the FVIII concentrates at the time of bleeding. However, emicizumab has strong pharmacodynamic effects on activated partial thromboplastin time (APTT)-based assay and chromogenic FVIII assays using human FX/FIXa, and can lead to misleading outcomes of coagulation assays in emicizumab-treated patients resulting in dosing difficulties. Two neutralizing anti-idiotype antibodies for emicizumab were developed to cancel its interference although their availability was limited.

Aims: We tried to create a regression equation for predicting the FVIII activity for HA patients without inhibitor who received emicizumab therapy.

Methods: Using archived 27 plasma samples from three HA patients without inhibitor who received emicizumab therapy, APTT, Ad|min1|, and Ad|min2|, were measured (Thrombocheck APTT-SLA, Sysmex). Then FVIII one-stage assay (OSA) (FVIII deficient plasma, SIEMENS) and chromogenic FVIII assay (CSA) using human FX/FIXa (Revohem FVIII chromogenic, Sysmex) were performed. These measured values were analyzed by both simple linear and multiple regression models to predict FVIII:C measured by OSA after

treatment with neutralizing antibody (Anti-emicizumab idiotype antibodies, Chugai Pharmaceutical). Since FVIII OSA exceeded the upper limit of measurement in many samples, the number of seconds in APTT after mixing patient plasma and FVIII-deficient plasma was used. In the multiple regression model, variables were selected based on the Akaike Information Criterion (AIC) using the stepwise method with both directions, and the significance level of each variable was set to 0.05. This study was approved by the Ethics Committee at Tottori University Faculty of Medicine (approval number: 19A056).

Results: In the simple linear model, Ad|min1|, Ad|min2|, FVIII OSA and FVIII CSA were significant variables, among which FVIII CSA was the most significant: the regression coefficient was 1.04 and the intercept was −14.55; the adjusted R-squared was 0.95 and $p < 0.001$. The absolute value of the intercept appeared to represent the equivalent FVIII activity of emicizumab. In the multiple regression model, FVIII OSA and FVIII CSA were selected as explanatory variables based on AIC using the stepwise method with both directions. The regression coefficient estimates of FVIII OSA and FVIIICSA were 1.74 and 1.15, respectively, and the intercept of this model was −92.03. This model had the smallest AIC and fitted well (adjusted R-squared 0.96, $p < 0.001$). Variance inflation factor of FVIII OSA and FVIII CSA were 2.05 and 2.05, respectively.

Summary/Conclusions: The simple linear model with FVIII CSA can well estimate FVIII:C after administration of FVIII concentrates in HA patients under emicizumab treatment. The addition of FVIII OSA just improved slightly the estimation.

P-161 | Audit on the use of fibrinogen concentrate within ICHNT major haemorrhage protocols

selected for main programme

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Background: Coagulopathy in major haemorrhage has different causes including dilutional coagulopathy, acute traumatic coagulopathy, hypothermia, acidosis, and hypocalcaemia. Management of this coagulopathy involves transfusion of platelets, FFP and typically cryoprecipitate. However, increasingly, fibrinogen concentrate has been used as an alternative to cryoprecipitate. Proposed advantages of fibrinogen concentrate include the standardised concentration of fibrinogen, viral inactivation, reconstitution within 10 minutes and not requiring consideration of blood group compatibility. Disadvantages however, include cost (more expensive than cryoprecipitate for comparable dose of fibrinogen), risk of thromboembolic events and current lack of evidence to support its use in preference to cryoprecipitate for acquired hypo/dysfibrinogenaemia. Currently, fibrinogen concentrate is only licensed in the U.K. for treatment of haemorrhage in congenital hypo/afibrinogenaemia. Nevertheless, it is used off-license for the treatment of acquired hypo/dysfibrinogenaemia. At Imperial College Healthcare NHS Trust (ICHNT), our major haemorrhage protocols initially recommend cryoprecipitate. However, after 10 units (or 80mls/kg if paediatric patients) of red blood cells (RBC) have been transfused, our protocol recommends considering fibrinogen concentrate (50mg/kg).

Aims: To review the use of fibrinogen concentrate as per current ICHNT major haemorrhage protocols (adult, obstetric, paediatric) that were updated in May 2019 to include fibrinogen concentrate.

Methods: Lists of all major haemorrhage calls activated between 1/7/2019 and 31/7/2020 were obtained from logs held by the blood transfusion laboratories. For each patient, data was collected from Telepath and Cerner.

Results: 457 major haemorrhage calls were activated between 1/7/2019 – 31/7/2020. In 9 cases, fibrinogen concentrate was given, but in 3/9 cases fibrinogen concentrate was given prior to transfusion of 10 units or 80mls/kg or more RBCs. There were 44 cases where 10 units or 80mls/kg or more RBCs were transfused but no fibrinogen was given. While fibrinogen concentrate may not have been appropriate in all these cases, it is unclear whether a conscious decision was made not to give, rather than simply failing to consider this treatment option. There was only one case of a possible, but not definite, adverse event related to fibrinogen concentrate; the affected patient had other risk factors for thromboembolic disease (extensive traumatic injuries) and the DVT was diagnosed 26 days after fibrinogen concentrate was given.

Summary/Conclusions: There is a discrepancy between the total number of cases where fibrinogen concentrate could have been indicated (n=53; 12%) and the number of cases in which fibrinogen was actually used (n=9; 2%). Furthermore, in a third of cases where fibrinogen concentrate was used, it was not given in accordance with current protocols. Possible explanations for failing to adhere to current protocols could be a lack of awareness of these recommendations, and also unfamiliarity in using fibrinogen concentrate given its relatively new addition to our protocols. In view of this, we plan further education of all staff involved in the management of major haemorrhage, specifically to highlight the role of fibrinogen concentrate in this context.

P-162 | The role of the transfusion coordinator in a major incident

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Background: The Emergency Planning Working Group (EPWG) is a multi-disciplinary working group for the National Blood Transfusion Committee (NBTC) for NHS England, with a remit to provide hospitals with guidance for transfusion emergency preparedness and response to Major Incidents and Mass Casualty Events (MCE) in consultation with a range of stakeholders (Doughty, Transfusion Medicine, 2020).

Transfusion risks during MCEs include delays in delivery, procedural errors, and inappropriate use of resources. We have identified that the informal development of a "Transfusion Coordinator" improved liaison and service delivery between clinical and laboratory areas. We propose the emerging role requires formalisation, tools, and training.

Aims: To define the role of the Transfusion Coordinator and develop an action card template that can be adapted for local use.

Methods: The EPWG collated experiences from their own networks during recent events to define the role. Other sources of information included a literature search, results from the UK haemovigilance organisation and developments in triage systems.

A subgroup working with the wider Transfusion Practitioners network drafted an Action Card which was then socialised within regional education days. The action card was then revised before ratification and posting as a tool on the website.

Results: The role summary was defined as: *To co-ordinate prompt and safe transfusion support during a Major Incident*. The role is designed to support liaison between clinical and laboratory areas and includes emergency blood issue, regulatory compliance, blood sample handling, blood collection and appropriate use of blood. Staff should be trained and rehearsed for their role as part of the wider hospital response.

The post-holder should be familiar with transfusion systems including specimen preparation, handling and use of blood components, and feel comfortable in the emergency clinical departments. The post holder in the UK is most likely to be a Transfusion Practitioner (TP) from either a Nursing or Biomedical Scientist background. Alternative arrangements may be needed especially outside of core hours.

The action card is an Aide Memoire for the Transfusion Co-ordinator. It was designed as a single page divided into three sections in chronological order. The information can be presented either as text or flow diagrams.

- **Incident Standby *The pre-planning phase*** Establish potential casualty load and implications for demand, hospital plan for planned procedures and implications for stock management and staffing.
- **Incident Declared *Response phase*** Report to the ED. Set up a "blood station" to issue blood and ensure traceability. Support clinical staff through transfusion triage and sample handling.
- **Incident Stand down *The closure of emergency activity with return to normal*** Return stock to laboratory, complete traceability and cold chain records, and incident report. Attend debriefs to acknowledge success and areas for service improvement.

The final document is available at https://www.transfusionguidelines.org/uk-transfusion-committees/national-blood-transfusion-committee/working-groups#Emergency.

Summary/Conclusions: A designated Transfusion Coordinator improves communication and co-ordination between the clinical and laboratory areas during MCEs. We have defined the novel role and present an example of an Action Card.

P-163 | Empirical versus ROTEM guided use of blood products and hemostatic agents during massive transfusions

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Background: Massive bleeding (MB) is associated with coagulopathy and high mortality. Currently, there are diverse approaches to the management of MB, depending on the cause and the transfusion packages, which include red blood cells (RBC), fresh frozen plasma (FFP) and platelet concentrates (PC). The use of hemostatic agents like fibrinogen concentrate (FC), tranexamic acid and prothrombin complex concentrate (PCC) has been shown to reduce mortality.

The use of viscoelastic tests such as ROTEM (Rotational thrombelastometry) is recommended in this setting, since they can help detect, control and orientate the treatment of coagulopathy associated to acute hemorrhage.

Our MB protocol utilizes a ratio of 4RBC:4FFP:1PC in transfusion packages. Our hospital administrates approx. 12000 blood products/year and since 2018 the anesthetics team is using ROTEM in the management of MB.

Aims: This study aimed to determine the use of blood components and hemostatic agents during MB in an empirical versus ROTEM guided setup.

Methods: We performed a retrospective review of massive transfusion requests and patients charts and separated them in 2 groups: empirical coagulopathy management (from January 2016 to December 2017) and ROTEM guided management (from January 2019 to December 2020). Patient demographics, indications and laboratory records were retrieved from the hospital system and analyzed.

Results: During this period, a total of 135 MB were detected, with an average of 33/year.

The 2 groups were analyzed separately and the demographic results are shown in Table 1. MB was associated with surgical interventions, cirrhotic complications, trauma and obstetric bleeding. We analyzed the outcome of these cases within 24 hours from the MB event.

P-163 Table 1. Demographics - CCP was used in 6 patients in the empirical management group and in 8 patients in the ROTEM guided group.

	Number of MB	Sex	Age	Surgical interventions	Cirrhotic complications	Trauma	Obstetric	Death within 24h
Empirical management (2016-2017)	65	43 males 22 females	57.81y (22-88)	41	6	13	5	14
ROTEM guided (2019-2020)	70	36 males 34 females	58.72y (21-88)	41	16	11	2	14

Summary/Conclusions: The optimal way to resuscitate patients with MB remains unclear, and clinical trials are difficult to perform in this setting. There is controversial data regarding the use of ROTEM in the management of MB but in our study we have noticed a slice decrease in the use of blood products since we have started using it and accordingly an increase in the use of hemostatic agents which led us to conclude that we need to adjust our internal MB protocol accordingly.

P-164 | Audit of massive transfusions protocol in a secondary hospital from Spain

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Background: Massive bleeding (MB) is associated with coagulopathy and high mortality. It is important that every hospital has a massive transfusion protocol (MTP) adapted to its level and the type of MB cases it attends.

The main difference between MTPs is in the number of blood products in the transfusion packages (red blood cells (RBC), fresh frozen plasma (FFP) and platelet concentrates (PC)). There is however important evidence for the use of hemostatic agents like fibrinogen concentrate (FC), tranexamic acid and prothrombin complex concentrate (PCC) since their use can reduce mortality.

Our MTP utilizes a ratio of 4RBC:4FFP:1PC in the transfusion packages. Our hospital administrates approx. 12000 blood products/year. Since 2018 the anesthetics team is using ROTEM in the management of MB.

Aims: This study aimed to determine the use of blood components and hemostatic agents during MB in our hospital in the past 5 years as well as to establish if the protocol was activated correctly.

Methods: We performed a retrospective review of massive transfusion requests and patients charts from January 2016 to December 2020. Patient demographics, indications and laboratory records were retrieved from the hospital system and analyzed.

ROTEM guided transfusion therapy was performed by the team of anesthesiologists managing the patient when considered necessary since 2018.

Results: During this period, a total of 173 MB were detected, with an average of 34/year. The medium age for these events was 57.42y (range of 18-88y) and included 71 females and 102 males. MB were associated with surgery (98), cirrhotic complications (33), trauma (33), and obstetric bleeding (9).The blood products used were 1395 RBC (average 8.06/patient) and 264 PC (average 1.52/patient). Of the 810 FFP units (average 4.68/patient) that were thawed, 681 (average 3.95/patient) were used. Of the remaining units 47 were used for other patients while 72 were discarded, resulting in a significant loss of blood products.

MTP was activated only in 101 cases (58.38%). The ratio of blood products used in massive transfusions was different from the established one and in was 5.28 RBC: 2.57 FFP: 1 PC.

Summary/Conclusions: We have detected that in our hospital exists a great variability in the management of massive bleeding and MTP activations.

We believe that MTP should function as defined by the Transfusion commission of the hospital, as a consensus-based interdisciplinary protocol. In our hospital the ratio of blood components used in the massive transfusions was different from the one established in the MTP which led us to change this protocol to a more realistic one and include only RBC and hemostatic agents in the initial transfusion pack and a ratio of 4RBC:2FFP:1PC in posterior transfusion packs. Modifying the MTP to emergency red cells first may reduce the over activations and better use of blood and blood products.

P-164 Table 1. We analyzed the use of blood products and hemostatic agents (tranexamic agent, FC and CCP) for each group separately.

		RBC	PC	Thawed FFP	Used FFP	Tranexamic acid (gr)	Fibrinogen concentrates (gr)
Empirical management (2016-2017)	Total units	545	95	300	270	30	87
	Average/patient	8.38	1.46	4.61	4.15	0.48	1.33
ROTEM guided (2019-2020)	Total units	544	95	304	245	59	120
	Average/patient	7.77	1.35	4.34	3.5	1.05	2.14

P-165 | Massive transfusion: Contents of the first labile blood product prescription

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Background: Massive transfusion is a life-saving therapy. In addition to red blood cells (RBC) transfusion, the immediate support of coagulation is crucial to maintain effective hemostasis.
Aims: The aim of our study was to analyze the contents of the first prescription of labile blood products (LBP) in the context of massive hemorrhage.
Methods: We conducted an observational, survey-based study. The survey was sent as a Google Forms link to doctors who had to deal with transfusion emergencies due to major hemorrhage.
Results: A total of 124 physicians, including 62% emergency and resuscitation specialists, responded to the questionnaire. More than two thirds (N=97, 78.2%) of the participants ordered fresh frozen plasma (FFP) from the initial command: The majority (N=69; 55.6%) asked only for red blood cell units (RBC) and FFP. 22.6% transfused platelets in addition to RBC and FFP. More than half of the participants (58%) prescribed two RBC units in the original order. For the ABO blood type of the requested RBC units, 77 physicians transfused in isogroup. In case of unknown ABO-blood type, 52 physicians had refrained from responding; while 70 transfused by O RH-1 and this regardless of the patient's gender.
54% of participants transfused FFP concurrently with RBC. 119 participants answered the question about the FFP: RBC ratio. Fifty-eight physicians (48.8%) advocated a high ratio (>1/2), while 61 (51%) opted for an initial ratio of 1/2.
Summary/Conclusions: Different blood transfusion strategies are adopted to manage massive hemorrhage. The content of the first LBP prescription varies in terms of nature, number of LBP and ratios used. A tight collaboration between resuscitation specialists and haemoglobologists is needed to define the best transfusion strategy.

Clinical transfusion – Haemovigilance and transfusion safety

P-166 | Audit of transfusion practice: Tunisian experience

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Background: Transfusion medicine requires not only theoretical and practical knowledge but also a considerable part of ethics. In Tunisia, the technical circuit of blood products is well organized and documented at the level of the Blood banks and blood transfusion departments, but gaps have been identified at the level of care units.
Aims: The purpose of this study was to evaluate certain transfusion practices in Tunisian clinicians.
Methods: A four-month prospective survey was conducted via an anonymous form based on the questionnaire (A), designed by the working group of the Réseau d'Experts d'Hémovigilance Aquitaine et Limousin «REHAL». Our questionnaire included 12 questions on the pre-transfusion and post-transfusion information provided to patients, as well as the fate of unused labile blood products (LBPs). The form was administered by e-mail to physicians involved in the transfusion.
Results: 158 clinicians completed the survey, mostly from the public sector, and 2/3 of them were from a medical specialty. Most participants (88%) reported that patients did not receive pre-transfusion information and that only half of respondents ensured that oral information was provided to the patient. In contrast, only 7% reported that they "always" share information about alternative therapies with patients. In addition, 86.7% reported that they have no document on the patient's post-transfusion information. However, in terms of blood product management, 46.2% of responses indicated a lack of a system to analyze the non-use causes of LBPs.
Summary/Conclusions: This survey identified areas where improvement measures are essential such as development and dissemination of pre-transfusion and post-transfusion information materials.

P-167 | Incidence, patterns and associated factors of acute reactions to blood transfusion in a tertiary hospital in Cameroon

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Background: Blood transfusion is an effective and lifesaving way of correcting hematological defects, but adverse effects which include transfusion reactions do occur during or after transfusion. The shift from infectious to non-infectious transfusion-related morbidity and mortality is a cause of concern. Developing powerful hemovigilance systems in areas with limited supply of blood products ensures

optimal and safe use of these blood products and thereby improving the patients' health outcome.

Aims: Thus, knowledge about the incidence, factors associated with these adverse reactions and clinical manifestations would improve our national hemovigilance protocols.

Methods: A prospective observational follow-up of 417 transfusion events among 212 patients was done at the Douala general hospital for 3months. The 3R (recognise, react and report) approach was used throughout the study wherein diagnoses of reactions were made using clinical signs and symptoms as described by the International Society of Blood Transfusion (ISBT). They were further confirmed by a physician who instituted the management. The associated factors were sort via history and assessment of blood cold chain. The data analysis was done using Stata IC version 13 and *P*-values ≤ 0.05 were regarded as significant.

Results: 63.7% of participants were female with ages ranging from 2 to 98 year-old. The mean hemoglobin level of transfusion was 6.4 ±1.6g/dL with whole blood(69.8%) being the most frequent transfused blood product. A total of 17 (4.1%) Acute Transfusion Reactions (ATRs) were recorded ranging from mild to moderate, with no case of severe reaction. These reactions occurred more with red blood cells (64.7%) than whole blood (29.4%). Allergic reactions (35.3%), was the most common encountered followed by febrile non-hemolytic transfusion reaction (23.5%) volume overload (17.6%), isolated hypotension (11.8%) and unclassifiable reaction (11.8%). The female gender ($p = 0.026$) was independently associated with ATRs in contrast with a history of transfusion. There was no significant difference between transfusion of stored blood(> 5days) and fresh blood(<5 days) on the incidences observed. All reactions had a favourable outcome, that is, symptoms were totally resolved after management without any extra hospital days required, no permanent disability was left and no death was encountered.

Summary/Conclusions: ATRs were not rare, as 4 out of every 100 transfusions resulted in an ATR. Transfusing females of reproductive age should be scrupulously monitored along the transfusion chain to lower the incidence of these reactions. Though no severe reaction was encountered, haemovigilance systems have to be intensified to limit as much as possible the number of reactions.

P-168 | Analysis of nonconformities at the institute of transfusion medicine of the Republic of North Macedonia

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Background: Quality management system (QMS) has a key role in ensuring the quality, safety and efficiency of blood components. With the introduction of the QMS, the Institute for Transfusion Medicine (ITM) of the Republic of North Macedonia strives for consistent implementation of nonconformities management in all its activities.

Aims: To analyze the reasons for the reported nonconformities, their frequency and seriousness, in the Quality Assurance and Quality Control (QAQC) Department at the Institute for Transfusion Medicine of the Republic of N.Macedonia during 2020.

Methods: This is a retrospective analysis of the data from the register of nonconformities in the QAQC Department at the ITM of the Republic of N. Macedonia during 2020.

Results: Total 169 nonconformities were reported in 2020 for various reasons (0.4% of the total number - 42983 produced red blood cell concentrates). The most common nonconformity 45 (26.6%) is due to a small volume of collected whole blood, 27 (15.9%) are due to a damaged bag of FFP, 14 (8.3%) due to incorrect registration of the blood donation in e-Delphyn, 12 (7.1%) – expired FFP, 10 (5.9%) – Covid19 positive blood donors, 8 (4.7%) - bloody FFP, 8 (4.7%) - wrong bar code on the blood bag, blood donor card or test tube), 7 (4.1%) - incomplete documentation of whole blood (missing blood donor card, bar code or test tube), 7 (4.1%) - inadequately sealed bag tubes after donation. Other reported nonconformities for various reasons are in very small numbers. Compared with the reported nonconformities in the previous years (2015-37, 2016-29, 2017-31, 2018-20, 2019-60, 2020-169), there is a significant growth trend, which is due to the increased activity and commitment of the QAQC Department at the ITM of the Republic of Macedonia in the consistent implementation of the Quality Management System.

Summary/Conclusions: There is a great variety in the reasons for the reported nonconformities. The most frequently reported nonconformities during 2020 are in the process of blood donation and production of blood components in ITM, and are a consequence of human error. This situation indicates and imposes the need for retraining and strict operation according to the existing Standard Operating Procedures. Greater engagement and activity of the Hospital Transfusion Committees and their cooperation with the QAQC Department is also necessary.

P-169 | Haemovigilance practice in Northern Zone-Tanzania from October 2018 to September 2020

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Background: Haemovigilance is set of surveillance processes involving monitoring, reporting, investigating and analysis of adverse events related to the donation, processing and transfusion of blood in order to improve quality and safety. Global Data Base System reported that 70/180 countries (39%) had a national Haemovigilance system. Africa had 12/42 countries (26%) with Haemovigilance system by 2013, whereby in Tanzania it was introduced in 2018. In Northern Zone-Tanzania, 2 hospitals and then 3 hospitals were enrolled in

Haemovigilance for phase one and two respectively. The selected hospitals send monthly Haemovigilance reports to the Northern Zone Blood Transfusion Centre.

Aims: To analyze haemovigilance data on the reporting practice including reporting pattern and transfusion adverse reactions.

Methods: It is descriptive cross-sectional study that analyzed the Haemovigilance data received from the five hospitals enrolled in Haemovigilance system from October 2018 to September 2020.

Results: Manyara Hospital and Arusha Lutheran Medical Centre (ALMC) send the reports consistently (24/24 and 15/15 months respectively) (100%), Mawenzi Hospital (17/24 months) (70.8%), Kilimanjaro Christian Medical Centre (KCMC) (5/15 months) (33.3%), and Tanga Hospital (10/15 months) (66.7%). During this period, a total of 7832 donated blood in these facilities, of which 43(0.5%) experienced donation adverse reaction, (4 from Tanga and 39 from ALMC). A total of 10242 patients received blood, of which 9447 (92.2%) consented to be transfused. All patients who did not consent were from ALMC. Cross match was performed before transfusion for all patients. A total of 14(0.14%) transfusion adverse reactions were reported (3 from ALMC, 3 from Tanga, 4 from Manyara and 4 from KCMC). Of the 14 reactions reported, 10 adverse reactions did not have event notification forms except 4 for Manyara. No adverse event/reaction has ever been reported by Mawenzi Hospital. No incidence, near miss, donor and recipients triggered look back event has ever been reported.

Summary/Conclusions: Haemovigilance reporting practice in Northern Zone is still inconsistent, with incomplete documentation, leading to inadequate details on the types, causes and severity of adverse events and who are the most affected population. We recommend further training to improve reporting behavior. We also recommend enrolling more facilities in Haemovigilance system to improve quality and safety of donors and patients.

P-170 | Preliminary assessment of the possibility of using universal pathogen reduction

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Background: Pathogen reduction technology is actively used in routine clinical practice to ensure the safety of transfusions. Moreover, the use of pathogen-reduced technologies makes it possible to increase the shelf life of pathogen-reduced platelets up to 7 days, as well as to release pathogen-reduced plasma immediately without having to quarantine it until the donor returns and is screened another time for HBV/HCV/ HIV and syphilis, as it is required for quarantine plasma.

Aims: Retrospective review of patients' case histories, who participated in the study of pathogen-reduced red blood cell suspension transfusions.

Methods: After the initial publication (Trakhtman, Vox.Sang 2019), we carried out an additional retrospective analysis of the patients' case histories who participating in the study and found, that two patients received exclusively pathogen-reduced blood products, i.e. fresh frozen plasma, red blood cell and platelet concentrates during one interval between chemotherapy courses (FFP and RBC at a dose 10-15ml/kg for each transfusion; PLT 5ml/kg for each transfusion). All blood products have been processed by pathogen reduction technology that acts on the combined action of riboflavin and ultraviolet light. Patient characteristics and the number of transfusions during the interval between chemotherapy courses are presented in Table 1.

Results: The mean duration of the transfusion-dependent period (the number of days from the first to the last transfusion) in these patients was 4±1 (3-5) days, while the mean interval between chemotherapy courses were 24.5±5 (21-28) days. All transfusions were effective (Table 2) and patients did not have any transfusion adverse effects.

Summary/Conclusions: We believe this approach for transfusion support of patients can be used in clinical practice. However, clinical trials are needed to determine the effectiveness and safety of this approach. We plan to conduct a clinical study of pathogen-reduced blood products transfusion versus standard transfusion practice in children with oncological and malignant hematological diseases to determine the clinical and cost-effectiveness of this approach.

P-170 Table 1. Characteristics of patients and transfusions.

	Gender	Age, years	Diagnosis	Number of RBC transfusions	Number of PLT transfusions	Number of plasma transfusions	Transfusion-dependent period duration, days	Interval between chemotherapy courses
Patient 1	male	1	Nephroblastoma	1	3	2	5	28
Patient 2	male	4	Rhabdoid tumor	1	3	1	7	21

P-170 Table 2. Transfusions efficacy.

RBC transfusions efficacy						
	Hb before transfusion, g\L	Hb after transfusion, g\L	Hb increment, g\L	Ht before transfusion,%	Ht after transfusions,%	Ht increment, %
Patient 1	80	93	13	22.7	26.3	3.6
Patient 2	77	104	27	22.2	29.5	7.3
PLT transfusions efficacy						
	PLT before transfusion		PLT after transfusions		PLT increment	
Patient 1	5; 11; 27		34; 27; 49		22.3±6.5	
Patient 2	17; 20; 15		45; 47; 54		31.3±6.6	
Plasma transfusions efficacy						
	Fbn before transfusion, g/L	Fbn after transfusion, g/L	Fbn increment,g/L	PT before transfusion, sec	PT after transfusion, sec	Decrease in PT, sec
Patient 1	1; 1,15;	2,15; 1,9;	0,95±0.3	22; 18,2;	18,6; 16,1;	2.75±1
Patient 2	1,5	2,5	1	15	13,8	1,2
	TT before transfusion, sec	TT after transfusions, sec	Decrease in TT, sec	aPTT before transfusion, sec	aPTT after transfusions, sec	Decrease in aPTT, sec
Patient 1	36,1; 31,2;	31,2; 30,1	3±2,7	47,7; 42,6;	42,6; 35;	6.25±1.6
Patient 2	27,6	25,6	2	38,5	37,5	1

aPTT, activated partial thromboplastin time; Fbn, fibrinogen; Hb, hemoglobin; Ht, hematocrit; PLT, platelets; PT, prothrombin time; RBC, red blood cells; TT, thrombin time.

P-171 | Bacterial contamination in low-titre group O whole blood

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Background: At our blood centre, we supply our air ambulance service, which is located 155 km (96 miles) from our production facility, with two units of low-titre group O whole blood (LTOWB). The quality control for measuring residual white cells in the product is sent to an external university hospital in our region. Due to logistical reasons and short shelf life, the product is distributed before the results are known. If the quality control does not fulfil criteria, we are contacted by telephone to evaluate possible recall of the product.

Aims: To evaluate and present an interesting case of non-conformity

Methods: Evaluation was done by sending the whole blood bag to the department of microbiology for aerobic and anaerobic culture, rechecking the donor questionnaire and routine lab results as well as transport temperature conditions.

Results: We were notified on day two about a possible increased leucocytes in the product, due to rejection of the technical validation, because of haemolysis and suspicion of bacteria in the sample. The product was recalled according to procedures. Temperature control showed no deviation during transport (between 2 and 6 degrees Celsius). Hence, the growth condition for bacteria was rightfully poor. Culture results revealed beta haemolytic Streptococcus group C, identified as Streptococcus dysgalactiae subspecies equisimilis in 1 out of 2 aerobic culture. The anaerobic culture was negative. The donor was a 54-year-old group O negative Caucasian male who met all donor acceptability criteria. His donation history included 70 donations in total including seven LTOWB. Pre-donation haemoglobin level was 13.8 g/dL, ferritin 51 µg/l, Platelet count 243 x10^9 /L, White blood cell count 6.85 x 10^9/L on the actual donation. One month after the donation he reported that he remained well and confirmed the history recorded at the time of donation.

Summary/Conclusions: Contamination from skin was unlikely. A possible explanation for the donor's bacteraemia could be transfer of bacteria from the oral cavity via breach in the oral mucous membrane caused by periodontitis. Beta haemolytic Streptococcus

group C can cause a chronic carrier state. The donor was recommended to see a physician to determine if he indeed is a chronic carrier. He was also recommended to see a dentist to sanitize his periodontitis. He has been deferred from further donations. To our knowledge, there have been no other reports on bacterial contamination of LTOWB. This case report demonstrates the importance of a thorough evaluation and the need to evaluate bacterial contamination in LTOWB.

P-172 | Haemovigilance system in the institute for transfusion medicine – 14 years Macedonian experience

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Background: One of the most important parts of the haemovigilance program is to improve reporting of transfusion related adverse events, assessing that information and providing data in order to improve the blood transfusion process.

Aims: Aim of this study was to analyze the adverse transfusion reactions that were reported to the Quality Assurance and Quality Control (QAQC) Department of the Institute for Transfusion Medicine of Republic of North Macedonia – Skopje (ITM).

Methods: retrospective analysis of the reported adverse reactions to the QAQC department in the period 2007-2020 from its monthly and yearly registries.

Results: The most frequent adverse events that were reported were mild allergic and febrile non-haemolytical transfusion reactions with urticarial rash, fever and vomiting. In 2007 were 2 reported adverse transfusion reactions (Cryoprecipitate and Fresh Frozen Plasma (FFP)), in 2008- 1(FFP), in 2009 – 1, in 2010 -1(FFP), 2011 – 3 (2 (RBC-SAG and 1 F.VIII conc.), in 2012 - 1 (Cryoprecipitate), in 2013- 2 (RBC-SAG and Cryoprecipitate), in 2014 – 1 (Cryoprecipitate), in 2015 – 1 (FFP), in 2018 - 4 (1-FFP and 3-RBC-SAG), in 2019 – 3 (FFP) and in 2020 - 4 (1-Platetelet concentrate, 3 RBC-SAG). There was no mortality associated with blood transfusion in the last 14 years.

Summary/Conclusions: The hospital transfusion committees as a link between clinical and transfusion medicine staff and an active surveillance program have a key role in enhancing patient safety by making changes to prevent reoccurrence and management of adverse reactions to blood transfusion. Analysis of the reports for the blood components use and adverse transfusion reactions will help us to focus on safe transfusion and upgrade of the legislative with a by-law for haemovigilance, with obligatory registration and report to the Institute of Transfusion Medicine of the outcome of every single transfusion of blood components and consequent functioning of the inspection system.

Donors and Blood Supply – Blood supply management and utilization

P-173 | Preventing alloimmunization using a new model for matching extensively typed red blood cells

selected for main programme

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Background: Alloimmunization is a well-known adverse event associated with red blood cell (RBC) transfusions, caused by phenotype incompatibilities between donor and patient RBCs that may lead to hemolytic transfusion reactions on subsequent transfusions. Alloimmunization can be prevented by transfusing fully compatible RBC units. Advances in RBC genotyping render the extensive typing of both donors and patients affordable in the foreseeable future. However, the exponential increase in the variety of extensively typed RBCs asks for a software-driven selection to determine the "best choice product for a given patient".

Aims: In this study we present a novel flexible issuing strategy that can be used to assign RBC units to patients. The aim of this issuing strategy is to provide all patients with suitable RBC units without introducing any additional shortages or outdating of RBCs.

Methods: We propose the MINRAR model for matching extensively typed RBC units to extensively typed patients to minimize the risk of alloimmunizations. The key idea behind this model is to use antigen immunogenicity to represent the clinical implication of a mismatch, which can then be used to determine the algorithm's penalty for mismatching on a particular antigen. Using simulations of Caucasian donor and patient populations, the MINRAR model is compared to a baseline model from the literature named FIFO/MROL ABOD, which matches antigens A, B and RhD only.

Results: Our simulations show that with the MINRAR model, even for very small inventories, the expected number of alloimmunizations can be reduced by 70% compared to a policy of only matching on antigens A, B and RhD. When matching is organized at a centralized level, a reduction of 93.7% can be realized. Note that this reduction is

P-173 Table 1.

Average number of RBCs demanded per day (inventory size)	FIFO/ MROL ABOD	MINRAR	Reduction of alloimmunization
25 (125)	3.135	0.680	78.3%
50 (250)	3.085	0.560	81.8%
100 (500)	3.115	0.415	86.7%
200 (1000)	3.110	0.310	90.0%
500 (2500)	3.110	0.195	93.7%

achieved without an increase in shortages or outdating. Furthermore, these reductions are concentrated on the more immunogenic antigens such as K, E and Jk[a], in line with the aim of the MINRAR issuing strategy which is to minimize the overall risk of alloimmunization.

Approximated expected number of alloimmunizations per 1000 transfused units using the FIFO/MROL ABOD issuing strategy and the MINRAR strategy for five different sized inventories.

Summary/Conclusions: Despite an exponential increase in phenotype variety, matching of extensively typed RBCs can be effectively implemented using the MINRAR model, effectuating a large reduction in alloimmunization risk without introducing additional outdating or shortages.

P-174 | Designing and testing an ethnic ancestry question for donors: Acceptability, feasibility, and understanding

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Background: Information on donor ethnicity is critically important in assisting blood collection agencies (BCAs) to adequately meet the demand for rare blood types. Australian Red Cross Lifeblood (Lifeblood) collects information on donors' county of birth; this information is not adequate for identifying which donations to perform extended phenotyping on. Ethnicity is a sensitive topic and prior to commencing routine collection of this information, we needed to ensure donors were comfortable providing this information, understood why it was required, and that the question and list of ethnicities were feasible.

Aims: The aim of the study was to assess the willingness of donors to provide their ethnic ancestry and to test the feasibility of the question. We also evaluated how much information donors required to adequately understand why we needed donors' ethnic ancestry details.

Methods: We conducted a test of the ethnic ancestry question by inviting 3000 donors (2,000 with a recorded birthplace being outside of Australia and 1,000 with any or no place of birth recorded) to provide their ethnic ancestry and complete a short survey. The list of ethnicities provided was predetermined by Lifeblood in consultation with Lifeblood's Red Cell Reference team. The list of ethnicities contained 53 choices divided into 7 ethnic regions and included a "prefer not to say" option. We also tested two preambles for the ethnicity question with half of the donors receiving a short version and half a more detailed version. The survey was both quantitative and qualitative and asked for donors' views on providing their ethnicity, their understanding of why the information was required, and their perspectives of the ethnicity choices provided.

Results: Overall, 504 (RR: 16.8%) donors completed the questionnaire. The majority of donors (97.8%) provided their ethnic ancestries, with only 2.2% selecting "prefer not to say". Donors reported being "very comfortable" about providing their ethnic ancestry and 91.3% reported finding an ethnicity option they were happy with. Only 10.7% of donors indicated that they did not understand why the information was important to Lifeblood. While a majority of donors receiving either of the question preambles indicated that they understood why ethnic information was important, those who received the shorter preamble were more likely to indicate they did not understand (16.1% v 8.9%): $^2(1, n=426)=4.920$, $p<0.027$. Lastly, those with non-European ancestries were more likely to state that they did not understand why the information was needed compared to those with European ancestry (25.5% v 7.1%): $^2(1, n=418)=25.943$, $p<0.001$.

Summary/Conclusions: The results from this study indicate that donors are willing to provide their ethnic ancestry to BCAs and the detailed list of ethnicities provided by Lifeblood was feasible. Providing a more detailed preamble to the question, increased the level of understanding on why the information is required. More education may be needed for donors from a non-European background to increase donor understanding on why BCAs may require this information. In light of these strong findings, Lifeblood has implemented the ethnicity question with the longer preamble and began collecting donors' ethnicity.

P-175 | An adaptive approach to forecasting blood demand selected for main programme

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Background: Blood supply chain reliability is largely dependent on the accuracy of the demand estimates. Shortages in fresh blood translate directly to potential loss of life, while oversupply means that blood and its procurement costs go to waste. Blood demand estimation on an operational level can be done using analytically derived forecasts, but they are often used only as a supplement to expert insight, due to insufficient method accuracy. Improving analytical demand forecasts is a requirement for the full automation of blood supply chain operations, which may result in fewer shortages and outdated blood products.

Aims: In this study, we aim to review the existing forecasting methods used at the Finnish Red Cross Blood Service (FRCBS), study the trends and changes in demand over time, and attempt to improve the forecasting methods and practices based on the observations. Additionally, a user interface is developed for accessing the forecasts.

Methods: Currently blood product forecasting at the FRCBS is carried out by two different methods. The first one is a modeler that measures the trend and seasonality of the series and by exponentially decreasing the importance of older observations, outputs a forecast for the next observation. The second method first tries to remove the seasonality from the data before using the same modeler. The seasonality component is then added back to generate a forecast. The end user then selects the method she believes provides the best prediction. The performances of these methods are estimated as is, using mean absolute percentage error (MAPE) and root-mean-square error (RMSE) and then compared to common benchmark methods. Next, we add a preprocessing step to the framework to see if adjusting for the number of work days in a given month affects the accuracy. To evaluate new methods and forecasting approaches, we aggregate the daily blood sales

data from the FRCBS to construct weekly and monthly time series of demand and explore their behavior with respect to time. We then test methods and modelers based on the existing literature, using the same training and testing periods as in the original framework. Finally, we build an automated method selection algorithm and test its performance.

Results: We find that the mean absolute percentage error of the current forecasting methods can be reduced by 9% with an additional data preprocessing step and by 53% when also selecting the best method. The automated method selection outperforms the original forecasting methods, but is left behind by some of the better single method approaches. We suspect this is a result of the size of the fixed analysis period. We also discovered that the nature of the weekly blood demand signal changes significantly around 2017, which underlines the need to develop forecasting systems with the capability to adapt to changes in demand.

Summary/Conclusions: We find that simple blood demand forecasts can be somewhat improved with careful data preprocessing and substantially improved by more appropriate method selection. We also find that the size of the fixed analysis period for the adaptive forecasting system needs to be optimized depending on the specific needs of the user. The balance between the ability of the system to adapt to changes and its forecasting accuracy invites further study, which we have begun by applying our prediction methods to the blood demand in a different setting, namely that of Sanquin blood supply in the Netherlands.

P-176 | Prediction and impact of personalised donation intervals selected for main programme

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Background: Deferral of blood donors due to low hemoglobin (Hb) is demotivating to donors, can be a sign for developing anemia, and incurs costs for blood establishments. To mitigate these negative effects, it would be beneficial to be able to predict donor's Hb value at a given date, or to directly predict whether the Hb will be below the deferral limit.

Aims: In this study, we aim to develop prediction methods for Hb/deferral in order to improve donor health and reduce costs due to deferrals without damaging blood supply. We essentially reimplement state-of-the-art methods and run them on our larger data sets with additional variables, such as genetic information. We also publish our model implementations in order to make it easier to build future research on our results.

Methods: Using donation history data (eProgesa) from last twenty years in Finland, the FinDonor blood donor cohort data, and Blood Service biobank genotyping data we build linear and non-linear predictors of hemoglobin deferral. As the donation history is a longitudinal data set, we can apply dynamic linear mixed models (DLMMs) to predict Hb. Our model has the form $y_{it}=x_{it}^{*}\beta + c_i^{*}\phi + b_i + \varepsilon_{it}$, where i refers to a donor and t to a donation time. The donation and donor specific variables are stored in matrices x_{it} and c_i, respectively, and the former includes previous Hb. The donor specific intercept b_i is the only random effect. To test whether the dependence of Hb is instead non-linear with respect to the predictors, we use a random forest (RF) model. Finally, based on financial data from the Finnish Red Cross Blood Service we then estimate economic impacts of deployment of such predictors. We study the effect of deferring a donor for 6 or 12 months, when our predictor indicates deferral. The entire economical effect E_{tot} is equal to $E_M - E_D$, where E_M is the effect on marketing cost needed to compensate the deferred donors, and E_D is the effect on economical savings due to avoided deferrals.

Results: We discover that while in general linear predictors predict hemoglobin relatively well, they fail to predict low hemoglobin values. Overall, we find that non-linear or linear predictors, with or without genetic data, perform only slightly better than a simple cut-off method based on previous hemoglobin. The effect of the "days to previous full blood donation" variable is so small that varying it will not affect deferral prediction to enable fully personalized donation intervals. However, if any of our deferral prediction methods is used for setting a fixed-length donation interval for donors that are predicted as deferred, our calculations still indicate cost savings while maintaining the blood supply levels.

The economic effects and their 95% confidence intervals in female data (value, low, high).

P-176 Table 1.

Id	euro/donation, 6 month deferral			euro/donation, 12 month deferral		
eProgesa DLMM	−0.75	−0.79	−0.70	−0.09	−0.14	−0.05
Biobank DLMM	−0.76	−0.95	−0.57	−0.17	−0.36	0.01
FinDonor DLMM	−0.85	−1.07	−0.63	−0.21	−0.47	0.04
eProgesa both genders RF	−0.92	−0.96	−0.89	−0.28	−0.32	−0.24
eProgesa cut-off	−0.60	−0.64	−0.56	0.12	0.09	0.16

Summary/Conclusions: We find that even though the prediction accuracy is not very high, the use of any predictor we implemented is still likely to bring benefits for blood donors and blood establishments alike. If the pre-donation Hb value is found to be below the threshold for positive economic effect, but above deferral limit, the donor can donate, but is deferred, for example, for 6 months.

P-177 | CMV screening of group specific orders- good stewardship

selected for main programme

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Background: In addition to antigen negative red blood cells (RBC), Immunohematology Reference Laboratories must provide RBC that are cytomegalovirus (CMV) negative. Due to the high percentage of CMV positive individuals, there is a challenge to find CMV negative, antigen negative RBC. Since almost half of the population in the United States is group O, the IRL selects predominantly group O donors that tested negative for CMV and these RBC are sometimes needed to fill orders for non-group O patients.

Aims: This study evaluated the number of units sent that were out-of-group used to fulfill CMV negative requests. The goal was to identify the percentage of non-group O blood requests that required the CMV attribute, determine the amount of group O RBCs used to fulfill those requests, and the effects the intervention by executive and laboratory management to increase CMV testing on group B units by 20% had on group O usage.

Methods: Requests for CMV negative and antigen negative RBC were divided into two periods. Period 1 (1 January 2019 – 29 February 2020) before intervention and Period 2 (1 March 2020- 31 May 2020) post-intervention were evaluated. ABO Rh units requested were compared to ABO Rh specific units provided.

Results: Period 1: 537 CMV negative RBC units were provided. 99/188(52.66%) group B positive requests were fulfilled using O RBCs. A total of 39/504 (7.74%) group O/D negative units were sent to fill D positive orders.

Period 2: 119 CMV negative RBC units were provided. 18/51 (35%) group B positive requests were fulfilled using O RBCs. Only 2/113 (1.77%) Group O/D negative units were sent to fill D positive orders. The smaller number of CMV negative units reflects that all elective procedures were suspended during this time for a period of approximately 6 weeks due to the COVID-19 pandemic.

P-177 Table 1 - Period 1.

Patient ABO	O+	O−	A+	A−	B+	Total units sent	Number Group/Rh specific units sent	Percentage of Group/Rh specific units sent	Number of Out of Group and/or Rh units sent	Percentage of Out of Group and/or Rh units sent	Number of Group O's sent	Percentage of Group O's sent
A-	0	1	0	8	0	9	8	88.89%	1	11.11%	1	11.11%
A+	14	1	51	2	0	68	51	75%	17	25%	15	22.06%
AB-	0	1	0	2	0	3	0	0	3	100%	1	33.33%
AB+	7	0	5	17	12	41	0	0	41	100%	7	17.07%
B-	0	1	0	0	0	1	0	0	1	100%	1	100%
B+	77	22	0	0	89	188	89	47.34%	99	52.66%	99	52.66%
O-	0	20	0	0	0	20	20	100%	0	0%	20	100%
O+	191	16	0	0	0	207	191	92.27%	16	7.73%	191	100%
Total	**289**	**62**	**56**	**29**	**101**	**537**	**359**	**66.8%**	**178**	**33.14%**	**335**	**62.38%**

*No Group B-, AB+ and AB- units were distributed.

P-177 Table 2 - Period 2.

Patient ABO/Rh	O+	O−	A+	A−	B+	Total units sent	Number Group/Rh specific units sent	Percentage of Group/Rh specific units sent	Number of Out of Group and/or Rh units sent	Percentage of Out of Group and/or Rh units sent	Number of Group O's sent	Percentage of Group O's sent
A-	0	3	0	0	0	3	0	0%	3	100%	3	100%
A+	2	0	7	3	0	12	7	58%	5	42%	2	17%
B+	17	1	0	0	33	51	33	65%	18	35%	18	35%
O-	0	3	0	0	0	3	3	100%	0	0%	3	100%
O+	31	0	0	0	0	31	31	100%	0	0%	31	100%
AB+	6	1	6	2	4	19	0	0%	19	100%	7	37%
Total	**56**	**8**	**13**	**5**	**37**	**119**	**74**	**62.18%**	**45**	**37.82%**	**64**	**53.78%**

*No Group B-, AB+, or AB- RBC units distributed.

Summary/Conclusions: 36.4% of CMV negative antigen orders during both periods were requested for B positive patients and 49.0% orders were filled with group O RBCs. To decrease unnecessary group O usage for non-group O patients, the IRL practice has changed to increase CMV testing for group B donors. Since the change in algorithm, there was a 17.66% decrease group O usage for group B patients allowing for better stewardship of the group O blood supply.

P-178 | The effect of COVID-19 on blood donation habits

selected for main programme

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Background: Blood centers (BC) rely on collections at schools and businesses. Shelter-in-place orders issued in 2020 due to COVID-19 closed these facilities. Additional donor campaigns were conducted to ensure adequate blood supplies during the pandemic. Testing for antibodies to SARS-CoV-2 for all allogenic donations was implemented 18 May 2021. This study was to determine how COVID-19 affected the donation habits of donors.

Aims: Determine how the COVID-19 pandemic and the addition of all donor testing for antibodies to SARS-CoV-2 affected donation habits.

Methods: The following time periods were reviewed: May to June of 2018 vs. 2019 (control) and May to June 2019 vs.2020 (study group). The following were reviewed: first-time, repeat, and lapsed donors (no donation > 2 years), gender, age, ethnicity and ABO blood groups. The addition of testing for antibodies to SARS-CoV-2 on all allogenic donation as an incentive was reviewed before [period 1 (1 May 2021 through 17 May 2021)] and after implementation [period 2 (18 May 2021 through 30 June 2021)] to determine if it increased number of donations.

Results: There were 264,593 donations in all periods. In 2020 there was a significant increase in total donations [2019-20 p=<.0001, 2018-19 p = 0.683] and by gender [2019-20 M p= 0.004, F p= <0.0001, 2018-19 M p=0.716, F p= 0.657]. In 2019-20 for the three largest ethnicities there was a significant decrease in Hispanic (p= 0.001) and African American (p <0.0001) and a significant increase in Caucasian (p<0.0001) donations. This was nonsignificant in 2018-19 [African American p=0.415, Hispanic p=0.620, Caucasian p = 0.685]. There was a significant increase in first-time (p<0.0001) and lapsed donors (p<0.0001) in 2019-20 compared with 2018-19 [first-time, p= 0.087, lapsed p=0.308] not seen in repeat donors [2018-19 p=0.730, 2019-20 p = 0.705]. The mean donor age increased significantly in 2019-20 (p=0.024) compared to 2018-19 (p=0.904). In 2019-20 there was a significant decrease in the percentage of donors < 30 years [2019-20 16-20 p<0.0001, 21-30 p<0.0001 vs. 2018-19 16-20 p=0.053, 21-30 p= 0.464]. There was also a significant increase in all blood types in 2020 [19-20 all blood types p<0.0001, 18-19 O+ p=0.621, O- p=0.724, A+ p=0.774, A- p=0.986, B+ p=0.176, B- p=0.665, AB+ p=0.840, AB- p=0.395]. During period 1 the mean daily donations was 2,578.71 compared to period 2 mean daily donations 3,227.18 (p < 0.0001).

Summary/Conclusions: Statistically significant changes occurred in the donation habits of donors in 2020 when compared to the control group. The addition of all donor testing for antibodies to SARS-CoV-2 significantly increased mean daily donations for the period evaluated. Additional donor campaigns may have also contributed to the increase in total donations as well as first-time and lapsed donors and among all blood types. The significant decrease in donors < age 30 years may be caused by the closure of educational facilities. Changes in the three largest ethnicities could not be explained.

P-179 | Use of new DXT 3.4 data management system to connect 18 Amicus and Aurora devices in 6 locations of the Madrid Blood Transfusion Center

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Background: The Madrid region has nearly 7 million inhabitants and is the most populated region in Spain. The Madrid Transfusion Center (MTC) collects and performs more than 4,000 multicomponent apheresis per year, 3,000 of them in the MTC facilities and the rest in other 5 external authorized centers. Traceability and security of collections must be insured according to the European guidelines (EDQM) and the blood donation and transfusion standards of the Spanish Foundation CAT (Comité de Acreditación Transfusional).

Aims: To implement the DXT 3.4 Data Management System (DXT) in order to capture procedure data from Amicus plateletpheresis and Aurora plasmapheresis devices in 6 MTC locations. To evaluate the abilities of DXT system for receiving, storing and transmitting the procedure information from the Fresenius Kabi devices to the external management e-Progesa software (MAK-SYSTEM). To detect opportunities for operational and processing improvements in terms of efficiency.

P-179 Table 1.

Year	Males*	Females*	African American	Hispanic	Caucasian	First-Time	Repeat	Lapsed	16-20 years	21-29 years	Mean Age
2018	43,645	38,126	7,076	14,102	56,994	16,202	54,878	10,695	6,003	12,276	45.08
2019	42,961	37,333	6,780	13,672	55,927	15,216	53,888	11,192	4,858	11,830	45.84
2020	47,745	54,768	3,555	11,076	83,931	30,745	52,999	18,778	2,547	9,036	50.07

*15 gender not provided.

Methods: 18 devices (13 Amicus + 5 Aurora) are located in the MTC and the 5 satellite centers: Hospital 12 Octubre, Hospital Ramón y Cajal, Hospital La Paz, Hospital Gregorio Marañón and Hospital Príncipe de Asturias. Due to COVID pandemic limitations, the implementation was made in 3 phases in 2020. In phase I (Feb 2020) the DXT software was installed on a server in MTC. In phase II (March 2020) 6 Amicus and 2 Aurora were connected in the main center and 1 Amicus in Hospital La Paz. In phase III (Sept 2020) 6 Amicus and 3 Aurora were connected to DXT in 4 other centers. Wi-Fi connection was used to transfer device data. Data were secured and encrypted using WPA2-PSK and MAC filter. Streamlined scanning of multi-part barcodes for automatic data assignment with a single scan was programmed.

Results: Operators find the DXT system easy to use, and their training took less than an hour. Procedure records contain details of all events required in quality standards, alerts, alarms and disposables used. Operators appreciate the reduction in barcode scans from 25 to 10, and automatic report printing. This fact helps to prevent mistakes and allows operator and donor interaction in the spare time. DXT reporting is easy to use for managers to review daily and to make monthly platelet and plasma production reports. Automatic Dashboard reports provide key metrics not previously available, such as donor disconnecting time and collection yield efficiency. Center or organization level KPIs can be easily tracked. From February 1, 2020 to February 28, 2021 the DXT system successfully captured data from 5.083 Amicus and Aurora procedures from all 6 locations.

Summary / Conclusions: DXT software saves time for operative tasks. Remote monitoring of devices in all locations is appreciated. Center supervisors have current, credible data to evaluate developing opportunities, detect inefficient processes, understand donor characteristics, and implement real-time changes. New KPIs (i.e., donor disconnecting time, yield efficiency) can be used to optimize procedures. With these positive results, Phase IV could be implemented to connect DXT to e-Progesa, permitting bidirectional communication. Achievement of paper-free documentation and time reduction for registers could be obtained, and permit exploring other DXT possibilities in the future.

P-180 | Red blood cells transfusion, where are we? – 20 years evolution of red blood cells consumption (2000-2020) in a Portuguese University Central Hospital

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Background: Strategies to improve blood usage in a central hospital are crucial. Centro Hospitalar e Universitário São João has a level 1 trauma center, a cardiothoracic surgery center, a hemato-oncology department which performs hematopoietic stem cell transplants and is a reference center of extracorporeal membrane oxygenation (ECMO). Consequently, the demand of red blood cells (RBC) is high and in order to minimize wrongful use, the Transfusion Committee created in 2012, has been taking measures to reduce the use of RBC through implementation of Maximum Surgical Blood Ordering Schedule (MSBOS), educational sessions with doctors and nurses, real time auditing of requests and promoting restrictive transfusion practices.

Aims: To analyze the evolution of red blood cells consumption in a tertiary hospital.

Methods: Data from January 2000 to December 2020 were collected from the blood bank database. Consumption was divided in categories: medical, surgical, day care, intensive care, pediatric and emergencies.

Results: There is a clear reduction (24.5%) in the use of RBC over the years (data detailed in Table 1). Day and critical care contradict this tendency, although some factors should be taken in consideration. ECMO patients have a high demand of transfusions and the number of beds has been increasing in critical care, mainly in 2020 due to the COVID-19 pandemic. The constant number of RBC may point to the fact that a restrictive policy has always been used in this setting. The increase in day care demand can also be attributed to a codification change that occurred when hematology day care increased its capacity. MSBOS seems to have an important part in reducing the number used in the surgery departments. Measures implemented in 2012 have shown positive results with an important decrease in consumption in all other departments.

P-180 Table 1.

Year	Surgery	Medical	Day Care	Critical Care	Pediatric	Emergencies	TOTAL
2000	7260	6155	503	1884	938	3958	20698
2001	7473	7099	495	1428	842	3878	21215
2002	6440	6765	483	3104	1093	3776	21661
2003	6192	7976	464	2701	1120	4247	22700
2004	6663	7492	633	2864	882	4649	23183
2005	6318	7020	695	2949	840	4913	22735
2006	6133	7132	757	3193	849	4763	22827
2007	6659	7752	681	2853	750	4835	23530
2008	8565	7999	771	2772	695	4826	25628
2009	8444	8291	879	2449	898	5198	26159
2010	7486	8056	789	2859	798	4229	24217
2011	7152	7662	973	2804	750	3353	22694
2012	6977	4072	3424	2840	854	2612	20779
2013	6827	3970	2840	2587	745	1998	18967
2014	6089	3563	3018	2501	505	1722	17398
2015	5868	3563	2683	2193	449	1758	16514
2016	5859	3692	2979	2372	552	1681	17135
2017	5452	3756	2630	2259	501	1866	16464
2018	4881	3217	2274	2047	702	1696	14817
2019	4868	3283	2876	2505	640	1863	16035
2020	4104	3230	3050	3061	550	1631	15626
Variation	−43,50%	−47,50%	+83,50%	+38,50%	-41,30%	−58,70%	−24,50%

Summary/Conclusions: The implemented measures seem to have been successful in reducing the RBC usage, highlighting the importance of a transfusion committee in the hospital setting. The shift from inpatient to outpatient care can justify our increasing numbers of transfusions in this setting. The mission of a transfusion committee never ceases. Through a constant evaluation and revision of transfusion policies, transfusion services can maintain an efficient and sustainable RBC usage.

P-181 | COVID-19 and impact on characteristics of the blood donor population in the Moscow City Blood Center

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Background: The Moscow city blood center (MCBC) is the largest blood provider in Russia. The COVID-19 outbreak has affected blood transfusion services at all levels. The Moscow lockdown lasted from March 28th to June 9th 2020. During this time all public events were canceled and travel restrictions were imposed, but blood donation was permitted. There was a significant decrease in demand for blood components in response to the shutdown of all elective surgeries and procedures.

Aims: to analyze the total number (TN) of donations, gender, age, number of first-time and repeat donors, distribution of blood group; to compare for two time periods [from January to December of 2019 vs 2020]; to assess the impact of the COVID-19 outbreak on the blood donors population.

Methods: The data from regional information system for blood service were retrospectively analyzed.

Results: There were 106740 and 93730 donations in 2019 and 2020, respectively. The TN of the donations within twelve months in 2020 decreased [p=0.082, -13,010 (12.2%)] compared with the same period of 2019. There was a statistically significant decrease in the number of first-time donors within twelve months in 2020 [p=0.025, -6,062 (24%)] compared with the same period of 2019. The number of repeat donors within twelve months in 2020 decreased [p=0.021, -562 (1.9%)] compared with the same period of 2019. Mean donor age were 30.99 years in 2020 compared with 29.99 in 2019. From the total number of donations in 2020: 75.89% were males and 24.11% were females. There was a statistically significant decrease in the number of female donations within twelve months in 2020 [p=0.021, -5,372 (19%)] compared with the same period of 2019. There was no a statistically significant decrease in the number of males donations within twelve months in 2020 [p=0.140, -7,638 (9.7%)] compared with the same period of 2019. There was distribution of donor age group in 2020: from 18 to 25 years 31.5%,

26-36 years group 40%, 36-45 years 21.4%, > 45 years 7.1%. There was a statistically significant decrease in donor age group from 18 to 25 years within twelve months in 2020 [p=0.010, -12,624 (30.1%)] compared with the same period of 2019. There was no a statistically significant decrease in the total number of donations in older donor age groups within twelve months in 2020 (26-36 years p=0.671; > 45 years p=0.191) compared with the same period of 2019. There was no a statistically significant increase in donor age group 36-45 years within twelve months in 2020 [p=0.637, +569 (2.8%)] compared with the same period of 2019. There was the ABO blood group distribution of blood donation in 2020: A 36%, O group 34.7%, B 20.5%, AB 8.8%. The was a statistically significant decrease in AB+ blood group, and all Rh negative blood groups in 2020 (O+ p=0.109, A+ p=0.181, B+ p=0.210, AB+ p=0.003, O neg p=0.001, A neg p=0.005, B neg p<0.001, AB neg p=0.008) compared with the same period of 2019.

Summary/Conclusions: our data demonstrate that the characteristics of the blood donor population in time of the COVID-19 outbreak had changed. The total number of donations decreased significantly: in first-time donor group, 18-25 age group, and females donations because of restrictions on citizens movement during lockdown and closed educational facilities when mobile blood collections were canceled.

P-182 | Addressing the availability of convalescent plasma during the COVID 19 pandemic period for COVID 19 patients

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Background: Covid 19 is an upper respiratory tract infection caused by the SarsCov2 virus that was first discovered in Wuhan, China. In Indonesia the case was first discovered in March 2020, where since then the case of covid 19 spread very massive in Indonesia. until now almost all regions of Indonesia found this case of covid 19. Therefore, the Indonesian government continues to find solutions in tackling and suppressing the number of covid 19 incidents in Indonesia. one of the steps taken by the Indonesian government through the Ministry of Health and BNPB in collaboration with the Indonesian Red Cross is the application of convalescent plasma clinical trials for covid 19 patients with mild and moderate degrees. Convalescent plasma is plasma that is taken by means of apheresis or conventional from prospective donors who have recovered from covid 19 that have antibodies titer >1/80 u/mL. Therefore, UTD PMI Cirebon district as one of those in charge of blood service conducts convalescent plasma services by complying with protocols and applicable rules. UTD PMI Cirebon district one of the UTD PMI that has been certified CPOB (How to Make Good and Correct Drugs) helped succeed the government's task by conducting convalescent plasma services in Indonesia. one of the challenges for UTD PMI Cirebon District is the lack of information related to convalescent plasma to the community or patients who have recovered from Covid 19. Therefore, we make various efforts in socializing to the people of Cirebon district who have recovered from covid 19 to want to donate their blood.

Aims: To describe the convalescent plasma service in UTD PMI Cirebon district in providing the needs of targeted convalescent plasma and assisting the Task of the Indonesian government in lowering the death rate of Covid 19.

Methods: This research method by analyzing the number of convalescent plasma donors before and after socialist held through zoom media, banners, electronic media and mass media from September 2020 to January 2021.

Results: From the analysis of the data we obtained there is an increase in awareness of convalescent plasma donors to donate their blood. It was seen where in September 2020 before socialization to the community related to plasma convalescent UTD PMI Cirebon district only get 2 donors of convalescent plasma. Therefore, UTD PMI Cirebon Regency held a socialization to the community in mid-September 2020, and this proved effective because there was an increase in the number of convalescent plasma donors in October 2020 – January 2021 with an increase of 5 donors each in October 2020, 18 donors in November 2020, 32 donors in December 2020 and 79 donors in January 2021. This increase is directly proportional to the increasing number of recruitment teams assisted by the Cirebon District Health Ministry in socializing to the public or patients who have recovered from covid 19 through zoom meetings, banners, electronic media and mass media.

Summary/Conclusions: From the results of our data analysis, it can be concluded that by using various media among them zoom media, social media, electronic media and mass media effective in inviting convalescent plasma donors to come and give their blood to patients covid 19.

P-183 | *Abstract withdrawn*

Donors and Blood Supply – Blood donor recruitment

P-184 | A simple reciprocal fairness message enhances non-donor's willingness to donate blood

selected for main programme

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Background: With growing demand from an ageing population and a decline in young blood donors, recruiting new donors is crucial for the continued effective operations of healthcare providers. Voluntary

Reciprocal Altruism (VRA) has been proposed as a blood donor recruitment intervention through asking potential donors 2 questions: one on potentially accepting a blood transfusion if required (*acceptance*) and one on willingness to donate (*willingness*).

Aims: We aim to determine whether VRA enhances non-donors' willingness to donate blood. Across three experiments, we used a *clinical trials* approach to determine whether a single component of the VRA intervention (i.e., *acceptance* or *willingness*) drives the proposed effect of the intervention on blood donation willingness. As trials often rely on self-reported willingness to donate blood, we also derived a correction factor through an initial cohort study to better estimate donation behaviour from willingness measures.

Methods: An initial longitudinal cohort study (N = 809 non-donors) was conducted over a 6-month time window to estimate a correction factor of how self-reported blood donation intentions translated to blood donation behaviour. This correction factor was then applied to the interpretation of results for subsequent experiments. Across 3 experiments (2 in the UK and 1 in Australia: Total N = 1,241 non-donors), we manipulated the presentation of the 2 VRA questions (*acceptance* and *willingness*), to explore how they influence both reported willingness to make a one-off or repeat blood donations. Specifically, Experiment 1 tested if the addition of the *acceptance* question, before the *willingness* question increased willingness to donate. Experiment 2 extended Experiment 1 by comparing the combination of the *acceptance and willingness* questions against both individual questions and a no-intervention control message. Finally, Experiment 3 explored whether participant response format, either through commitment (Yes or No) or endorsement (Agreeableness on a 7-point Likert scale), influenced the effectiveness of VRA.

Results: From the initial cohort study, we report a behavioural correction factor of.10. Results from Experiment 1 show that those exposed to both the *acceptance and willingness* questions, compared to only the *willingness* question, were 9 times more willing to make a single donation and 3.5 times more willing to indicate that they would make repeat blood donations. Results from Experiment 2, extended Experiment 1 by showing that the *acceptance* question is the primary active ingredient of VRA. Those exposed to the *acceptance* question only were 3 times more likely to indicate a willingness to make a one-off donation compared to the control. Additionally, compared to the control, those exposed to both VRA questions were 6 times more likely to indicate a willingness to make a one-off donation. However, directly comparing only the *acceptance* question to both VRA questions showed no significant difference. Experiment 3 showed that the effectiveness of VRA was not influenced by participant response format.

Summary/Conclusions: Across three experiments, we show that asking potential donors a simple question about whether they would accept a future blood transfusion could be a viable tool for recruiting new blood donors. VRA thus has the potential to be a cost-effective strategy to enhance new donor recruitment.

P-185 | Increasing donor return after deferral: Results from a field randomized controlled trial testing alternative good deeds and additional information

selected for main programme

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Background: Like in many other countries, the blood supply in the Netherlands relies on voluntary, non-remunerated blood donations. Sanquin, the sole Dutch blood supply organization, employs a deferral policy to safeguard the safety of both donors and recipients. Thus, donors may be deferred at the donation site. Previous research shows that many donors do not return for a new donation attempt after an on-site deferral. To encourage deferred donors to return, Clement and colleagues (Advances in Consumer Research, 2016) offered donors at the German Red Cross blood bank an alternative good deed. This increased donor return from 50% to 70%.

Aims: We studied whether offering an alternative good deed or providing more information to deferred donors in the Netherlands increased their return after expiry of the deferral.

Methods: All deferred whole blood (WB) and plasma donors who attempted to donate between January-September 2019 at one of the ten participating donation centers were invited to participate in the Randomized Controlled Trial. Participants were randomly assigned to one of five conditions:

- Write/draw a postcard (to help a patient in a different way); or
- Participate in research (to help Sanquin); or
- Receive extra information about deferral; or
- A choice between the first three options; or
- Control group.

We also included an unobtrusive control group with donors who were eligible but not invited to participate. We checked whether donors returned for a next donation attempt after expiry of their deferral and within four weeks of receiving a new invitation to donate (i.e. after four weeks, donors may receive a second invite), or until the end of the follow-up period (June 1, 2020).

Results: In total, 660 donors participated in the study. Of 619 donors, we had all the required information and they had had the opportunity to return for a donation attempt before June 1, 2020. Most participants were female, donated WB, and were deferred for low hemoglobin (see Table 1). 59% of participants returned within four weeks after the invitation, compared to 57% of the unobtrusive control group. Plasma donors returned more often than WB donors (91% vs. 50%). When we analyzed WB donors separately and controlled for the effect of age, we found that offering additional information led to higher return (60%) compared to the control group (45%). Return in the other conditions (45-53%) was not significantly higher than in the control group.

P-185 Table 1. Characteristics of RCT participants (N = 619).

Average age (*SD*)	35.7 (15.1)
Number of female donors (%)	450 (73%)
Number of WB donors (%)	497 (80%)
Average deferral duration, in days (*SD*)	77.2 (46.4)
Deferral reason (%)	
Short-term medical	111 (18%)
Long-term medical	18 (3%)
Low Hb	390 (63%)
Travel	70 (11%)
Miscellaneous	28 (5%)

Summary/Conclusions: Despite promising results from earlier work, we found no evidence that offering an alternative good deed led to higher return after deferral. We did however find that offering additional information about deferral had a positive effect on the return of WB donors in particular. Thus, to increase donor retention, more attention should be paid to properly informing donors about what deferral means, and that donors are welcome to donate again.

P-186 | The Postponement Study: A cluster randomised trial of deferral educational materials to increase donor return

selected for main programme

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Background: The application of a temporary deferral negatively impacts on the retention of donors, with reports suggesting that it may be due to donors not understanding why they are deferred or when they can return. Interventions to mitigate the negative impact of deferrals on donor retention are thus required. Tailored in-centre deferral materials, providing education to donors about the nature of their deferral, may improve their understanding and increase their likelihood of returning at the end of the deferral period.

Aims: The aim of this study was to evaluate the effectiveness of in-centre deferral educational materials on donor retention, three months following the end of the deferral period.

Methods: A three-arm multi-centre cluster randomised trial was conducted at 30 donor centres across Australia. Centres were randomly allocated to one of three conditions:

1. Incentre brochure plus email: In-centre collections staff provided a brochure to donors at the point of deferral. Staff were asked to fill in the brochure with the reason why the donor was deferred and the date they could return to donate. A "conversation guide" was developed to assist staff in having a conversation about the deferral that addressed donors' needs. Donors also received a follow-up email within one week of their deferral which provided information about deferrals and the date they could return to donate.
2. Email only: Donors received the business as usual process in the donor centre. Staff in the donor centre were unaware of the trial. Additionally, donors received the follow-up email within a week of being deferred.
3. Control: Donors received standard business as usual process in the donor centre and no additional communications. Staff in the donor centre were unaware of the trial.

Donors were eligible to be involved in the trial if they were aged between 18-79 years, had attended an appointment to donate at one of the study sites and had received a deferral ranging from 1-365 days in length. Donors were excluded if they received a "sensitive" deferral (e.g. male to male sex) or if they converted to another donation type allowing them to donate on the day despite a deferral being applied.

Data on donors' attempted return behaviour (subsequent deferrals and attendance at a donor centre) at three months after the deferral end date were collected and analysed using logistic regression.

Results: The final sample consisted of 6,110 donation attempts. Compared to the control condition, donors in the incentre brochure plus email condition had increased odds of return at 3 months after their deferral ended (OR:1.16, 95% CI 1.00-1.33). Novice (1-5 prior donations; OR:1.38, 95% CI 1.04-1.83) and established donors (OR:1.36, 95% CI 1.13-1.64) also had increased odds if they received the incentre brochure plus email condition compared to the control. Lastly, donors who received a donor safety deferral (for example low haemoglobin) had increased odds if they received the incentre brochure plus email (OR:1.28, 95% CI 1.03-1.60).

Summary/Conclusions: This study demonstrated a positive effect of providing tailored educational materials on deferrals to ineligible donors attempting to donate. This supports the use of evidence-based interventions in donor centres and post-donation to retain donors and mitigate the adverse effects of deferrals.

P-187 | Blood donors aged 50+: Their reasons for becoming a donor

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Background: Current generations of older adults have longer life-expectancies and most can expect to be healthier than previous generations. As a result, this group could make substantial contributions to the blood supply. Recent studies have demonstrated that concerns about the safety of blood donation for older adults were unwarranted. In 2019 Australian Red Cross Lifeblood (Lifeblood) removed the upper

age limit for existing whole blood (WB) and apheresis donors and raised the age for first-time donors from 71 to 75. This change in policy presents an opportunity to recruit more older donors. Where eligible, older donors make a substantial contribution to blood supplies. In Australia donors aged 50 and older comprise one-third of the donor panel. Even among first-time donors, older whole-blood donors are more likely to return and do so sooner than younger donors. However, little is known about why people donate blood in older age. Understanding how motivations to donate blood change with age can provide insights to develop strategies to engage, recruit and retain older donors.

Aims: To explore older donors' reasons for becoming and remain a blood donor and what blood donation means to them in the context of their age and life-stage.

Methods: This study used a qualitative approach. Semi-structured telephone interviews focussed on becoming and remaining a donor, donating in later life, and intentions to continue donating, were conducted with 34 donors aged 50 and over. Interviews were recorded and transcribed verbatim. Two researchers created a descriptive coding framework based on the research questions and literature. Higher level thematic codes were created by combining codes that described reasons for starting to donate and changes over time. Participants were categorised into three groups based on their age at the time of first donation: (1) younger (started donating as teenagers or in their twenties [n=13]); (2) in mid-life (30s-40s [n=9]); or (3) in later life (50+ [n=12]).

Results: Family was an important influence for those who started donating at a young age, with half of these donors mentioning parents or family members who donated. Younger donors also mentioned donating because of friends or colleagues. These participants saw donating blood as an ordinary, everyday activity. In contrast, family influence seemed less important in late life. Those who started donating in mid-life and in later life tended to discuss becoming donors in contexts related to their ages and life-stage, for example knowing someone who required blood, having greater understanding of the need for blood products, or having more awareness of the significance of having a rare blood group in older age. Other age-related influences for becoming a donor were having more time or flexibility, for example reducing work hours or having children who were grown up. A small number of older donors started donating after being advised to for elevated iron levels, although they were not therapeutic donors.

Summary/Conclusions: Reasons for starting to donate blood often change with age as a result of family, life-stage influences, accumulated knowledge and experiences. To optimise the recruitment and retention of donors aged 50 and over, it may be useful to acknowledge and address the life-stage experiences of mid-life and older donors more explicitly in acquisition campaigns. Recruitment approaches could identify ways to make it easy for donors to introduce their family and others they know to blood donation.

P-188 | Knowledge, attitude, perception, motives, barriers, and practice of voluntary blood donation among health science students at the University of Buea, Cameroon

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Background: Blood transfusion can save millions of lives if readily available and provided safely. Hemovigilance and quality assurance are key elements in this process of providing safe blood transfusions. The blood transfusion chain begins with blood donors, constituting a major pillar in the safety of blood and its products. Of the three main types of blood donors; voluntary non-remunerated blood donors (VNRBD), replacement and remunerated, VNRBD donors are the safest. With limitations from screening for blood borne infections and pathogen reduction therapy, it is vital to comprehend factors that affect voluntary non-remunerated blood donation.

Aims: As such, we sought to assess the knowledge, attitude, perception, motives, barriers, and practice regarding voluntary blood donation among health science students in Cameroon.

Methods: This was a cross-sectional study conducted at the Faculty of Health Sciences (FHS) university of Buea, Cameroon in June 2018 during a mobile blood donation campaign. Data was collected by use of self-administered questionnaires on the subject. Knowledge on a subject was considered good if over 70% of students answered the concerned question correctly. Also, if more than 70% of participants responded positively to an attitude question, the group was said to have a positive attitude towards blood donation about the said question. A p-value of <0.05 was considered statistically significant. Ethical approval was sought, and confidentiality maintained throughout the study.

Results: We recruited 193 students predominantly females (65.8%) among which 126 students in medicine, 35 nursing students, and 32 medical laboratory science students with a mean age of 20 ±2.25 years. 76.2% of students had good knowledge on basic eligibility criteria for blood donation. Sixty-five participants (33.6%) had donated blood at least once with 93.8% donating on a voluntary basis. Altruism was the main motivation for participating in the blood drive (84.6%) with 66.2% agreeing to donate if more blood drives were organized. Among the 128 donors who had never donated before,

60 (46.9%) responded to the fact that, they had never been approached to donate before and 15 (11.7%) were discouraged by previous donors with a negative experience. Blood donation was perceived as an altruistic act (67.9%), a healthy habit (44.6%) and religious duty (15.5%) by the participants. Blood donors were older than non-donors (OR=1.476, p=0.001, CI=1.23-1.78) and males were twice as likely to donate as females (OR=2.423, p=0.008, CI=1.30-4.52).

Summary/Conclusions: Health science students have good knowledge on blood donation, their attitude is positive although the rate of blood donation is low. A good majority of those who had never donated before were never approached to do so whereas some were influenced by negative perceptions. Hence, investing in communication strategies and frequent blood drives could scale up quality blood products in our setting.

P-189 | Recruiting voluntary blood donors to join the China marrow donor program register by using questionnaire: A Randomized Controlled Pilot Study

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Background: Hematopoietic stem cell (HSC) transplantation is an important measure of treatment of hematological system diseases. Because of the family planning policy (one-child policy) in China, the rate of population with highly HLA-matched (human leucocyte antigen) decreased, HSC transplantation mainly relies on the unrelated donors to find a match. Therefore, the supply of HSCs in China cannot satisfy the patients' need.

Aims: The barriers of people not becoming HSC volunteers mainly included the lack of awareness of the importance of HSC transplantation, being unaware of HSC donation methods and process, and considering HSC donation would affect health. HSC donation is similar with blood donation, especially with apheresis platelet donation. The number of blood donors in China is enormous that recruiting blood donors as HSC volunteers is convenient and feasible. The aims of this study were to examine the effects of recruiting blood donors to join the China Marrow Donor Program (CMDP) register by inviting them to complete a questionnaire.

Methods: This single-center, non-blinded, parallel randomized controlled trial in Guangzhou, China included 3000 blood donors who have donated blood during December 1 to 31, 2020. Both whole blood and apheresis platelet donors with qualified blood test results were eligible. The donors were randomly assigned to two groups (1500 for each): the intervention group completed Questionnaire 1, which contained knowledge about HSC, including the importance of HSC transplantation, donation progress, and the impact of health etc.; the control group completed Questionnaire 2, which did not include this knowledge. We measure blood donors' pure altruism, the level of perceiving demand of HSC, and the level of perceiving HSC donation risk. They were all asked whether they would join the CMDP register at the end of the questionnaires.

Results: There were 274 (18.3%) blood donors completed Questionnaire 1, whilst 257 (17.1%) completed Questionnaire 2. Blood donors in the intervention group expressed more willingness to join the CMDP register than those who in the control group ($M_{intervention} = 4.32$, SD = 0.87; $M_{control} = 4.02$, SD = 0.93, $P < 0.001$). Linear regression model showed that being apheresis platelet donors ($P = 0.007$), pure altruism ($P < 0.001$), and perceiving demand of HSC ($P = 0.003$) were positively associated with the intention of joining the CMDP register; whereas perception of HSC donation risk ($P < 0.001$) was negatively associated with joining intention. Perceiving demand of HSC was a moderator of Questionnaire 1 and intention of joining the CMDP register. Completing Questionnaire 1 made participants who perceived higher HSC demand expressed higher intention of joining the CMDP register. Perception of HSC donation risk was a partially mediator, that participants completed Questionnaire 1 perceived lower HSC donation risk, thereby expressed higher joining intention.

Summary/Conclusions: Using questionnaire which contained knowledge about HSC to recruit HSC volunteer donors was simple and effective. Further studies are required to measure the actual joining rates of CMDP register among blood donors.

P-190 | Maintaining a panel of anti-D donors: Insights from staff and donors

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Background: In Australia, Australian Red Cross Lifeblood (Lifeblood) is the sole organisation that provides Anti-D immunoglobulin for the prevention of haemolytic disease of the foetus and newborn (HDFN). Maintaining a panel of volunteer donors who donate a specific product can be challenging, and in recent years loss of active donors and a decline in recruitment has led to a need to develop strategies to improve recruitment and retention of anti-D donors. Dutch research found that a lack of information, time and travel were the main barriers to becoming an anti-D donor, while wanting to give back, wanting to prevent the incidence of HDFN and knowledge that anti-D was needed were the strongest motivators (Slootweg et al, 2018). While these findings are valuable, the anti-D program in Australia is different and the experiences of donors in the Netherlands may not be applicable.

Aims: To explore staff and donor perspectives on motivators and barriers to becoming, and remaining, an anti-D donor.

Methods: A qualitative approach was used for this study. Staff working in the anti-D program and anti-D donors at Lifeblood were invited to participate. Nine staff participated in an online focus group about recruitment strategies and perceived barriers and motivators to donors joining the program. Donors participated in either an online discussion forum (13 donors) or a semi-structured

telephone interview (10 donors) about their experiences of the anti-D program, motivators and barriers to joining and remaining in the program and recommendations for recruiting new donors. Interviews were transcribed and a draft coding framework developed based on the literature and discussion topics. Higher level themes were identified through re-reading the data and discussions between the researchers.

Results: Staff perceived the main barriers for donors were related to committing to regular and frequent plasma donations for number of years. As such they highlighted the strict screening criteria and process used to recruit donors into the panel, including ensuring prospective donors understand the commitment and are well educated about anti-D. Staff discussed making donation easier for these special donors through scheduling donation appointments for them and being directly available for donors to contact them. The majority of donors were motivated through having a personal connection to anti-D either through being a recipient themselves or knowing a recipient. For many, donating specifically to help mothers and babies was also highly motivating. All participants expressed a sense of obligation to remain in the program and to donate frequently, as they perceived the anti-D panel to be small. Barriers identified were time waiting in centre, deferrals and physical impacts of frequent donations. Donors suggested that recruitment could be improved through education in the community about anti-D and mentoring potential future anti-D donors.

Summary/Conclusions: Anti D donors in Australia are highly dedicated rare-product donors who identify few barriers to remaining in the program. Staff management of the program appears to be central to the effective recruitment and retention of these donors; however, current criteria may also limit the number of donors who join. As donors are motivated largely through a personal connection to recipients, inclusion criteria could be broadened to include other donors who share this motivation, while using existing donors to mentor new donors could help to support new donors.

P-191 | Knowledge, attitudes, practices (KAP) survey of potential blood donors in Libya

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Background: Libya's blood transfusion system suffers from chronic blood shortages due to low rates of voluntary, non-remunerated blood donors (VNRBDs). Over 90% come from individuals donating on behalf of a family member or friend; women are almost entirely absent.

Aims: To understand barriers and motivations for blood donation in Libya to design blood donation sensitization campaigns.

Methods: Mixed-methodology included Knowledge, Attitudes, Practices (KAP) survey - 896 people in 7 Libyan cities, July 23 - August 26, 2020; 6 key informant interviews (KIIs) conducted with blood bank managers in these 7 cities. Thematic content analysis and SPSS were used.

Results: A surprisingly strong awareness of blood donation, high knowledge blood use, and a high interest in donating were shown. People strongly appreciated altruistic and religious value of helping those in need. An unexpected motivating factor: association between blood donation and *hijama*, the traditional medical bloodletting practice promoted in Islamic hadith. Over half non-donor respondents had considered donating. Majority of individuals who had not donated blood cited not having considered donating as primary barrier. Other common barriers included need for additional information about blood donation process to dispel misinformation, and fears related to blood donation experience -fainting or contracting disease.

Distinct barriers identified for female donors including misconceptions about female eligibility, social acceptability to donate, and constraints based on husband opinions. These barriers were observed in both the respondents as well as blood bank managers. Additionally, there is no consistent follow-up with former donors and no standardized data collection during donation process. This combined with low awareness about frequency of donation hampers encouraging people to become regular donors.

Summary/Conclusions: Sensitization campaigns must encourage people to donate in order to dispel fears, and reinforce the connection between blood donation and altruism/religion. Religious institutions (mosques, *hijama* centers) and universities must be engaged to help reinforce the idea that blood donation is ethically compatible with Islam and has commonalities with *hijama*. Special attention should be placed on educating the public about eligibility of female donors. The potential for adult family members to donate together could be promoted since there was significant hesitation from both male and female respondents about women donating blood without support from their families. Education should also focus on staff to ensure sharing accurate information about female eligibility and other blood donation topics. Blood banks must establish information management systems, social media presence, standardized hospitality practices to create and maintain relationships and promote regular donation.

P-192 | Evaluation of knowledge about blood donation among a military population

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Background: The military population is theoretically above the average civilian population regarding to physical health in general, as it is

mostly young and highly charged in terms of health and fitness, factors that even determine the permanence in the Armed Forces. These factors make the military quarters, especially the Military Training Schools, an important niche to be encouraged and made aware by specific campaigns, educational programs and targeted recruiting blood donors processes, in order to maintain an adequate and loyal proportion of military volunteer blood donors.

Aims: The aim of the present study was to evaluate the knowledge of the military population of the Pirassununga Aeronautics Garrison (GUARNAE-YS) about voluntary blood donation, allowing the definition of strategies for the loyalty of blood donors in this population.

Methods: This study evaluated the knowledge of the military population of GUARNAE-YS about voluntary blood donation, through a cross-sectional, quantitative study, developed through analysis responses to a questionnaire, with 35 objective questions on the subject. The characterization data of the respondents were correlated with the answers given and with the characteristic of correct or incorrect and then analyzed statistically using Fisher's exact test, Wilcoxon rank sum test and Kruskal-Wallis rank sum test. The results were considered significant for p values ≤ 0.05.

Results: A total of 992 military personnel from the GUARNAE-YS answered all questions in this study's questionnaire. The analysis of the answers showed that the levels of knowledge about blood donation among the military personnel ranged from moderate to high, with hit rate of MED = 13 (range: 0 - 25) and a higher level of knowledge among female sex (MED = 17, range = 0 - 24 and p ≤ 0,001), higher education levels (postgraduate, MED = 18 (5 - 25); complete university education, MED = 15 (0 - 24); incomplete university education, MED = 13 (0 - 23); complete high school, MED = 12 (0 - 23); incomplete high school, MED = 9 (2 - 10); p ≤ 0,001) and people who have already applied for blood donation (MED = 15 (0 - 25); p ≤ 0,001). In the analyzed sample, 624 military personnel (62.90%) have never applied for blood donation. The reasons that prevent the majority of the military population from donating blood frequently and regularly were: lack of knowledge about the importance of donation (34.07%), lack of time (27.52%), frequent trips to endemic or epidemic regions (13.81%), fear of having their military routine compromised (13.51%), sexual behavior (6.85%) or other unspecified reasons (4.23%).

Summary/Conclusions: A significant portion of the study population has never applied for blood donation, most of them due to their lack of knowledge about the importance of that. This demonstrates the relevance of strengthening the permanent education process, expanding the possibilities to recruit and retain more military volunteers for regular blood donation. The highest medians of correct answers or expected responses were detected among females, individuals with higher education levels, hierarchical degrees with longer military service and individuals who have already applied for blood donation. Finally, despite the good level of knowledge about blood donation among the military, it is important that the weak points of knowledge about blood donation, pointed out in this study, are discussed to support future educational strategies aimed at recruiting and retaining that specific target audience.

P-193 | Evaluation of effect of COVID 19 pandemic on voluntary blood donors and their donation practices: A survey based cross sectional study

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Background: Maintaining adequate inventory of blood and various components had been a great challenge for transfusion services during Covid 19 pandemic. Voluntary blood donors are always the backbone of transfusion service which were negatively impacted during pandemic.

Aims: To evaluate the effect of COVID-19 pandemic on voluntary donors and donation practices through an online survey.

Methods: This was a survey based cross sectional study, conducted at a Government Paediatric Superspeciality Hospital in North India. Study was designed using a structured digital questionnaire (Google Forms) sent to voluntary blood donors of our blood centre in Noida and Delhi NCR region as research population during July-August 2020. The study was initiated after taking appropriate institutional ethics committee approval.

Voluntary donors who donated whole blood or platelpheresis atleast twice during March 2019- Feb 2020 were included while Voluntary donors who suffered or became close contact of Covid 19 were excluded.

Eligible respondents were categorized into two groups: Group A: Donors who have donated at our centre during pandemic. Group B: Donors who did not donated during pandemic or refused to donate on repeated calls from the blood centre. The target was to enroll minimum 50 voluntary donors in each group.

Statistical analysis-The responses were automatically embedded in MS office Excel sheets from Google forms. All the categorical variables were summarized as proportions and frequencies and were analysed using Chi Square test and Fisher's exact test. Continuous variables were expressed as mean or median All the statistics was carried out using Microsoft Excel and SPSS-20 (SPSS IBM Corp. Ltd. Armonk, NY).

Results: Out of 146 respondents, only 106 met inclusion/exclusion criteria and these responses were taken further for analysis. Out of 106, 52 donors were part of group A and 54 donors were part of group B. Though demographic details of two groups were statistically similar, but group A donors were more affirmative for blood donation as exhibited by their past donation practices (Mean donation Group A:B- 12.64:6.05). Group A participants had better knowledge regarding mode of transmission and risk of spread of COVID-19 infection with blood donation (Group A:B −9.6%:29.6%), the updated donor selection criteria with respect to COVID-19.

Awareness of scarcity of blood during pandemic and hurdles faced for donation (viz travel and family restrictions) were similar in two groups but perception of acquiring covid 19 due to blood donation was higher in group B.

Summary/Conclusions: Our study highlighted that past donation practices and dissemination of appropriate updated knowledge directly influence donor behavior during adverse situations. Therefore emphasis should be laid on retention of existing donor pool and their education to donate in routine and adverse scenario.

P-194 | Insights from analysis of blood donor deferral in pursuit of donor and patient safety

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Background: Blood donor deferral is part and parcel of this commitment to assure the safety of the health of the blood donor as well as the recipient of the blood. The deferral has a negative impact on the psyche of temporarily deferred donor in terms of the return for blood donation.

Aims: 1. Understanding the common causes of deferral in the demographic zone.

2. Help plan interventions to recruit and retain healthy blood donors keeping donor and patient safety in mind.

Methods: A retrospective analysis of deferral amongst blood donors who presented at the blood centre over a span of nine years (2011-2019). The donors were selected in accordance with the Drugs and Cosmetics Act of 1940 and the rules therein as amended from time to time. The reasons were classified into four categories, i.e. donor safety, patient safety, donor and patient safety, and miscellaneous reasons.

Results: Overall 14.6% of prospective donors were deferred from donating blood. Majority (87.7%) donors were temporarily deferred. Almost equal number of donors (49.1 % & 48.5%) were deferred for donor safety and patient safety reasons respectively. Overall, low hemoglobin (21.6%), hypertension (11.4%) and history of jaundice of unknown origin (9%) were the three most common reasons for deferral. In donor safety, low hemoglobin (43.4%), hypertension (22.9%) and low blood pressure (4.5%), and for patient safety, history of jaundice of unknown origin (18.6%), common cold (15.8%), and high-risk behavior (8.8%) emerged as the three most common reasons for deferral respectively. In males, low hemoglobin (14.5%), hypertension (12.7%), history of jaundice of unknown origin (10%), and in females, low hemoglobin (76.8%), menstruation (2.7%), underweight (1.9%) emerged as the three most common reasons for deferral respectively. In donors above 40 years of age, low hemoglobin (23.6%), hypertension (21%) and history of jaundice of unknown origin (5.6%) emerged as the most common reasons for deferral. Notably, high-risk behavior (4.4%) is consistently observed a common reason for deferral in donors less than 50 years age.

Summary / Conclusions: Health initiatives towards fortification of diet with iron, school health programs like mid-day meal for provision of protein in diet, and screening for early detection of iron deficiency, hypertension and education about high-risk behavior would be the foundation pillars for the realization of better health for all and will help in provision of a safe and sustainable blood supplies.

P-195 | Plasmapheresis; the experience of the Iranian Blood Transfusion Organization

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Background: Plasmapheresis is the removal of a weight-dependent volume of plasma and the return of *cellular elements* to the donor. This plasma is used to purify proteins such as clotting factors and intravenous immunoglobulin (IVIg) for medical use. Therefore, the need for source plasma as starting material for manufacturing plasma derivatives is growing. Iran as a country with increasing demand for plasma-derived medicines has considered plasmapheresis for the production of sufficient quantities of plasma. Therefore, the Iranian Blood Transfusion Organization (IBTO) as a single national authority of blood transfusion set up plasmapheresis centers in different parts of Iran.

Aims: The aim of this study was to evaluate the four-year policies of the Iranian Blood Transfusion Organization (IBTO) in terms of plasmapheresis recruitment of first-time donors and its effect on plasmapheresis outcome.

Methods: This research was a retrospective observational, cross-section study. Plasmapheresis data related to 16 centers from 2016 to 2019 obtained from IBTO software. This information includes (1) blood donation number, (2) plasmapheresis donation number, (3) number of plasmapheresis donors, (4) plasmapheresis donor's demographic data, (5) plasmapheresis donor's status, (6) frequency of plasma donation for each donor, (7) volume of plasma donation, and (8) HIV & HCV frequency in plasmapheresis donor's population.

Results: Result demonstrated that plasmapheresis centers (sixteen centers in eleven cities) have recruited 85515 first-time and 9453 regular donors during 4 years. A percentage comparison of plasmapheresis donation for each year showed that higher than 80% of recruited donors were first-time donors. Moreover, 78,152 units of plasma were collected without obvious serious events. Plasma volume that was collected during this time was 49203 Liter. Demographic data showed that most plasmapheresis donors were male (P value = 0.043) with a mean age of 36.4 years. Individuals *weighing more than 70 kg* and less than 70 kg, donated 700 ml and 500 ml of plasma per session, respectively. The mean number of donations was 2 times for each donor per year. The percentage of plasmapheresis to blood donation was increased from 0.2% in 2016 to 4.9% in 2019. Furthermore, results indicated that during 2016 to 2019, the HIV prevalence in repeat and regular donors was significantly higher than first-time donors (P value = 0.0001), while the prevalence of HCV was significantly higher in the first-time donor population compared with repeat and regular donors (P value = 0.0001).

Summary/Conclusions: It is concluded that during four years, significant progress has been made in the process of donor recruitment and increase of plasma donation statistics in Iran. However, selecting and recruiting first-time donors can be associated with challenges such as increasing screening costs and compromising the safety of plasma resources.

P-196 | Analysis on the situation of unpaid blood donation in Zhejiang Province from 2013 to 2020

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Background: Since the promulgation of the Blood Donation Law of the People's Republic of China in 1998 to implement the system of non-remunerated blood donation, the blood industry has made great progress and development. Since January 1, 2014, Zhejiang Province has implemented the "Zhejiang Province Implementation of the Blood Donation Law of the People's Republic of China > Measures", the cause of blood donation without compensation in Zhejiang Province has been booming, and the team of blood donation without compensation has also been stable development and growing. With the continuous improvement of the medical and health level, the scale of large public hospitals in the urban area of Hangzhou continues to expand, the development of private hospitals increases year by year, the number of patients in the provincial capital also increases year by year, and the demand for clinical blood increases accordingly year by year. Therefore, it is of great significance to analyze and understand the development trend, population distribution and blood safety situation of voluntary blood donation in this region, take measures to respond to the trend change, optimize recruitment strategy and establish a stable contingent of voluntary blood donation in this region. Taking Hangzhou City as an example, this paper retrospectively analyzed the changes in the distribution of blood donation in Zhejiang Province from 2013 to 2020 based on the number and quantity of blood donation without compensation.

Aims: To explore the development trend of blood donation in Zhejiang province and provide the basis for further improving the recruitment strategy of non-gratuitous blood donation.

Methods: The number of blood donations and blood donations from 2013 to 2020 in Zhejiang province were counted, and Hangzhou city was used as an example to analyze the composition of the total blood donation and blood donation, including street, city, University and others ， Statistics were made on HIV positive cases from 2013 to 2020.

Results: The number of blood donations and blood donations in Zhejiang Province increased by 27.1% and 26.2% respectively from 2013 to 2020. The number of blood donations and blood donations in the streets of Hangzhou decreased by 5.74% and increased by 0.52% respectively, and the number of team blood donations and blood donations increased by 43.15% and 49.41% respectively. The number of confirmed HIV positive cases in 2020 was 13.04 percent lower than that in 2013.

Summary/Conclusions: The development of blood donation in Zhejiang province is healthy, but the trend of blood donation has changed from the street to the team. The blood donation volume of the team has increased year by year. It is necessary to further increase the publicity of blood donation in the street and improve the long-term mechanism of blood donation recruitment.

P-197 | Study preliminar: Knowledge about requirements blood donation in university students

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Background: In Latin America, it is evident that voluntary blood donation and not paid is still a matter of concern in recent times, because donates are less than half. Several authors agree that our population has little and erroneous knowledge about the requirements and processes of blood donation that influence over blood units collected every year.

Aims: Assess knowledge level of the blood donation requirements in university students about blood donation in 2021.

Methods: Is a cross-sectional prospective study, the data was collected from February to march 20th. Instrument was Blood Donation Knowledge Questionnaire is composed of 23 items based blood donation requirements and was administered an online, self-report questionnaire. The questionnaire covered the following items: socio demographic variables and blood donation requirements.

For the case of this questionnaire, the number of correct answers in the instrument is a variable ranging from 0 to 23 For the knowledge level variable, the cut-off points adopted for the classification of the participants were: with a best level of knowledge greater than ≥19 correct answers; with an average knowledge of 14 to 18 correct answers and with a worst level of knowledge than ≤13 correct answers

Descriptive statistical analyses were used to report frequencies with percentages for categorical variables and means with standard deviations for continuous variables.

All data analysis will be carried out using Stata version 14 statistical software (StataCorp, Texas, USA).

Results: A total of 100 university student answered all the questions of the questionnaire and were included in the study, 77% were female; and mean age was 19.9 ± 2.39 years. The all participants only 16% had already donated blood and 25% students weren´t aware of their blood groups.

All total students, 73% was knowledge low level, 26% medium level and 1% high level, but on the other hand, 34% answered that women who are breastfeeding can donate blood, also 17% answered that people need blood have to pay.

Summary/Conclusions: Our study reflects the low level of knowledge regarding the requirements blood donation and this may be related to low levels of donation in our country.

Donors and Blood Supply – Blood collection including apheresis

P-198 | An electronic donor health questionnaire may decrease blood donor deferral rate

selected for main programme

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Background: Effective and adequate blood donor selection processes and methods are essential in order to safeguard safe and sustainable blood supply. Decreased deferral rates, increased donor return rates, and cost savings for blood establishments can be achieved by optimizing the pre-selection process of donors.

Aims: In this study we investigated the impact of the implementation of an electronic donor health questionnaire (eDHQ) to the deferral rate of donors.

Methods: Finnish Red Cross Blood Service implemented in May 2020 a new Blood Service ICT-system to the whole chain of operations from donor selection to product storage and distribution. The donor health questionnaire in paper format was replaced by an eDHQ, which consist of secure web-based questionnaire for donors (Deltagon, Finland) and customer relationship management part component (Microsoft Dynamics CRM 2016, Microsoft Corporation, USA) for the staff. We analyzed the donor deferral data in 2019 and 2020. The only process change – new malaria testing algorithm - that had an impact on donor deferrals as such was included as an explanatory factor in the results.

Results: After the implementation of the eDHQ (May 2020) a declining tendency of deferrals is observed (deferral rate in 2019: 9.5 %, from 1/2020 to 4/2020: 9.2 % and from 5/2020 to 12/2020: 6.3 %). In Table 1 an illustrated deferral rate is shown where the of process change of malaria testing is included. The analysis of the reasons for donor deferrals shows a proportional increase of deferrals due to low hemoglobin among all deferral reasons (Table 1).

Summary/Conclusions: We have observed a decrease in donor deferrals after eDHQ has replaced the paper format questionnaire. Due to the concurrent outbreak of the Covid19 pandemic it is hard to separate the pandemic's impact to donor deferrals from effects of the eDHQ, for example due to travel restrictions. A longer period of observation and data from other countries is needed to confirm our results.

P-198 Table 1. Deferral rate and proportion of hemoglobin deferrals 1/2019-12/2020.

Year/Month	Deferral rate (%)*	Proportion of Hb deferrals (%)
2019/01	9.7	21.1
2019/02	9.8	20.5
2019/03	10.1	19.0
2019/04	9.5	22.5
2019/05	9.9	23.4
2019/06	9.6	25.4
2019/07	9.2	22.0
2019/08	10.4	24.6
2019/09	10.5	19.5
2019/10	10.1	17.5
2019/11	9.2	17.7
2019/12	8.7	19.8
2020/01	8.8	19.4
2020/02	10.3	18.4
2020/03	9.7	19.9
2020/04	7.9	22.3
eDHQ implemented	7.0	30.7
2020/06	8.0	34.9
2020/07	7.4	31.5
2020/08	7.3	30.4
2020/09	6.6	28.9
2020/10	6.4	29.1
2020/11	5.9	26.9
2020/12	5.6	29.1

*Illustrated deferral rate where deferral policy change concerning donor testing for with malaria-antibodies is taken to account.

P-199 | Donating blood or plasma during the COVID-19 pandemic: Donor motivations

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Background: The COVID-19 pandemic and subsequent infection preventive measures, such as physical distancing and stay at home

orders, have a large impact on the health care system, the economy, and society. However, people are often inclined to show prosocial behavior in times of crisis. In the Netherlands, a call for blood donations during the first wave of the pandemic resulted in a significant increase in new blood donor registrations. While donating blood during the pandemic offers individuals a possibility to show solidarity and contribute to society, it also poses a risk, since physical distancing is impossible during a donation process.

Aims: In this study, we explore what motivated blood and plasma donors in the Netherlands to donate during a pandemic. Additionally, we study if motivations differ between donation types and age groups.

Methods: Dutch donors donating plasma or whole blood between April 1st and April 7th 2020 were invited to participate in an online questionnaire. The questionnaire contained questions about opinions on the pandemic and motivations for blood donation based on known motivators. Because Sanquin announced to test antibodies for research purposes, we also included test-seeking motivations. In an open text field, donors could write down other motivations. Demographic data were obtained from the blood bank information system. We used chi^2-tests to test for differences between donation types and age groups.

Results: The analysis included 3,175 donors (response rate: 42.6%), including 10.6% first-time donors, 18.9% whole blood donors, and 70.5% plasma donors. About 80% of all donors wanted to help COVID-19 patients. Whole blood donors were more often motivated by a call for donations (90.8%) than first-time (61.9%) and plasma donors (77.5%, $p<0.001$). Plasma donors more often hoped to get tested for SARS-CoV-2 antibodies (27.7%), than first-time (20.6%) and whole blood donors (26.5%, $p=0.012$). Disregarding donation type, older donors (aged ≥40) more often indicated they hoped to get tested, wanted to help COVID-19 patients, and thought it was not possible to get infected during the donation process, compared to younger donors ($p<0.001$). Younger donors (aged ≤39) more often indicated that getting out of the house and not having to go to work was a motivator to donate ($p<0.001$). Donors using the free text space ($n=839$) mostly indicated that they always donate when they get an invitation, and the pandemic did not influence their donation behavior.

Summary/Conclusions: The majority of donors making a donation attempt during the first wave of the pandemic indicated that they were mainly motivated by the possibility of helping COVID-19 patients. Test-seeking behavior played a minor role as motivation, and possibly was underestimated due to socially desirable answers. Even though we have no information on donors who cancelled donation appointments or did not show, we found that donors who did donate mainly had altruistic motivations and indicated the pandemic did not influence their willingness to donate.

P-200 | Preliminary characterisation of blood donation practices and donor demographics in six Asian regions

selected for main programme

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Background: Haemoglobin (Hb) levels are a crucial measure to assess the suitability of an individual to donate blood. Individuals with a decreased Hb level may be at risk of anaemia and would not be a suitable blood donor. The World Health Organization defines anaemia as Hb levels of <12.0 g/dL in adult females and <13.0 g/dL in adult males, but there are no specifications for the minimum Hb level to donate blood. While Hb thresholds are known to vary with age, sex, elevation above sea level, smoking and pregnancy status, there is increasing evidence that ethnicity and race also impact these thresholds.

Aims: The aim of the project was to characterise and compare the blood donor profile from six Asian blood collection services, and to explore the blood collection practices, including the Hb criteria and screening strategies.

Methods: This was a retrospective multi-centre study. All six sites provided data on blood donations collected for a 12-month period between 2017 and 2020. Descriptive statistical analysis was undertaken to present mean (SD), frequencies and percentages. Possible associations between age and sex were analysed using bi-variate tests for significance. Data was analysed by R version 4.0.3 (2020-10-10).

Results: A total of four national (Hong Kong, Japan, Mongolia and Taiwan) and two hospital based (Indonesia and Malaysia) blood collection services participated. Data from 6,859,498 donations was analysed (Table 1).

Predominantly more males donated blood in all sites except Mongolia. Group O was the most common blood group for all sites except Japan where group A was the most common. The mean Hb of male donors decreased with age, in contrast the mean Hb increased with age for female donors.

P-200 Table 1. Donations, blood groups, Hb method, Hb threshold and Hb means of 6 blood collection services

		Hong Kong	Indonesia	Japan	Malaysia	Mongolia	Taiwan
Donations (n)		**207,655**	**20,410**	**4,812,307**	**21,494**	**25,889**	**1,771,743**
Total Donations (%)	Males	51.88	78.67	71.87	59.72	37.39	63.77
	Females	48.12	21.33	28.13	40.28	62.61	36.23
ABO groups (%)	Grp A	25.46	24.01	37.69	24.10	21.37	26.65
	Grp AB	6.26	7.47	10.25	7.05	9.37	5.92
	Grp B	26.38	28.50	21.49	27.82	29.63	23.51
	Grp O	41.90	40.02	30.57	41.03	39.64	43.92
Hb screening method		**Haemocue**	**ComboLab Fresenius Kabi**	**Sysmex Haematology Analyser**	**Copper sulphate**	**Copper sulphate**	**Copper sulphate**
Minimum male Hb (g/dL)		13.0	12.5	13.0 (12.5*)	12.5	12.5	13.0
Male Hb mean (SD)	<40yr	14.9 (0.98)	15.1 (1.15)	15.1 (0.97)	NA	NA	NA
	40-59yr	14.7 (0.94)	14.9 (1.13)	14.8 (1.02)			
	60+yr	14.6 (0.92)	14.7 (1.29)	14.6 (1.04)			
Minimum female Hb g/dL)		11.5	12.5	12.5 (12.0*)	12.5	12.0	12.0
Female Hb mean (SD)	<40yr	13.0 (0.85)	13.5 (0.79)	13.3 (0.72)	NA	NA	NA
	40-59yr	13.0 (0.88)	13.6 (0.91)	13.4 (0.78)			
	60+yr	13.2 (0.88)	13.9 (0.73)	13.5 (0.78)			

*lower Hb level for small volume whole blood collection. NA = not available.

Summary/Conclusions: This preliminary study shows that donation practices and donor demographics vary among blood collection services in Asia. Among the six Asian regions participating in this study variability was observed in the proportion of male to female donors, the methods applied to assess the Hb levels and the minimum Hb levels required for donation. The most common blood group for Japan was group A which was not consistent across the other Asian regions. Interestingly, the Hb trends after the age of 50 years varied between males and females. The data suggest that older female donors may be able to continue donating to a later age but this remains to be investigated.

P-201 | Impact of RT-needle plus in whole blood donation time: A 4-year experience

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Background: In our Blood Bank, 3 different Blood Donation Systems (BDS) were used. We did a retrospective analysis of almost 4 years of collection data to determine the impact of a modified version of a standard 16G needle called RT-Needle Plus developed by Fresenius Kabi (Germany). This patented design facilitates higher flow between the needle and the tubing, allowing a faster donation time. The RT-Needle Plus has an integrated needle protector, tamper-proof cap, ergonomic hub design and bevel indicator for correct needle positioning at venipuncture.

Aims: To verify by large data set analysis that the RT Needle Plus allows faster donation, less clotting and consequently less units discarded.

Methods: 45.337 donations with a target volume of 450±10% ml in 63 ml CPD were collected in 3 different BDSs. Male and female donors were randomly assigned to BDS groups A, B and C. Groups A and B used BDS with standard 16G needle configuration, and Group C used Fresenius Kabi C3321AA CompoFlow® T&B system with RT-Needle Plus. All collections were performed using CompoGuard blood mixers (Fresenius Kabi). A fixed flow alarm was set at 30 ml/min for all procedures to ensure donation time within 15 min. This allows WB-derived plasma to be used for direct transfusion or the preparation of coagulation factors (EDQM guidelines 20th Ed. par 3.6.2.6). Various 1D barcodes are scanned with a barcode reader and donation data collected using DonationMasterNet (DMNet) data management software. DMNet performs automatic checks such as volume collected, target flow, if the donation was stopped by the operator, and donation time.

Results: Mean donation times were: 6'10"±75" Group A, 6'32"±70" Group B and 5'03"±68" Group C with RT-Needle Plus. 88% of the donations with RT-Needle Plus were completed within 6 min and ≥ 50% were in the range 4-5 min, while the distribution curves for Groups A and B show more procedures with longer donation times. Donations >12 minutes were almost 0 in Group B and C, and 0,1% in Group A. Donations with flow alarms were 4,4% in Group A, 3,4% Group B and 2,2% Group C. Percent of collections not achieving minimum target volume of 405 ml was 0,77% Group A, 0,88% Group B and 0,55% Group C. No differences in donor reactions were observed between the 3 groups.

P-201 Table 1.

Donation time (min)	RT-Needle+ n=16.294	Group An=12.801	Group Bn=16.242
3-4	8%	0%	0%
4-5	55%	12%	4%
5-6	25%	43%	33%
6-7	7%	27%	38%
7-8	3%	10%	16%
8-9	2%	4%	5%
9-12	1%	4%	4%
>12	0.0%	0.1%	0.0%

Summary/Conclusions: Donations performed in BDS equipped with RT-Needle Plus provide a faster flow and a shorter mean donation time of ca. 5 minutes compared to 6:20 min for BDS with standard needles, with no impact to donor reactions. Consistent donation times allow personnel to more easily recognize donors with disturbed flow unrelated to the BDS needle. The tight donation time distribution indicates that RT-Needle Plus allows good and consistent flow independently from vein access. As a result, we have now switched to 100% usage of BDS with RT-Needle Plus.

P-202 | Overview of automated plateletpheresis in the institute for transfusion medicine of RNM

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Background: Platelet concentrates collected from single donor by plateletpheresis are preferable in terms of reducing the risks of adverse reactions in platelet transfusion when compared to random donor platelet concentrates.
Aims: The aim of this study is to present our experience in automated collection of single donor platelets with apheresis.
Methods: This is a retrospective study performed in the Institute for Transfusion Medicine of Republic of North Macedonia from 2010 till 2021. All donors were fully informed on the donation procedure and signed an informed consent for donation. The optimal platelet count that we want to achieve was ≥ $3.0x10^{11}$ equal to 5 random donor platelet doses. Minimum preapheresis platelet count in donors requested to start the apheresis collection was 150.000/μl. Platelet collection was performed using flow cell separators Haemonetics MCS+ and Terumo BCT Trima Accel. Acid Citrate Dextrose formula A was used for anticoagulation.
Results: There were 1850 apheresis platelet collections for the mentioned period, median 168 per year. There were 49 apheresis collection in 2010, 66 collections in 2011, 78 collections in 2012, 97 collections in 2013, 105 collections in 2014, 108 collections in 2015, 120 collections in 2016, 208 collections in 2017, 356 in 2018, 364 in 2019 and 299 in 2020. The number of apheresis collections is increasing from year to year with almost double increase in 2017 and quadruple in 2018 and 2019. There is a slight decrease in the number of the collections in 2020 due to COVID-19 pandemic. Median precollection platelet count of donors was 257.000/μl, with range from 150.000/μl to 397.000/μl. Male were 75% of the donors and females were 25%. The single procedure usually took 45-70 minutes. The median platelet count collected was $4.0x10^{11}$, range $2\text{-}6.5x10^{11}$. The median processed blood volume was 3117ml and median used ACD-A was 335ml. Mean total volume of collected product was 302ml. The adverse effects included vein perforation and the numbness of the extremities as reaction of ACD-A (hypocalcemia), which occur rarely and was very mild.
Summary/Conclusions: Apheresis technologies support the best management of blood supply due to substantial improving of productivity and quality of component collection.

P-203 | Quality of platelet and C-plasma products collected on AmiCORE 2.1 device

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Background: Clinical Institute of Transfusion Medicine (CITM), University Hospital Osijek, is a facility of the Faculty of Medicine, Josip Juraj Strossmayer University Osijek, and the second largest blood collection establishment in Croatia. CITM analyzed blood components, platelets in additive solution (PAS) and concurrent plasma (cPlasma), collected on AmiCORE® Apheresis System (Fresenius Kabi, Germany), software 2.1. Although the AmiCORE offers features like reduced size, simplified handling, process leukoreduction and saline infusion for donor comfort, the quality and consistency of the products is of utmost importance for collection centers.
Aims: The evaluation intended to demonstrate AmiCORE's ability to deliver high product quality under routine conditions for the collection of double and single dose platelets in PAS and cPlasma.
Methods: In total 36 plateletpheresis procedures were performed, 30 collected double dose platelets (DDP) and 6 collected single dose platelets (SDP). All collections were in PAS. Regular donors fulfilling the general requirements of donor eligibility on the day of donation and a minimum platelet precount of 250×10^{9}/L were selected to donate DDP (6.0×10^{11}). Donors with platelet precount lower than 250×10^{9}/L were selected to donate SDP ($3.6x10^{11}$). In all procedures, 200ml cPlasma was collected. All procedures were performed with a citrate infusion rate setting of 1.4 mg/kg/min and a fixed AC ratio of 9:1. Standard settings for inlet and return flow rates were applied to start at 110ml/min and 150ml/min, respectively. Flow rates were adjusted only if required due to donor's vein condition. AmiCORE's automated PAS

addition feature was used to store platelets in 65% InterSol and 35% plasma. Platelet products were analyzed for yield, ratio of actual platelet yield/target platelet yield (A/T), Collection Efficiency, storage volume, and residual WBCs. The cPlasma products were analyzed for volume and total protein. Data are given as mean values with standard deviation (±) of results measured or recorded. All products were for transfusion.

Results: Mean donor platelet precount in DDP procedures was 276×10^9/L with mean hematocrit of 0.44. In SDP procedures, mean donor platelet precount was 226×10^9 with mean hematocrit 0.46. Achieved mean platelet yield in DDP was $5.98\times10^{11} \pm 0{,}46$ in total storage volume of 587 ml, procedure time was on average 70 minutes. In SDP mean platelet yield was $3.57\times10^{11} \pm 0.33$ in total storage volume of 312 ml and average procedure time of 52 minutes. In all plateletpheresis products (n=36), A/T ratio was 1.0 ± 0.08. Average WBC value was $0.2\times10^6 \pm 0{,}17$ (DDP 0.22×10^6 and SDP 0.09×10^6). Mean collection efficiency in all procedures was 72.4%. Swirling was observed in all products, there were no aggregates in the final platelet products. In all collected cPlasma products mean volume was 203 ml, total protein >50 g/l and residual RBCs and WBCs met EDQM guidelines levels.

Summary/Conclusions: Platelet and cPlasma collected on AmiCORE device met all regulatory requirements and CITM's internal standards. Achieved WBC values were remarkably below the EDQM recommended limits, consistently under half of the limit. Our data demonstrate that high platelet and cPlasma product quality was achieved, with high uniformity and consistency of results.

P-204 | Donor comfort on AmiCORE 2.1 apheresis system in University Hospital Osijek, Croatia

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Background: Blood and blood components in Republic Croatia are supplied through regular donations by voluntary, unpaid blood donors. Therefore, it is important to insure donor comfort and safety during donation in order to retain donors. At the Clinical Institute for Transfusion Medicine (CITM), University Hospital Osijek we apply this approach especially for apheresis procedures, resulting in regular response in donor recruitment. When evaluating new apheresis technologies, it is also important to assess donor comfort to confirm that platelet yield performance does not come at the expense of the good donation experience.

Aims: The aim of the study was to assess donors' feeling of comfort during plateletpheresis procedures on the new AmiCORE 2.1 apheresis system in a study demonstrating high platelet collection yield.

Methods: In the period from 2018 until February 2021, we performed 6 evaluations (average 2-3 weeks each) on the new apheresis system AmiCORE 2.1, Fresenius Kabi Germany. A survey was developed to measure donor comfort and donation satisfaction, and it was administered for all 156 procedures performed. Regular donors fulfilling the general requirements of donor eligibility on the day of donation and a minimum platelet precount of 250x109/L were selected to donate $6{,}0\text{x}10^{11}$ platelets and 200ml cPlasma. This study design required a much higher platelet yield than CITM donors were used to, as the standard donation is $3{,}6\text{x}10^{11}$ platelets. During the procedure, donor indicator lights and built-in pressure cuff, inform the donor when to squeeze/release and indicate procedure progress. The operator provides the donor with information and guidance throughout the procedure to put the donor at ease and insure comfort.

Results: All recruited donors were able to donate blood on the AmiCORE 2.1 apheresis device. The device is designed to provide a positive donor experience to help retain donors. In this study, 75.7% of donors had 1-5 years of plateletpheresis experience, 13.8% <1 year, and 11.2% > 5 years. Satisfaction with overall experience donating on AmiCORE 2.1 was: 96,7% satisfied, 2.6% neutral, and 0.7% negative. 90.2% of donors said they would like to donate again on the same device, 6.5% had a neutral opinion, and 3.3% were dissatisfied and would not donate on the same device. Donor satisfaction with procedure time was: 80.9% satisfied, 11.2% neutral and 7,9% not satisfied. In all procedures, operators did not observe any weakness, collapse, or interrupted puncture. No citrate-triggered symptoms were reported. Saline prime and infusion during the procedure helped provide additional comfort and mitigate possible unpleasant reactions by minimizing intravascular fluid deficit.

Summary/Conclusions: Donor satisfaction and comfort is associated with the intention of donor to return for future donations. In these times of corona crisis and blood shortage, it is very important to insure donor satisfaction and comfort to secure sufficient and consistent blood supply. Reliable apheresis devices and well-trained personnel can largely affect the feeling of donor comfort during plateletpheresis procedures. Donors reported a highly positive donation experience on the AmiCORE 2.1 device and >90% indicated a willingness for repeated donations.

P-205 | A 7-year retrospective analysis of the quality of apheresis platelets in platelet additive solution and concurrent plasma products collected on the Amicus Separator

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Background: Clinical Institute of Transfusion Medicine (CITM), University Hospital Osijek, is an educational and scientific facility of the Faculty of Medicine, Josip Juraj Strossmayer University Osijek, Croatia. Osijek is a city in eastern part of Croatia, and thus responsible for the supply of blood products to the associated centers in the area. Collection and distribution of platelet concentrates in sufficient numbers is of high importance. CITM performs platelet apheresis

procedures since 2008 and follows all guidelines and recommendations from Council of Europe (EDQM).

Aims: Our aim was to analyze the product quality of apheresis platelets collected using the Amicus Separator (Fresenius Kabi, Germany) in the period 2014 through 2020, and quantify the increase in platelet and concurrent plasma (cPlasma) products provided by apheresis and the use of Platelet Additive Solution (PAS) as a storage solution.

Methods: All donor room staff were certified as Amicus Operators by Fresenius Kabi instructors prior to operating the device. In addition to fulfilling all donor eligibility requirements on day of donation, first-time donors on apheresis were required to complete a minimum of 5 whole blood donations before selection for Amicus donation. The target yield for all donors was single dose platelets (SDP) $3.0\text{-}3.6\times10^{11}$ in total storage volume 290 - 335mL, and 200mL cPlasma when PAS (InterSol, Fresenius Kabi) was used. The Amicus automated PAS addition feature was used to store platelets in 65% InterSol and 35% plasma. 100% of platelet products are controlled by the CITM Quality department using Mindray 3600 BC hematology analyzer and ADAM for residual leukocytes. Data for this analysis was obtained from e-Delphyn, National Blood Banking Information System from 2014 to 2020.

Results: In the analyzed period, CITM produced in total 27.936 platelet products, of which 23.7% apheresis platelets were collected on Amicus. In 2014, the apheresis platelet production on Amicus was 16.28%, and successively grew to reach 26.78% today. 99.37% of platelet products achieved the target platelet yield with a mean yield of 4.01×10^{11} ±0.1. Storage volume was met in 94.41% products. 99.47% of platelet products met the EDQM limit for WBC/unit ($<1\times10^{6}$) with an average of 0.15×10^{6} $\pm$ 0.06. All platelet products are tested for pH, and all had pH>6.4. Collection of apheresis platelets in PAS was started in 2017 with 67% of collections in PAS, until reaching 100% in 2020. The use of PAS allowed CITM to begin cPlasma collection in plateletpheresis procedures. The cPlasma product validation confirmed total protein >50 g/l. Since 2017, more than 4.000 cPlasma products have been collected. Pathogen inactivation was introduced at the end of 2019, with no impact to the quality of apheresis platelet products.

Summary/Conclusions: Based on this analysis, we conclude that the CITM collected high quality apheresis platelets on the Amicus since implementing in 2014. The introduction of PAS enabled CITM to collect cPlasma products in 100% of plateletpheresis procedures. The collection of apheresis platelets in PAS allows CITM to efficiently meet the requirement of associated centers for high quality platelet and plasma products that are moreover safer for patient.

P-206 | Evaluation of AmiCORE apheresis system software version 2.1: A close look at platelet yield, collection efficiency and product quality

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Background: The AmiCORE Apheresis System is a centrifuge-based apheresis system that utilizes continuous-flow centrifugation to separate whole blood into its components. AmiCORE has been CE marked and is used in Europe, Middle East, Asia and Latin America. The Fresenius Kabi Donor Room is a clinical research site that supports several clinical studies, including the study protocol "Optimization of the AmiCORE Apheresis System," approved by the institutional review board. Current evaluation of the AmiCORE study utilizes a device containing a new software version 2.1, with the ability to collect leukoreduced platelets in platelet additive solution (PAS) and concurrent plasma (cPlasma), while returning the remaining blood components to the donor. Single and double platelet collections were completed across a range of targeted yields representative of the EU market settings.

Aims: To evaluate platelet yield, collection efficiency and product quality of a new AmiCORE SW 2.1 on single and double platelet collections in PAS with cPlasma product under routine conditions using the new AmiCORE SW 2.1.

Methods: A total of 20 platelet collections were completed, 7 single and 13 double collections. All donors met current safety guidelines for whole blood, plasma and plateletpheresis donations as set forth by the FDA, the AABB and the participating institution. The minimum acceptance criteria on the day of collection included a platelet precount of 150,000/μL for single dose platelets (3.0×10^{11}) and 250,000/μL for double dose platelets ($6.0\text{x}10^{11}$) and a 200 ml cPlasma product. Citrate infusion rate (CIR) was set at 1.4 mg/kg/min with a fixed AC ratio of 9:1. Settings for inlet and return flow rates were 110 ml/min and 130 ml/min, respectively. AmiCORE's automated PAS addition feature was used to store platelets in 65% InterSol and 35% plasma.

This was an exploratory study and no statistical hypotheses were intended to be tested. Standard summary statistics (e.g. mean, standard deviation, minimum, median and maximum values, and confidence intervals) were reported for subject, procedure and product parameters.

Results: No adverse events were reported. The CIR was lowered to 1.15 mg/kg/min for 3 donors with known citrate sensitivities. Occurrence of flow alerts was reduced with AmiCORE's Intelligent Flow Control (IFC), a feature that automatically adjusts to an optimal draw rate and cuff pressure based on venous pressure. Only 1 out of

20 collections required operator intervention where the inlet and return flow rates were lowered to 100 ml/min. Measured pH, pCO_2 and pO_2 values were at expected levels on Day 0 and Day 1. Platelet swirl was observed in all platelet products throughout storage and only 1 out of 20 was aggregated on Day 1. The actual mean platelet yield was higher than the targeted yield: single dose was 3.7×10^{11} vs. 3.0×10^{11}, and double dose was 6.8×10^{11} vs. 6.0×10^{11}. Collection efficiency was 78% in both groups combined. The mean collection time for single dose was 49 minutes and 66 minutes for double dose group. All platelet units met the leukoreduction standard of $<1.0\times10^{6}$ leukocytes, with a mean of $6.9x10^{4}$.

Summary/Conclusions: AmiCORE met all criteria for evaluable platelet products per regulatory requirements and institute's internal standards. This evaluation demonstrated yield consistency, achieved the targeted platelet yields, and provided quality platelet products using the new software 2.1.

P-207 | Collection and ensuring safety of platelet concentrates in the Russian Federation

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Background: Platelet concentrates (PCs) are essential components of transfusion therapy for patients with hematological disorders, severely thrombocytopenia. Platelet transfusions are effectively used in patients undergoing chemotherapy, bone marrow transplant recipients. Advanced medical technologies allow to ensure the quality and safety of blood components. In the Russian Federation, the collection and ensuring safety of PCs is carried out by state blood service establishments located in all regions of the country.

Aims: The aim of the work was to analyze the indicators of collection and ensuring safety of platelet concentrates in the blood service establishments in the Russian Federation in 2015-2019.

Methods: The indicators of blood service establishments activity in the Russian Federation for 2015-2019 were analyzed. The number of units of collected PCs, the number of donors, the use of apheresis, PCs pathogen reduction and leukoreduction methods were studied. The analytic data is presented according to the Russian Federation administrative division into federal districts (FD).

Results: Since 2015, the number of collected PCs has increased by 40% and reached 1012883 units in 2019. The number of donors who donate mainly platelets increased by 21%, and their percentage in the donation structure increased by 1.4 times. The highest proportion of platelet donors was registered in the blood service establishments of the Central FD. PCs were produced from whole blood donations (using the buffy coat or platelet rich plasma method) or using apheresis technology. Automated processing of whole blood to increase the production of PCs is used in some blood centers. The percentage of PCs produced by apheresis increased from 56.1% to 74.6%. In 2019, the blood service establishments collected the largest number of PCs in Central FD, where the leading medical centers providing care in oncology, hematology and surgery are located. Various methods of ensuring safety were effectively used in the production of PCs in the blood service establishments. Thus, the proportion of leucoreduced PCs increased from 50.8% to 59.2% and reaches 100% in some regions of Russia. The percentage of pathogen-reduced PCs was 15.8%, but it was 19.5% in the Ural FD. About 7.9% of PCs was exposed to irradiation for use in recipients. Multicomponent apheresis has been implemented in some regional blood centers.

Summary/Conclusions: The demand for transfusion of PCs increased by 40% in the Russian Federation over the period 2015-2019. Platelet apheresis was the main method of PCs collection in the blood service establishments. Advanced technologies were implemented and used for ensuring the safety of PCs.

Donors and Blood Supply – Donors adverse events

P-208 | Willingness of blood donors in Australia to extend the gift of blood for health research

selected for main programme

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Background: Recent technological advances and efficiencies in genomic methods and data linkages provide an opportunity to develop new programs of research that will enhance our understanding of the health and well-being of donors and the quality and safety of donated products. Lifeblood envisages establishing a large longitudinal cohort of blood, plasma, and platelet donors (collectively referred as blood donors) in Australia – known as the Australian Blood Donor Study (ABDS).

Aims: To assess the feasibility of recruitment and measure the willingness of Australian donors to participate in long-term cohort studies.

Methods: We conducted a pilot study surveying donors using four methods of invitation: (1) postal letter; (2) postal letter and email; (3) email only; and (4) In centre recruitment. We invited 500 donors with at least one successful donation in the last 6 months to participate through the first three methods and a further 517 donors were recruited in person by Lifeblood staff at donor centres. The survey questionnaire asked for information on demographics, life-style, health, and multiple psychological aspects of blood donation. The

survey also contained a set of questions measuring the willingness of donors to provide information, including blood samples for future research, and for their information to be used in external data linkage for research in donor and transfusion safety as well as other health and medical research.

Results: The response rates in the survey ranged from 23.8% for the email only invitation to 75.8% for the in-centre recruitment. Overall, of the 2017 invited donors, 827 (41.0%, 95% Confidence Interval (CI) 38.8-43.2) completed the survey. Of the total 827 respondents, 790 (95.5%) indicated they were willing to provide extra blood samples for donation and transfusion-related health research. Of those willing to provide blood samples, >90% were also willing to provide blood for use in other population health research, as well as research that involves genetic testing, and agreed to linkage of their information to external health databases for research purposes.

Summary/Conclusions: The results from this study demonstrate Australian donors' support for a large cohort study of healthy individuals that will contribute to health and medical research. Given these encouraging findings, Lifeblood plans to implement the ABDS in phases. In the first phase, we will establish a cohort of several thousand whole blood donors, collecting questionnaire data and blood samples, and we will conduct several small-scale research projects in the area of donor health and donation behaviour to demonstrate the value and impact of the cohort as a research asset.

P-209 | Individual and environmental determinants of serum ferritin levels: A structural equation model

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Background: Serum ferritin levels are increasingly being used to assess iron stores in blood donors. Considerable variation in ferritin levels within and between individuals has been observed, but our current understanding of factors that explain this variation is far from complete.

Aims: Many studies have investigated associations with ferritin levels, but most are restricted to variables from a single area of interest, e.g. donation history. We aimed to create a model that includes variables from different areas, by combining multiple potential determinants in an integrative model that shows relative importance and potential interactions.

Methods: A structural equation model with three latent constructs (individual characteristics, donation history, environmental factors) was fit to data on newly registered (N = 59,596) and active whole blood donors (N = 78,318) in the Netherlands. Separate models were made for new and active donors, and parameters were estimated separately for each sex.

Results: The model explained 25% of ferritin variance in new donors, and 40% in active donors. Individual characteristics and donation history were the most important determinants of ferritin levels in active donors. Their relative importance is dependent on the donor's sex: in women, individual characteristics explain 1.4 times more variation in ferritin levels than donation history does, while in men this is the other way around. The association between ferritin and environmental factors was smaller than the other two constructs, but still substantial; higher exposure to air pollution is associated with higher ferritin levels. This association is considerably stronger for active blood donors than for new donors.

P-209 Table 1. Regression coefficients of latent constructs on ferritin levels. Note that row-wise comparisons are not possible due to variance scaling within groups.

	Active donors		New donors	
Construct	Women	Men	Women	Men
Individual characteristics (age, weight, BMI)	+0.62	+0.46	+0.50	+0.49
Donation history (number of donations, time since previous donation)	+0.43	+0.65	N/A	N/A
Environmental factors (population density, ozone, NO_2, soot, $PM_{2.5}$, PM_{10})	+0.14	+0.08	+0.06	+0.02

Summary/Conclusions: This study shows that up to 40% of variation in ferritin levels can be explained by individual and environmental factors. Our model presents some known ferritin determinants in a broader perspective, allowing for comparison with other determinants as well as between new and active donors, or between men and women. It also shows that ferritin levels are associated with environmental factors. This is important to take into account when ferritin levels are analysed over a geographical area with different levels of air pollution. It is uncertain whether ferritin levels are still a good proxy for total body iron in conditions with high air pollution, or that they are elevated due to (low-grade) inflammation, while total body iron has not changed. Interaction effects across different constructs were found, which would go unnoticed in models investigating a single area of interest. The current model can also be extended with additional constructs which may increase the proportion of explained variance even further.

P-210 | Visits to general practitioners after low haemoglobin and/or serum ferritin deferrals in middle-aged and older Australian donors: Do they return to donate?

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Background: Lifeblood advises donors to visit their General Practitioner (GP) for medical follow-up if they are deferred from donating due to having lower than acceptable level of haemoglobin (Hb) and/or serum ferritin levels. These donors receive a letter from Lifeblood by postal mail within 2 weeks of their recent attempt to donate advising them of their Hb and/or ferritin levels at the time of their deferral and requesting them to visit their GP as soon as they can.

Aims: To examine the pattern of visits to General Practitioners (GPs) after a low haemoglobin and/or serum ferritin deferral and investigate whether an early visit to a GP (visit within 30 days following the deferral) has an impact on donors returning to donate within 12, 18, and 24 months after the deferral compared to a late or no GP visit.

Methods: We used the Sax Institute's 45 and Up cohort study data linked to administrative data sets that provide information on blood donation, interaction with GPs, medications, hospital attendances, and mortality. We used cox-proportional hazard models to examine the likelihood of donors returning to donate.

Results: The rate of visits to GPs doubled in the first month after deferral compared to the rate observed a month prior. However, of the 1928 deferred donors, only 52.4% visited a GP within four weeks of the deferral, with slightly more than half of those (53.7%) receiving iron monitoring tests. The 24-month return rate was lower in donors visiting their GPs early after deferral (adjusted Hazard Ratio 0.84, 95%CI 0.75-0.94). Early GP visitors were likely to be less healthy, as indicated by their higher likelihood of hospital admission within 24 months of deferral and an indication of higher risk of mortality.

Summary / Conclusions: To our knowledge this is the first study in blood donors that examines the GP visit pattern in deferred donors using recorded events of contact with healthcare providers and examines whether this behaviour has any impact on donor return rate. This highlights the need for more effective strategies to encourage immediate seeking of medical investigation by donors after iron-related deferral. As the donors of our study who visited a GP early after deferral had a relatively less favourable health prospect after the deferral, it is not surprising to observe a lower return to donate in these donors compared to other donors.

P-211 | Association between psychosocial factors and vasovagal reactions amongst blood donors in a tertiary care hospital in Southern India

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Background: Though the vasovagal reactions to donation are mild and account for roughly around 1% of donations, the embarrassment caused to the donors, lower likely return rates for future donations, workforce hours for attending those who reacted etc. warrants that these reactions should always be brought to a minimum as far as possible. There are various factors, both modifiable and non-modifiable, involved in the causation of such reactions.

Aims: In this study, we sought to find if various psychosocial factors are associated with these reactions.

Methods: This was a cross-sectional comparative study conducted by an interview-based survey administered to donors who had vasovagal reactions (cases) over one year (Jan 2018 to Dec 2018). The questionnaire was validated, and the person administering it was trained in using it. The survey included questions, a few of which are listed in the Table 1 under results. Age and sex-matched donors who did not have vasovagal reactions (controls) were also surveyed using the same questionnaire on similar occasions. The responses were recorded and statistically analyzed by IBM SPSS Statistics Version 19.

Results: A total of 177(1.01%) donors had vasovagal reactions amongst 17,575 donors who donated whole blood during the study period. 157 age and sex-matched donors who did not have vasovagal reactions were administered the questionnaire as controls. Both the groups were comparable for Height, whereas the mean weight of people who reacted was 65.6, whereas it was 71.3 in the control group. The variables like admitted fear of the needle, voluntariness to donate, preference, state of the mind and quality and duration of sleep the previous day were all significantly different between groups by chi-square test with a p<.05. The salient findings of the study are summarized in the table below.

P-211 Table 1.

Character	Cases (n=177)	Controls (n=157)	p value
Uncomfortable with the needle prick	95.5%	73.5%	<.01
Scared at the sight of blood being drawn	91%	64.6%	<.01
Aware of who the recipient is	95.5%	85.7%	<.01
Wish to register as a regular donor	26.6%	44.9%	<.01
Admission of being in good spirits	4.5%	17%	<.01
Admitted of a less than usual duration of sleep	22.6%	12.9%	<.01
Uninterrupted or sleep without breaks	24.9%	57.1%	<.01
Chose a smiling smiley( ) after donation	0	19.2%	NA

Summary/Conclusions: The psychosocial factors like fear of the needle, the sight of the blood, state of mind, quality and duration of sleep seem to have an association, adversely impacting the donors resulting in vasovagal reactions after/during blood donation.

P-212 | Blood donation adverse reactions and complications

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Background: Introduction. Blood transfusion therapy is an integral part of the treatment process and its relevance remains at a high level, especially in medical industries, where new tertiary medical technologies are being introduced. The banking of donated blood and its components is based on donation. Only the broadest community participation in the donor movement will make it possible to meet the growing needs of clinics for blood components and blood products. A comprehensive in-depth-study and evaluation of the many aspects of medical and non-medical donation is very important for practitioners of the blood transfusion, and can be factored into developing an effective program of motivation for repeated donations.

Aims: To characterize various adverse reactions and complications and determine the frequency of their occurrence in blood donors in the Scientific-Production Center of Transfusiology" (hereinafter - the Center) of the city of Nur-Sultan during 2020.

Methods: The subjects of the study are whole blood donors donated blood at the Center. Donors were qualified for blood donation after being examined by a general practitioner. The whole blood was collected into blood bags with a standard 450 mL volume. The donors' health condition was monitored during blood collection.

Results: In 2020, the number of donations registered in the Info Donor database was 22,949, including 3265 (14.2%) on-site donations.

Adverse reactions and complications in blood donors were observed in 172 cases, which amounted to 0.7% of all donations.

In 142 cases (82.6%), vasovagal reactions were collaptoid states and syncope of varying severity. The major part of adverse reactions occurred in primary donors - 80 cases (56.3%). In repeated and regular donors, vasovagal reactions were observed in 62 cases (43.7%). The puncture of the veins was noted in 30 cases (17.4%).

In all cases, medical assistance was delivered in full.

Summary/Conclusions: The risk of adverse reactions and complications associated with blood donation is low. However, attention needs to be paid to donor selection at the pre-donation screening stage, and to monitoring donors conditions during donation. Since the negative donation experience of donors can have a negative impact on their subsequent repeated blood donation, in order to psychologically prepare potential blood donors, it is critical to conduct more in-depth informing and counseling at the stage of selection.

COVID – PCR Testing

P-213 | The tendency for the persistence of the SARS-CoV-2 virus in patients with COVID-19 during the outbreak in NUR-SULTAN, KAZAKHSTAN

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Background: In 2019, the world was faced with a new coronavirus infection (CVI) triggerred by Coronavirus-2 (SARS-CoV-2), which causes severe acute respiratory syndrome.

In Kazakhstan, the first cases of coronavirus infection COVID-19 were registered on March 13, 2020 in two citizens of Kazakhstan who arrived from Germany. As of March 09, 2021, 220,018 cases of Covid-19 coronavirus infection were recorded in Kazakhstan. The total number of deaths from coronavirus infection in Kazakhstan is 2,821 cases. The mortality rate was 1.28%. Confirmed cases of full recovery from the virus as of March 09, 2021 in Kazakhstan: 203 467. The peak of the increase in COVID-19 cases in Kazakhstan was at the end of June - beginning of July 2020.

Aims: To study the persistence of the SARS-CoV-2 virus shedding in patients with COVID-19 until negative results of a PCR study are obtained.

Methods: The material of the study was a nasal smear taken in a test tube with a transport medium for viruses from patients of provisional hospitals and polyclinics. To detect the RNA of the SARS-CoV-2 virus, the PCR method was used on an automatic analyzer Cobas 6800 (Roche) involving Cobas SARS-CoV-2 reagents, which selectively amplify the target nucleic acid from the sample by using target-specific forward and reverse primers for non-structural region of ORF1a/b; and a conserved region in the E-gene of the structural protein coat. Test sensitivity: 0.007 TCID50/ml. The studies were carried out in the Molecular Biological Laboratory of the Research and Production Center for Transfusiology, Nur-Sultan.

Results: In the June-July 2020 period, 9684 patients were tested for the detection of SARS-CoV-2 virus RNA by PCR. RNA of the SARS-CoV-2 virus was detected in 4,864 patients, which amounted to 50.22%.

To determine the tendency of the persistence of the SARS-CoV-2 virus shedding in COVID-19 patients, 305 patients were selected with positive results and had two or more studies in our Laboratory in the period June - July 2020.

In 305 patients, the shortest period of negative PCR results for SARS-CoV-2 RNA was day 2, and a longest was on day 44. The largest number of negative results for SARS-CoV-2 RNA was noted from the 5th to the 8th and from the 11th to the 17th days. On average, on the 14th day, half of the patients had negative results. In 154 patients, virus shedding persisted after 14 days and negative results were obtained within 15 to 44 days.

14 days after receiving the first positive result in 161 patients (53%), the test showed a negative result. Twenty-one days after the first positive result, 247 (81%) patients had a negative test. However, in 58 (19%) patients, the virus continued to be detected in the upper respiratory tract.

Summary/Conclusions:

1. PCR testing in reliable systems provides reproducible, high-quality results, allowing reliable data to be obtained.
2. The disappearance of the virus after 14 days in 161 out of 305 patients shows that the adopted isolation period of two weeks is only 50% effective.
3. The minimum isolation period for infected patients should be 21 days or end earlier, but only if a negative PCR test for SARS-CoV-2 RNA is obtained.

COVID - Antibody testing

P-214 | Tracking SARS-CoV-2 seroprevalence among Canadian blood donors through two pandemic waves

selected for main programme

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Background: Tracking the proportion of the population exposed to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) over time enhances our understanding of population-level immunity, the burden of infection and guides public health policies.

Aims: Using residual blood from healthy blood donors we estimated SARS-CoV-2 seroprevalence rates by sociodemographic factors over two pandemic waves in Canada.

Methods: This serial cross-sectional study was conducted between April 6, 2020 and January 27, 2021 from people donating at all Canadian Blood Services (CBS) locations. CBS collects approximately 850,000 blood donations per year from a combination of fixed and mobile sites in all larger cities and most urban areas from all provinces in Canada except Quebec. We used the Abbott Architect assay to detect SARS-CoV-2 IgG (targeting nucleocapsid) antibodies from retention plasma. Seroprevalence was standardized to population-level demographics and the assay characteristics were adjusted using the Rogan-Gladen equation. Over the study periods seroprevalence rates were stratified by region, age, ABO blood groups, ethnicity and quintiles of material and social deprivation indices (Q1 [least deprived] -Q5 [most deprived]). Predictors of reactivity were evaluated using hierarchical multivariate logistic regression models during wave 1 (April-August 2020) and wave 2 (October 2020-January 2021).

Results: Overall 179,473 retention samples were tested. Adjusted seroprevalence increased significantly from 0.70% (95% CI 0.63, 0.77) between April and August 2020 (Wave 1) to 1.51% (95% CI 1.31, 1.71) in November 2020 (Wave 2) and 1.99% (95% CI 1.84, 2.15) in January 2021 (Wave 2). Based on the most recent survey in January 2021, there were significant variations by regions and age groups. Across Canada seroprevalence remained the highest in Manitoba (3.92% [95% CI 2.92, 4.93]) and as low as 0.5% (95% CI 0.09, 1.03) in Atlantic Canada. Donors aged 17-24 years old had the highest seroprevalence rate (3.45% [95% CI 2.87, 4.02]) compared to all other age groups. Disparities by socioeconomic status and racialized groups widened over time. Donors living in the most materially deprived neighborhoods were nearly four-times more likely to be seropositive than those living in affluent neighborhoods (4.04% vs.1.17%). Racialized groups were two times more likely to be seropositive compared to self-identified white donors (3.37% vs.1.66%). During Wave 1, only ethnicity and living in the most populous province in Canada were significant predictors of SARS-CoV-2 infection. In Wave 2, in addition to regional differences there was a significant dose response association between living in material deprived neighborhoods and SARS-CoV-2 infection (adjusted odds ratio (aOR); 95% CI) Q2 (aOR 1.5; 1.2, 1.9); Q3: (aOR 1.7; 1.4, 2.2); Q4: (aOR 1.9; 1.5, 2.4); Q5 (most deprived) (aOR 2.7; 2.1, 3.5) compared to Q1 (least deprived).

Summary/Conclusions: Worldwide, blood services have leveraged their operational capacity to inform public health policies. SARS-CoV-2 seroprevalence remained low in Canada but there were significant variations by regions and sociodemographic factors. Our results may be underestimated due to waning antibodies. Widescale seroprevalence studies will continue to play a pivotal role in helping governments and public health monitor disparities of SARS-CoV-2 infection and identify at-risk populations for improved health care and vaccine delivery.

P-215 | Current challenges of SARS-CoV-2 seroprevalence studies among blood donors: A scoping review

selected for main programme

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Background: Given the unprecedented urgency to evaluate the true burden of COVID-19, SARS-CoV-2 seroprevalence studies were mobilized quickly. Worldwide blood services leveraged their operational capacity to lead SARS-CoV-2 seroprevalence studies with the intent of informing public health. While blood donors are a common and convenient population to conduct seroprevalence studies, challenges exist.

Aims: We conducted a scoping review to summarize SARS-CoV-2 seroprevalence rates specifically from blood donor populations, evaluate methodology and provide epidemiological guidance for future research.

Methods: PubMed and MedRvix databases were systematically reviewed for seroprevalence studies among blood donors between January 2020 to 2021. Using standardized forms, two reviewers extracted seroprevalence rates and methodology pertaining to population sampling, periodicity, assay characteristics and antibody kinetics. Data on cumulative incidence and social distancing policies at the country-level were extracted from publicly available sources.

Cumulative case detection was extracted two-weeks from the end of each study period to calculate case identification ratios.

Results: From 157 articles, 33 studies representing 1,323,307 blood donors from 20 countries were included. The majority were from Europe (n=8), followed by North America (n=4), Asia (n=4), Africa (n=2), South America (n=1) and Australia (n=1). Seroprevalence studies from low- and middle-income countries were limited. The majority (94%) of the studies initiated sero-surveys within three months of the WHO pandemic declaration. Overall 79% of the studies found seroprevalence to be less than 10%. However, there was significant variability between countries from as high as 76% in parts of Brazil (adjusting for waning antibodies), to as low as 0% in Saudi Arabia. We also found significant heterogeneity by study design and methodology. 52% of studies reported seroprevalence at a single time point. Stratification by age and sex were most common (64%), followed by region (48%). Overall less than 1 in 3 studies, standardized seroprevalence rates to reflect the demographics of the target population. From 34 studies, 27 unique assay combinations were identified; 74% of studies used a single assay to determine reactive samples. Less than half of the studies adjusted rates for imperfect test performance. We evaluated the association between cumulative incidence and seroprevalence and found case detection was significantly under reported in Kenya, USA (NYC), Pakistan, Italy, and China. The median case identification ratio was 1:11.4 but as high as 1:1262 in Kenya. We found no association between seroprevalence rates and policy stringency.

Summary/Conclusions: Overall the majority of the studies reported prevalence rates <10%; levels far from reaching herd immunity. In addition to differences in community transmission and diverse public health policies, study designs and methodology were likely contributing factors associated with seroprevalence heterogeneity. Despite limitations, blood donors will continue to play a vital role in facilitating seroprevalence studies to assess and monitor the burden of COVID-19.

P-216 | Anti SARS-CoV-2 antibodies prevalence in Madrid blood donors prior to first outbreak

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Background: Our region is the Spanish autonomous community with the highest population density that globally reaches 676 inhabitants per Km2, seven times much higher in the municipality. From March 16th to 31st, 10%>70% of the hospital beds were occupied and on March 20th 100% of ICU beds were saturated by COVID-19 patients. The abrupt increase in severe COVID-19 cases, as well as the accelerated way in which community transmission was achieved, could suggest that the SARS-CoV-2 virus could be present in the region several months before without being detected.

Aims: To determine if SARS-CoV-2 antibodies were present in blood donor plasma samples collected between January, (prior to the first identified case in Madrid on February 25) and the third week of March, before the first outbreak and rapid spread of the infection in the region

Methods: On October 2020 a total of 2,419 residual archived EDTA plasma samples, collected from blood donors that donate blood from January 1 to March 22, 2020 and stored at -40°C, in the blood transfusion center, were analyzed for total qualitative (IgM+IgG) anti-SARS-COV-2 antibodies (MosaiQ COVID-19 Antibody Magazine, Quotient Suisse SA) (n= 800, 1071 and 548, collected on January, February and March 2020, respectively).

Results:

We have not found the presence of IgG + IgM anti-SARS-CoV-2 antibodies in the samples collected on January and February 2020. Only two positive donations were detected in the samples collected on the first and second week of March. They represent 0.36% of the total donations analyzed on March and 0.08% of the total included in the study.

P-216 Table 1.

	Samples analysed		Anti-SARS-CoV-2 positive samples	
Date of donation	Number	% versus total	Number	%
1° week January	88	(3.64%)	0	
2° week January	178	(7.36%)	0	
3° week January	183	(7.56%)	0	
4° week January	179	(7.40%)	0	
5° week January	172	(7.11%)	0	
Total January	**800**	**(33.07%)**	**0**	
1° week February	91	(3.76)	0	
2° week February	387	(16.00)	0	
3° week February	227	(9.38)	0	
4° week February	366	(15.13)	0	
Total February	**1071**	**(44.27%)**	**0**	
1° week March	276	(11.41%)	1	(0.40%)
2° week March	183	(7.56%)	1	(0.50%)
3° week March	89	(3.68%)	0	
Total March	**548**	**(22.65%)**	**2**	**(0.36%)**
TOTAL STUDY	**2419**		**2**	**(0.08%)**

Summary/Conclusions: Our results show a very low anti-SARS-CoV-2 antibody prevalence rate among the total number of analyzed samples. These data would suggest that only a few infected cases are needed to trigger an outbreak as the observed in Madrid during March 2020, in contrast to expected, confirming the high infectivity of SARS COV-2 virus. This would be in accordance with the rapid increase in cases that has been observed in the following outbreak. Also confirm the high infectivity of SARS COV-2 virus and the importance of the rapidly introduction of protective measures in order to prevent the transmission.

P-217 | SARS-CoV-2 neutralising antibody capacity among asymptomatic whole blood donors vs convalescent plasma donors: A comparative study

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Background: Covid-Convalescent plasma therapy is passive transfer of SARS-CoV-2 neutralizing antibodies obtained from individuals recovered from Covid-19 infection.

Aims: The study Aims to compare the neutralising antibody inhibition levels between asymptomatic whole blood donors (no prior Covid-19 positive diagnosis/symptoms) and Convalescent plasma donors with Covid-19 symptomatic history and positive diagnosis.

Methods: Observational study.

Study Set 1: 43 Covid-19 Convalescent plasma (CCP) donors-individuals recovered from Covid-19 infection (symptoms+/positive RTPCR report).

Study Set 2: 47 asymptomatic whole blood donors-no prior Covid-19 positive diagnosis/symptoms.

Screening for SARS-CoV-2-IgG antibodies: Chemiluminescence (CLIA) assay (Ortho Vitros);samples with S/C OD value of 6 or greater were preserved for Neutralisation Assay.

Neutralisation Assay: SARS-CoV-2 Surrogate Virus Neutralization Test (GenScript USA);neutralisation inhibition capacity was expressed in %; ≥20% inhibition was correlated to high titres of NAb.

Results: ***Sociodemography***: Distribution of Age, gender, blood groups and donation status between the two groups of donors were similar with no statistical difference.

SARS-CoV-2-IgG CLIA assay: Among 90, 33 donors had mean OD S/Co value of 7.4 (range 6.1-11.8) and 57 had mean OD S/Co value of 24.3 (range 12.1-42).

Neutralising Antibodies (NAb) assay: Neutralization Inhibition of 90 SARS-CoV-2-IgG positive samples among both the groups was >20%, with mean of 77.4% among the CCP donors and 75.2% among the whole blood donors (p=0.157). Also, Inhibition % appeared similar in two groups with respect to age, gender, blood group, donation status without any statistical significance (Table 1).

Summary/Conclusions: No significance difference in neutralising antibody capacity was observed in asymptomatic whole blood donors and Convalescent plasma donors. Therefore, donors having adequate levels of SARS-CoV-2-IgG antibody levels on screening can be considered for Convalescent plasma donation irrespective of prior Covid-19 diagnosis or Covid-related symptoms. Whole blood derived Covid Convalescent plasma with adequate SARS-CoV-2 antibodies makes the Convalescent Plasma therapy an economical, affordable and readily available alternative among the developing countries where facilities of apheresis technology are not widely established.

P-217 Table 1. Detailed neutralization assay results in both the groups

1A. Comparison of Chemilunescence assay OD S/Co with Neutralisation Inhibition percentage among two groups					
Chemiluminescence assay (CLIA)		**Total N=90** Mean (Range)	**CCP Donors N=43** Mean (Range)	**WB Donors N=47** Mean (Range)	**P value**
CLIA OD S/Co 6-12 (N=33)			(n=28)	(n=5)	
	CLIA OD	7.4 (6-12)	7.53 (6-12)	6.68 (6-7)	0.157
	Inhibition %	76.16 (41-95)	76.35 (41-95)		75.1 (69-83)
CLIA OD S/Co ≥12 (N=57)			(n=15)	(n=42)	
	CLIA OD	24.37 (12-42)	29.09 (12-42)	22.69 (12-40)	0.153
	Inhibition %	76.38 (21-95)	79.37 (21-95)		75.31(44-94)
1B. Demographic parameters and Inhibition percentage among two groups					
Demographic parameters					P value
Age Groups (years)	18-29	34; 76 (45-94)	8; 77.5 (45-93)	26; 75.8 (48-94)	0.19
	30-45	52; 76.6 (21-95)	33; 78.2 (21-95)	19; 73.9 (44-90)	
	46-60	4; 73.4 (45-89)	2; 65.6 (45-85)	2; 81.2 (73-89)	
Blood Groups	A	19; 77.7 (58-94)	10; 82.3 (65-94)	9; 72.5 (58-83)	0.213
	AB	11; 70.6 (44-93)	2; 80.4 (75-85)	9; 68.4 (44-93)	
	B	19; 74.5 (21-95)	10; 72.9 (21-95)	9; 76.3 (50-90)	
	O	41; 77.9 (45-94)	21; 76.8 (45-94)	20; 79.1 (48-94)	
Donation Status	First Time Donor	15; 75.7 (50-94)	5; 83.4 (65-94)	10; 71.8 (50-93)	0.157
	Repeat Donation	75; 76.4 (21-95)	38; 76.6 (21-95)	37; 76.2 (44-94)	

P-218 | Seroprevalence of SARS-CoV-2 among blood donors

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Background: According to the current knowledge, SARS-CoV-2 serology tests serve to determine the seroprevalence in a given population, to identify potential donors of Covid convalescent plasma (CCP), to complement molecular testing in diagnosis of acute infection, vaccine evaluation studies etc.
Aims: To determine SARS-CoV-2 seroprevalence in blood donors in order to assess natural immunity in the general population and to identify potential donors of convalescent plasma. The second aim was to determine SARS-CoV-2 seroprevalence in the group of medical workers in our blood transfusion facility.
Methods: In the period from November, 2020 to March, 2021 (before the start of vaccination), besides the routine TTI testing, we performed SARS-CoV-2 IgG test in every blood donor who met the usual donor selection criteria, as well as the additional criteria concerning the coronavirus pandemic, such as normal body temperature, no signs of infection, no recent contact etc. We used Abbott SARS-CoV-2 IgG assay on Architect 2000 platform.
Results: We tested 9773 blood donors out of which 2437(25%) had SARS-CoV-2 IgG antibodies. The index of the test > 5 S/C was observed in 813 (33.6%) of the positive donors whose samples were further subjected to a SARS-Cov-2 RBD-IgG test in order to identify plasma units as CCP. The seropositivity among blood donors was increasing, being 12.4%, 25.0%, 28.8% and 29.5% in the months from November until the end of February. The highest seroprevalence of 33.0% was observed in the blood donors from the western part of the country. The overall seropositivity in blood donors was significantly lower (25.0%) in comparison to blood facility medical workers (35.2%).
Summary/Conclusions: According to our opinion, blood donors are excellent representative of the general population, so the estimated seroprevalence of SARS-CoV-2 IgG can be considered as a seroprevalence in the population. These data can be used for further planning of the COVID-19 protective measures and vaccination programme.

P-219 | Analysis of the retention of anti-SARS-CoV-2.0 antibodies in convalescent plasma donors after 4-6 months

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Background: Severe Acute Respiratory Syndrome – Coronavirus 2 (SARS-CoV-2), which causes the 2019 Coronavirus Disease (COVID-19), has become a global pandemic. When infected, SARS-CoV-2 causes humoral responses, and within 3 weeks, almost all infected patients develop antibodies against the receptor-binding domain (RBD) and the S1 and S2 domains of the spike (S) glycoprotein, as well as against the nucleocapsid protein (N). Gudbjartsson et al showed that there was no sign of decreased antibody levels up to 4 months after infection.
Aims: Assessment of the concentration of antibodies to SARS-CoV-2 4-6 months after the first wave of the pandemic in convalescent plasma donors with predominantly moderate and severe COVID-19.
Methods: Convalescent plasma donors 4-6 months after the first donation of convalescent plasma were invited for laboratory serological testing for the presence of antibodies to SARS-CoV-2. The first donation from donors was collected 14-16 days after recovery. All participants signed informed consent and donated venous blood. The level of class G antibodies to the SARS-CoV-2 nucleocapsid protein in blood serum was determined using reagents manufactured by ABBOTT and Architect i2000sr equipment (ABBOTT, USA) in our own laboratory.
Results: The research involved 52 participants, 94.2% of whom were men. The average age was 41.0±9.6. The number of days after the first assay (mean±SD) – 162.7±31.8. The index (S/C) after the first assay was (mean±SD) 5.8±1.6, the second (mean±SD) – 2.2±1.6, decrease (mean±SD) by 3.6±1.6. In 22 (42.3%) participants, the assay gave a negative result.
Summary/Conclusions: An association was established with a decrease in antibody concentration after 4-6 months (r -0.54; 95% CI: -0.03; p <0.001). The decrease in antibodies did not depend on the age of the participants (r 0.06; 95% CI: 0.01; p 0.66). This gives a hope, but the antibody concentrations required to protect against reinfection have not yet been established. Consequently, large follow-up studies are needed to establish protection against reinfection and/or disease and duration of protection with antibodies.

P-220 | Dynamics of antibodies to SARS-CoV-2 in convalescent plasma donors

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Background: Characterization of the human antibody response to SARS-CoV-2 infection is vital for serosurveillance purposes as well for treatment options such as transfusion with convalescent plasma or immunoglobin products derived from convalescent plasma.
Aims: In this study, we longitudinally and quantitatively analyzed antibody responses in RT-PCR positive SARS-CoV-2 convalescent adults during the first 34 weeks after onset of symptoms.
Methods: We measured antibody responses to the receptor binding domain (RBD) of the spike protein and the nucleocapsid protein of Sars-CoV-2 in 844 longitudinal samples from 151 RT-PCR positive SARS-

CoV-2 convalescent adults. With a median of 5 (range 2 – 18) samples per individual, this allowed analysis of individual longitudinal antibody profiles. Kinetic profiles were analyzed by mixed effects modelling.

Results: All donors were seropositive at the first sampling moment, and only one donor seroreverted during follow-up analysis. Anti-RBD IgG and anti-nucleocapsid IgG levels declined with median half-life's of 62 and 59 days, respectively, between 2-5 months after symptom onset, and several-fold variation in individual half-lifes was observed. The rate of decline of antibody levels diminished during extended follow-up, which points towards long-term immunological memory. The magnitude of the anti-RBD IgG response correlated well with neutralization capacity measured in a classic plaque reduction assay as well in an in-house developed competition assay.

Summary/Conclusions: The result of this study gives valuable insight into the long-term longitudinal response of antibodies to SARS-CoV-2.

P-221 | Study on antibodies against SARS-CoV-2 IN convalescent plasma for COVID-19: Based on the data of 123 convalescent plasma for COVID-19

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Background: Previous studies have shown that convalescent plasma treatment (CPT) can effectively reduce the mortality of patients with severe influenza A or SARS-CoV virus infection. Which disease classification of the convalescents for COVID-19, and how long from onset is the best condition to collect convalescent plasma to ensure that it has adequate antibody value?

Aims: Analyze the situation of plasma donated by COVID-19 convalescents in Zhejiang Province, exploring associated factors of antibody values.

Methods: Before donate, the RNA and related antibodies were tested and evaluated. A total of 123 convalescents with previously confirmed SAR-CoV-2 infections in Zhejiang Province were included in the study. The basic demographics were included in this study. We detected the Ab, IgM, IgG and S-IgG antibodies values by two methods (chemiluminescence, immunofluorescence), which were evaluated as negative if S/CO value was less than 1.0 (u/L).Procedures were carried out in strict accordance with the manufacturer's instructions. This study was approved by Medical Ethics Committee of Blood Centre of Zhejiang Province(No.5.5-2018).T-test, one-way ANOVA and Pearson correlation analysis were used to test the associations between antibody values and factors by SPSS 20.0.The p level of statistical significance was 0.05.

Results: 73.2% aged 31-50 years old, 53.7% were males, blood type O was the majority (44.7%).The majority of plasma donors (57.7%) were mild symptoms, 25.2% were common symptoms, 5.7% with atypical symptom, 2.4% donors were asymptomatic infections, and 8.9% donor were severe symptom. Among the plasma donors, the average hospitalization duration was 14.36 $\pm$ 5.7 days (min to max was 5days to 30 days). The average time interval of plasma donation from onset was 37.47 $\pm$ 8 days (min to max was 22 days 60 days). The seropositive rate for Ab, IgM, IgG, S-IgG antibody against SARS-CoV-2 were 97.6%, 9.5%, 95.7%, 51%, respectively. 18 samples detected seropositive of both IgG and IgM antibodies, and 4 were seronegative both of IgG and IgM antibodies. one of four with 37 years old was tracked. She donated plasma and detected on day-40 from onset, and collected and detected again on day-81 from onset. The results showed that the nucleic acid test was negative. IgM and IgG antibodies were both evaluated as weak positive (S/CO value = 2.2, 1.21), S-IgG antibody was evaluated as negative (S/CO value = 0.44). The S-IgG antibodies values for females were significantly higher than males (p=0.006).The Ab values was significantly associated with age (Pearson=0.222, p=0.015). The time interval from onset was significantly associated with severity of symptom (F=3.070, $p = 0.019$).The time interval since onset was significantly associated with hospital duration (Pearson=0.646, $p= 0.000$). IgG antibody value was associated with time interval since onset (Pearson=0.191, $p = 0.041$). For three asymptomatic infections who were all females, aged 36-39 years old, the values of IgG antibodies ranging from 4.63-49.53(u/L), but IgM and S-IgM antibodies were all seronegative.

Summary/Conclusions: **A**ntibodies against SARS-CoV-2 including Ab, IgM, IgG seroconversion occur and exist in convalescents for COVID-19 from day-22 to day 60 since onset. High antibodies values including Ab and IgG antibodies tend to appear in convalescents over the 30 years old. Given the importance of S-IgG antibodies, an increasing S-IgG antibody detection is required.

P-222 | Seroprevalence to SARS-CoV-2 at the blood banking center in Nur-Sultan, Kazakhstan

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Background: When a new disease such as COVID-19 emerges, epidemiological surveillance and testing strategies initially focus on patients with severe disease and the use of molecular diagnostics to detect acute infections as they seek and need care. With this approach, some patients with mild or asymptomatic infection who do not require medical attention may not be identified, with the result that the true extent of the infection remains unknown.

"Antibodies" are part of the body's immune response to infection. Antibodies that work against SARS-CoV-2, the virus that causes COVID-19, are usually detected in the first few weeks after infection. The presence of antibodies indicates that a person has been infected with SARS-CoV-2, regardless of whether the infection is severe, mild, or asymptomatic.

Aims: Determine the seroprevalence to SARS-COV-2 among the employees of the organization.

Methods: From January 18 to February 1, 2021, employees of the Scientific-Production Center for Transfusiology (Nur-Sultan, Kazakhstan) underwent laboratory serological testing for the presence of antibodies to SARS-CoV-2. Employees signed informed consent and donated venous blood. The level of antibodies to SARS-CoV-2 in blood serum was determined using test systems manufactured by ABBOTT on Architect i2000sr device (ABBOTT, USA) in our own laboratory.

Results: Of the 270 employees, 222 (82.2%) donated venous blood for research. The average age of employees was 41 ± 11 years, of which 80.2% were women. A positive result of studies of antibodies to SARS-CoV-2 was found in 50 employees (22.5%). Of these, 26 employees (11.7%) did not previously have a verified diagnosis and history of complaints, did not seek medical help. In addition, another 29 employees (13.1%), who also did not contract the disease in an active form, had "traces" of antibodies to SARS-CoV-2 (positivity coefficient from 0.3 to 1.3).

Summary/Conclusions: Serological testing helps to retrospectively determine the extent of an outbreak or the prevalence of infection in the population of interest. Seroprevalence studies give a more complete picture of which part of the population is infected with SARS-CoV-2, allowing to identify cases of infection that were not detected during routine or active epidemiological control, as well as to determine the immune status of a particular population before the onset of a new wave.

P-223 | Results of confirmatory testing of convalescent anti-covid plasma donors for antibodies to spike and nucleocapsid antigens

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Background: According to computer modeling data, the genome of the SARS-CoV-2 virus encodes 4 structural proteins (N, E, M, S) and 20 non-structural proteins. The cell receptor used by the SARS-CoV-2 virus to enter target cells is angiotension-converting enzyme 2 (ACE-2).

A study by Chinese scientists revealed that ACE-2 is an endogenous spike protein (spike glycoprotein with the S-domain) SARS-CoV-2, which, as part of the ACE-2 + SARS-CoV-2 complex, binds to the ACE-2 receptor located on the target-cell membrane. Neutralizing protective antibodies are directed against the receptor-binding domain of protein S, which interacts with ACE-2 and CD26 receptors upon entry of viruses into the cell.

Aims: To determine the presence or absence of neutralizing antibodies to Spike-antigens of SARS-CoV-2 in donors of convalescent anti-COVID plasma (CCP) who had class G antibodies to the nucleocapsid antigen in screening qualitative tests.

Methods: For the study, we used frozen serum samples of CCP donors, tested before donation with reagents manufactured by ABBOTT, on Architect i2000sr immunoassay analyzer (ABBOTT, USA), and having class G antibodies to the SARS-CoV-2 nucleocapsid antigen.

Confirmatory testing was performed on semi-automated equipment (TECAN) for ELISA assays. The study was carried out using ELISA reagents: "GA CoV-2 IgG +" (GA Generic Assays GmbH, Germany). We carried out the determination of the specificity of antibodies against the main immunodominant antigens SARS-CoV-2 (Spike Glycoprotein 1, Spike Glycoprotein 2, Nucleocapsid).

Results: The study tested 33 samples of CCP. Antibodies to the Nucleocapsid antigen were detected in all samples (index (S/C) = 10.83 - 12.97). Antibodies to Spike Glycoprotein 1 were also detected in all samples (index (S/C) = 1.20-12.73). Antibodies to Spike Glycoprotein 2 were detected in 17 (52%) samples (index (S/C) = 1.04 - 12.81).

Antibodies to Spike Glycoprotein 2 were detected in samples of CCP with an index (S/C) = 3.0 - 4.5 in the primary screening for IgG antibodies in qualitative CLIA (Architect, ABBOTT) in four (67%) of the six tested samples.

Antibodies to Spike Glycoprotein 2 were detected in 13 (48%) of 27 tested samples in CCP samples with an index (S/C) ≥ 4.5 in the primary screening for IgG antibodies in qualitative CLIA (Architect, ABBOTT).

Summary/Conclusions: All CCP samples had antibodies to the Nucleocapsid and Spike Glycoprotein 1 antigens, consequently, the CCP contains virus neutralizing antibodies that block the interaction of the SARS-CoV-2 protein receptor-binding domain S with target cells.

A complete set of antibodies to the three main structural antigens of SARS-CoV-2 is present in 17 (52%) CCP samples.

The presence of a complete set of antibodies to SARS-CoV-2 antigens does not depend on the index (S/C) value (with CP >3.0 or >4.5) when screening IgG antibodies in qualitative tests.

P-224 | Seroprevalence of SARS-CoV-2 in blood donors during the second wave of COVID-19 pandemic in Peshawar, Pakistan

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Background: COVID-19 (coronavirus disease 2019), first reported in December 2019, has spread across the globe resulting in an ongoing pandemic affecting healthcare systems and the economy across all continents. It is uncertain how many individuals have acquired the SARS-CoV-2 virus without knowing and are asymptomatic. Hence, the number of COVID-19 reported cases do not accurately reflect the precise number of those affected. Antibody-based screening assays for SARS-CoV-2 can prove useful to assess the magnitude of asymptomatic SARS-CoV-2 infections and to monitor the overall pandemic.

In Pakistan, scanty data exist about the antibody response against SARS-CoV-2 in asymptomatic individuals.

Aims: To determine the seroprevalence of anti-SARS-CoV-2 antibodies in healthy blood donors at a Regional Blood Centre in Peshawar, Pakistan.

Methods: This single-centre cross-sectional study was conducted from November 2020 to February 2021, at the Regional Blood Centre, Peshawar, Pakistan. A total of 2,147 blood donors were tested for qualitative detection of SARS-CoV-2 antibodies through chemiluminescence immunoassay (CLIA) from Roche Diagnostics International Ltd. (Rotkreuz, Switzerland). The result of a sample was given either as positive for SARS-CoV-2 (if the result's cut-off index is ≥1.0) or negative for SARS-CoV-2 (if the result's cut-off index is <1.0). Statistical analysis was done by SPSS version 22.0. The study was endorsed by the ethical committee of Khyber Medical University, Peshawar.

Results: Among 2,147 blood donors, 23 (1%) were females and 2,124 (99%) were males of different age groups ranging from 18-50 years of age. The mean age of the donors was 25.63 ± 7.05 years. Among the 2,147 blood donors tested, 1,159 (54%) were reactive for antibodies to SARS-CoV-2.

Summary/Conclusions: This present study assessed the prevalence of antibodies in those healthy blood donors having no previous record of infection. The findings showed a high prevalence rate of SARS-CoV-2 antibodies (54%) in blood donors. With the introduction of the vaccine to SARS-CoV-2, the serologic testing may need to be carefully performed and observed.

P-225 | Analysis of antibody in convalescent plasma from patients with COVID-19

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Background: The novel coronavirus pneumonia is a disease caused by New Coronavirus, which has been showing a global epidemic. Up to November 14, 2020, 53638475 cases were diagnosed globally and 1306593 cases died of [8]. The new coronavirus encodes more than 20 kinds of proteins, which will be quickly recognized by the human immune system and produce a large number of antibodies. In order to further novel coronavirus pneumonia patients with severe or critical illness, the New Coronavirus pneumonia treatment plan (trial version 7) has treated the rehabilitation plasma as a routine treatment for patients with faster, heavier and more severe disease. Serum antibody is one of the important methods to evaluate the effectiveness of convalescent plasma in patients with covid-19.

Aims: To investigate the antibody levels in convalescent plasma from patients with covid-19.

Methods: The antibody levels in convalescent plasma from patients with covid-19 were detected by kit of IgG,IgM, S protein IgG and S protein IgM. The results of IgG, IgM, S IgG and S protein were analyzed statistically according to gender, blood type, age and discharge time.

Results: The samples of convalescent plasma were collected from 72 cases, the positive rates of IgG antibody, IgM antibody, S protein IgG antibody and S protein IgM antibody were 80.56%, 81.94%, 46.58%, 29.17% respectively. There was no significant difference in sex, blood type and discharge time of IgG, but there was significant difference in age group ، the positive rate of 40-49 years old group was the highest (100%),and that of 50-54 years old group was the lowest (42.86%). There was no significant difference in IgM, S-protein IgG, S-protein of IgM, gender, blood type, discharge time and age groups.

Summary/Conclusions: There is no significant difference in plasma antibody of convalescent patients with cowid-19 except IgG.

COVID-19 - Convalescent Plasma (CCP)

P-226 | Associations between symptoms, donor characteristics and IGG antibody response in 2082 COVID-19 convalescent plasma donors

selected for main programme

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Background: Convalescent plasma collected from donors recovered from COVID-19 with high neutralizing antibody titres could be used as a potential therapy for patients. Selecting donors requires a thorough understanding of the kinetics of the antibody response. Previous studies identified considerable variation in antibody response between patients.

Aims: We aim to identify predictors for variation in antibody levels and rate of decline in COVID-19 convalescent plasma donors using donor characteristics and clinical symptoms.

Methods: Plasma samples (N=1118))were collected from COVID-19 recovered adults (N=2082) who enrolled in the convalescent plasma programme at Sanquin Blood Supply (the Netherlands). To obtain insight in their clinical symptoms, participants completed an exhaustive questionnaire. Plasma was sampled every 2-4 weeks, until anti-RBD IgG antibody titres dropped below a certain limit or 6 months had passed since recovery. The median number of samples per donor was 5 (total range 3-18). Antibody levels were measured using a quantitative IgG anti-RBD ELISA, results were expressed in AU/ml. Longitudinal trends in log-transformed antibody titres were analysed with a linear mixed-effect model, allowing random slope and intercept for each donor to account for individual differences in antibody response and clearance rates.

Results: Most variation is present in the distribution of the fitted random intercepts. These follow a lognormal distribution with a median value of 15 AU/ml (IQR 7–33 AU/ml, total range 1–300 AU/ml). The variance in the random slopes is smaller, corresponding to a median half-life of 51 days (IQR 31 – 82 days). The residual variance represents the within-donor variance and is very small, indicating that the individual decline in antibody titre is stable over time.

Of the variance in highest antibody level (intercept), 23% could be explained by several donor characteristics, which are all known to be related to increased risk for more severe disease. Sex was associated with both the intercept and slope: initial antibody titre is higher in men than in women, and the rate of decay is faster for men. Age and BMI are both positively correlated with the intercept: donors of higher age and/or BMI tend to have higher antibody levels. No associations were found for differences in ABO or RhD blood groups. A correlation of +0.16 was estimated between intercept and slope.

Out of eighteen symptoms queried in the questionnaire, nine showed a significant association with antibody levels ($p<0.05$). Interestingly, presence of nasal cold, headache and anosmia were associated with lower antibody levels, whereas presence of dry cough, fatigue, fever, dyspnoea, diarrhoea and muscle weakness were associated with higher levels. Donors who had been admitted to the hospital (N=63) had substantially higher antibody levels and a higher rate of decay. No associations between presence of symptoms and the rate of antibody decay were found.

Summary/Conclusions: Most variation in antibody levels is between-donor variation, and more variation is present in the height of antibody level than in the rate of decay. Part of this variance can be explained by donor characteristics and presence of particular symptoms, as donors showing upper airway complaints including anosmia had lower antibody responses. The associations we found can be used to improve the selection of potential convalescent plasma donors based on how long their antibody levels are expected to remain above a certain threshold value.

P-227 | Transfusion adverse events associated with COVID-19 convalescent plasma compassionate use therapy for SARS-CoV-2 *selected for main programme*

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Background: COVID-19 convalescent plasma (CCP) has been effective in the prevention of severe COVID-19 in older patients. In most published studies, CCP transfusion was considered safe with very few adverse events (AE), whereas some have shown a high incidence of AE (12.9%) attributed to PCC (3,1%) "Nguyen, Transfusion, 2020".

Aims: To evaluate the incidence of AE, we performed a review of AE's occurrence reported through our national haemovigilance network.

Methods: As recommended by the French competent authority (CA) ANSM, each patient was deemed eligible for CCP transfusion after approval by multidisciplinary meeting and informed consent of the patient. CCP was most often administered as two units of 200 ml of PCC on day 1 and two units on day 2 (4 units usually issued from different donors).

From May 7 2020 to January 18 2021, 305 patients had received at least one cycle of CCP treatment. Any AE related to plasma transfusion was notified to the CA in compliance with the on-going national regulation. In addition, a specific follow-up of AE was performed by the person responsible for the issuing of plasma with the support of the practitioner in charge of the patient, all along the study.

Types of transfusion reactions are defined as: allergic reactions; fever-chills, or febrile non hemolytic transfusion reaction; Transfusion Acute Circulatory Overload (TACO); Transfusion Related Acute Lung Injury

(TRALI); systemic or local (pulmonary) inflammatory reaction and unknown adverse reactions should be also declared.

Results: Thirty one AE occurred in 27 patients among 305 patients transfused (incidence 10,2%), 2 of them had 2 AE (twice fever, twice allergic reaction) and 1 of them had 3 AE (TACO).

Among the 31 AE, 10 (32%) were classified as transient worsening of respiratory symptoms that did not last; 10 (32 %) as fever, sometimes in addition to various symptoms (1 chills, 1 leukopenia, 1 hyperlactatemia); 7 (23%) were allergic reactions and 4 (13%) were TACO.

The causality of PCC transfusion in AE occurrence was as follows:

- Transient worsening of respiratory symptoms: 4 not evaluable, 4 likely, 1 possible, 1 excluded
- Fever: 6 possible, 1 uncertain, 1 not evaluable, 2 data missing,
- Allergy: 5 likely and 1 data missing
- TACO: 3 not evaluable, 1 likely

The severity of AE was as follows

- Transient worsening of respiratory symptoms: 2 mild, 2 moderate, 6 severe
- Fever: 4 mild, 4 moderate, 2 severe,
- Allergy: 4 mild, 1 moderate, 1 severe
- TACO: 4 moderate

All patients (27) with an AE had co-morbidities described in Table 1.

For patients have been followed, they evolved as follows: 9 deaths; 9 leaving hospital after improvements; 3 improvements under following; 2 clinical stability; 1 worsening; 1 had a second PCC transfusion; 3 data missing.

P-227 Table 1.

Co-Morbidity	Malignant hemopathy	Auto-immune disease	Transplant organ/ hematopoietic cells graft
AE type			
Worsening of respiratory symptoms	5	3	2 (kidney, heart)
Fever	8	1	0
Allergic reactions	4	1	1 (Kidney)
TACO	1	1	0
Total	18	6	3

P-227 Table 2.

Age	N	Mean	SD	Error Deviation
≥46	395	5,33	3,24	0,16
< 46	385	3,89	2,74	0,14

Summary/Conclusions: AE incidence in COVID-19 patients transfused with PCC (11%) is higher than AE incidence in non-COVID-19 patients transfused with non-convalescent plasma (hemovigilance data of INH 0,047%).

P-228 | Association of age with anti-SARS-CoV-2 IGG antibody titers in convalescent plasma donors

selected for main programme

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Background: Plasma of patients that have survived after COVID-19 has been proposed as a passive immunotherapy for acute patients. The level of neutralizing antibodies may vary depending on different factors.

Older people are at high risk of developing severe acute respiratory syndrome caused by SARS-CoV-2. Previous studies showed an association between the severity of the disease and the titer and durability of specific antibodies. A recent study found an association of age with the level of anti-SARS-CoV-2 antibodies in the pediatric setting.

Aims: This study aims to evaluate whether there is a relation between the anti-SARS-CoV-2 IgG antibody titers and the age in a cohort of convalescent plasma donors.

Methods: The study participants were individuals that had suffered the COVID-19 and presented to become convalescent plasma donors. Only males are accepted as plasma donors in our Blood Centre, following a widespread policy to prevent TRALI. All study participants were submitted to regular blood donor testing (ABO, RhD typing, and infectious disease screening) and were also screened for anti-SARS-Cov2 IgG antibodies.

The antibody assay employed was the Euroimmun anti-SARS-CoV-2 IgG ELISA (Mountain Lakes, NJ). The ratio between the optical density of the sample and the cut-off (S/CO) was considered as the antibody titer. The cut-off was established at =/>1.1.

Eligible individuals were required to have a minimum titer of antibodies of 3.5. Although most of them have donated convalescent plasma (CCP) several times, only the first sample was included in the study. Samples of not eligible individuals, because of low antibody titers, were also included in the study group.

Statistical analysis:

Age was measured in years and antibodies in S/CO ratio. Sample characteristics were described for the total sample using means and standard deviations for continuous variables. Student's t-test for continuous variables was used to examine antibodies' mean by age. Given that the median of the entire population was 46 years, two age groups were established, with a cut-off point at 46 years. P-values <0.05 were considered to be significant. All statistical procedures were performed using SPSS v.20.0 software (SPSS, Chicago, IL.).

Results: A total of 787 CCP donors were evaluated. Donors older than 46 years old, showed a significant (P<0.001) higher level of antibodies than their younger counterparts.

Summary/Conclusions: The results of this study suggests that SARS-CoV-2 viral-specific antibody response levels are distinct in different age groups. Most laboratories rely on ELISA assays for detection and quantification of IgG anti-SARS-Cov-2 antibodies, due to the technical

difficulties that encompass determining the neutralizing antibodies (NtAb). The identification of correlates of high NtAb titers would eliminate the need for slow, time-consuming screening procedures and streamline plasma donor selection. Previous comparative studies have determined that IgG antibody titers above 3.5 correlate well with adequate NtAb levels. The correlation found here suggests that an age-targeted strategy for CCP donors may be of interest.

P-229 | Evaluation of SARS-CoV-2 antibody concentrations in different convalescent plasma donors' subgroups

selected for main programme

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Background: Therapy with passive antibodies has been in use for over a century for both postexposure prophylaxis (rabies, polio) and treatment (SARS-CoV-1, Middle East respiratory syndrome, Ebola).

Aims: The aim of our study is to evaluate the SARS-CoV-2 antibody concentration in different convalescent plasma donors' subgroups.

Methods: This is a prospective study organized in the Institute for Transfusion Medicine of Republic of North Macedonia since 30 April 2020. Antibody testing was performed at the Institute for Immunobiology and Human Genetics in Skopje using CLIA method with Snibe Maglumi 2019-nCoV IgM and 2019-nCoV IgG (qualitative) in the beginning and continued and retested with Snibe Maglumi SARS-CoV-2 S-RBD IgG (quantitative) with IgG cut-off larger than 5 AU/ml. Preferred method for plasma collection was plasmapheresis which was performed with Terumo BCT Trima Accel and donation of whole blood, depending on the donor preference and venous access. The following criteria for potential CCP donors applied: a prior diagnosis of COVID-19 documented by a positive RT-PCR or a positive test for SARS-CoV-2 antigen or a positive test for SARS-CoV-2 antibodies,whether the individual had symptoms or not. A deferral period of at least 28 days was applied after symptom resolution. Prospective donors that had no clinical signs of the disease were accepted for donation at least 21days after: laboratory evidence of viral RNA clearance from the upper respiratory tract or being tested positive for the presence of anti-SARS-CoV-2 antibodies. All donors signed inform consent for donation and inclusion in the study.

Results: There were 361 males (68.8%) and 164 females (31.2%) out of 525 total CCP donors. Mean age of the donors was 43 years, with range from 18-63. Mean value of SARS-CoV-2 S-RBD IgG in our study was 33.56 AU/ml, with range from 5.1 AU/ml to >100 AU/ml. There were 63 donors that were previously hospitalized with mean value of SARS-CoV-2 S-RBD IgG = 51.97AU/ml (range 5.5-100AU/ml), and 462 that were treated at home with mean value of SARS-CoV-2 S-RBD IgG = 30.85AU/ml (range5.1-100), of which 427 had symptoms with mean value of SARS-CoV-2 S-RBD IgG = 28.84AU/ml (range 5.1-100) and 35 were asymptomatic with mean value of SARS-CoV-2 S-RBD IgG = 32.87AU/ml. The CCP donors had the following distribution according to the age: 7 donors in the 18-20 age group with median value of SARS-CoV-2 S-RBD IgG = 29.85AU/ml (range 9-100), 103 donors in the 21-30 age group with mean value of SARS-CoV-2 S-RBD IgG = 23.33AU/ml (range 5.2-100), 144 donors in the 31-40 age group with mean value of SARS-CoV-2 S-RBD IgG= 26.08AU/ml (range 5.1-100), 165 donors in the 41-50 age group mean value of SARS-CoV-2 S-RBD IgG = 38.39AU/ml (range 5.2-100), 96 donors in the 51-60 age group mean value of SARS-CoV-2 S-RBD IgG = 43.63AU/ml (range 5.7-100) and 10 donors in the 61-65 age group mean value of SARS-CoV-2 S-RBD IgG = 41.25AU/ml (12.7-100).

Summary/Conclusions: The collected units of CCP in our Institute were with high concentration and quality. The concentration of SARS-CoV-2 S-RBD IgG in CCP obtained from previously hospitalized patients is almost double of the ones that were treated at home. Interestingly, the concentration of SARS-CoV-2 S-RBD IgG in asymptomatic patients is larger than in ones with symptoms treated at home. The concentration of SARS-CoV-2 S-RBD IgG was higher in advanced age group. The further studies are needed to clarify the impact of different variables on antibodies concentration/titer in donors.

P-230 | Assessment of the impact of pathogen-inactivation treatment with three different commercially available technologies on the Anti-SARS-CoV-2 N-protein IGG antibody titer of COVID-19 convalescent plasma

selected for main programme

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Background: Convalescent plasma is considered as rapidly available, promising treatment option for COVID-19 patients in many regions of the world. Its therapeutic potential is currently assessed in many countries, also in Belarus, under local emergency use approval. To mitigate the risk of transfusion-transmitted infections, especially HIV/HBV/HCV in the window-period, and to address potential concerns about SARS-CoV-2 transmission, pathogen-inactivation technologies are used COVID-19 convalescent plasma (CCP). Recent studies suggest that the neutralizing antibody quantity and quality significantly affects the therapeutic efficacy of CCP. In many blood centers only serology tests are available as surrogate marker for the neutralizing activity, recently assay-specific values for high titers have been defined to allow the selection of clinically effective high-titer plasma.

Aims: Comparative assessment of the impact of pathogen-inactivation treatment on the anti-SARS-CoV-2 N-protein IgG titer.

Methods: 600 mL plasma donations were collected from convalescent COVID-19 patients with a plasmapheresis device (Haemonetics, Fresenius) and subsequently pathogen-reduced with three different methods: amotosalen/UVA (INTERCEPT Blood System, Cerus) (AS), methylene blue/visible light (Theraflex MB, Macopharma) (MB) and riboflavin/UVB/UVC (Mirasol, Terumo BCT) (RF). Samples were taken before- and after the pathogen-inactivation treatment. Quality control of the plasma was carried out in accordance with the requirements of the order of the Ministry of Health of the Republic of Belarus dated 06.04.2018 No. 325. The antibody titer was assessed with an anti-SARS-CoV-2 N-protein IgG CMIA (ARCHITECT, Abbott), high titer plasma was defined by the US FDA for that assay as index S/C ≥4.5.

Results: 44 plasma units were treated with AS, 22 with MB and 20 with RF. Pre- AS-treatment the antibody titer was 4.68 ± 2.19, post treatment 4.60 ± 2.12; an average loss of 0.18 (95% CI: 0.12-0.26) corresponding to 3.77% ± 3.93 (p-value: <0.001). Pre-MB-treatment the antibody titer was 3.56 ± 1.60, post treatment 3.40 ± 1.58; an average loss of 0.16 (95% CI: 0.09-0.23) corresponding to 4.81% ± 4.61 (p-value: <0.001). Pre- RF-treatment the antibody titer was 4.52 ± 1.57, post treatment 3.96 ± 1.48; an average loss of 0.56 (95% CI: 0.41-0.71) corresponding to 12.71% ± 7.25 (two-tailed p-value: <0.001). The treatment impact on high-titer plasma was slightly lower compared to low-titer plasma.

Summary/Conclusions: The pathogen-inactivation treatment impacted the SARS-CoV-2 N-protein IgG titer significantly with all 3 technologies. AS-treatment had the lowest impact, followed by MB which also had a relatively low impact. The antibody loss after RB-treatment was ≈3-fold higher compared to the other technologies assessed in that study.

P-231 | The results of HLA antibodies testing in COVID-19 convalescent plasma female donors in Moscow City Blood Center *selected for main programme*

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Background: Since the beginning of Covid-19 pandemic Convalescent plasma (CP) transfusion has been used for treatment of Covid-19 patients. HLA and HNA antibodies in CP can cause Transfusion-related acute lung injury (TRALI) that results in an additional risk of acute respiratory distress syndrome in these cases. As the main reasons of HLA antibodies formation are previous transfusions and pregnancies, careful donor selection along with the testing CP female donors for HLA antibodies seems to be useful approach as a part of strategy for TRALI prevention.

Aims: To assess the results of anti-HLA testing in Covid-19 CP female donors in Moscow City Blood Center according to the pregnancy background.

Methods: HLA Class I and II (IgG) screening tests were performed for 1647 Covid-19 CP female donors using Luminex technology (Immucor Inc.). Controls included 30 sera samples with negative screening and single-antigen bead (SAB) assay results. The history of donor pregnancy was collected. Then, the prevalence of HLA-antibodies was evaluated in different female donor groups according to the presented information. The comparison of HLA-alloimmunization in groups was assessed using chi-square test. Correlation model was used for the association of parity with HLA-alloimmunization rate. Statistical significance was assigned to p values <0.05.

Results: Among 1647 CP female donors overall alloimmunization rate was 25.3%, herewith HLA class I antibodies was observed in 8.3%, class II in 10.6% and both classes I&II in 6.3%. We got the history of pregnancy from 1542 women: 587 were never pregnant and 955 had 1 or more pregnancies. Alloimmunization rate even in nulliparous women (13.3%) was higher than in control group (0%, p=0.02). However, in alloimmunized never pregnant females the median MFI was less than 1400 and 1000 for anti-HLAI and II class respectively that might be result of obscure early pregnancy loss. The real clinical significance of this data remains a question of future investigations. Parous females had statistically higher prevalence of HLA antibodies (32.3%) than nulliparous ones (13.3%, OR 3.11, CI 2.36-4.09, p=0.000001).Correlation model showed that the presence of HLA-antibodies increased with parity:13.3%(0), 22.8%(1), 33.3%(2), 38.3%(3 or more), p = <0.05. The effect of the last pregnancy resulting in a delivery or pregnancy loss (miscarriage or termination) on anti-HLA prevalence was also evaluated: 35.4% vs. 21.9% respectively (OR 1.95, CI 1.32-2.9, p=0.0007). Interestingly, the time from the last pregnancy didn't affect alloimmunization rate that was 35.9% in females with pregnancy within the last 5 years, 30.2% - from 5 to 10 years and 32% - more than 10 years. Also we compared the ratio of class I, class II and both classes I&II HLA-antibodies among alloimmunized individuals in presented groups. It turned out that there wasn't any difference in this ratio, except the group of nulliparous women. There, among 78 females with anti-HLA, class II alloimmunization rate was remarkably higher (85.9%) compared to class I only (5.1%) and both classes I&II (8.9%), p = <0.05.

Summary/Conclusions: The prevalence of HLA-antibodies in CP female donors increases with parity regardless the time from the last pregnancy, although alloimmunization in nulliparous females requires future investigation. Also, the outcome of the last pregnancy affects alloimmunization rate. Our data allow to focus on anti-HLA testing in particular CP donor population as a potential TRALI risk-reduction measure.

P-232 | Complications post donation in COVID-19 convalescent plasma donors – NHSBT experience

selected for main programme

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Background: Convalescent plasma, donated by persons who have recently recovered from COVID-19, is the acellular component of blood that contains antibodies, including those that specifically recognise SARS-CoV-2 virus. Safety and efficacy of COVID-19 Convalescent Plasma (CCP) was tested as part of two large randomised controlled trials in UK (REMAP-CAP and RECOVERY). NHS Blood and Transplant (NHSBT) is a Special Health Authority in England and ensures a safe and reliable supply of blood components. CCP collections by apheresis were started across NHSBT from early in the pandemic to support the trials. Apheresis collections avoid unnecessary red cell loss in the donor and optimise the volume of plasma that can be collected. Donors could donate at 2 weekly intervals for several months before antibody titres begin decreasing provided, they passed the donor health screen and haemoglobin check.

Aims: Cumulative data regarding post donation complications seen in CCP donors was evaluated to identify common themes and risk factors for adverse events.

Methods: Data regarding adverse events of donation were collated and analysed from CCP donors who had donated at least once in the period between April 2020- Feb 2021(inclusive).

Results: There were 50,562 attendances for convalescent plasma donation in the period 21/04/2020–28/02/2021. Of these, 6,153 (12.2%) resulted in at least one adverse event, reported within 7days of attendance. Those experiencing an adverse event tended to be younger than those who did not (56% of the group experiencing an adverse event were aged under 45, compared with 43% of the group who did not). The M/F split was very similar in both groups. No strong relationship between ethnicity and risk of adverse events was evident. Donors experiencing an adverse event were more likely to be first-time donors than donors with no adverse event. The risk of having any adverse event falls from 15% for first-time donors to 7% for repeat donors. CCP donors experienced lower rates of mild vasovagal reaction (VVR) to new/returning whole blood donors overall but appear to be at higher risk after stratifying by sex and age. They are significantly more likely to feel faint than new/returning apheresis donors. Bruising and arm pain are more likely in CP donors compared to new/returning apheresis donors, and moderate VVR are nearly twice as common. However, CCP donors are at lower risk of citrate toxicity. The trends over time in adverse event rates in CCP donors have been broadly stable. One serious adverse event of donation was recorded in a new male CCP donor in his mid-40s who had severe immediate VVR with hypotensive seizure requiring hospitalisation following his first CCP donation. He recovered subsequently and was withdrawn from donation.

Summary/Conclusions: Donating CCP was largely safe but complications were seen following donation in 12% with VVR, bruising and arm pain being the most reported donor adverse events. VVR could be multifactorial with increased anxiety, new/first time donors, vascular dysregulation or subclinical cardiac dysfunction secondary to recent COVID-19 infection possibly contributory. Donor haemovigilance is particularly important given donors were recovering from an emerging potentially life-threatening illness. As understanding about long-COVID evolved, donor health screen and selection guidelines were updated to ensure donor safety was optimised. It is encouraging to see that the risk of having any adverse event halved with repeat donations.

P-233 | Plasma donated by persons vaccinated against SARS-CoV-2 is new source for passive immunotherapy

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Background: Plasma with antibodies to SARS-CoV-2 for passive immunotherapy is collected now from convalescents (CCP, anticovid plasma). The convalescents are mostly first-time donors with high risk of conventional blood-transmitted infection. That is why in accordance with Russian guidelines for COVID-19 CCP must be pathogen-reduced with methylene blue or amotosalen. On 14th of March, 2021 there are 4,0 mlns of convalescents and 7,7 mlns of vaccinated people in our country.

Aims: To assess the possibility of anticovid plasma collection from donors vaccinated against COVID-19 infection.

Methods: We observed a cohort of 42 healthy people who received the Russian vaccine "Gam-COVID-Vac" (also known as Sputnik V), solution for intramuscular administration according to the prime-boost scheme: the introduction of component 1 was carried out on the 1st day, and component 2 - on the 21st day. Reciprocal titers of 2 antigen-specific antibodies (anti-S1 and anti-RBD) measured by two different ELISAs were monitored. Reciprocal anti-RBD titer 1:3200 corresponds to the titer of virus-neutralizing antibodies 1: 160.

Results: We collected plasma (median – 600 ml) by apheresis on the days after vaccination 21-30 from 7 donors and on the days 42-50 from 11 donors. SARS-CoV-2 S1-specific and RBD-specific IgGs were detected in 100% of our vaccinated plasma donors. All our donors were healthy and met the criteria for plasma donors. A week after donation, the median titers of both antibodies increased from 6400 to 12800. No adverse reactions were observed in donors. The prepared plasma met all generally accepted quality standards.

Summary/Conclusions: With the introduction of vaccination against COVID-19, a new category of anti-SARS-CoV-2 plasma donors appears.

P-234 | The titer of anti-SARS-CoV-2 neutralizing antibodies in pathogen-reduced convalescent plasma is related to donor age

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Background: COVID-19 convalescent plasma (CCP) may be clinically efficacious to prevent severe COVID-19 disease progression when administered early after infection. There is evidence that treatment efficacy is linked to high anti-SARS-CoV-2 neutralizing antibody (NAb) titers, and various studies have attempted to correlate donor characteristics with NAb titers in CCP donations.

Aims: To assess the correlation of CCP donor characteristics and NAb titers facilitating the selection of high-titer CCP donors.

Methods: Eligible CCP donors met the criteria of the Polish Ministry of Health for plasma donors and the following additional criteria: age 18-65 years, in case of history of transfusions or previous pregnancy anti-HLA testing, confirmed COVID-19 infection and a minimum of 14 days post last negative SARS-CoV-2 PCR. Plasma collection (600 mL) was performed using an automated plasma separator (Aurora™ or Autopheresis C, Fresenius-Kabi). The plasma units were subsequently treated with amotosalen/UVA pathogen reduction (INTERCEPT™ Blood System for Plasma, Cerus Corporation), split into three 200 mL therapeutic units and immediately frozen. The NAb titer was determined with a SARS-CoV-2 S-RBD IgG CLIA test (MAGLUMI™, Snibe Diagnostic), titers >500 (corresponding to >27.4 AU/mL) were considered sufficiently efficacious. The two-sample t-test was used for statistical analysis, p-values <0.05 were considered statistically significant.

Results: We collected 145 CCP donations to date, 17.9% had a low titer (<500), 43.3% a high titer (500-1999) and 38.6% a very high titer (≥2000). We had 121 male and 24 female donors, the average age of the male donors was significantly higher (male donors 41.6 ± 10.3 years, female donors 35.1 ± 10.7 years, p-value 0.01). We did not find a correlation between the average antibody titer and sex (average female: male 1145.8 ± 650.7: 1257.9 ± 712.6, p-value: 0.45). There was also no significant difference between ABO blood group and the average NAb titer (p-values for average titer difference between blood groups: O:A 0.944, O:B 0.317, O:AB 0.905, A:B 0.842, A:AB 0.376, B:AB 0.521). However, we found a correlation between donor age and NAb titer, the plasma of donors above the age of 50 years had a significantly higher antibody titer compared to the group of younger donors (p-value: 0.001). The <50 years average titer was 1140.7 ± 705.1, median 1000 (100-2000); the ≥50 years average titer was 1680.0 ± 522.8, median: 2000 (400-2000). The median time between onset of symptoms and remission was 10 (7-31) days, there was no significant difference between the average antibody titer of donors with a shorter symptomatic phase (7-10 days, 1289.7 ± 704.6) and a longer symptomatic phase (10-37 days, 1177.4 ± 700.8), p-value: 0.454. The median time between remission and donation was 59 (17-279) days, there was no significant difference between the average antibody titer of donors with a shorter time (<43 days, 1351.7 ± 686.4), a medium time (43-84 days, 1172.7 ± 740.4, p-value 0.266) and a longer time (>84 days, 1396.6 ± 665.5, 0.802) between remission and donation.

Summary/Conclusions: Severity of disease has been reported previously as strongest correlation with high NAb titers, followed by male sex and blood group B. In our donor population we only found a correlation between older age and higher antibody titers, which may be linked to disease severity.

P-235 | Establishment of methodology for COVID-19 convalescent plasma collection and testing at Croatian Institute of Transfusion Medicine

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Background: Passive immunotherapy is the century-old practice of administering antibodies from an exposed convalescent or vaccinated person to a patient susceptible to the disease in question. Experience from prior outbreaks with other coronaviruses (SARS-CoV-1) shows that such convalescent sera contain neutralizing antibodies (NAb) against relevant virus and that their use was beneficial in the treated patients. Collection of COVID-19 convalescent plasma (CCP) at Croatian Institute of Transfusion Medicine (CITM) started in July 2020 and first unit for clinical use was issued in December. Clinicians in Croatia started using CCP in second wave of pandemics, mostly for patients with haematological malignancies. Such patients are not able to fight the SARS-CoV-2 infection by producing their own antibodies. CCP with individually checked levels of NAb, as an external source of antibodies, showed promising results in improving condition of these patients during SARS-CoV-2 infection.

Aims: To present the establishment of relevant methodology to properly evaluate SARS-CoV-2 neutralization capacity testing and to assess the correlation of donor disease severity and demographic characteristics with antibody titre level.

Methods: Each donor had a documented history of laboratory-confirmed SARS-CoV-2 infection. All plasma was donated by recovered and healthy COVID-19 patients and collected by apheresis.

In the beginning of the collection, titre of NAb was measured with the SARS-CoV-2 neutralization assay for quantification of SARS-CoV-2 NAb on Vero E6 cell suspensions and with home working stocks of SARS-CoV-2 virus prepared from a clinical isolate of the Laboratory working stock label SARS-CoV-2 297/20 Zagreb virus. In the meantime, the regression analysis for Vidas SARS-CoV-2 IgG

test was done. This resulted in high correlation coefficient with neutralization assay and the regression equation has been used for the estimation of cut-off NAb level. High titre was determined as ED_{50}/ml ≥ 1200. Data were collected from national transfusion IT system (e-Delphyn) and CCP donor checklist. Chi-squared, Kruskal-Wallis and Mann-Whitney tests were performed in MedCalc software for statistical analysis.

Results: We collected data for donations during the eight months period (28th July 2020 to 28th March 2021). 256 CCP units have been collected from 162 apheresis procedures. The donors' age median was 36 (18-62) years. 30.4 % procedures were collected from women. The NAb median level of CCP for clinical use was 2560 (274 - 145,218). The most common blood type was A (43%), followed by O (33%), B (12%) and AB (12%). COVID-19 symptoms in donors varied: asymptomatic (2.5%), mild (75%), medium (19.4%) and severe (3.1%). 75.4% of all donations had high NAb titre.

Titre level did not statistically differ between genders ($P = 0.262$, $Z = 1.12$), severity of symptoms ($P = 0.072$, $\chi^2 = 6.99$) nor blood types ($P = 0.842$, $\chi^2 = 0.83$). Severity of disease did not statistically differ between genders ($P = 0.23$, $\chi^2 = 4.26$) nor blood types ($P = 0.803$, $\chi^2 = 5.34$).

Summary/Conclusions: 3/4 of collected CCP had high NAb titre. In rare cases, when the CCP supplies were scarce, physicians required issuing of CCP with lower titres. NAb titre was not dependent on gender, symptom severity nor blood type. Application of serological test performed in CITM highly correlated with the neutralization assay and allowed quicker issuing of CCP for clinical use which is very important for due time therapy of COVID-19 patients.

P-236 | The impact of convalescent plasma on the clinical outcome of COVID-19 patients

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Background: According to FDA, covid convalescent plasma (CCP) treatment is approved as a potential therapeutic option for patients with COVID-19. In April 2020, the Institute of transfusion medicine in Skopje started a project for collection of CCP from individuals who recovered from COVID-19, according to the pre-established protocol.

Aims: To evaluate the efficacy of convalescent plasma treatment in COVID-19 patients.

Methods: We evaluated 80 COVID-19 patients who received 200ml CCP (IgG > 5 AU/mL -SARS-CoV-2 RBD IgG) during their hospitalization. We also evaluated 114 COVID-19 hospital patients who did not receive CCP as a control group. Patients were divided in three groups according to the severity of the disease and the type of the oxygen support: Low flow oxygen support group (LFOS), High flow oxygen support devices group (HFOSD) and Invasive mechanical ventilation group (IMV). Patient age, sex, CCP administration after hospitalization (≤3 days, >3 days) and comorbidities (CMB) were correlated to the survival. Blood group distribution of the patients was also assessed.

Results: The average age of the CCP patients was 53 years, 58 (70.2%) were male and 22 (28.8%) were female, 51 (63.8%) have comorbidities, the average hospitalization was 14.6 days. Out of 80 patients, 55 (68.8%) survived and 25 (31.2%) did not survive. Age, CCP administration, comorbidities and outcome for each of the patient group is shown on Table 1.

There was no significant difference between the control group and CCP patients according to the age, sex, comorbidities and disease severity distribution. The outcome of the COVID-19 in the control group is shown on Table 2.

Out of 47 patients who received CCP before day 3, 36.2% did not survive, also 24.2% out of 33 patients who received CCP after day 3 did not survive. Survival rates across the groups according to CCP transfusion did not show statistical difference ($z = 1.13$; $z > -1.64$, $p=0.87$). Also, there is no significant difference in the survival between COVID-19 patients who received CCP and the control group ($X^2 = 0.119$, $p = 0.73$).

P-236 Table 2. Outcome of COVID-19 in control group

	Control group N (%)	Survived No (%)	Non-survived No (%)
LFOS	78 (68.4)	75(96.2)	3 (3.8)
HFOSD	16 (14.0)	6 (37.5)	10 (62.5)
IMV	20 (17.6)	0 (0.0)	20 (100.0)
Total	114 (100.0)	81 (71.1)	33 (28.9)

Summary/Conclusions: CCP administration early after hospitalization statistically does not influence the mortality rate in COVID-19 patients. According to the control group results, CCP treatment has no impact on the survival of COVID-19 patients.

P-236 Table 1. Age, CCP administration, comorbidities and the outcome in COVID-19 patients

Oxygen support	Patient No (%)	Mean age (years)	CCP (days) Patient No (%)		CMB No (%)	Survived	Non-survived
			≤ 3	> 3			
LFOS	57 (71.3)	52	30 (52.6)	27 (47.4)	34 (59.6)	54 (94.7)	3 (5.3)
HFOSD	12 (15.0)	60	9 (75.0)	3 (25.0)	10 (83.3)	1 (8.3)	11 (91.7)
IMV	11 (13.7)	53	8 (72.7)	3 (27.3)	7 (63.6)	0 (0.0)	11 (100)
Total	80 (100)	53	47 (58.8)	33 (41.2)	51 (63.8)	55 (68.8)	25 (31.2)

P-237 | There is no association between anti- SARS-CoV-2 antibody titers and ABO blood type

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Background: Blood group A individuals had been suggested to be at higher risk of SARS-Cov-2 infection. Previous studies regarding ABO type an antibody titres association gave discrepant results.

Aims: This study aims to evaluate antibody titres in convalescent plasma (CCP) compared to ABO type.

Methods: Material and Methods

The study participants were individuals that had suffered the COVID-19, and presented to become CCP donors. As a policy to prevent TRALI, only males are accepted as plasma donors in our Blood Centre. All study participants were submitted to regular blood donor testing (ABO, RhD and infectious disease's screening) and anti-SARS-Cov2 IgG antibodies (ELISA. Euroimmun. Mountain Lakes, NJ) against the S protein. The ratio between optical density of the sample and the cut-off (S/CO) was considered as the antibody titer.

Neutralizing anti-SARS-Cov_2 antibodies titers (NtAb50) were assessed using a vesicular stomatitis virus pseudo typed with the SARS-CoV-2 Spike protein. Dilutions from1:20 to 1:12500 were assayed. The reciprocal of the serum dilution resulting in 50% virus inhibition was considered as the antibody titer.

Eligible individuals were required to have a minimum titer of IgG antibodies of 3.5. Although most of them have CCP several times, only the first sample was evaluated. Samples of non-eligible individuals were also included.

Statistical analysis: Sample characteristics were described for the total sample using means and standard deviations for continuous variables and frequencies for categorical variables. Student's t-test for continuous variables and Chi-Square tests for categorical variables were used to examine mean and proportional differences of the select characteristics of the sample by group. Data were analyzed using a one-way ANOVA, followed by the Tamhane T2 post-hoc test. P-values <0.05 were considered significant. SPSS v.20.0 software (SPSS, Chicago, IL.) was used.

Results: 780 COVID-19 CCP donor candidates were evaluated. ABO blood types distribution among the study and general population is shown in Table 1. Although not statistically significant, there were more type A individuals in the CCP group compared to the general population, while type O was under-represented.

IgG anti spike antibody titers ranged between 0.09 and 13.99 (Table 2). There were no statistically significant differences between ABO group means as determined by one-way ANOVA ($F = 0.404$; $p = 0.75$)

In 34 samples NtAb50 titres against the S protein were also measured and once again no statistically significant differences between ABO group means as determined by one-way ANOVA ($F = 0.785$; $p = 0.465$) were found.

Summary/Conclusions: The study includes a high number of prospective CCP donors. We did not found any relationship between ABO blood types and SARS-CoV-2 antibody titers, of both classes (IgG and NtABs).

P-237 Table 1. ABO type distribution

ABO Type	General population (%)	CCP donors N (%)
O	43.6	310 (39.7)
A	43.9	386 (49.5)
B	9.5	62 (7.9)
AB	3.0	2.8 (2.8)

P-237 Table 2. Antibody Titers by ABO type

					95% CI			
	N	Mean	SD	Error Dev	Inf. limit	Sup. limit	Mínimum	Máximum
O	310	4.52	3.11	0.18	4.17	4.87	0.11	12.59
A	386	4.69	3.06	0.15	4.39	5.00	0.09	13.99
B	62	4.63	3.14	0.39	3.84	5.43	0.13	11.10
AB	22	4.07	3.27	0.69	2.62	5.52	0.16	11.10
Total	780	4.60	3.09	0.11	4.38	4.82	0.09	13.99

P-238 | Convalescent COVID-19 plasma: HLA and HNA antibodies screening assay validation

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Background: COVID-19 pandemic is a serious global health threat and the use of convalescent covid-19 plasma (CCP) against SARS coronavirus 2 (SARS-CoV-2) have been tested In several clinical trials. The assure safety Human Leukocyte Antigens (HLA) and Human Neutrophil Antigens (HNA) are screening to avoid Transfusion-related acute lung injury (TRALI).

Aims: As such the aim of our study was the validation of LABScreen® Multi test to assess HLA and HNA antibodies in human plasma.

Methods: LABScreen® Multi kit is a bead-based assay for antibody detection that utilize a panel of color-coded beads that are coated with purified HLA or HNA antigens HNA-1a/b/c, HNA-2, HNA-3a/b, HNA-4a,and HNA-5a/b as well as differentiating antibodies against HLA classes I and II. Reference sera with known HNA antibodies, provided by immunohematology laboratories from Banc de Sang i Teixits de Barcelona (BSTB) and German Red Cross (GRC), were used. For HLA, recipients within deceased kidney waiting list, tested continuously with cytotoxicity and LABScreen® Mixed and Single Antigen Bead assays, were included in the validation assay. After incubation of beads with sera, bound antibodies were detected with phycoerythrin (PE)-conjugated anti-Human IgG, fluorescence detected with a FLEXMAP 3D analyzer and final analysis performed using HLA Fusion v4.4. software (One Lambda, Inc). Cut-off values were set using ratio and MFI values according to manufacturer instructions.

Results: HLA and HNA antibodies were confirmed correctly in the total of 27 samples were tested covering all HLA and HNA possibilities with exception of HNA-5a e HNA-5b: 2 negative samples for HLA and HNA antibodies, 2 positive samples for HLA class I alone, 2 positive samples for HLA class II, and 2 positive samples for HLA class I and class II. Five samples received from BSTB had neonatal alloimmune neutropenia (NAIN) with anti-FcIII, anti-HNA-1a, anti-HNA-1b, anti-HNA-1d and anti-HNA-3b, three with TRALI diagnosis with anti-HNA-2, anti-HNA-3a and anti-HNA-4a, and finally a sample anti-HNA-1c from an external quality control. GRC samples were anti-HNA-1b, anti-HNA-2, anti-HNA-3a, anti-HNA-3b and anti-HNA-4a.

Summary/Conclusions: The LABScreen® multi is a high throughput and sensitive method that allows HLA and HNA antibodies detection simultaneously, assuring efficacy and safety within CCP programs.

P-239 | Experience with the production of COVID-19 convalescent plasma in a tertiary hospital

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Background: The current status of professional knowledge indicates the possibility of a favourable effect of early application of COVID-19 convalescent plasma (CCP). In our department, we produced CCP for this purpose.

Aims: To assess the experience with the production of CCP and to establish the criteria for the release of CCP.

Methods: CCP was collected using apheresis from donors who had suffered from COVID-19 and complied with eligibility requirements recommended by the Society for Transfusion Medicine and the European Commission. Antibody levels were determined using 2 tests: LIAISON® SARS-CoV-2 S1/S2 IgG (Diasorin) and Elecsys® Anti-SARS-CoV-2 S (Roche Diagnostics). SARS-CoV-2 virus neutralization (VN) titres (Army Health Institute, Techonin and Health Institute, Ostrava) were added in 64 %. The examinations were not performed at each collection due to their limited availability. CCP was released with confirmed or expected VN titre ≥ 160. The dependence between antibody levels and VN titre, antibody levels and age was evaluated using linear regression.

Results: CCP was collected from April 24, 2020 to March 23, 2021. As anti-SARS-CoV-2 IgG levels in plasma units were insufficient, the antibodies were examined before collections of CCP - a total of 312 applicants, VN titre was added in 13 (4%). At the same time, 12 applicants were disqualified for: venous insufficiency, florid eczema, faintness during sampling, contraindicating medication or surgery. A total of 92 donors attended donations: 79 males and 13 females. In 65 donors, anti-SARS-CoV-2 antibody levels were examined before the CCP collection. The vast majority of donors suffered from mild and 5 from moderate COVID-19. Anti-HLA antibodies were examined in one male donor with a history of transfusion and all female donors to reduce the risk of TRALI, 2 females with positive anti-HLA antibodies were deferred. A total of 292 plasmaphereses was performed. 810 units of plasma were produced, 564 units released as CCP, 29 units for fractionation and 111 units for clinical use. In each donor, 1-8 collections were performed, a median of 3 collections, 4 donors donated 8 times. There occurred adverse reactions during collections: vasovagal reactions 8, complications with veins 11. Antibody test LIAISON® SARS-CoV-2 S1/S2 IgG correlated with VN titre in Techonin (R^2=0,32, P < 0,001) and Ostrava (R^2=0,38, P < 0,001)

and Elecsys® Anti-SARS-CoV-2 correlated with VN titre in Techonin (R^2=0,22, P < 0,001) and Ostrava (R^2=0,05, P < 0,022). CCP was released with the values of LIAISON® SARS-CoV-2 S1/S2 IgG > 90 AU/ml, 55 % donors complied, and/or Elecsys® Anti-SARS-CoV-2 S > 200 AU/ml, 62 % donors complied. At the value of Elecsys ≥ 132 AU/ml, VN titre was ≥ 160: 82 % collections (VN titre Techonin) and 74 % collections (VN titre Ostrava). After vaccination against COVID-19 with an mRNA vaccine, antibody levels of LIAISON increased above the measurable range (> 400 AU/ml) just as with Elecsys (> 250 AU/ml) and VN titres were in high values (1:1280 a 1:2560). The correlation of antibodies in LIAISON with age was at the limit of statistical significance (R^2=0,11, P = 0,0577).

Summary/Conclusions: The examinations of antibody levels and VN titre show satisfactory correlation and their combination enables the release of CCP with a sufficient quantity of antibodies. On the basis of these results, the FDA criterion did not always correspond to our VN titre ≥ 160. Therefore, CCP was released according to VN titre or antibody levels meeting our criteria.

P-240 | Anticovid antibodies are perfectly preserved in cryosupernatant plasma

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Background: Anticovid plasma is recommended as an etiotropic treatment of new coronavirus infection. The therapeutic effect of plasma is associated with the level of virus-neutralizing antibodies. The prothrombotic effect common to fresh frozen plasma (FFP) can become a side effect, potentiating coagulopathy, which is characterized by infection with COVID-19. Thus, among the new tasks of the blood service to provide clinics with anticovid plasma, it is important to reduce its thrombogenicity. A way to reduce plasma thrombogenicity is the release of cryoprecipitate, which is becoming increasingly popular as a means of correcting hypofibrinogenemia. Hypothesis: if antibodies are retained in cryosupernatant plasma, it can become an alternative to the clinical use of anticovid FFP.

Aims: To investigate the level of anticovid antibodies in the plasma of donors before and after the isolation of cryoprecipitate.

Methods: Cryoprecipitate was isolated from the plasma of 6 convalescent donors after slow thawing of FFP at temperatures from +2°C to +6°C by hard centrifugation.

In the initial blood plasma of donors and CSP, the following was determined:

- general antibodies to SARS-CoV-2 coronavirus by ELISA (Platelia SARS-CoV-2 Total Ab test system, BIO-RAD, France) on an automatic enzyme-linked immunosorbent analyzer "Evolis" (BIO-RAD LABORATORIES SAS, France); the result of the study was taken into account as a coefficient of positivity (S/CO) - the ratio of the sample signal value to the cut-off;
- IgG to the receptor-binding domain (RBD) of the surface glycoprotein S (spike) of the SARS-CoV-2 coronavirus (GAM-COVID-anti-RBD test system, NF Gamaleya Research Center of the Ministry of Health of Russia). After determining the S/CO of the samples, the antibody titer was established based on the manufacturer's recommendations.

Statistical processing of the results was carried out using descriptive statistics at a significance level of p < 0.05.

Results: The S/CO of total antibodies and the anti-RBD titer did not change at all in the anticovid CSP compared to the initial plasma:

- S/CO of common antibodies - 9.0;
- titer in five samples - 1: 1600 and in one - 1: 3200.

The anti-RBD S/CO also did not change significantly (p>0.05):

- in the original plasma - 2.64 ± 0.33,
- in the original plasma - 2.64 ± 0.33,

Summary/Conclusions: After the isolation of cryoprecipitate in cryosupernatant plasma (CSP), there is no decrease in the level of both total antibodies and specific to the RBD domain of the SARS-CoV-2 coronavirus. It is advisable to include the anticovid CSP in the recommendations for etiotropic treatment of patients with new coronavirus infection, to conduct clinical studies of the thrombogenicity of 2 types of anticovid plasma: conventional and CSP. In addition to the hypothetical decrease in the thrombogenicity of the anticovid CSP, its additional advantage is the production of quite real cryoprecipitate.

P-241 | The Greek experience of pathogen-reduced COVID-19 convalescent plasma and pooled-platelet concentrates (P-PCS) using the intercept pathogen inactivation (PI) system

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Background: The pathogen reduction technologies (PRTs) for blood components enhance the blood safety by minimizing the risks of transfusion transmitted infection, the transfusion–related adverse events (TRAEs) and increase the life duration of P-PCs. Currently, we are confronted with a pandemic disease, the COVID-19, caused by the SARS-CoV-2 virus, which is characterized by high morbidity and mortality and limited therapeutic approaches. The convalescent plasma (CP), containing antibodies for SARS-CoV-2 virus is one of these options with therapeutic efficacy. Although the PI of CP is not mandatory the ISBT recommends PRTs as "high desirable".

Aims: Our aim was to present our experience and data of the pathogen reduction technology of INTERCEPT (CERUS) Blood System (IBS) regarding the technique, the application and the quality of the treated products in our institute as well as the TRAEs to the recipients.

Methods: The COVID-19 CPs were collected by apheresis using Terumo BCT Trima Accel Collection System V 6.0. Twenty-five CP

collections from 22 donors, who fulfilled the criteria of ECDC for COVID-19 CP donors, were performed. The volume of each CP after apheresis was 600 to 800 ml. IBS was used as PI method. Afterwards, the CPs were split in doses of 200 ml and were stored in freezer at -80°C until administration. The IBS was applied in 10% of the annual production of P-PCs, displacing the ionizing irradiation. In a 9 months' period, 100 P-PCs from 4 or 5 buffy coats were treated. For PI treatment each unit of P-PC had to fulfill the following criteria: 1) additive solution (PAS) / plasma ratio: 65/35 (%), 2) PLT yield >2,5X 10^{11}, 3) RBCs' concentration < 4X10^{6} / μl, 4) volume: 300-420 ml. Quality control was conducted on every treated component and they were transfused to haematological patients aged between 17 and 60 years old, with good life expectancy.

Results: The IBS was easily applied in the daily routine of our department and the staff's training was simple and fast. All plasma collections were performed without adverse events to the donors, except one in which a female donor fainted and exhibited seizure movements. PI treatment was performed in the 60/93 plasma products. Thirty-eight of the inactivated CPs were transfused to the patients at the COVID Department (CD) and 8 to the patients at the ICU. In one COVID-19 CP recipient, an adverse event characterized by erythema and exacerbation of respiratory failure was observed 2 hours after transfusion. The patient was admitted in the ICU for 24 hours and he was treated as Transfusion–related Acute Lung Injury (TRALI). As the CP was donated from a male donor with no history of transfusions and negative anti-HLA and anti-granulocyte testing the TRALI syndrome was not demonstrated. With regard to P-PCs the mean PLT yield per product was 3.17 (±0.58) 10^{11} while the loss of PLT in processing was 8% (±0.4%). All PI treated P-PCs were transfused to the group of patients that was scheduled and no TRAEs were observed.

Summary/Conclusions: The application of IBS in COVID-19 CPs and P-PCs is simple and can be easily introduced in a regional institute. In addition, adequate platelet and plasma doses were performed after PI processing and no significant adverse events were observed to the recipients. While the PI implementation will result in additional costs we believe that the method is worth applying as it enhances transfusion safety, especially in frail COVID-19 CP recipients.

P-242 | Experience in organizing the procurement and monitoring of the use of immune anti-COVID-19 plasma in blood centers in Kazakhstan

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Background: With COVID-19, traditional methods used to treat viral diseases have proven ineffective. An alternative method of treatment is fresh frozen plasma obtained from convalescent donors.

In Kazakhstan, measures to organize the production of convalescent plasma began in March 2020 with a search for diagnostic systems to detect antibodies in the blood of convalescents.

In April 2020, convalescent plasma was included in the Clinical Protocol for the Diagnosis and Treatment of COVID-19.

Severe or rapidly developing life-threatening COVID-19 has been identified as indications for use in accordance with the FDA (Food and Drug Administration, USA) Convalescent Plasma Production Guidelines

Aims: Analysis of immune plasma production and monitoring of its use in Kazakhstan.

Methods: Screening of donors for the presence and titer of anti SARS-Cov2 antibodies was carried out on test systems manufactured by Epitope Diagnostics Inc., USA, as well as test systems manufactured by ABBOTT Laboratories (USA).

As a threshold for the sufficiency of the level of antibodies, the value of index (s/c) adopted as 3.0.

An assessment of the neutralizing activity of the convalescent plasma was carried out using 10 samples, of which 1 has a titer of 1:50, 2 samples – 1:100, 1 – 1:400, the remaining 6 – 1:1600.

Also, the presence of virus neutralizing antibodies was assessed using "GA CoV-2 IgG+" (Generic Assays, Germany) confirmatory test systems, which detects antibodies to 3 immunodominate antigens of the new coronavirus (Spike Glycoprotein 1, Spike Glycoprotein 2).

Results: As a result, it was established that antibodies to the Spike Glycoprotein 1 antigen were detected in 100% of the samples, and in 52% of the samples, antibodies to the Spike Glycoprotein 2 antigen were detected.

As of March 25, 2021, 2,032 people became convalescent plasma donors, of whom 424 donated plasma twice. 7215 doses of IP received, 3152 doses issued to medical organizations.

Convalescent plasma was used in the treatment of 1128 patients, 2168 doses were transfused. Of the total number of recipients, 214 keep continue treatment, of which 181 with improvement. 666 patients were discharged, 248 people died.

To assess the CP effectiveness, a special questionnaire was developed, which made it possible to assess the state of health of patients as per the WHO progression scale (from 1 to 9) at the beginning of treatment and at certain intervals.

Questionnaires of 375 patients from 13 regions were studied. Analysis of the questionnaires showed that the most effective was the use of plasma in the first two weeks from the onset of the disease. On average, convalescent plasma was applied on the 11th day of hospitalization, while in patients with recovery – on day 10, in patients with fatal outcome – on day 12.

At the beginning of treatment, the health state according to the WHO scale was estimated at 4-7 points in 338 patients, in 37 – 8-9 points. It was noted that patients who corresponded to 4-7 points on the WHO scale were discharged in 69.2% of cases, patients with an assessment of 8-9 points – in 29.7%. The proportion of patients discharged with recovery was 58.6%.

Summary/Conclusions: With the respect to the severity of the disease, the most effective can be considered the use of plasma at stage 6 of the progression scale (43% of recipients were discharged who required high-flow oxygen therapy) and at stage 7 (33.3% of recipients were discharged requiring intubation and mechanical ventilation).

P-243 | Collection of COVID-19 convalescent plasma in the institute for transfusion medicine of Republic of North Macedonia

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Background: Plasma collected from patients that have recovered from an infectious disease has been transfused over many decades for the prophylaxis and/or treatment of various infectious diseases. Taking into consideration the expansion of COVID-19 pandemics we started the COVID-19 convalescent plasma programme.

Aims: The aim of our study is to show our experience with collecting the COVID-19 Convalescent Plasma (CCP).

Methods: This is a prospective study organized in the Institute for Transfusion Medicine of Republic of North Macedonia since 30 April 2020. Donors eligible for the study were donors without a history of blood transfusion, female donors who have never been pregnant, or who have been tested and found negative for anti-HLA antibodies, age 18-65, in good health that fulfil all other criteria for regular blood donors. All potential donor were tested for: negative RT-PCR for SARS-CoV-2 before donation, anti-SARS-CoV-2 antibodies, anti-HLA antibodies (where applicable), blood count, blood group, TTI and biochemistry. Preferred method for plasma collection was plasmapheresis which was performed with Terumo BCT Trima Accel and donation of whole blood, depending on the donor preference and venous access. Antibody testing was performed at the Institute for Immunobiology and Human Genetics in Skopje using CLIA method with Snibe Maglumi SARS-CoV-2 S-RBD IgG with IgG cut-off larger than 5 AU/ml. All donors signed inform consent for donation and inclusion in the study.

Results: There were 525 donor that fulfil all the criteria and obtained 607 units of COVID-19 convalescent plasma; 471 (77.6%) units from whole blood donors and 136 CCP units from 54 donors donated with plasmapheresis (22.4%). There were 361 males (68.8%) and 164 females (31.2%). Mean age of the donors was 43 years, with range from 18-63. Mean value of SARS-CoV-2 S-RBD IgG in our study was 33.56 AU/ml, with range from 5.1 AU/ml to >100 AU/ml. Distribution of CCP donors according to the ABO blood group was: 234 donors had blood group A (44,57%), 159 donors had blood group O (30,28%), 91 donor had blood group B (17.33%) and 41 donor had blood group AB (7.8%). There were 63 donors that were previously hospitalized with mean value of SARS-CoV-2 S-RBD IgG=51.97AU/ml (range 5.5-100AU/ml), and 462 that were treated at home with mean value of SARS-CoV-2 S-RBD IgG=30.85AU/ml (range5.1-100), of which 427 had symptoms with mean value of SARS-CoV-2 S-RBD IgG=28.84AU/ml (range 5.1-100) and 35 were asymptomatic with mean value of SARS-CoV-2 S-RBD IgG=32.87AU/ml. Distribution of 63 CCP previously hospitalized donors according to the ABO blood group was: 26 donors had blood group A (41.26%), 18 donors had blood group O (28.57%), 13 donors had blood group B (20.63%) and 6 donors had blood group AB (9.52%). There were 5 donors that donated CCP with plasmapheresis twice. Only two donors (0.4%) had mild vasovagal reactions, when donating whole blood.

Summary/Conclusions: Starting of COVID-19 convalescent plasma program was a big success for our institution and our country. The procedures are safe and effective and collected CCP units were with high quality. Taking into consideration that convalescent plasma is one of the best therapies currently available to treat COVID-19 we should continue our valuable work in obtaining more CCP for our patients.

COVID-19 – Clinical

P-244 | Profile of COVID-19 patients that need blood transfusion

selected for main programme

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Background: COVID-19 is a real challenge for transfusion, not only because of the increased consumption of resources, but also because of the decrease in the availability of blood components due to the decrease in donations.

Aims: In this study, we analyze the transfusion profile in COVID-19 patients in our environment, with the aim of identifying areas for improvement that allow optimizing it and distributing resources in the most appropriate way.

Methods: We conducted a retrospective, single-center case series study in patients with COVID-19 (confirmed by PCR) who required admission between March 2020 and February 2021.Discrete variables are summarized as median (IQR) values, categorical variables as whole numbers with percentages.

Results: A total of 1993 patients with diagnosis of COVID-19 were admitted to our center. Of them, 70 patients (3.5%) required transfusion, 285 blood components (235 red blood cells units, 47 platelets units, 3 units of convalescence plasma). Of these blood components, 64% have been consumed in intensive care units (ICU) (184 units in 23 patients). During this period of time in our center, a total of 35,034 blood components have been transfused to 5,447 patients (6.4 units/patient versus 4.1 units/patient in COVID-19 patients; ratio in COVID-19 patients ICU 8 vs no ICU 2.15).

The median age of the transfused patients was 69 years (IQR 58.8-77 years), 37% of them women. 67% of the patients had anemia prior to admission (chronic disorder in 53% of cases, iron deficiency in 6%). Thrombocytopenia was present in 29% patients prior to admission (hypersplenism 20%, chemotherapy toxicity 10%). 91% of the patients presented comorbidities on admission, the most frequent hypertension (63%, treated in 44% of the cases with ACE inhibitors), diabetes (44%) and malignant oncohematological disease (34%).

The median D dimer were 2770ng/ml (IQR, 1370-4980ng/ml) and ferritin levels were 802 μg/l (543-1822μg/l) 48 hours prior to transfusion. A total of 23 patients required ICU admission (32%), 21 presented respiratory

P-244 Table 1.

	Pretransfusional Global	Pretransfusional No ICU	Pretransfusional ICU	Postransfusional Global
Hemoglobin (Hb) gr/L, Median, IQR	76 (72-82)	75 (73-83)	77 (62-80)	91 (82-97)
Platelets $\times 10^9$/L Median, IQR	28,5 (16.5-41.2)	17,5 (9.75-39)	69 (35-89)	50 (23.5-75.3)

distress and 19 of them invasive respiratory support. Most of these patients were under anticoagulant treatment (85%), prophylactic dose 73%, therapeutic dose the rest. The median time from admission to transfusion was 13 days (IQR, 10-19.8 days). There were reported 2 transfusion reactions, all of them mild and with a degree 3 of imputability.

Despite thromboembolic prophylaxis, 7 events have occurred (4 venous, 3 arterial). Bleeding complications occurred in 14 patients (20%), 9 of them WHO grade 3 or more. Mortality of the series was 27%, median time of hospitalization of 27 days (IQR, 11.5-44), ICU of 17 days (IQR, 13-33.5 days).

Summary/Conclusions: Transfusion requirements in patients with COVID-19 is usually related to other criteria of severe infections, most of them occurring in the ICU. More than half of these patients have anemia at the time of admission and are not transfused until the second week of admission. The number of serious bleeding complications is high, probably due to the coexistence of anticoagulation and thrombocytopenia in many of them. Defining clear criteria for platelet transfusion in these circumstances based on clinical evidence is crucial.

P-245 | The impact of COVID-19 pandemic on young professionals in transfusion medicine: A global perspective

selected for main programme

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Background: The coronavirus disease (COVID-19) pandemic brought about changes to daily life as measures to contain the spread of the virus increased across the world. Young professionals (YPs) are some of those most affected by these changes.

Aims: To assess the impact of the COVID-19 pandemic on YPs in transfusion medicine around the globe.

Methods: A cross-sectional web-based survey was distributed electronically to ISBT members inviting YPs (≤ 40 yrs) to participate. Statistical analysis was performed using the EpiData software.

Results: A total of 283 YPs completed the survey with representation from all World Health Organization regions. The majority of the participants were in the age groups 35-40 years (34%). 31% of the participants were based in countries in the second wave of COVID-19, with an increasing number of cases, while 27% were based in countries in the declining phase of the first wave.

41% of the participants indicated increased work due to COVID-19. For the majority (76%), this stemmed from staff absences due to COVID-19 infection. Further, 55% indicated that the option to work from home was not offered to them. For those undertaking study (n=85), COVID-19 impacted their experience. For the majority of students (94.1%) colleges and universities closed. This led to different responses, including shifting to online education (n=67, 78.8%), complete suspension (6%) or reduction (4.8%) of academic activities, and cancellation of assessments and exams (3.6%). Fortunately, 95.3% of the learners reported access to online education.

In terms of perceptions of the risk of COVID-19, 195 (68.9%) respondents considered themselves to be working in a high-risk setting or have vulnerable family members, and 46% of these respondents indicated that they applied social distancing measures at home. Within the sample were 124 clinicians &/or nurses; only half of these (54%) indicated that they often had sufficient Personal Protective Equipment to protect themselves during work. The majority of these respondents (77.5%) had family/household members living with them, and 61.3% indicated that they were worried about infecting them because of the nature of their work. More broadly, over half of the participants (57%) wore a facemask at work because it was mandatory at their workplace, while 2.8% indicated that they did not have access to facemasks due to shortages.

In terms of the psychosocial impact of the changes brought about by COVID-19, almost half of the participants indicated increased stress levels, 16% indicated being depressed, and 2.9% recorded dysfunctional levels of anxiety on the Coronavirus Anxiety Scale (Lee, Sherman, Death Studies 44.7 2020 393-401). While some of this was attributed to work-related factors such as staff shortages, concerns about infection of themselves and family members, lack of childcare and homeschooling, respondents also highlighted the loss of social engagement with peers and colleagues (58%), increased pressure from information seen on media (36%), and difficulties in accessing basic supplies (22.3%) as factors that negatively impacted their psychological wellbeing.

Summary/Conclusions: COVID-19 pandemic had a major impact on young professionals globally. Measures are required to ensure that young professionals are protected and mentally supported while undertaking their duties in current and future pandemics.

P-246 | Effect of the COVID-19 pandemic on blood donation and transfusion in Nigeria – A multi-facility study of 34 tertiary hospitals

selected for main programme

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Background: The COVID-19 pandemic affectedblood donation activities. For countries like Nigeria that were already struggling with meeting blood needs, the possible impact on national blood supplies was terrifying. Mobile blood drive campaigns halted, and voluntary blood donations reduces, challenging available blood supplies. Furthermore, fears of the of virus transmission led to deferrals of elective surgeries and non-urgent clinical procedures with npoticeable declines in blood donations and transfusions.

Aims: We aimed to assess the effect of the COVID-19 pandemic on the number of blood donations and transfusions across the country by blood product type across departments including accident and emergency, obstetrics and gynaecology, paediatrics, surgery and internal medicine.

Methods: A retrospective descriptive study was conducted to determine the impact of the COVID-19 pandemic on blood services in thirty-four (34) tertiary hospitals in Nigeria, comparing January to July 2019 (pre-COVID-19) to January to July 2020 (peri-COVID-19). Data was collected from the country's web-based software District Health Information System, Version 2 (DHIS2), the platform for the National Health Management Information System (HMIS) and analysed using SPSS Version 25.

Results: A 17.1% decline in numbers of blood donations was observed over the study period, especially in April 2020 (44.3%). Similarly, a 21.7% decline was observed in numbers of blood transfusions over the same period, with the month of April 2020 experiencing the sharpest declines (44.3%). The highest declines in transfusion were noted in surgery department for fresh frozen plasm (80.1%) $p = 0.012$ and accident and emergency department transfusion of platelets (78.3%) $p = 0.005$. The least decline of statistical significance was observed in internal medicine transfusions of whole blood (19.6%) $p = 0.011$.

Summary/Conclusions: The COVID-19 pandemic significantly affected the numbers of blood donations and transfusions in Nigeria. STrengthening blood services to provide various blood components and secure safe blood supplies during public health emergencies is therefore critical.

P-247 | A Scottish Collaborative Effort supported by National Data and Local Intelligence from Clinical and Laboratory Teams to inform demand planning for blood components during the COVID-19 pandemic

selected for main programme

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Background: In an immediate response to the coronavirus pandemic, the Scottish National Blood Transfusion Service (SNBTS) SNBTS Transfusion Team (SNBTS TT) joined forces with SNBTS Supply Chain Analytics and hospital-based colleagues to gather rich and diverse data to understand the demand for blood in an unknown and drastically changing landscape of clinical activity, transfusion needs and blood donation.

Aims: To utilise comprehensive national data sources supported by local intelligence from clinical and laboratory teams to inform demand planning during the COVID-19 pandemic and meet the needs of Scotland's patients.

Methods: Scotland holds a wealth of exemplary national data sources relating to healthcare. The electronic Data Research and Innovation Service (eDRIS) updated data previously provided to SNBTS relating to clinical indications for blood component use, in which inpatient, outpatient, maternity, birth records and emergency admission data was linked with transfusion records from the SNBTS data mart Account for Blood to gain a comprehensive picture of recent clinical transfusion demand. Access was obtained to System Watch, the Scottish national tool for predicting and monitoring urgent care and emergency services across Scotland, and subsequently live, daily admissions data was provided directly from the Rapid Preliminary Inpatient Data (RAPID) data mart. This enabled SNBTS to identify trends in hospital activity and map that information to data on blood usage in specific clinical areas of interest.

National data was supplemented with local intelligence provided by hospital transfusion teams (HTT) via Situation Reports, rapidly developed and introduced by SNBTS TT. These "SitReps" alerted SNBTS centrally to actual and expected changes in hospital clinical activity, as well as observations on blood component stockholding, usage, and wastage, laboratory issues such as staffing, and changes in transfusion policy. Latterly the SitReps also collected information about participation in convalescent plasma trials and significant clinical events impacting demand such as major haemorrhages.

SitRep completion was supported by the network of SNBTS Transfusion Practitioners (TPs) who work across both local board and SNBTS services. Their input included collaboration with the local HTTs to review the interactive SNBTS Blood Bank Dashboard which provides real time component activity data, and providing key links to pass local intelligence between clinical teams and SNBTS. The TPs were ideally placed to influence review of local data and provide subject matter expertise which enabled local HTTs and clinical teams to focus on key clinical priorities and the most appropriate use of blood for patients.

Results: The combined intelligence was quickly embedded via data dashboards into routine Senior Management Team and demand planning meetings to inform donor collection strategy. While stocks generally remained sufficient to meet demand in the early phases of the pandemic, the data has helped to prevent over-collection and is now being used to enable SNBTS to respond to unpredictable and often rapid changes in demand in a tailored approach as clinical services continue to resume over the coming weeks and months.

Summary/Conclusions: During the pandemic the accessibility to real time data and local transfusion intelligence enhanced the national and local communications and partnership with the overall aim of ensuring supply met demand for the provision of blood for the patients in Scotland.

P-248 | Clinical case: Treatment of SARS-CoV-2 pneumonia in a child after HSCT using hyperimmune plasma of convalescents

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Background: Patients with severe acquired immune deficiencies may have a more severe and atypical course of COVID-19. Treatment of such patients is difficult because of patient's inability to develop a pathogen-specific immune response and it is can be a predictor of a poor prognosis for COVID-19.

Aims: To demonstrate the successful treatment of SARS-CoV-2 pneumonia by means of hyperimmune convalescent plasma.

Methods: We described a clinical case of SARS-CoV-2 pneumonia in a child after HSCT that was treated with convalescents hyperimmune plasma.

Results: SARS-CoV-2 was detected during routine virologic monitoring in throat swab without any clinical symptoms in 9-month-old girl with juvenile myelomonocytic leukemia 3 months (+99 days) after haploidentical HSCT with TCRαβ+ and CD19+ graft depletion (Miltenyi Biotec, Bergisch Gladbach, Germany). For the treatment of GVHD (intestinal (++)), the patient received budenoside and etanercept. Budesonide therapy was discontinued.

On the 144th day, during immuno-suppressive therapy (metylprednisolon) for reactivated GVHD (grade III; skin (++), intestinal (+++)), the child developed a dry cough, shortness of breath, $SaO_2 = 86\%$. Computed tomography revealed polysegmental bilateral viral pneumonia with areas of "ground glass" affecting 60% of the lung area. SARS-CoV-2 was positive in pharyngeal swab. The patient after HSCT had no T- and B-lymphocytes, had agammaglobulinemia and also needed immunosuppressive therapy for GVHD with methylprednisolone at the dose 1 mg/kg. The patient was administered tocilizumab (10 mg/kg), intravenous immunoglobulin (5g), antibiotic therapy (Piperacillin/Tazobactam). Also a dose of fresh frozen plasma (10 mL/kg) obtained from SARS-CoV-2 convalescent donor (titer of specific SARS-CoV-2Ab 1:160) was transfused on day+146.

After 14 days marked improvement in the patient's clinical condition and lung lesions was revealed: lesion of the lung parenchyma by bilateral polysegmental pneumonia is less than 25%, at the stage of current resolution. Later was performed 2 transfusions of convalescents' fresh frozen plasma obtained from different donors at a dose 10 mL/kg; (titers of specific SARS-CoV-2Ab - 1:160 and 1:80). After that, there was a complete clinical and radiological resolution of pneumonia.

5 days after the last convalescents hyperimmune plasma transfusion IgG antibodies specific to SARS-CoV-2 was detected with chemiluminescent microparticle immunoassay (ARCHITECT, Abbott, USA) with the complete absence of the B-lymphocytes and lack of endogenous immunoglobulin synthesis. Additionally, we did not detect SARS-CoV-2-specific T-lymphocytes 90 days after initial virus detection (Elispot reader, ImmunoSpot Series 5 UV Analyzer; C.T.L, Cleveland, OH).

Summary/Conclusions: This clinical case demonstrates that time to pulmonary damage by SARS-CoV-2 may be delayed in patients with immune insufficiency. Convalescent plasma therapy with high titers of pathogen-specific antibodies is one of the possible and effective options in patients with immunodeficient and COVID-19. This kind of therapy should be a curative option in patients with no prospects for the formation of an adaptive response to the pathogen.

P-249 | Therapeutic plasma exchange in COVID-19: A case series

selected for main programme

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Background: Coronavirus Disease (COVID-19) has become a serious health crisis with many different therapeutic modalities being investigated. Therapeutic plasmapheresis (TPE) was reported as an effective treatment modality in patients with 2009 pH1N1 influenza A virus. TPE has also been used in patients with sepsis with multiorgan failure, and this entity has been categorized by the guidelines of the American Society for Apheresis (ASFA) as category III indication (grade 2B), with optimum role of apheresis therapy not yet established. Emergency Use Authorizations was issued by FDA in April 2020 for use of apheresis technology combined with adsorption cartridge to treat adult critically ill patients with confirmed or imminent respiratory failure secondary to COVID-19. Various reports suggest benefit of performing TPE in COVID-19 with cytokine release syndrome.

Aims: To describe the experience of our center performing TPE for patients with severe COVID-19 and cytokine release syndrome.

Methods: During May and June 2020, critically ill adult patients with COVID-19 and cytokine release syndrome were evaluated by the intensive care and apheresis teams for consideration of therapeutic plasma exchange. Cytokine release syndrome was defined as elevated ferritin, C-reactive protein (CRP), D-dimer, LDH, triglycerides or a combination of them. TPE was performed using Spectra Optia® Apheresis System and albumin 5% was used as replacement fluid. TPE was performed in addition to conventional management.

Results: Among COVID-19 patients admitted to the intensive care unit in King Abdulaziz University Hospital in May and June 2020, five patients were included in this case series, including 1 female. The mean age was 45.8 years (39-71). Three patients had no previous comorbidities, while one had diabetes and hypertension, and one had hypertension and heart failure. The mean APACHE score was 7 (4-10), and the mean SOFA score was 4.4 (2-17). Average body mass index 30.2 (23-44). All patients were on mechanical ventilation. Each patient underwent 3-5 sessions of TPE and 1-1.5 plasma volumes were processed during each session. Some procedures were complicated by hypotension. Average values of D-dimer, CRP, and ferritin were reduced after conclusion of all procedures. Two patients died (40%) while 3 survived to hospital discharge. Mortality among other critically ill patients on mechanical ventilation admitted to our center during the same period was 87%.

Summary/Conclusions: TPE is a feasible low risk therapeutic option for patients with COVID-19 and cytokine storm. Further research is required to further clarify its role in such patients.

P-250 | Coagulopathy in COVID - 19 patients – Macedonian experience

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Background: COVID-19 is a disease, which in some cases may induce a cytokine storm with acute respiratory distress syndrome, systemic inflammatory response syndrome (SIRS) and coagulopathy that can have a poor prognosis. COVID-induced coagulopathy, causes imune-mediated hypercoagulable response that can lead to arterial and venous thrombo-embolic complications, and consecutive fatal outcome. In approximately 30% of COVID-19 cases, the patients are

developing venous thrombo-embolism due to inadequate thromboprophylaxis.

Aims: of the study was to analyze the haemostatic parameters of the hospitalized COVID-19 patients at the Clinic for Infectious Diseases and Febrile States in Skopje.

Methods: In a retrospective study, data was analyzed for 1728 patients with COVID -19 infection hospitalized at the Clinic for Infectious Diseases and Febrile States with COVID -19 from March-September 2020. The haemostatic parameters (platelet count, prothrombin time, activated partial thromboplastin time, thrombin time, D-dimers, fibrinogen and anti–factor Xa assay) were measured at the laboratory in the Department for haemostatic and thrombotic disorders at the Institute for Transfusion Medicine of the Republic of Macedonia-Skopje. The total platelet count was measured with Medonic-M51, the other haemostatic parameters were analyzed with the coagulometer Dade Behring BCS XP-Siemens with commercial reagents from Siemens.

Results: The analyzed data from 1728 patients showed the following average values: platelet count 232 x 10^9 (150 – 450 x 10^9); prothrombin time 11.7 seconds (9,8 – 14,2 sec.); activated partial thromboplastin time 30,89 seconds (27,9 – 37,7 sec.); thrombin time 19,8 seconds (16,1 – 24,1 sec.); D-dimers 1974 ng/ mL (0 – 500 ng/ mL). In 25 patients the average fibrinogen level was 2.62 g/L (2,3 – 3,5g/L). The anti–factor Xa assay was analized in 9 patients, 3 of them were in the prophylactic range (0,2-0,5 IE), and 6 were in the therapeutic range (0,5 – 1,2 IE).

Summary/Conclusions: Our data suggests increased levels of D-dimers in hospitalized patients with severe COVID-19 that may lead to procoagulant state. The anticoagulant prophylaxis is crucial and the dosage regimen of low molecular weight heparin must be evaluated in every patient, especially in patients with severe renal impairment, low platelet count and weight disproportions. Further randomized controlled studies should be done in order to determine the benefit from anticoagulation therapy.

P-251 | The impact of COVID-19 in the blood transfusion chain in Greece: Clinical and ethical dilemmas

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Background: Maintaining a safe and accessible supply of substances of human origin during the COVID-19 pandemic is of vital importance to public health.COVID-19 is not believed to threaten blood safety directly, given lack ofevidence or precedent for parenteral transmission of coronaviruses. However, the pandemic has led to significant shortages of blood, due to donors' fear of exposure to the virus in a medical facility and to the general disruption of civil society. In the first pandemic wave in Greece, blood collection fell by 36%overall and by 65% on blood services' (BS) premises. To avoid shortages, the Ministry of Health decreed that blood transfusions should be carried out only for the management of emergency cases, multi-transfused patients with thalassaemia, and selected ontological and haematological cases. A vital element in encouraging blood donations was the "All Together We Can" campaign.

Aims: As part of the assessment of the impact of COVID-19 on public health and the general population, we investigate blood collection and blood use during the second and third pandemic waves1/6/2020 to 19/3/2021.Surveillance of Blood Transfusion Services(BTS) operation and arising clinical and ethical issues are also discussed.

Methods: BS reported aggregate data on blood collection, broken down by month and collection site, to the Coordinating Haemovigilance Centre and Surveillance of Transfusion. Five large BS provided data on red blood cells (RBC), Fresh Frozen Plasma(FFP), Common Plasma (CP), Whole blood derived Platelets(WBDP) and Apheresis Platelets(AphP) issued, by month. Weekly case incidence of COVID-19 and case fatality rate were examined by the National Public Health Organisation.

Results: In the first 6 months from Feb 2020, weekly COVID-19 incidence averaged 20 / 1,000,000 population, increasing to 582 in the next 6 months and 1056 in Jan-Feb 2021. In the same periods, the death rate was 1, 20 and 23 / 1,000,000 respectively and the case fatality rate 6.17%, 3.08% and 3.66%, respectively.

The intense effort to restore blood collection in the first pandemic wave resulted in monthly collection of over 6000 units in April to June under "All Together We Can". After the expected reduction in the summer, collection was recovering, to 4480 units in October, when the second wave struck and lockdown was reimposed in November. Total collection through the study was 46772 units, with only 29% since October (42% of the duration). The proportions of blood collected outside and inside hospitals were exactly reversed during the course of the pandemic: out-of hospital collection was 58% in the first wave, but in-hospital was 58% thereafter. Five BS issued 51547 units

of RBC in the study period, 18340 units of FFP, 26898 of CP and 2751 of AphP. Usage of RBC peaked in June-July 2020 at just over 5000 units per month, falling steadily to 4000 in February 2021. Monthly use of other products was steady from June 2002 after large fluctuations earlier in the study: FFP averaged around 1450 units, CP 23315 and AphP236 units.

Summary/Conclusions: Impacts of the pandemic on blood collection and use are clear. Reassignment to duties related to COVID-19 has reduced staff availability for off-site blood collection. Reallocation of beds to COVID-19 patients has reduced availability for others. Clinicians and staff of BS face increased clinical and ethical dilemmas in prioritising the use of diminished resources under increasing pressure from the pandemic.

P-252 | ABO blood types and Covid-19: A pilot study to assess the risk of infection

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Background: The ongoing pandemic of novel Coronavirus disease (COVID-19), caused by SARS-CoV-2, has brought a global health emergency. Published data suggest that certain factors including age, sex, and chronic medical conditions play a role in acquiring infection and disease severity. There is currently no biological marker known to predict the susceptibility to SARS-CoV-2 infection. Earlier studies have proven that the ABO blood group system is related to many bacterial and viral infections. Some preliminary research data on the ongoing pandemic point out the fact that the ABO blood group can play a role in the immunopathogenesis of COVID-19.

Aims: To assess the association of ABO blood groups to COVID-19 as a biomarker.

Methods: This single-center cross-sectional study was performed at the Peshawar Regional Blood Centre in Khyber Pakhtunkhwa province, Pakistan, over a period of six months (September 2020 to February 2021). Blood groups of 2,140 patients infected with SARS-CoV-2 (confirmed by real-time RT-PCR) were performed by standard tube technique using anti-sera (Lorne Laboratories Ltd, Berkshire, UK). Besides, 2,450 blood donors (negative for SARS-CoV-2) were used as controls. Statistical analysis was done using Statistical Package for the Social Sciences (version 22.0). The odds ratio and 95% confidence interval were also calculated. The study was endorsed by the ethical committee of the Regional Blood Centre.

Results: Among the 2,450 blood donors (control group), ABO blood group percentage distribution was 28.36%, 32.19%, 28.34%, and 11.11% for A, B, O, and AB, respectively. On the other hand, 2,140 SARS-CoV-2 patients displayed a distribution of ABO blood groups as 34.14%, 33.32%, 20.88%, and 11.66% for A, B, O, and AB, respectively. The percentage of "A" blood group in SARS-CoV-2 infected patients was considerably higher compared to the control group of donors (34.14% versus 28.36%) with a p-value of < 0.001. Conversely, the percentage of "O" blood group in SARS-CoV-2 infected patients was considerably lower compared to the control group of donors (20.88% versus 28.34%) with a p-value of < 0.001. The findings exhibited a considerably higher risk of acquiring SARS-CoV-2 for blood group "A" individuals (95% CI: 1.134-1.439) (Odds ratio: 1.269). The blood group "O" individuals on the other hand displayed a lower risk of acquiring SARS-CoV-2 (95% CI: 0.589-0.761) (Odds ratio: 0.678).

Summary/Conclusions: Blood group "A" has a higher risk of acquiring the infection while blood group "O" has the lowest risk of acquiring the infection in comparison with A, B, and AB groups. The study had limitations concerning the sample size and other factors. It is premature to use this study to guide clinical practice at this time. If verified by further studies with a larger sample size, it may contribute to understanding the susceptibility to SARS-CoV-2.

P-253 | COVID-19 and direct antiglobulin test positivity

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Background: The DAT detects the *in vivo* presence of immunoglobulin and/or complement bound to red blood cell membrane. Since long ago, a DAT positivity has been reported in various viral infections. Coronavirus disease 2019 (COVID-19) is a recently emerged disease caused by the novel Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). With time, the scientific community becomes aware of multiple clinical and laboratory aspects of this new infectious clinical entity. Recent literature data report an increasing frequency of DAT positivity in COVID-19.

Aims: To determine the prevalence of a positive DAT result in hospitalized patients positive for COVID-19 whose blood samples were sent to the laboratory for the routine immuno-hematological pre-transfusion testing.

Methods: This is an observational study conducted in the Blood Unit of AHEPA University Hospital of Thessaloniki, Greece. We collected data prospectively during a 5-month-period. The data collected include: age, gender, DAT result: positivity and grade. Our study included hospitalized patients with confirmed COVID-19, whose blood specimens were sent to our laboratory for the ordinary immuno-hematological pre-transfusion testing. The patients were receiving medical treatment with a combination of drugs. RBCs from the patients were used from EDTA specimens after washing a red cell aliquot once with normal saline. In the initial screening we used the polyspecific reagent (which simultaneously detects IgG and/or C3c)

and when positivity was confirmed we proceeded into the use of monospecific reagents (IgG, IgA, IgM, C3c, C3d). The positivity was graded 0 up to +4 (when found 0: it was regarded as negative).

Results: A positive DAT was found in 44 out of 144 (31%) COVID-19 patients. 27 (61%) were men and 17 (39%) were female. Gender does not seem to affect DAT's positivity (p: 0.68). The mean age of all patients was 69 years old, whereas the mean age of the patients with a positive DAT was 77 years old. On determining the type of DAT: 33 (100%) of patients tested with monospecific reagents had IgG only and the intensity of reactions ranged between weak and 3+ (w: 24%, 1+: 45%, 2+: 21%, 3+: 9%).

Summary/Conclusions: In acute illness the percentage of patients with a positive DAT is higher, with studies showing a 1% up to 15% incidence of a positive DAT finding in hospitalized patients. Hospitalized patients may have various factors associated with DAT positivity (medications, infection, hypergammaglobulinemia, formation of rouleaux, antiphospholipid antibodies). According to published data the mechanism provoking DAT positivity with SARS-CoV2 may be attributed to modifications of the RBC surface during the acute course. The hyper-inflammation may enhance the accumulation of C3 and the binding of IgG autoantibodies to RBC membrane, which promotes the clearance of RBCs by macrophages. One major limitation of our study is that our data on drugs administered to the patients were not complete. Therefore the possible contribution of drug-induced mechanisms could not be ruled-out. In conclusion, a positive DAT was detected in the blood of almost one third of the patients with COVID-19 referred to our Blood Unit. Our data reinforce the already published reports that COVID-19 is associated with an increased prevalence of DAT positivity. It still remains to be investigated whether RBC membrane injury is related to DAT positivity and if this injury could promote the thrombotic complications frequently observed in COVID-19 patients.

P-254 | Blood group antigens in ABO - Lewis - P1PK systems in hospitalized patients with COVID-19

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Background: COVID-19 presents with a wide spectrum of severity and this may indicate that certain environmental and/or host risk factors may play a role. Traditionally ABO blood group has been linked to susceptibility to various infections. The presence/absence of A/B antigens and correspondent anti-A/B antibodies may provide protection against infection. Preliminary data about the association between SARS-CoV-2 infection and ABO blood group are controversial.

Aims: To determine the association between several blood groups and COVID-19.

Methods: This is a prospective observational study conducted in our Blood Unit from mid-November 2020 until March 2021. All hospitalized patients positive for COVID-19 who needed blood transfusion were included. We collected data about the age, gender and blood group type. Comparative data on the prevalence of different blood groups in Greece were screened through PubMed.

Results: 147 patients were included. 87 (59%) were male and 60 (41%) were female. The frequencies of blood types A, B, AB, and O were: 50%, 12%, 5%, and 33%, respectively. Blood Group O was proportionately less represented and blood Group A more represented, respectively, in the hospitalized patients in comparison to the Greek general population. Moreover antigens Le^a and P_1 were tested. Le^a was found positive in 18% of patients (23/130) and P_1 was found positive in 75% of patients (98/131). These percentages coincide with literature reports on Caucasian populations.

Summary/Conclusions: ABO blood type has been suggested as a cause for predisposition to COVID-19. Studies are not consistent in their findings. Our findings suggest that there might be a linkage between COVID-19 and ABO blood groups. According to published data the blood type distribution in Greece is: O 44%, A 38%, B 13% and AB 5%. Several mechanisms may explain the association observed in our study. The exact mechanisms that would explain the associations between blood group antigens and disease have not yet been fully elucidated. Different carbohydrate epitopes could serve as receptors for pathogen lectins and also different naturally-occurring antibodies neutralizing pathogens with ABO-mimicking sugar coats on their surfaces could play a role in the evolutionary conservation of certain ABO phenotypes observed in certain populations. Natural anti-A antibodies circulating in the blood may block the interaction between SARS-CoV-2 and its receptor, providing protection, explaining why patients with blood group A seem more susceptible to SARS-CoV-2 infection, while patients with blood group O are not. Additionally the immunoglobulin predominant isotype of anti-B/anti-A is IgM in serum from group A and B individuals respectively, but IgG in O group serum. However, other mechanisms may be implicated and further study is required. The percentages of Lea and P1 found in COVID-19 patients were consistent with literature concerning healthy Caucasian population. Our study has several limitations. First, not all the patients admitted with COVID-19 were tested to determine their blood type, with exception of the ones in need for blood transfusion. Secondly, this is a single center study. In conclusion, the blood group might be one risk factor for COVID-19. Blood group should be checked for every infected individual positive for COVID-19. Further large scale collaborative studies are needed on ABO linkage to the prevalence and mortality of COVID-19. Their findings could shed further light in understanding the disease's pathophysiology.

Blood components/products – Blood processing, storage and release

P-255 | Costs and outcomes associated with large volume delayed sampling and pathogen reduction technology processing *selected for main programme*

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Background: Large volume delayed sampling (LVDS) and pathogen reduction technology (PRT) are two strategies for platelet processing to control risk of contamination prior to transfusion. LVDS and PRT have different processing methods and result in platelets having different risks, viability, and shelf life. Each of which can affect platelet costs and availability to the healthcare system.

Aims: This study compares the economic and clinical impact of LVDS and PRT strategies while considering a holistic view of processing/testing through patient treatment.

Methods: A decision model was constructed to simulate the collection, processing, and use of platelets for four platelet processing strategies: PRT with shelf life of 5 days (PRT5), PRT with shelf life of 7 days (PRT7), LVDS with shelf life of 7 days (LVDS7), and LVDS with initial shelf life of 5 days extended to 7 days with secondary testing (LVDS5/2). Target population were adults requiring two or more transfusions such as patients with hemato-oncological disease or receiving hemopoietic stem cell transplantation. Platelet collection, processing, storage, and distribution data were obtained from the National Blood Collection and Utilization Survey and published literature. Patient outcomes associated with transfusions (adverse events, number of units needed per transfusion, total number of transfusions needed, interval between transfusions, and platelet counts resulting from transfusing) were obtained from AABB guidelines, meta-analyses, and other published clinical studies. Per patient reimbursement costs (2020 USD) were obtained from reimbursement schedules and other published sources.

Results: Based on shelf life and average contamination and expiration rates, for 10,000 donated platelet units, 9,512, 9,878, 9,511, and 9,651 units of PRT5, PRT7, LVDS5/2, and LVDS7 platelets would be available for transfusion, respectively. With these units, 1,502, 1,560, 2,172, and 2,329 corresponding transfusions can be performed where platelet-related adverse events are expected to occur 10.7%, 10.7%, 10.1%, and 10.1% of the time. Hospitals will need to have 72,882, 70,181, 50,406, and 47,022 PRT5, PRT7, LVDS5/2, and LVDS7 platelet units on hand in order to fulfill transfusion demand in a year. The resulting per-patient reimbursement cost per transfusion and per transfusion cycle is higher with PRT units.

Per-Patient Costs

[1]Per-patient reimbursement costs for PRT do not differ based on shelf-life availability because patients do not absorb the cost of expired platelet.

[2]Cycle refers to a series of transfusions to satisfy a full treatment.

Summary/Conclusions: The findings of this holistic view of platelet processing show that compared to PRT, LVDS strategies are associated with lower costs and higher platelet availability for transfusions while patients experience similar levels of adverse events. Increased utilization of LVDS has the potential to improve efficiency, patient access to platelets, and reduce healthcare costs.

P-255 Table 1.

Outcome	PRT (95% Percentile Interval)[1]	LVDS5/2 (95% Percentile Interval)	LVDS7 (95% Percentile Interval)
Cost per transfusion	$10,740 ($5,160 – $30,264)	$6,294 ($3,613 – $10,039)	$5,957 ($3,425 – $9,493)
Cost per full cycle[2]	$71,092 ($30,731 –$205,458)	$33,519 ($16,428 – $60,405)	$31,725 ($15,527 – $57,078)

P-256 | Validation study of top & bottom in-line RBC sets with DEHP-free bags and DEHP-free RBC leukoreduction filter

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Background: PVC plasticized with di(2-ethylhexyl)phthalate (DEHP) is commonly used in the market. However, DEHP will be replaced to other plasticizer, due to toxicological concerns and following REACH restrictions. Platelets are currently stored in PVC plasticized with trioctyl trimellitate (TOTM), which has a higher gas permeability, and allows a prolonged platelet storage compared to DEHP PVC. Replacing DEHP in red blood cell (RBC) bags with DEHP-free PVC plasticizer is challenging since DEHP has been shown to improve stored RBCs morphology, deformability and osmotic fragility, without affecting 2,3-DPG and ATP levels. Therefore, the storage characteristics of DEHP-free PVC needs to be evaluated.

Aims: This study assessed the performance of Leucored CPD-SAG-M® T&B system (Grifols) with a new DEHP-free PVC plasticizer (di-isononyl-cyclohexane-1,2-dicarboxylate, DINCH), a soft housing filter (Sepacell™ RS2, Asahi Kasei Medical) as well as the quality of platelets stored with PVC bags using TOTM or DINCH as plasticizer.

Methods: Whole blood was collected into four types of Leucored CPD-SAG-M® T&B in-line RBC systems: DINCH PVC bag and filter (Sepacell™ RS2) with PAGGS-M (Sample-A); DINCH PVC bag and filter (Sepacell™ RS2) with SAG-M(Sample-B); DEHP PVC bag, DINCH PVC filter (Sepacell™ RS2) with SAG-M (Sample-C); and DEHP PVC bag and filter (Sepacell™ R-S11) with SAG-M (Sample-D). Bags were stored overnight at room temperature and were separated into RBCs, plasma and buffy coats (BC). Then, filter was primed with additive solution and RBCs were mixed. RBCs were filtrated at 110 cm head height between pre-filtration and filtrate bags. Then, hematology, blood-gas and metabolism analyses were performed weekly until 49-day storage.

The obtained four pools of five ABO matched BC units and 300 mL of PAS III M (Grifols) as additive solution were pooled using the TACSI (Terumo BCT). Two pools of Leukoreduced platelets concentrates were stored in TOTM PVC bags and the other two were stored in DINCH PVC bags. Hematology, blood-gas analyses and other parameters were evaluated on 1, 3 and 7-day storage.

Results: Data were shown as mean ± standard deviation. Residual leukocyte count (units) of Sample A, B, C and D was 4.89± 0.49, 4.75±0.02, 4.74± 0.03 and 4.74± 0.02, respectively. Filtration time (min) was 19.9±4.2, 18.8± 3.1, 18.0±4.7 and 28.0±4.6, respectively. Filtration time of Sample A, B and C (Sepacell™ RS2) was significantly shorter than sample-D (Sepacell™ R-S11) ($p<0.01$), without significant differences in residual leukocyte counts.

RBC hemolysis of Samples A, B, C and D at 49-day storage was 0.38±0.07, 0.51±0.14, 0.58±0.33 and 0.43±0.09, respectively. RBC quality with Sample-A after 49-day storage was equivalent to Sample-D. Platelets stored in DINCH PVC bags showed slightly higher pH at Day3 and Day7 than those stored in TOTM, but differences were small and limited, and may be related to gas permeability properties of the bags.

Summary/Conclusions: This study showed that storage performance of DINCH PVC bag and PAGGS-M was equivalent to the current DEHP PVC bag with SAG-M. Sepacell™ RS2 filter performance was better than Sepacell™ R-S11. Platelets storage performance of DINCH PVC and TOTM PVC bags showed almost the same properties for quality control. However, further studies with more samples would be needed.

P-257 | RFID technology for blood components safe management

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Background: Our Centre performs qualification tests for 40.000 blood units a year. In 2020 we decided to introduce an RFID technology system to improve safety and to reduce our staff work load.

Aims: The choice of introducing RFID technology to manage the delivery of blood components, has the aim to improve the traceability, to reduce human error, to lighten the work load of technical staff and to accelerate the processes, maintaining the same level of safety for patients, with specific regard to emergency and distance assignment.

Methods: Considering that our structure produces blood components for the five centers in North-East Piedmont, we have chosen to introduce RFID technology by TAG RFID integrated in the UNI label of final validation and directly codified during the printing process by printers able to print the validation label and contextually to read and write the expected data on the TAG RFID. The features of the TAG have been identified so to grant the complete correspondence to the standard ISO 15693, with 2048 memory bits, 13,56 MHz (type HF) frequency and the guarantee of keeping the data for exercise temperatures from -40°C to +37°C. To make sure the process of blood components delivery, we implement an "intelligent RFID based technology drawers" that, integrated with our informatics System, is able to assign a specific blood bag to a recipient by reading the code of a specific request. The system has required the indispensable management software modifications with the relative approval. A "middleware" integrated with our Informatics System, controls both the drawer and a specific "reading RFID plate". Once the blood bag has been extracted from the drawer, the RFID tag reading by the plate, will confirm the correct blood bag assignment to the correct recipient.

Results: This solution permits the urgency delivery even from remote without the presence of any member of the staff. The system has demonstrated such a high level of security and manageability that we have decided to use it even in our daily routine

without any support from our staff. In about six months from September 2020 we have delivered about 2500 blood units with only 50 returned blood units.

Summary/Conclusions: Thanks to this system we have reduced the number of units which remains out of our service and without temperature check as well as our staff work hours committed to the delivery of the blood bags. The experience has been very positive and has offered a lot of possible uses of the TAG itself. Now we are thinking of using the RFID reading technology for the control at the patient's bedside. Actually it is already managed by matching patient bracelet barcode with the assigned blood bag. Considering that in writing phase inside the drawer the patient's data are recorded on the TAG RFID, at patient's bedside the TAG reading by portable devices, might give his/her data not encoded, offering a further possibility to confirm even in case of temporary absence of online connection with the management software.

P-258 | Whole blood optimization algorithm: Management of platelet inventory to avoid expiry and maximize the volume of plasma recovered

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Background: The growing need for blood products forces centres to optimize the production of plasma, platelet concentrates (PCs) and red blood cell concentrates (RCCs) to adapt it to the needs of the population. This difficult process leads to concentrate expiry, stockouts and economic losses due to the highly variable growing demand for PCs, which is difficult to manage because of their short shelf life. The *Reveos* automatic fractionation system (Terumo BCT) allows to produce, with the same set of bags, either three components (RCCs, plasma and PC) or two components (RCCs and plasma of larger volume) with a difference of 34ml of plasma between the two protocols. This decision has to be taken just before splitting the units.

Aims: To develop an optimization algorithm that provides on a daily basis routine the optimal number of WB units needed for the production of PCs with the objective of maintaining an adequate stock without expiry or stockouts. As a consequence, to assign the highest percentage of WB units to process with the two-component protocol in order to increase the annual plasma volume. To establish a robust mathematical model underlying the algorithm to withstand sudden changes in donations or demand.

Methods: A statistical study including data from WB estimates, donations and hospital demand for PCs of years 2018 and 2020 in BSTA to analyse also changes due to the pandemic. The mathematical model is based on a combination of the *periodical review model* adapted to BSTA and an optimization problem. Only 50% of the PCs were subject to pathogen reduction and its self-life was 7 days. With this approach an algorithm was developed to determine the daily production decision. This algorithm was integrated into a user-friendly software that takes into account and anticipates special holidays. For each day, only the number of WB units, PCs from apheresis pending of analysis and the PCs stock by age must be entered in an Excel sheet. The software returns the amount of WB to be processed with the three-component protocol. The algorithm incorporates a security parameter k which depending on its value adjusts the model to be closer to the stock-out and therefore the PCs expiry rate will be lower, or to move away from the stock-out with a higher PCs expiry rate.

Results: The algorithm has been validated via simulation with the empirical data of the first half of year 2019 in BSTA.

The annual approximate plasma increase is 500 litres. To check that the mathematical model is robust a simulation has been carried out with the empirical data between January and November of 2020 in BSTA (See Table 1). Preliminary statistical analysis showed significant changes in donations and demand due to the pandemic. Therefore, compared to the previous simulation an additional safety margin in production was introduced.

P-258 Table 1.

	BSTA	*Algorithm (k=2.5)*	*Algorithm (k=2)*
PCs expired	75	22	7
PCs stockouts	1	0	0
WB in three components	19350	12400	12311
WB in two components	1207	8157	8246

Summary/Conclusions: The algorithm significantly reduces the number of expired PCs, keeps an optimal stock and increases the annual volume of plasma. The software guides production decisions daily and allows the process to be automated. This tool can be very helpful in situations of uncertainty, whether for several holidays or for changes in demand or donations such as fluctuations during the covid-19 pandemic.

P-259 | Evaluation of whole blood flex filter in routine setting of a regional blood bank

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Background: White blood cell (WBC) depletion of whole blood is a well-established process and used in different variations. If buffy coats are not needed for pool platelet production, whole blood inline

filtration is used in many blood banks for component preparation with the advantage of WBC and platelet removal combined with lower loss of red blood cells (RBC) and plasma and time saving by singular filtration of two components.

Aims: As WBC may cause several unwanted side-effects in patients receiving blood components, HLA antibodies formation ahead, depletion of WBC below $1x10^6$ cells/µl (critical immunogenic leucocyte load; CILL-value) is the goal of filtration. Whereas national and international guidelines require a 90% conformity of the used filters with respect of residual WBC $<1\times10^6$ cells/µl our intention is to come close to 100% and keep variation of the efficacy exceptionally low. Also, negative aspects of filtration like RBC and plasma loss inside the filter, hemolysis, and loss of units due to filter blockages should be kept low. Therefore, we evaluated our quality control data for filter performance, consistency of values, hemolysis, and filtration time.

Methods: For the time period between October 2019 and November 2020, 420 controls out of 34.403 whole blood units collected with a quadruple top&top donation system PQ31555 (Composelect® 4F 63 ml CPD/100 ml SAG-M, Fresenius Kabi) equipped with whole blood filter Bioflex WB and filtered as routine setting at room temperature were evaluated for [DJ1] residual leucocytes, red cell damage resulting in hemolysis (%) and total hemoglobin of the RBC component and filter blockage rate. Filtration time was calculated out of 1.374 procedures during routine processing, because filtration time is not included in our quality control program. Data were expressed as mean±SD or in %.

Results: 98% of the 1.374 filtration processes were finished within 20 minutes, [DJ1] no one lasted longer than 30 minutes. For the whole observation period we did not find a single blocked filter out of 34.403 filtration processes. Evaluating our 420 registered quality controls residual leucocytes were $0{,}18\times10^6 \pm 0{,}19\times10^6$ cells/µl and only 4 tested units met (1) or exceeded (3) the borderline of 1×10^6 cells/µl (0,95%) with a maximum value of 1.72×10^6 cells/u. Total hemoglobin of our filtered red blood cell units was 58.57g/unit ± 6,64g/unit, only one unit was below the limit of 40g/unit, which was caused by an unexpected low hemoglobin level of the donor. Hemolysis of RBC units in SAG-M in % after 6 weeks of storage was 0,26% ± 0,14% and not a single unit reached or exceeded the borderline of 0,8%.

Summary/Conclusions: Routine use of the soft whole blood inline-filter Bioflex WB from Fresenius Kabi results in high filtration efficacy and a noticeably short filtration time, which will help centers to optimize their separation process. Hemolysis at the end of storage was consistently low, total hemoglobin is consistently high above quality requirements, and with zero filter blockages resulting in loss of units, representing a stable process. Remarkable is also that our results represent data from a routine process and not a perfectly controlled study setting.

P-260 | The quality of red blood cells isolated from cord blood during storage

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Background: Standard leukocyte-depleted red cell concentrates (RCC) are currently used in treating anemia in prematurely born neonates. Red blood cells (RBC) of neonates contain over 90% HbF, where adult RBCs contain over 95% HbA. Because HbA has a lower oxygen affinity for oxygen than HbF, higher oxygen release can occur after a transfusion with adult RBCs. As higher oxygen release in the retina is associated with retinopathy, transfusion with HbF containing RCC may prove superior.

Aims: The aim of this study was to find the optimal cord blood (CB) processing and storage conditions.

Methods: For the preparation of CB into an RCC, CB was diluted tree times with SAGM or PAGGSM and filtered with a standard whole blood (WB) filter for leukodepletion and platelet removal. The CB-RCC hematocrit was adjusted to between 50 and 65%. 20ml of CB-RCC was stored for 35 days in a DEHP plasticizer-free transfer bag, for standard pediatric transfusion, or a DEHP transfer bag. On days 1, 7, 21 and 35 the blood gasses, metabolism, hematological parameters, deformability, hemolysis and HbF were determent. Adult RCCs served as a control.

Results: The CB-RCCs were found to consume less glucose and produce less lactate than WB-RCCs. The fraction of non-deformable cells, measured with the Automated Rheoscope and Cell Analyzer (ARCA), of the CB-RCCS was very high on day 35 (SAGM: 11,28% ± 5,67, PAGGSM: 23,93% ± 9,57), compared to the WB-RCC (SAGM 3,45% ± 1,51%). Hemolysis of the CB-RCCs was higher than that of the adult RCCs from day 21 to day 35. On day 35, hemolysis of the CB-RCCs for both storage solutions no longer met the target value of <0.8%. CB-RCC stored in a DEHP-free transfer bag did not result in higher hemolysis as compared to the DEHP-containing storage bag. Follow-up research into volume/ surface ratio for now shows that storage a volume of 50ml instead of 20ml reduces hemolysis.

Summary/Conclusions: In this pilot the quality of CB-RCCs stored in both SAGM and PAGGSM quickly deteriorated after day 21. During this period, no negative effect of the absence of the DEHP plasticizer was found. However, preliminary data shows that increasing the RCC volume may lead to lower levels of hemolysis during storage and stabilize other parameters as well. Further advancements may open a path for use of relatively short-stored CB-RCC in clinical trials to assess the impact of CB-RCC transfusion on retinopathy in prematurely born neonates.

P-261 | Using smart RFID solutions to improve RBC units stock management, transfusion safety and blood unit traceability with clear benefits to the patient

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Background: The biolog-id Solution connects healthcare professionals to essential product information with precise real-time RBC unit location. This traceability allows unused RBC units to be reliably returned to the Blood Bank for potential new patient assignation before expiration date.

We implemented the Biolog Transfusion Solution, using RFID verification, to improve our blood supply chain, at our 419-bed Santarém District Hospital (HDS), delivering more than 6,000 blood transfusions a year.

Project started in November 2020 for all RBC units received at the HDS' Blood Bank from donor centers.

Aims: Evaluate the performance of the Biolog Transfusion Solution for:

- Ease of use and implementation;
- Reliability and robustness of the equipment used;
- Benefit and security of the RFID solution for RBC reception, inventory management, patients' unit's assignment, release for transfusion & returns;
- Evaluation of the RFID solution to ensure complete traceability of all operations of the workflow.

Methods: To ensure full traceability of RBC, RFID tags placed on each blood bag were encoded with product and patient information. The information in the RFID tag was obtained from the Blood Bank Management Software (ASIS) interface.

A RFID Smart Storage cabinet (SST-R) (180 RBC units' capacity) was installed and adapted to our Blood Bank refrigerator.

Retrospective analysis of reception, inventory management and release for transfusion of RBC units was carried out from November 26, 2020 to March 10, 2021, at the HDS' Blood Bank. Collected data were exported through the BDS-Inventory–ASIS interface.

Biolog-id's Data Analytics Application was used to transform data generated by the RFID solution within a period, into comprehensive & dynamic graphical dashboards and reports.

Results: RBC Units received: 1474;
Average volume (ml): 249,68;
RBC units assigned: 1404;
Total Patients: 582 (Women:699 - 47,65%; Men:735 - 52,35%);
Average RBC Units assigned per patient: 2,8 (Women:2,5; Men:3,3);
Average age of patients (years): 75 (Women:77; Men:74);
RBC units released for transfusion: 1246.

The biolog-id Solution provided a real-time visibility of the physical stock of RBC units, their exact location in the refrigerator and the availability of Rh/Kell Phenotypes, impacting the search time of blood units. In addition, there was no electronic physical confirmation of the blood units released according to the information in the ASIS software.

Over the period, the Biolog Transfusion Solution for RBC inventory management, demonstrated substantial improvement in RBC units' movements traceability, assignments of units and their release for transfusion. These represent major benefits in the quality and safety of the HDS' Blood Bank operational procedures.

Summary/Conclusions: The safety of the Solution and the ease of the process have been demonstrated. The RFID equipment installed has demonstrated reliability and robustness, with no major issues detected. The Biolog Transfusion Solution has proven to be a good tool, improving transfusion process traceability and, consequently, the quality of the service provided by the blood transfusion department. The capacity to locate blood units in real-time and to monitor transfusion processes, are the main achievements made possible by using RFID technology. That is really a forwarding security step.

This RFID technology may allow us, in the future, to trace blood components from the donor to the recipient. We plan to continue using the biolog-id RFID solution in our hospital.

P-262 | Effects on biochemical parameters of the additive solutions for platelet concentrates and pathogen inactivation (PI) with intercept®: Do they accomplish requisites for adequate storage?

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Background: The platelet additive solutions (PAS) are employed to improve the storage conditions of platelet concentrates (PC), the effect of PAS has reported a slight decrease in the number of platelets stored, an improvement of its metabolic conditions and decreased adverse events after transfusion of PC. INTERCEPT Blood System for platelets is intended for the ex vivo preparation and storage PC and is used to inactivate a broad spectrum of pathogens as well as contaminating donor leukocytes in platelet products. The device uses amotosalen HCl (a photoactive compound) and long-wavelength ultraviolet (UVA) illumination to photochemically treat platelets.

P-262 Table 1.

	N=125	COMPOSOL (25)	PASIII (25)	PASIII-PI (25)	PAS E (25)	PAS E-PI (25)
Pool volume	MEAN	389.48	405.47	412.00	376.86	384.92
	SD	13.44	18.05	10.43	13.10	19.15
P-Day 1	MEAN	3.29	3.42	3.41	3.27	3.43
	SD	0.67	0.42	0.53	0.51	0.46
P-Day 5(7)	MEAN	3.29	3.31	2.61	3.26	2.64
	SD	0.57	0.57	0.29	0.57	0.48
pH-Day 1	MEAN	7.25	7.16	7.16	7.15	7.16
	SD	0.05	0.03	0.03	0.04	0.03
pH-Day 5 (7)	MEAN	7.07	7.01	7.00	7.01	6.97
	SD	0.08	0.04	0.02	0.04	0.07
Glucose-Day 1	MEAN	109.64	98.80	101.66	98.14	102.83
	SD	14.22	14.44	15.70	15.18	13.77
Glucose-Day 5 (7)	MEAN	49.00	40.50	27.55	51.04	32.74
	SD	25.04	15.58	19.72	80.07	17.14
Lactate-Day 1	MEAN	31.24	32.07	31.93	33.29	33.38
	SD	5.03	6.68	5.59	7.62	6.00
Lactate-Day 5 (7)	MEAN	99.76	103.60	104.00	105.79	106.52
	SD	22.40	17.99	16.30	15.31	14.47

Aims: The main goal of our study was to analyze distinct biochemical and metabolic parameters at days 1 and 5, with three PAS of different composition: Composol (Fresenius-Kabi®), corresponding to the PAS-D group; and PAS III M (Grifols®) and PAS-E (Terumo®), matching the PAS-E or PAS5 group; and at days 7 and 7 in the PI group with PAS III M and PAS-E.

Methods: We employed Leucored Grifols T&B (Grifols®) blood collection bags and FRACTIOMATIC X2 (Grifols®) system to obtain different components. We obtained 25 PC in each branch from a 5 Buffy Coats pool after centrifugation with TACSI (Terumo-BCT®). Afterwards, PAS (300 mL) was added to achieve a 70% PAS and 30% plasma ratio. At days 1,5 and 7 of storage we analyzed the volume(mL), platelet yield($\times 10^{11}$/unit), residual leucocytes, swirling, presence of aggregates, pH, pO2 and pCO2(mmHg), glucose(mg/dL), lactate(mg/dL) and microbiological contamination. We used Coulter DX800 to assess haematological parameters, flow cytometry (FACSCalibur 255(CellQuest) to determine residual leucocytes, blood gas analyzer (GEM premier 4000) for metabolic parameters and blood culture in agar(Beckton & Dickinson) for microbiological controls. Statistical analysis was performed with MedCalc 19.1.7.

Results: We obtained the following results in the different groups of analysis:

In none of the PC groups we detected aggregates, leucocyte count was <1x10^6/unit in all of them, swirling was 4+ and microbiological controls resulted negative for all of them. We observed a reduction in the number of platelets in the PC from all groups: 0.15% Composol, 3.28% PASIIIM and 0.28 PAS-E; and near 20% in the PI groups. Composol and PAS-E groups achieves better glucose results. There was no statistical difference in the number of platelets in the PC, lactate and pH, and all of them had similar variations in pO2 and pCO2.

Summary/Conclusions: These PAS preserve adequate parameters in the PC regarding the EU Guidelines and CAT (Spain) quality requests. In this study, we have detected a lesser reduction in the number of platelets in the Composol and PAS-E groups, as well better glucose levels. In the PI groups there was a drop in PC close to 20%.

P-263 | Comparative study of two pathogen reduction systems for platelets

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Background: The Luxemburgish Red Cross (LRC) is the single blood establishment in charge of the collection, preparation and distribution of blood products in the Grand-Duchy of Luxemburg. Since 2014, platelets products are pathogen reduced by using Riboflavin and UV light. As the LRC should ensure the self-sufficiency and be able to respond to the hospital needs at any time, the platelet production is highly superior to the demand, generating discard rate of 17.8% of the platelet production. In order to reduce this rate, we considered extending the storage time from 5 to 7 days, which is allowed if platelets are pathogen reduced (PR). The two PR technologies available in routine were tested (MIRASOL (MIR) and INTERCEPT (INT)) on Reveos platelet pools (RPPs) and apheresis platelets from TRIMA (APTs)

P-263 Table 1.

Mean and standard deviation. N=6	APTs (Trima)				RPPs (Reveos)			
	Day 0 before PRT	Day 1	Day 5	Day 7	Day 1 before PRT	Day 2	Day 5	Day 7
pH (22°C)								
Mirasol	7.16 ± 0.02	7.14 ± 0.02	6.72 ± 0.10	6.71 ± 0.05	7.19 ±0.01	7.12 ± 0.02	6.80 ± 0.04	6.83 ± 0.01
Intercept		7.05 ± 0.01	7.03 ± 0.05	6.82 ± 0.08		7.08 ± 0.02	7.12 ± 0.02	7.08 ± 0.03
Glucose (mg/dl)								
Mirasol	131.7 ± 6.1	107.3 ± 4.8	23.0 ± 6.0	<20.00	105.5 ± 3.2	85.8 ± 3.4	≤20	≤20
Intercept		116.8 ± 5.6	62.8 ± 8.1	24.0 ± 5.9		93.8 ± 3.1	61.8 ± 4.8	40.8 ± 6.3
Lactate (mg/dl)								
Mirasol	8.2 ± 2.4	17.1 ± 2.1	108.6 ± 12.2	123.1 ± 6.5	25.8 ± 1.4	31.8 ± 2.2	108.3 ± 7.6	119.4 ± 2.1
Intercept		15.3 ± 2.5	65.9 ± 7.4	104.3 ± 9.5		29.0 ± 1.7	58.7 ± 4.1	78.0 ± 4.6
Annexin V binding (%)								
Mirasol	2.3 ± 0.6	2.6 ± 1.4	6.5 ± 3.7	22.2 ± 14.8	6.9 ± 1.9	9.5 ± 2.0	15.0 ± 2.0	26.9 ± 5.2
Intercept		3.3 ± 1.1	5.2 ± 2.4	7.2 ± 3.5		6.8 ± 1.4	8.8 ± 0.8	8.2 ± 2.5
Swirl								
Mirasol	2.00	1.80	1.70	0.00	2.00	1.90	1.20	0.20
Intercept		2.00	2.00	2.00		2.00	2.00	2.00

Aims: To study the in vitro quality of platelets treated by MIR or INT and stored until day 7.

Methods: For each product type, 6 double platelet concentrates were prepared and equally divided into 2 units, one was treated by MIR and the other one by INT (Amotosalen and UVA light). Several parameters were followed, before and after PR, at day 5 and 7: platelet concentration, pH, glucose and lactate concentration, pCO_2, pO_2, sCD62p, Annexin V binding, MPV, swirling and morphology scoring.

Results: A decrease of the platelet quality parameters was observed in both types of platelet products (APTs and RPPs) with both PR methods and 7-day storage.Nevertheless, we found some differences between the two PR techniques, particularly a higher increase of lactate and glucose depletion, suggesting a higher stimulation of the glycolytic pathway, a higher Annexin V binding and a loss of swirling in the MIR treated units from day 5 (Table 1). On the other hand, the platelet loss was significantly higher in the INT compared with the MIR units: 12% and 5.3% in APTs and RPPs treated by INT vs 5.3% and 1.5 % in those treated by MIR respectively.

Summary/Conclusions: Results suggest that MIR treatment has a higher deleterious impact on in vitro platelet quality than INT, but we observe a more important loss of platelet with INT, the process including an amotosalen adsorption step post-illumination. 7-day storage is confirmed with INT.

Blood components/products – Blood components

P-264 | Antioxidants supplementation effects on red blood cell storage lesion

selected for main programme

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Background: Stored red blood cells (RBCs) undergo a series of time-dependent changes in their quantity and quality, altogether known as storage lesion. Imbalance between oxidative insults and antioxidant defenses has been considered a critical underlying factor for those defects. Moreover, there is evidence that the *in vivo* levels of uric acid, which is the main antioxidant in the plasma, have an impact upon both the structural and the functional

adequacy of stored RBCs, by restraining in part the accumulation of storage-related phenotypes.

Aims: The aim of this study was the effect of uric acid (UA) and/or ascorbic acid (AA) supplementation on the quality and redox parameters of RBC units stored under standard conditions in the cold.

Methods: Packed RBCs from twenty eligible blood donors in CPD/SAGM were split in 4 sub-units that were individually supplemented with UA (8.0 mg/dL), AA (2.2 mg/mL), a mixture of UA/AA or nothing at all (control). The concentration of the additive solutions (UA and AA) was established by preliminary analysis for possible cytotoxic effects. Quality (hemolysis, pH, calcium homeostasis) and redox [intracellular reactive oxygen species (ROS), antioxidant capacity, oxidative hemolysis, lipid peroxidation] parameters were assayed throughout storage.

Results: UA, AA and UA/AA supplementations of storage medium did not affect pH, calcium accumulation, storage and mechanical hemolysis, but slightly altered the resistance to osmotic stress, resulting in a not clinically relevant elevation of mean corpuscular fragility values in the RBCs of the modified units (e.g., day 21: 0.448±0.022 vs 0.444±0.022 %NaCl, p=0.0004, AA vs control). Nevertheless, most redox-related parameters were different in the supplemented units. More specifically, tBHP-induced intracellular ROS (e.g., day 42: 2393±370 vs 2907±647 RFU/mg of protein, p=0.0019, UA vs control) and phenylhydrazine-induced hemolysis were lower throughout storage in all test units, while endogenous ROS levels were either equal or slightly higher (UA units, p>0.05) than control. Peroxidation of membrane lipids was universally improved in all time points and conditions tested (e.g., 29.90±13.10 vs 42.50±11.90 μM TBA/mL RBCs, p=0.022, mix vs control). In the case of units' supernatant, total antioxidant capacity was increased in all modified units (e.g., day 7: 571±71 vs 273±55 μM Fe^{2+}, p=6x10^{-14}, UA vs control), while the UA-dependent and UA-independent activities were increased in the UA and AA units, respectively, as well as in the mixed UA/AA units.

Summary/Conclusions: Uric acid and ascorbic acid supplementation did not affect the storage quality parameters of the RBC units. On the contrary, it seemed to protect the stored RBCs from oxidative insults by enhancing the antioxidant potential. The slight increase (or trend for increase) in the intracellular levels of ROS observed in the UA units needs to be re-examined by future studies, since it may be indicative of backstage side effects on RBC metabolism.

P-265 | Irreversible processes in the cytoskeleton of packed red blood cells during prolonged storage

selected for main programme

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Background: Filtered packed red blood cells (pRBCs) used for blood transfusion can be stored at 4^{0}C in special preservative solution for 42 days. Storage-related processes initiate a cascade of metabolic, structural and morphological changes of PRBCs which in turn lead to impairment in functionality and viability after transfusion. These lesions are caused by oxidative stress and taken place on specific time-scale. Transfusion of "old" (more than three weeks) pRBCs leads to increase risk of post-transfusion complications.

Aims: to analyze changes occurring in the cytoskeleton of pRBCs during their storage and to establish the relationship with the timing of these processes.

Methods: Leukocyte-depleted pRBCs in air-tight bags containing SAGM additive solution and the CPD anticoagulant were obtained from the clinical centers of blood transfusion Moscow, Russian Federation. Samples from pRBC were withdrawn on days 3, 12, 19, 21, 24, 28, 35 and 42. Atomic force microscope NTEGRA Prima (NT-MDT Spectrum Instruments, Moscow, Russian Federation) was used to obtain cytoskeleton images in different control days of storage. The topological parameters of pRBC cytoskeleton were calculated by the Image Analysis P9 software from SPM Nova software (NT-MDT Spectrum Instruments, Moscow, Russian Federation). Stiffness of the native cell membranes was studied by using atomic force spectroscopy.

Results: During prolonged storage the cytoskeleton and stiffness of pRBC membranes undergone detrimental changes. Up to day 21 of storage these parameters are almost unchanged. In the middle of the storage time the main dominant event was ruptures of cytoskeleton filaments and transformation of small pores into large. On day 19-21 all alterations in membranes grow with maximum rate and become irreversible. Irreversible clusterisation of protein components in the cytoskeleton began. By the end of the storage period the average pore length L increased by 1.8±0.55 times, and the average pore area S – by 3.2±1.4 times. At the same time, stiffness of pRBC membranes increased non-linearly. By day 42 it became 2.4±0.2 times higher than the initial. The high correlation between the dynamics of significant changes in the cytoskeleton configuration L, S and stiffness of the membranes at the level of p<0.05 was obtained: r_{EL}= 0.87, r_{ES}= 0.88. The equally high correlation was obtained between pH and cytoskeleton parameters L, S at p<0.05:, r_{LpH}= -0.80, r_{SpH}= -0.82; between pH and E: r_{EpH}= -0.88; between K^{+} and cytoskeleton parameters and stiffness:r_{LK}= 0.85, r_{SK}= 0.87, r_{EK}= 0.86; between lactate and cytoskeleton parameters and stiffness: r_{LLac}= 0.75, r_{SLac}= 0.78, r_{ELac}= 0.83.

Summary/Conclusions: This work shows study of the relationship between the processes occurring in red blood cells during their storage and the timing of these processes. Destruction of pRBC cytoskeletal causes irreversible impairments of morphology and nanostructure, increase stiffness of the pRBC membranes. We observed increasing in rate of cytoskeletal changes and membrane stiffness after days 19–21. These processes can lead to poor transfusion outcomes. Our findings can be used for pRBC quality assessment in clinical practice and in research of the molecular mechanisms of the structural disorders of pRBCs.

P-266 | In vivo hemostatic function of cold-stored platelets in platelet additive solution evaluated in a thrombocytopenic rabbit bleeding model

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Background: Cold storage of platelets (PLTs) in PLT additive solution (PAS) can extend the shelf life of PLT concentrates (PCs) beyond 7 days, preventing bacterial growth and aggregate formation. Growing 4wevidence suggests that transfusion of cold-stored (CS) PLTs is effective in patients with massive bleeding due to rapid and efficient hemostatic function. Few studies have evaluated the *in vivo* hemostatic function of CS PLTs in PAS, especially in severe thrombocytopenia.

Aims: To compare the hemostatic efficacy of CS PLTs in PAS with that of room temperature-stored (RTS) PLTs in PAS in a thrombocytopenic rabbit bleeding model.

Methods: To induce thrombocytopenia, busulfan was injected twice into rabbits. Two weeks later, rabbits that developed thrombocytopenia with prolonged bleeding time (>600 seconds) after incision of an ear fine vein were used for the study. Blockage of the reticuloendothelial system with ethyl palmitate and washing out plasma in PCs prior to PC transfusion allowed for the assessment of the *in vivo* function of human PLTs in this rabbit bleeding assay. This assay might mimic PC transfusion intended for mild bleeding in patients with thrombocytopenia during chemotherapy. Human PLTs in PAS-E were prepared from an apheresis PC on the day after collection, split to form matched pairs, and stored for up to 9 days (in total) at RT or in cold storage. CS or RTS PLTs stored for 3, 6, or 9 days (4.0×10^9/kg) were transfused into the rabbits. Bleeding time was measured at two incisions in the ear fine veins of 2 to 4 rabbits, depending on how many rabbits developed severe thrombocytopenia among 5 rabbits. The rate of hemostasis was defined as the percentage of rabbits that achieved cessation of bleeding within 600 seconds at one or more incision sites. This assay was performed 5 times using PCs stored for different durations.

Results: Mean PLT count of rabbits before PC transfusion was 8.6±5.2 x 10^3/μL, with no significant differences across assays. The rate of hemostasis with RTS PLTs (5 PCs transfused into total of 14 rabbits) and CS PLTs (5 PCs in 14 rabbits) on day 3 was each 100%. On day 6, the rates of hemostasis with RTS PLTs (5 PCs in 14 rabbits) and CS PLTs (5 PCs in 15 rabbits) were 93±15% and 73±15%, respectively. On day 9, the rates with RTS PLTs (5 PCs to 14 rabbits) and CS PLTs (5 PCs to 13 rabbits) were 65±36% and 73±37%, respectively. There were no statistical differences between the groups on day 6 (p=0.07) or 9 (p=0.27). PLT count increments tended to decrease as the storage period increased under both storage conditions. Total PLT counts after transfusion of CS PLTs were significantly lower than after transfusion of RTS PLTs for all days (67.6±8.8 vs. 76.1±9.5 x 10^3/μL, p=0.022 on day 3; 56.2±11.5 vs. 64.4±7.9 $\times 10^3$/μL, p=0.035 on day 6; and 42.4±14.7 vs. 58.7±5.7 $\times 10^3$/μL, p=0.0007 on day 9, respectively). Total PLT counts after transfusion did not reach 50 x 10^3/μL, the ideal target post-transfusion PLT count for acute bleeding, in several experiments when we transfused RTS PLTs stored for 9 days or CS PLTs stored for 6 or 9 days. Furthermore, PLT count increments were significantly correlated with *in vivo* hemostatic efficacy only with CS PLT transfusion. Thus, hemostatic efficacy of PCs could be improved if more PLTs, especially CS PLTs, are transfused.

Summary/Conclusions: CS PLTs might be effective for achieving hemostasis to the same extent as RTS PLTs in patients with thrombocytopenia and mild bleeding.

P-267 | Near infrared low-level light improves quality of platelets in vitro

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Background: The storage lesion of platelets seems to be determined mainly by the functional properties of their mitochondria. Mitochondrial functions could be improved by near infrared (NIR) low-level light treatment, because it is thought that the primary photon acceptor is Complex IV of the mitochondrial membrane. Zhang et al (J Biophotonics, 2019) demonstrated significant improvement of stored murine platelets after illumination with NIR light. In a pilot study, we observed that the glycolysis rate in human platelets can be lowered, suggesting an increase of aerobic metabolism by mitochondria.

Aims: To investigate the effect of NIR light treatment on routinely prepared buffy coat-derived platelet concentrates (PCs).

Methods: Twenty-two buffy coats were pooled, and from this, two pairs of 2 PCs in PAS-E and 2 PCs in plasma were prepared (n=6). One unit of both pairs was illuminated with 830 nm light (LED, 5 minutes, 15 J/cm^2). PCs were stored for 10 days on a flatbed shaker at 22°C and sampled regularly for analysis of *in vitro* quality. Data of the illuminated groups were compared with their corresponding controls with a paired two-sided t-test.

Results: All PCs fulfilled European Guidelines on Day 1 and both groups of each pair had comparable volume and cellular composition. On Day 6 and thereafter, the illuminated PCs in PAS-E had lower lactate concentrations (6.6±0.6 vs 6.8±0.7 mmol/L, p<0.05), and corresponding higher pH. On Day 8, also the percentage CD62P positive cells was lower (11.2±2.1 vs. 12.8±2.8%, p<0.05) and on Day 10 the MPV (9.5±0.2 vs 9.6±0.3 fL, p<0.05) was lower in the illuminated PCs. On Day 6 and thereafter, average Annexin A5 binding (phosphatidylserine exposure) was slightly lower in illuminated units. ATP content and mitochondrial membrane potential (JC-1 ratio) were not different between the groups. Although the trends

were the same, the differences for the illuminated PCs in plasma did not reach statistical difference as compared to the control group.

Summary / Conclusions: Illumination with 830 nm NIR light on Day 1 has a small positive effect on the *in vitro* quality of PCs in PAS-E, but did not reach statistical significance for PCs in plasma. Further research is needed to optimize the timing and energy density of the illumination process. Implementation of this technique to improve storage conditions of PCs is attractive because of its simple, non-invasive and additive-free character.

P-268 | Apheresis platelets at reduced dose as a contingency measure for covid19 and other pandemics – A laboratory evaluation

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Background: The COVID19 pandemic has challenged Blood Services to strengthen contingency plans in case demand for platelet components outstrips supply. Resilience options include increasing donations, accepting alternative ABO groups and reducing prophylactic use of platelets (Stanworth et al., Lancet Haematol., 2020). To augment such plans, we investigated component development options that could be implemented quickly to effectively boost platelet supply, whilst minimising additional validation work in a processing environment already occupied with response management.

Aims: To evaluate the impact on supply, platelet yield and quality, of a reduced dose of apheresis units in plasma.

Methods: We assessed splitting apheresis double donations into three (yielding ⅔ doses) or standard dose units in half. The impact of these changes on unit numbers, counts and volumes was modelled using production figures. To assess quality, eight pools of three ABO/Rh-matched apheresis (Trima Accel) double donations in plasma were split to ⅔ and ½ volumes in both Terumo and Fresenius storage bags (both in use for standard storage). These were irradiated and subject to maximal permitted periods of non-agitation (3x8h) before comparing platelet quality markers (including pH, CD62P expression) to day 9 of storage.

Results: Splitting all double donations into three was predicted to expand NHSBT inventory by 23% overall (as 50% of platelets are whole blood derived), whereas halving units clearly doubles stock. In our study, ⅔ doses contained 153±15x10^9 platelets (~138mL) whereas ½ doses contained 113±11x10^9 (~102mL) platelets. Following storage, ⅔ doses showed higher pH than ½ doses in each pack type, with units in Terumo packs significantly higher than Fresenius at either dose. This better platelet quality in larger volumes/doses and in Terumo packs was reflected in lower CD62P expression and other markers. Nevertheless, by pH monitoring at expiry (set as Day 8), all ⅔ doses and most ½ doses were at least pH6.4.

Summary/Conclusions: Apheresis platelets in plasma, split to lower doses, appears feasible and maintains acceptable platelet quality. Clinical use may need to be tailored, however this approach offers a viable contingency option to maintain supply when faced with extreme shortages.

P-269 | Post-transfusion efficacy of RBCS from heterozygous beta-thalassemia donors

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Background: Recent studies of our team revealed that red blood cell (RBC) units from eligible blood donors with beta-thalassemia traits (βThal$^+$) cope better with storage stress that induces hemolysis and redox disequilibrium, while their metabolic and proteome profile are indicative of enhanced antioxidant potential. Certain superior characteristics of stored βThal$^+$ RBCs, like exceptional resistance to lysis and phosphatidylserine (PS) exposure, may be linked to better post-transfusion performance.

Aims: The aim of this study was to examine the recovery and other physiological aspects of βThal$^+$ RBCs post transfusion *in vitro*, as well as *in vivo*, by using animal models.

Methods: RBCs from βThal$^+$ (n=6) and control (n=6) donors stored in CPD-SAGM were reconstituted with plasma from healthy (n=3) or transfusion-dependent thalassemic (n=3) recipients before incubation for 24 hours at body temperature. Reconstituted and control samples were then assayed for storage and stress-induced (osmotic, mechanical, oxidative) hemolysis, endogenous and stress-triggered reactive oxygen species (ROS) levels and PS exposure. Fresh (non-stored) RBCs from βThal$^+$ and control subjects (n=3 per group) marked with fluorescent dyes were transfused to C57BL/6J and NOD/SCID recipient-mice (n=3 per group) and their 24-hour post-transfusion recovery was evaluated by flow cytometry. In both models of transfusion (*in vitro* and *in vivo*) the volume of transfused RBCs was equivalent to two RBC units. Significance was accepted at p<0.05 and data are shown as mean±SD.

Results: Our results showed that βThal$^+$ RBCs reconstituted with control or thalassemic plasma were more resistant to spontaneous (e.g., late storage – control plasma: 14.69±9.93 vs 42.94±23.54 mg Hb/dL, p=0.022, βThal$^+$ vs control), osmotic and mechanical (e.g., early storage – thalassemic plasma: 0.747±0.259% vs 1.271±0.158%, p=0.0017, βThal$^+$ vs control) hemolysis, by

presenting lower levels (or a trend for that) compared to reconstituted control RBCs. PS exposure and redox related parameters were equal between the "transfused" RBCs of the two groups, apart from oxidative hemolysis of old stored βThal$^+$ RBCs (showing a trend for lower levels in both recipient backgrounds) and ROS levels of the same cells (lower in thalassemic plasma, 1342±413 vs 2546±1142 RFU/mg of protein, p=0.036, βThal$^+$ vs control). Regarding the xenobiotic *in vivo* model of transfusion, βThal$^+$ RBCs exhibited either equal (p>0.05; NOD/SCID) or an interesting trend for higher (p=0.058; C57BL/6) levels of post-transfusion recovery.

Summary/Conclusions: Stored RBCs from carriers of beta-thalassemia traits maintain their intrinsic resistance to spontaneous, mechanical and osmotic lysis post *in vitro* transfusion and are equal or slightly superior – at late storage – in redox-related parameters in comparison to controls. Transfusion of non-stored human βThal$^+$ RBCs in recipient mice, via a xenobiotic model, results in not inferior levels of post-transfusion RBC survival. However, this finding needs expansion by using RBCs collected from blood units intended for transfusion.

P-270 | Mitochondrial respiration of single-donor platelets in PAS-E

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Background: Previously it was shown that donors could be classified as having platelets (PLT) with good, average or poor storage properties. PLT differed in metabolic activity, resulting in rapid acidification of "poor" storing PLT concentrates (PCs). In PAS-E the risk of acidification is lower, but the risk for depletion of glucose, an important substrate for PLT, is higher. Some observations pointed towards mitochondrial activity, indicating that PLT with a higher glycolysis rate also had a higher demand for oxygen.

Aims: To characterize buffy coat-derived single-donor PCs in PAS-E during storage and to assess PLT oxygen consumption as a measure for mitochondrial activity.

Methods: Whole blood donations of 24 donors >45 years were selected. The next day, single-donor PC in PAS-E were prepared from the buffy coats. PC were stored for 9 days at 22±2°C in a 600 ml PVC-DEHP container and regularly sampled for analysis. On Day 2 and 9 PLT oxygen consumption was assessed in an Oroboros oxygraph (300×10^9/L PLT, medium 65% PAS-E/35% plasma, 37°C), and basal levels and levels after addition of oligomycin, antimycin (respiration inhibitors) and FCCP (decoupling agent) were determined. Based on glucose consumption, the group was divided in quartiles. Subsequently, quartiles of each parameter were analysed with ANOVA followed by a Tukey-Kramer post-test (GraphPad Instat).

Results: See Table 1

Quartiles were not different in volume and cellular composition. PLT in Q4 with the highest glucose consumption (and thus glucose depleted on Day 8) had a higher mean platelet volume (MPV), already on Day 1. On Day 1 and 2, also the respiration rate was higher in Q4, according to the oxygen tension in the storage container at 22^{0}C as well in the oxygraph measurements at 37^{0}C. Basal, mitochondrial and ATP-linked respiration of PLT on Day 2 correlated well with MPV (R^2>0.71), indicating that the larger PLT used more ATP. The storage lesion was more severe (more lactate, lower pH and more activation) in Q4, corresponding with glucose consumption.

Summary/Conclusions: Glucose depletion of PLT in PAS-E seems to be caused by PLTs that are on average larger and have a higher metabolic activity, both in glycolysis rate as in mitochondrial respiration. From literature it is known that a high MPV is associated with health issues like Type 2 diabetes and cardiovascular diseases and that young PLT are metabolically more active than older PLT. So, the observation that PCs of some donors have inferior storage properties may be related to their higher PLT turnover, and further studies are ongoing.

P-270 Table 1.

Glucose consumption Day 1-4 (mmol/day/10^{11}PLT)	Q1 0.038-0.061	Q2 0.062-0.072	Q3 0.082-0.097	Q4 0.105-0.164
MPV Day 1, fL	8.8 ± 1.0	9.5 ± 0.5	9.6 ± 0.4	10.0 ± 0.6*
ΔpO_2 Day1-2, mm Hg	73 ± 13	74 ± 7	85 ± 5	95 ± 5**
Basal respiration Day 2, pmol/(sec mL)	30.9 ± 4.0	32.3 ± 2.3	34.1 ± 3.5	34.5 ± 2.6

*p<0.05 vs Q1; **p<0.01 vs Q1 and Q2

P-271 | Evaluation of single & double dose platelet procedures with saline reinfusion for donor comfort during collection with amicore apheresis system at Cho Ray Blood Transfusion Center-Viet Nam

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Background: Cho Ray Blood Transfusion Center (Cho Ray BTC) is one of the five biggest blood transfusion centers in Vietnam. We take responsibility to collect and supply safe and quality blood components for patients in 72 hospitals in South-Eastern area of Vietnam with a population of over 10 million people. Platelet apheresis is the main product of Cho Ray BTC with a collection average of around 15,000 units per year. The AmiCORE Apheresis System was newly installed in our center in 2020. One of the major advantages of using AmiCORE is the saline replacement to donors during single and double dose platelet unit collections.

Aims: The aim of this study was to evaluate the performance of AmiCORE Apheresis System (Fresenius Kabi AG, Germany) for both single and double dose collections and to investigate if the products meet the quality and safety requirements of Vietnamese guidelines for platelet product and donor.

Methods: Total 348 healthy donors were included in this cross-sectional descriptive study. Of these, 127 donors (88 male and 39 female) were recruited to donate platelet apheresis single dose unit (SDU) (3 x 10^{11} platelets per unit) and 221 donors (132 male and 89 female) were recruited to donate double dose units (DDU) (6 x 10^{11} platelets per unit). Donors for SDU and DDU had average platelet pre-counts of 270 $\pm$ 50.95 x 10^3/µl and 335 $\pm$ 48.62 x 10^3/µl, respectively. All donors met Vietnamese eligibility requirements. The quality characteristics of collected platelet units (SDU & DDU) and safety of donors were measured. The performance data were reported as mean values.

Results: All platelets were suspended in 100% plasma, specifically 265 mL in SDU and 505 mL in DDU. The actual vs. target platelet yields were 3.11 $\times$ 10^{11} vs 3.0 $\times$ 10^{11} for SDU, and 6.18 x 10^{11} vs 6.0 $\times$ 10^{11} for DDU. The procedure time and whole blood processed were 46 minutes and 2,175 mL for SDU, and 74 minutes and 3,627 mL for DDU, respectively. The anticoagulant (ACD-A) used was 230 mL for SDU and 367 mL for DDU. The WB:ACD ratio used was 10:1. The provision of Saline Prime and Saline Reinfusion ensured volume replacement for donor comfort. The total saline used was 300 mL for both SDU and DDU. The leucocyte reduction resulted in less than 1 $\times$ 10^6 leucocytes per SDU and DDU in random quality control check, leukoreduction is achieved by in-process without a leucocyte filter during the procedure. The microbiological culture recorded negative results over 5 days for both SDU and DDU. All parameters on quality and safety of SDU and DDU were met according to the standards of Vietnamese guidelines for platelet apheresis units. There was no adverse reaction recorded from any donor. Donors were totally satisfied with the AmiCORE Apheresis System for both SDU and DDU collections.

Summary/Conclusions: The platelet collection by SDU and DDU using AmiCORE Apheresis System totally fulfilled quality and safety requirements of Vietnamese guidelines for platelet apheresis unit. The actual platelet yields were higher than the target yields in both SDU and DDU. The saline replacement for donors ensured donor comfort and the in-process leucocyte reduction during the procedure provided a product without the need for any additional filtration steps. Thus, the quality of platelet collection procedure and safety for donors were maintained with the AmiCORE Apheresis System.

P-272 | Comparative evaluation of new non-DEHP platelet pooling kit to PVC-DEHP version

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Background: DEHP (di-(2-ethylhexyl) phthalate) is a plasticizer widely used to give PVC material the required flexibility. However, growing concerns about the long-term health effects of DEHP triggered an increasingly stringent regulation for use in medical devices. In 2019, Fresenius Kabi launched pediatric storage bags made of non-DEHP components, and in 2020 introduced non-DEHP versions of the CompoStop™ portfolio, the first commercially available pooling systems. The new pooling systems are made entirely with non-DEHP components, with materials changed for the tubing (PVC-DEHT), the sampling and pooling bags (PVC-DINCH) and the filter housing (PVC-DEHT). The platelet storage bag remains unchanged, as it has always been made of PVC-BTHC. All the tubing component dimensions (i.e., lengths, diameters) and bag volumes remain the same.

Aims: Evaluate the performance of the new non-DEHP pooling kit compared to the same configuration pooling kit in PVC-DEHP.

Methods: 144 whole blood collections (Day 0) in quadruple top & bottom InLine CQ32255 (CompoFlow® 4F T&B 63 ml CPD, Fresenius Kabi) were performed on CompoGuard (Fresenius Kabi). Platelet pools (PP) were manufactured from 6 buffy coats (BC) on Day 1: 12 with the standard CompoStop CT52600 (Flow-Flex 3F T&B) and 12 with the non-DEHP Compostop GT52600 (Flow-Flex 3F T&B). Pools were made by ABO group as we do in routine. Both configurations use the CompoFlow technology that enables automatic breaker opening during processing on the CompoMat G5 Plus (Fresenius Kabi). After adding 280 mL of PAS (Intersol, Fresenius Kabi) all PP were centrifuged in the Hettich Rotosilenta 360 centrifuge (348g, 1041rpm, 10 min, acc 8 brake B2 at 22°C) and separated immediately after. Samples were tested for PLT content PRE- and POST-filtration, as well as residual WBCs and RBCs on Day 1 using BD Facscanto II and Mindrain BC6800 Plus. On Day 2 we measured pH, pO_2, pCO_2, HCO3- and glucose following the routine procedures. Sterile connections were performed using the Fresenius Kabi CompoDock.

Results: The 24 PLT pools, expressed as mean value +/- st. dev, show a POST mean volume of 371±14,6 ml (369ml CT52600, 373ml GT52600), and mean PLT yield 3,34±0,06x10^6 cells/u. GT52600 had mean PLT yield 3,31x10^6 cells/u with 73.9% recovery, and CT52600 had mean PLT yield 3,36x10^6 cells/u with 73.0% recovery (Pvalue 0,824, 2 sample t test). Residual leukocytes were all below 60.000 cells/u. No significant difference was found between CT56200 (mean rWBC 0,021x10^6 cells/u) and GT52600 (mean rWBC 0,019x10^6cells/u): Pvalue 0,819 (2 sample t test). Processing time was evaluated on CompoMat G5 Plus using the same program used in routine. For a mean PRE volume of 519,7ml in CT52600 we had a mean processing time of 4m29sec; using the GT52600 the separation mean time was 4m31sec for a mean PRE volume of 524,7ml. Mean pH for the 24 PP was 7,06±1,3, with a mean pH of 7,03 in GT52600 and mean pH 7,10 in CT52600. No significant differences in the metabolic parameters were observed between the two sets. We observed no impact to sealing/docking procedures with new non-DEHP material.

Summary/Conclusions: The non-DEHP CompoStop GT52600 achieves the same PLT quality as the same configuration in PVC-DEHP, while keeping the same beneficial CompoFlow automatic opening. All measured parameters complied with EDQM requirements and we observed no impact in non-DEHP system handling in terms of centrifugation, sealing or kinking of tubings.

P-273 | Study of the relationship of the storage lesion and oxygen unloading kinetics of red cells

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Background: Red cell storage is associated with measurable quality changes, physical (progressive loss of membrane, rounding) and metabolic, which are predicted to affect O_2 transport capacity and exchange kinetics. For example, the drop in 2,3-diphosphoglycerate (2,3-DPG) concentration is expected to raise the oxygen affinity of haemoglobin, thereby reducing oxygen release at tissues. However, the overall effect of storage on O_2 release rates has not been measured, despite this being important, particularly in critically ill patients receiving large volume transfusion. The process of rejuvenation has been trialled in an attempt to restore the physiological quality of stored red cells, but the effect these treatments have on O_2 release are also not known. However, these factors are now amenable to study, as single red cell O_2 saturation kinetics can now be measured by live imaging fluorescent microscopy combined with ultrarapid solution switching of oxygenated and deoxygenated buffers (Richardson, SL et al, PNAS, 2020).

Aims: The aim of the study was to examine the changes in red cell O_2 exchange over the course of standard blood bank red cell storage and upon rejuvenation; and examine their relation to standard measures of red cell quality.

Methods: Twelve red cell concentrates (RCC) in Saline Glucose Adenine Mannitol (SAGM) were produced under standard NHS Blood and Transplant procedures and stored at 4 ± 2°C. Six were tested early and late in storage on days 2, 7, 35, 42 and 49. The other 6 RCC were pooled in 2 ABO matched pools of 3 and split into 3 paired RCC. Each RCC was rejuvenated on days 7, 21 and 35 of storage and tested before and after rejuvenation (n = 2). All RCC were tested for O_2 unloading kinetics and standard storage parameters full blood count, haemolysis, supernatant potassium, lactate and glucose, ATP, 2,3-DPG and red cell microvesicles.

Results: With storage, cellular O_2 unloading became slower (increase of 0.1s in mean time constant for complete O_2 unloading per 10 days in storage), and correlated with 2,3-DPG levels (r2 = 0.51) in the early phase of physiological remodelling. Intriguingly, exchange kinetics continued to decrease, even after 2,3-DPG levels became depleted, suggesting a role for another, possibly geometric, factor in slowing gas exchange. Cellular O_2 binding capacity decreased over storage to a lesser degree. Rejuvenation of stored cells restored ATP and 2,3-DPG back to levels of fresh whole blood, and fully restored O_2 release kinetics.

Summary/Conclusions: There is a degradation of O_2 exchange kinetics and capacity over standard red cell storage, associated with reversible metabolic changes, that may have *in vivo* consequences immediately post-transfusion.

P-274 | Operational validation of double dose buffy coat platelet concentrates prepared with the CompoStop CI pooling and leukodepletion set

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Background: Double dose (DD) leukodepleted platelet concentrates (PC) can be obtained from pools of 8 buffy coats (BC) supplemented by a Platelet Additive Solution (PAS). A platelet Pooling Set (CompoStop™CI) has been developed by Fresenius Kabi (Germany) to perform this preparation with a semi-automatic method using centrifuges and presses. The obtained DD-BC-PC are intended to be pathogen reduced (PR) with the INTERCEPT™ (Cerus) Dual Storage (DS) processing set including two platelet storage containers.

Aims: The objective was to perform a routine operational validation of the process to support an application for regulatory approval by French authorities (ANSM).

Methods: 100 evaluable DD-BC-PC were prepared with CompoStop™CI at each EFS site (EFS1=Strasbourg and EFS2=Toulouse) at day 1 after whole blood collection. Eight BC and 280 mL of PAS InterSol (Fresenius Kabi) (EFS1) or 300 mL of PAS SSP+ (Macopharma, France) (EFS2) were sterile docked to the Octopus harness and combined into a 600 mL pooling bag. Pools are constituted

P-274 Table 1.

	Volume (mL)	Plt Conc. G/L	Plt content 10^{11}/U	Res. WBC 10^6/U	Plasma/PAS ratio %
Mean	393	1738	6.8	0.17	39.7
Standard deviation	10	149	0.6	0.13	2.4
Min	346	1052	4.0	0.05	32.5
Max	424	2003	7.9	0.73	44.3
Requirement / Target	300-420 (375-420)	-	2.5-7.0 7.1-8.0	≤1,0	32–47
% conformity	98 %		100%	100%	100%

so that the addition of the 8 donors pre-counts falls within a range of approximately 1800-2200 platelets.10^9/L. Each pool is centrifuged in a Cryofuge 6000i (Heraeus, Germany) at 2000 rpm / 8 min (EFS1) and at 1100 rpm / 13 min (EFS2) and the PC supernatant expressed through a leukodepletion filter (A2CC0070, Fresenius Kabi) into a temporary platelet storage container. The obtained DD-BC-PC were tested within one hour of preparation for compliance with the INTERCEPT process entry (guard bands) and regulatory requirements for volume (300-420 mL), platelet content (2,5-8,0.10^{11}), residual leukocytes (WBC, ≤1.10^6), residual red blood cells (≤4.10^6/mL, visual assessment) plasma ratio (32-47%) and swirling.

Results: Results were similar for both EFS and therefore combined. 202 units were performed: 2 units were excluded after visual assessment for red blood cells contamination observed after extraction. For the selected 200 DD-BC-PC, all units exhibited maximum swirling after preparation. All INTERCEPT process guard bands for the DS set were met (Table 1) except for one unit with a volume of 424 mL, which could be volume reduced before INTERCEPT treatment.

Summary/Conclusions: Leukocyte-depleted "double dose" buffy coat platelets with a high platelet content and ready for pathogen reduction with the INTERCEPT DS processing set, can be routinely produced with the CompoStop™CI pooling and leukodepletion set

P-275 | Quality assessment of Mirasol PRT-treated platelet concentrates after 5 and 7 days of storage

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Background: The reduction of pathogen and leukocyte transmission risk via Platelet Concentrates (PC) is one of the major challenges for transfusion medicine today. The Mirasol Pathogen Reduction System (Mirasol PRT) has been developed to reduce the transmission risk of pathogens and inactivate white blood cells in PC using a combination of Riboflavin and UV-illumination. This Pathogen Reduction Technology is able to inactivate a variety of pathogens, those that are screened for or others that are not, including gram-negative/positive bacteria, enveloped/non-enveloped viruses, parasites and emerging pathogens like MERS-CoV and SARS-CoV-2.Aim.

Aims: In this report we compare the impact of the Mirasol PRT on the quality of whole blood-derived PC on day 5 and day 7 after donation.

Methods: Seventy-five PC were produced by pooling 5 buffy coats with 250 ml PAS-E solution according to our institution's SOP. Mirasol PRT treatment of these PC was performed in accordance to the manufacturer's instructions for use. Treated PC were separated into two Groups: 46 PC were stored up to day 5 (EOS5), 29 PC were stored up to day 7 (EOS7). Quality parameters such as: pH, platelet content, $CD62P^+$ with and without TRAP-6 on day 1 post production (PP) and at EOS5 or EOS7 were analysed. To test the statistical significance the Mann-Whitney-U test was used. A p value of ≤0.05 was considered statistically significant.

Results: EOS5 Mirasol PC showed on average pH 7.3±0 PP (n=27) and 7.2±0.1 at EOS5 (n=46); platelet yield (n=46) of 2.9±0.3 ×10^{11}/unit PP and 3.0±0.3 ×10^{11}/unit at EOS5; $CD62P^+$ without TRAP-6 (n=24) of 24.2±3.8 % PP and 46.9±5.0 % at EOS5; $CD62P^+$ with TRAP-6 (n=24) of 76.9±4.9 % PP and 72.3±4.7 % at EOS5. By adding TRAP-6 the percentage of $CD62P^+$ increased by 25.3±7.6 % at EOS5. EOS7 Mirasol PC (n=29) showed on average pH 7.3±0 PP and 7.0±0.1 at EOS7; platelet yield (n=29) of 2.9±0.3 ×10^{11}/unit PP and 2.9±0.3 ×10^{11}/unit at EOS7; $CD62P^+$ without TRAP-6 (n=15) of 28.6±4.8 % PP and 58.9±4.1 % at EOS7; $CD62P^+$ with TRAP-6 (n=15) of 87.4±2.6 % PP and 72.5±6.9 % at EOS7. By adding TRAP-6 the percentage $CD62P^+$ increased by 13.6±7.9 % at EOS7. No bacteria growth was observed in all PC at EOS.

Summary/Conclusions: Quality control results of Mirasol-treated Platelet Concentrates showed promising results at end of day 7. Values of pH value at EOS remained far above minimal threshold of 6.4. Platelet yields did not change after treatment till EOS in both groups. CD62+ values without TRAP-6 activation were significantly higher at EOS in both groups, yet no statistically significant difference was observed in respect to $CD62P^+$ response to TRAP-6 between the two groups at EOS. In fact, one could still observe upregulation of CD62P+ by TRAP-6 in Mirasol treated PCs at EOS7 (+13.6±7.9 %). This indicates Mirasol treated PC do not loss their activation ability by adding two more days of storage.

P-276 | A comparative analysis of hemolysis and potassium levels in pre-storage leucocyte depleted RBC units with and without gamma irradiation

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Background: There is a constant debate between clinicians and Transfusion Medicine physicians about the efficacy and safety of stored blood. Less data is available regarding the effects of gamma irradiation on pre-storage leucocyte depleted packed RBC units.

Aims: To study the trend of hemolysis and potassium accumulation in pre-storage leucocyte depleted packed RBC units with and without gamma irradiation.

Methods: Plasma free hemoglobin was measured using the Hemocue Plasma/low hemoglobinometer in N=230 packed RBC units each with and without gamma irradiation. Serum potassium was measured using an automated biochemistry analyzer (Ion selective electrode method) in N=276 units. The data was analyzed after grouping the results at an interval of 7 days.

Results: The mean percentage hemolysis in non-irradiated PRBC units was 0.25 and in irradiated PRBC units was 0.27. The extent of hemolysis percentage at outdate for non-irradiated units was 0.48 % whereas, hemolysis upto 0.3% was noted in units stored for 14 days after gamma irradiation. 6/460 units studied for hemolysis showed >0.8 % hemolysis. Two of these units were returned back after being issued for transfusion and the other 4 units were tested on their date of expiry.

The mean potassium level in stored PRBC units was 4.13 mmol/L. The mean potassium level in non-gamma irradiated units (N=143) was 3.98 mmol/L and the same in gamma irradiated units was 4.25 mmol/L. In gamma irradiated PRBC units, potassium levels were found to increase significantly when tested on Day 14 after gamma irradiation (9.78 mmol/L).

Summary/Conclusions: This data establishes the safety of stored PRBC units without and after gamma irradiation in terms of acceptance level of hemolysis and extracellular potassium. Multi-centric data of this type can be used to establish benchmarks for optimal practices.

P-277 | Routine quality control for leukodepleted blood components using a portable microscopic cell counter in a regional blood bank in Greece

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Background: Since 2015 all platelet concentrates (PCs) and 40% of red blood cells (RBC) are leukodepleted in our blood bank. Flow cytometry (FCM) is the more accurate technique for residual WBC (rWBC) measurements but the application of the FCM in a regional blood bank is difficult. The ADAM-rWBC microscopic cell counter measures leukocytes after their fluorescent staining with propidium iodide which stains only cells containing DNA and constitutes an alternative to FCM.

Aims: The aim of our study is to present a 6 month period experience and data of the introduction of ADAM-rWBC automated instrument (NanoEnTek, N. Korea) to our daily practice for quality assessment of leukodepleted blood products.

Methods: According to our quality control protocol, 1% of leukodepleted RBCs, 10 units of 4 or 5-buffy coat pooled PCs (p-PCs) and 10% of apheresis platelet concentrates (A-PCs) of our monthly production were measured for rWBC using the ADAM-rWBC device. For the leukodepletion of RBCs we used the Composelect prestorage system (Fresenius, KABI) and the BPF4 ARLD system (Hemonetics). For apheresis platelets we used the Spectra Optia apheresis system (Terumo BCT). Finally, for leukoreduction of p-PCs we utilize the BIOP FLEX pool (Fresenius, KABI) and Autostop BC (Hemonetics) in-line filtration. All rWBC measurements were made during the first 10 and 2 days of storage for RBCs and PCs, respectively. Residual leukocytes were measured in 35 RBC units, 25 A-PCs and 75 p-PCs. All calculations with the ADAM-rWBC device were performed according to manufacturer's instructions.

Results: The use of ADAM instrument needed minimum training for the staff and the time for each test did not exceed the ten minutes. So, it was very easily incorporated in the daily routine of our blood bank. The data of the quality assessment of leukodepleted blood products in the last six-month period are presented in Table 1. As shown, the 100% of both measured RBCs and A-PCs were efficiently leukoreduced as they were found to contain less than $1X10^6$ rWBCs. However, 29/62 and 6/13 of the pPCs that where leukoreduced with BIOP FLEX pool and Autostop BC in-line filter respectively, were found to container WBCs above recommended limits according to the R(95) 15 of the Council of Europe (19^{th} Ed. 2017).

Summary/Conclusions: The ADAM-rWBC easily incorporates in the routine of a regional blood bank and constitutes an especially useful option for screening of leukocyte contamination of blood units. All tested RBCs and A-PCs were found sufficiently leukodepleted according to the recommendations of the Council of Europe but approximately 30% of pPCs were found to have rWBCs slightly above $1X10^6$ per unit regardless of the leukodepletion filter that was used. However, it has been noted elsewhere that the rWBC values obtained by the ADAM-rWBC are significantly higher compared to FCM so the danger of misclassification of borderline products exists. Further research in leukodepleted pPCs comparing FCM and ADAM-rWBC technique would be useful. In conclusion, the ADAM-rWBC device makes the leukoreduction quality control routine very easy in hospital blood banks.

P-277 Table 1. Quality assessment of leukodepleted blood products using the ADAM-rWBC cell counter.

Blood component		Leukodepletion system/ apheresis device	Number of blood products	Mean rWBC±SD *10^6/unit
PCs	5 bc pooled	BIOP FLEX pool	62	1.1 ± 0.7
		Autostop BC	13	1.3 ± 0.99
	apheresis	Optia, Terumo BCD	25	0.4 ± 0.2
RBCs		Composelect prestorage system	25	0.28 ± 0.26
		BPF4 ARLD system	10	0.2 ± 0.18
Total			115	

P-278 | The feasibility of transfusing ABO and *RHD* identical platelet concentrates

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Background: Platelet concentrates (PCs) are a common, essential therapy used for a prevention and management of bleeding in thrombocytopenic patients. A growing demand for PCs, combined with a relatively short shelf life and geographical distance from blood suppliers compromise PCs availability, particularly ABO and RhD identical PCs. Consequently, PCs are often transfused without regard to ABO and RhD compatibility. ABO non-identical PCs may contain anti-A and anti-B antibodies, as well as soluble ABH antigens that can cause an immune complex formation, a various degree of haemolysis, and a lower platelet count increment. On the other hand, RhD+ PCs may contain residual red blood cells that can cause anti-D alloimmunization if they are given to RhD- recipients.

Aims: The aim of the study was to retrospectively analyse PCs use over a 5-year period in order to determine the overall rate of ABO and RhD PCs identical and non-identical transfusions.

Methods: University Hospital Centre Zagreb is a large academic hospital. Our transfusion service does not stock PCs on the inventory, except for specific platelet transfusions planned in advance prior to certain procedures. PCs are delivered on demand from our blood supplier, the Croatian Institute of Transfusion Medicine (CITM). Our policy is to provide ABO and RhD identical transfusions whenever blood supplier's inventory allows. When this is not feasible, we try to postpone the transfusion until ABO and RhD identical PCs are available. Only if the patient's clinical condition does not allow postponing platelet transfusion, ABO and RhD non-identical PC are given. When ABO minor incompatible PCs in plasma are given, the volume of incompatible plasma is reduced.

In CITM, PCs are obtained either from a single donor apheresis or pooled from 4 units of random donor whole blood. Platelets were stored in plasma until the beginning of 2016 when 70-75% of plasma was replaced with platelet additive solution. Platelet production planning is based on the replenishment model, but it is also adjusted by the daily demands for certain blood group PCs.

A retrospective analysis of all platelet transfusions in UHC Zagreb from 2014 to 2018 was performed. ABO and RhD group of transfused PCs and recipients, as well as specific requirements regarding ABO and RhD group for allogenic ABO and RhD mismatched hematopoietic stem cell transplanted (HSCT) patients were extracted from a comprehensive database of the transfusion service.

Platelet transfusions were categorized as ABO identical if ABO and RhD type of the patient and PC were identical and in case of ABO HSCT, when the ABO and RhD type of the PC was compatible with both the HSCT donor and the recipient.

Results: A total of 46,351 PCs were transfused to 3,776 recipients. Overall, 98.0% (45,433) of all transfused PCs were transfused to ABO identical recipient and only 2.0% (918) to ABO non-identical recipients. Of these ABO non-identical, 59.5% (546) were minor mismatched, 33.6% (308) major mismatched and 7.0% (64) bidirectional mismatched platelet transfusion. Out of all transfused PCs 94.7% (46,351) were Rh D identical and 5.3% (2,476) Rh D non-identical. Of those Rh D non-identical only 2.7% (1,265) were indeed incompatible, when Rh D+ PCs were transfused to Rh D- recipients.

Summary/Conclusions: In our settings, transfusion of ABO and RhD identical PCs was challenging, but feasible, since 98.0% of all transfused PCs were transfused to ABO identical recipients and only 2.7% RhD+ PCs were transfused to RhD- recipients.

P-278-B Table 1.

Outcomes	No of studies	No. of Participants (albumin/no albumin)	Risk Difference (RD)/ SMD (95% CI)	GRADE level of Evidence
Mortality	8	606/617	RD: 0.01 (-0.01, 0.02)	○○LOW
Kidney failure	3	169/170	RD: -0.03 (-0.22, 0.15	HIGH
ICU length of stay	9	510/529	SMD: -0.04 (-0.20, 0.12)	○○○VERY LOW
Hospital length of stay	4	250/259	SMD: 0.02 (-0.20, 0.24)	○○○VERY LOW
Blood Loss	10	515/543	SMD: -0.16 (-0.34, 0.01)	○○○VERY LOW

P-278-B | Intravenous albumin in adult cardiac surgery: a meta-analysis

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Background: Intravenous albumin is often used to prime the cardiopulmonary circuit and for volume resuscitation in adult patients undergoing cardiothoracic surgery. The effectiveness and the safety of intravenous albumin has not been synthesized.

Aims: This systematic review examines the effectiveness of intravenous albumin in reducing mortality and other clinically important outcomes compared to no albumin or alternative solutions for adult patients undergoing cardiothoracic surgery.

Methods: MEDLINE, Embase, CCRT, Transfusion Evidence Library from inception (1946) to December, 19th 2019 as well as, reference lists of eligible systematic reviews were searched. Randomized controlled trials (RCTs) or observational comparative studies published in English were included. The primary outcome was mortality. Secondary outcomes included kidney failure, hospital and intensive care unit (ICU) length of stay, and blood loss. Risk of bias for the RCTs was assessed using the Cochrane Risk of Bias tool and overall quality of outcomes was assessed using the GRADE criteria (GRADEproGDT). Random effects models were used to calculate the pooled risk difference and 95% confidence intervals (CI) for binary outcomes, and the standardized mean difference (SMD) with 95% CIs to estimate the effect on the continuous outcomes using Review Manager 5.4.1.

Results: A total of 1435 references were retrieved, and 48 RCTs met inclusion criteria (Table 1). Intravenous albumin compared to no albumin did not significantly reduce mortality, kidney failure, length of hospital stay, length of ICU stay, or blood loss (Table 1).

Summary/Conclusions: The use of intravenous albumin did not reduce mortality or other clinically important outcomes when used for priming of the cardiopulmonary circuit or volume resuscitation in adult patients undergoing cardiothoracic surgery.

Blood components/products – Plasma products

P-279 | Comparison of factor viii activity at different storage intervals in fresh frozen plasma produced at 8 hours and 24 hours after blood donation

selected for main programme

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Background: Guidelines for the preparation of fresh frozen plasma (FFP) are somewhat different between AABB and ISBT. The main difference is that the AABB requires FFP to be generated within 8 hours after blood collection and stored below -18°C. However, according to ISBT, FFP can be produced within 24 hours after collection if whole blood is quickly placed at 20°C to 24°C and frozen to -30°C in 1 hour during FFP preparation.

Aims: The purpose of this study was to evaluate and compare the differences of factor VIII activity at different storage intervals in FFP produced at 8 hours (FFP-8h) and 24 hours (FFP-24h) after blood collection.

Methods: This study was approved by the IRB Committee of Taiwan Blood Services Foundation and performed at the Hsinchu Blood Center. In the study, a total of 30 blood samples were randomly selected, in which blood types A, B, and O were chosen in the ratio 3:3:4. Each sample was about 5mL. After centrifuge, the plasma was divided into 5 aliquots. The samples were then completely frozen, and divided into two groups. Group 1: FFP samples were frozen at -20°C in the air-cooled freezer (HLU-500-3D5H, Taiwan) for 4 hours. Group 2: FFP samples were frozen at -30°C in the contact freezer (Plasmafrost I.Te.

M.4, Angelantoni Life Science, Italy) for 1 h. Factor VIII activity was measured by KC-4 Delta coagulation analyzer (Diagnostica Stago) in each group and compared at different storage periods of 3, 6, 9, and 12 months after freezing. The data before freezing were used as control.

Results: In group 1, the average factor VIII activity of FFP-8h before freezing, and stored for 3, 6, 9 and 12 months after freezing were 94.6%, 91.9%, 90.7%, 87% and 80%, respectively. For FFP-24h samples, their factor VIII activity were 77.4%, 76.3%, 75.4%, 74.4%, and 68.9%, respectively. In group 2, the average factor VIII activity of FFP-8h at 3, 6, 9 and 12 months after freezing were 93.3%, 94.3%, 94.0%, and 82.4%, respectively. For FFP-24h samples, their factor VIII activity were 76.0%, 76.0%,73.7%, and 66.1%, respectively. The results showed that factor VIII activity of FFP prepared within 8 hours after blood collection was significantly higher than that prepared within 24 hours ($p < 0.001$). In addition, the data showed only a slight decrease in storing samples from 3 months to 9 months, but a sharp decrease in the samples stored for 12 months. Also, the data demonstrated that there was no difference between the samples frozen under the conditions of -20°C and -30°C, regardless of whether the FFP were prepared within 8 hours or 24 hours after collection.

Summary/Conclusions: From the results of the study, the factor VIII activity in the FFP rapidly frozen below -30°C was not better than that of FFP frozen at -20°C. No matter the FFP prepared within 8 hours or 24 hours after blood collection, the decrease of factor VIII activity in the first nine months of storage was not obvious ($p>0.05$). However, the activity of the factor VIII declined dramatically from 9 to 12 months after storage. In conclusion, FFP prepared by both methods in this study, whether frozen at -20°C or -30°C, met the Council of Europe's standard that the level of factor VIII in stored FFP should be equal to or greater than 70% of freshly collected plasma unit.

P-280 | Validation of the preparation of amotosalen/UVA light pathogen reduced plasma from previously quarantined units *selected for main programme*

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Background: Two types of therapeutic plasmas are currently produced by the French Blood Service (EFS), plasma secured by quarantine (Q) and plasma pathogen reduced (PR) by amotosalen/UVA light treatment (A-UVA) (INTERCEPT™ Blood System, Cerus). Both can be produced from whole blood or apheresis collections. About one third of Q plasma cannot be released for transfusion because the donors do not come back and are not re-tested for viral markers.

Aims: To validate a process consisting after a frozen Q period of 30 weeks in thawing the plasma, A-UVA treating and refreezing for storage up to 3 years from collection. The A-UVA plasma quality before and after treatment was evaluated. This process is not approved in France.

Methods: 18 apheresis ACD-A plasmas of at least 640 mL were collected, split in three sub-units, frozen with 24 hours and stored at <-25°C for 30 weeks. After thawing in a water bath at 37°C, pools of triplets were reconstituted and submitted to the A-UVA treatment including addition of 15 ml of 6 mM amotosalen solution, exposure to 3J/cm^2 UVA light, removal of the active compound (≤2,0 µM) by filtration through a compound adsorption device (CAD), split in 3 units and refreezing within 6 hours for <-25°C storage. Similarly 18 groups of 5 CPD whole blood derived plasma units were frozen and stored at <-25°C for 30 weeks. After thawing, pools of 5 units were constituted, split in 2× 640 mL minimum. Each of the two sub-pools was A-UVA treated, split in three units and refrozen within 6 hours in the same way. All plasma units were non-O. A panel of plasma proteins including coagulation factors and inhibitors and global coagulation tests were measured in the thawed plasma pools after the Q period (pre) and in the plasma units after A-UVA treatment and up to 14-day frozen storage (post).

Results: The results of a selection of plasma parameters for the 36 combined replicates are reported in the Table 1 below. The requirements from the French Official Journal (JO), at least 70 % of the units with FVIII ≥ 0.5 IU m/L and Fibrinogen ≥ 2.0 g/L were met. Residual amotosalen concentration was 0.69 ± 0.11 µM.

P-280 Table 1.

N=36	Pre (after 30-week ≤-25°C storage)	Post (after thawing / A-UVA PR / 14-day frozen storage)
Total proteins (g/L)	58.6 ± 3.1	56.9 ± 3.0
Albumin (g/L)	37.2 ± 1.6	35.9 ± 1.7
IgA (g/L)	1.70 ± 0.45	1.66 ± 0.44
IgG (g/L)	8.44 ± 1.42	8.11 ± 1.33
IgM (g/L)	0.78 ± 0.32	0.76 ± 0.31
APTT (ratio)	1.02 ± 0.06	1.24 ± 0.00
PTT (%)	95 ± 7	82 ± 6
Fibrinogen (g/L)	2.81 ± 0.43	2.54 ± 0.37
FVIII (IU/mL)	1.22 ± 0.29	0.86 ± 0.18
FII (%)	89 ± 7	80 ± 6
F V (%)	93 ± 13	86 ± 12
FVII (%)	103 ± 17	80 ±14
FIX (%)	116 ± 19	93 ± 12
FX (%)	91 ± 9	80 ± 9
FXI (%)	108 ± 16	92 ± 14
VWF Ag (%)	117 ± 27	114 ± 26
AT-III (%)	97 ± 7	92 ± 7
Protein C (%)	97 ± 14	85 ± 12
Protein-S (%)	84 ± 15	78 ± 13
Plasminogen (%)	93 ± 9	84 ± 9
D-Dimers (µg/mL)	0.32 ± 0.08	0.31 ± 0.06
α2-antiplasmin (%)	99 ± 7	82 ± 6

Summary/Conclusions: Having the possibility to pathogen reduce plasma units not released after a quarantine period of 30 weeks offers an additional source of therapeutic plasma. Previously frozen plasma treated post-thawing with A-UVA retained sufficient levels of plasma proteins, coagulation factors and inhibitors and normal clotting time. This process can also be applied in first intention to optimize production efficiency by batch processing of frozen plasma units.

Blood components/products – Novel blood products

P-281 | Optimizing red cell production from umbilical cord blood with whole blood filtration

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Background: For neonates and preterm infants, where the transfusion dose is low (20 mL/Kg), the use of red blood cells (RBC) from umbilical cord blood (UCB) appears to be feasible. Some aspects need to be evaluated before introducing this transfusion practice on a routine basis. First, clinical studies are needed to demonstrate the efficacy of these RBC products. Secondly, the technical limitation is obtaining an adequate volume of red cell from cord blood units, even when the UCB collection volume is not so high (i.e. 60-80 mL).

Aims: In order to produce RBC with adequate volume, we evaluated the efficiency of a whole cord blood filtration and subsequent automated fractionation to obtain pre-storage filtered RBC from UCB units.

Methods: We evaluated UCB units not suitable for transplantation collected at UNICATT Cord Blood Bank. Eligible UCB units with more than 60 mL of volume (including the anticoagulant), haematocrit (Hct) ≥ 33%, and no apparent clots or haemolysis underwent whole cord blood filtration using a standard soft RCC filter (BioR Flex 01 BBS, Fresenius Kabi, Germany). Then, filtered UCB units were centrifuged and fractionated with Compomat G5® (Fresenius Kabi, Germany). Packed RBC were suspended in SAG-M solution (ratio blood: SAG-M=2:1) in non-DEHP, Di2-ethylhexyl phthalate, bags (CompoFlex® Pediatric RCC Storage System, Fresenius Kabi, Germany). We assessed the following parameters on whole UCB units and UCB-RBC: volume, haematocrit, RBC mass, haemoglobin/unit, residual WBC after filtration and microbial contamination. Technical parameters such as filtration time and automated fractionation time were also recorded.

Results: Data relative to 11 UCB units were evaluated with a median collection volume of 71 mL (63-93): 10 out 11 were between 60-80 mL. Median Hct was 36.5% (33-39.5) and median red cell mass was 26.2 mL (21.1-34.1). After filtration and fractionation, we obtained UCB-RBC with a median volume of 23 mL (18-38), median Hct of 63.3% (61.7-65.9) and red cell mass of 14.8 mL (11.5-24.1). Red cell mass recovery was almost 60%. Haemoglobin content was 11.5 gr/unit (10.4-12.3) before filtration and 4.6 gr/unit (3.6-7.6) in UCB-RBC. Filtration was efficient with median residual WBC contamination of 0.01 $x10^6$/unit (0.0-0.04). Filtration and automated fractionation allow obtaining units quickly in less than five minutes in both phases. Blood cultures were negative in all units.

Summary/Conclusions: These preliminary data show that whole UCB filtration and automated fractionation is feasible resulting in optimizing RBC production, even when collection volume in less than 80 mL. As reported in a previous study, filtration of RBC after fractionating UCB units less than 80 mL results in packed RBC with a median volume of 17 mL, which is not sufficient to guarantee a single transfusion dose. After filtration of whole UCB, RBC-UCB units appear to be compliant in terms of transfusion dose and quality of the units, with adequate haematocrit, haemoglobin and residual WBC content, despite such low collection volume. The soft filter technology allowed a better and consistent red cell mass recovery. In the future, development of a filter for whole UCB red cell production would further permit to ameliorate this process.

P-282 | *Abstract withdrawn*

P-283 | Effect of delayed cold storage on platelet aggregation and hemostatic response

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Background: Previous studies have demonstrated that cold stored platelets exhibit enhanced surface activation markers and maintain equivalent to better aggregation and hemostatic responses relative to room temperature platelets, especially when stored in InterSol platelet additive solution. Short duration storage at room temperature (≤24 hours) prior to cold storage may affect these properties.

Aims: To compare in vitro quality parameters, aggregation and coagulation responses of cold stored Amicus InterSol platelets following overnight room temperature agitated hold (D1 CS) to immediate post-collection cold storage (D0 CS).

Methods: Double dose InterSol platelets (n=6; 65% InterSol, 35% plasma) were collected from healthy subjects using the Amicus Separator. Products were split equally between two Amicus platelet storage containers following a 1 hr rest on the benchtop. One container was sampled immediately for test parameters, then stored at 1-6°C (D0 CS). The other container was stored at 20–24°C with agitation until next day (approx 24 hrs), then sampled and placed in cold storage (D1 CS). Pairs were sampled and tested on Days 7, 14, and 21 post collection. Clot formation was assessed by thromboelastography both with and without dilution in platelet-poor, autologous fresh frozen plasma (FP-PPP). Agonist based aggregation responses were tested using collagen, ADP, and TRP. In addition, storage parameters including platelet counts, glucose, lactate, supernatant LDH, pH, gas analysis, and activation markers (CD62, PS expression) were evaluated.

Results: D0 CS platelets had significantly lower mean platelet volume (MPV), less phosphatidylserine expression, and lower supernatant LDH

than the D1 CS platelets throughout storage. Platelet counts remained comparable between conditions at all-time points ($p>0.05$). Bicarbonate and pH trended lower on D7 for the D1 CS condition but were not statistically different beyond this point. pCO_2 was lower for D1 CS platelets at D14 and D21 ($p<0.05$), but pO_2 remained comparable to the D0 CS condition for the storage duration. Glucose consumption was lower in D0 CS platelets up to D7; differences beyond this point were not significant. Lactate production remained comparable through D14, with D1 CS platelets yielding less at end of storage. CD62 expression was higher in D1 CS platelets at D7, but comparable for later time points. D0 CS platelets had superior agonist induced aggregation responses (Collagen, ADP, TRP) to D1 CS pairs. Native TEG responses (no dilution) for clot strength (MA) and crosslinking (angle) were comparable on D7 but degraded at later time points, trending lower for the D1 CS condition on D14, but without difference by D21 ($p>0.05$). In contrast, no statistical difference between the D0 and D1 CS conditions was observed for TEG responses following dilution with FP-PPP; data remained within normal range through D21.

P-283 Table 1.

Parameter (Mean ± SD)	CS Day	Pre CS	D14	D21
PLT Ct. ($\times 10^3$/uL)	0	1427 ± 66	1195 ± 93	1179 ± 96
	1	1401 ± 75	1243 ± 46	1205 ± 26
MA (mm), Native	0	76 ± 3	64 ± 13*	23 ± 22
	1	75 ± 2	43 ± 22*	22 ± 19
MA (mm), FP-PPP Diluted	0	71 ± 3	69 ± 3	62 ± 5
	1	71 ± 1	69 ± 3	61 ± 7

*p<0.05.

Summary/Conclusions: Platelets stored for up to 24 hours under standard room temperature agitated conditions prior to cold storage have reduced aggregation responses to specific agonists but maintain similar hemostatic properties to those stored cold immediately after collection. Under both storage conditions, TEG response after D7 was restored to normal levels when coagulation factors were replenished with FP-PPP.

P-284 | The effect of downscaling platelet cryopreservation on the phenotype and function of thawed platelets

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Background: Platelet components can be cryopreserved to extend the shelf-life from 7 days to at least 2 years. However, platelets are altered during the freezing and thawing process. Downscaling platelet cryopreservation (freezing in tubes) would support rapid screening of novel strategies to improve the quality of cryopreserved platelets (CPPs), such as alternative cryoprotectants or protective compounds. However, substantially reducing the freezing volume may alter the dynamics of the freezing process, thus changing the platelet characteristics, compared to standard freezing protocols.

Aims: The aim of this study was to characterise the effect of freezing conditions on the *in vitro* phenotype and function of platelets frozen in a low volume compared to standard CPPs.

Methods: Buffy coat-derived platelets were prepared for cryopreservation using 5-6% DMSO and were processed using standard protocols or were aliquoted into 2mL plastic tubes. Platelets were hyperconcentrated to 25mL (standard CPPs) or 200µL (tubes) before freezing at −80°C (n=8). Six insulators/controlled rate freezing containers were used to vary the freezing rate of platelets in tubes (Table 1). A temperature logger was used to continuously monitor the freezing rate of platelets (n=3 per condition). Platelets were thawed and resuspended in plasma at volumes relevant to the volume frozen, and then assessed by flow cytometry and thromboelastograhpy (TEG).

Results: The use of different insulators for tubes varied the freezing rate of platelets compared to platelets frozen using the standard protocol (Table 2). However, this had no impact on recovery of the platelets, which remained similar between groups. The proportion of platelets expressing GPIbα (CD42b) or GPVI was similar between tubes and standard CPPs, while platelets frozen in tubes had a lower proportion of platelets with externalised phosphatidylserine, compared to platelets frozen with the standard protocol. The clot forming ability (TEG) of platelets was similar between groups.

Mean±SD; *indicates $p<0.05$ compared to standard CPPs, as determined using one-way ANOVA with *post-hoc* Bonferroni tests.

P-284 Table 1.

Insulator	Justification/rationale
Tissue paper	Readily available materials, historically used to slow freezing rate of cells
Flexible foam	
Corflute cardboard	Standard material used to store cryopreserved platelets
Corflute plastic	
CoolCell	Commercially available container for controlled rate freezing
Mr Frosty	

Summary/Conclusions: Modifying the freezing rate and volume by freezing platelets in tubes altered some platelet characteristics. Importantly, platelet recovery, the proportion of platelets expressing receptors highly sensitive to cryopreservation-induced damage (GPIbα and GPVI), and platelet function remained comparable to platelets frozen using the standard protocol regardless of the insulator used. This demonstrates the feasibility of downscaling platelet cryopreservation for future exploratory investigations.

P-284 Table 2.

Parameter	Standard CPPs	Tissue paper	Flexible foam	Corflute cardboard	Corflute plastic	CoolCell	Mr Frosty
Freezing rate (°C/minute)	−1.7 ± 0.2	−3.7 ± 0.4*	−3.9 ± 0.2*	−4.1 ± 0.2*	−4.0 ± 0.2*	−0.8 ± 0.0*	−0.8 ± 0.0*
Recovery (%)	82 ± 7	80 ± 7	80 ± 9	79 ± 7	76 ± 11	79 ± 8	80 ± 7
CD42b (% positive)	80 ± 7	84 ± 3	83 ± 3	82 ± 4	83 ± 3	83 ± 3	82 ± 2
GPVI (% positive)	25 ± 5	32 ± 4	30 ± 5	31 ± 7	32 ± 6	28 ± 5	26 ± 6
Annexin V (% positive)	69 ± 4	48 ± 6*	50 ± 6*	49 ± 7*	47 ± 7*	51 ± 7*	53 ± 9*
TEG R-time (minutes)	5.2 ± 0.3	5.4 ± 0.2	5.3 ± 0.2	5.4 ± 0.3	5.6 ± 0.4	5.3 ± 0.2	5.3 ± 0.1
TEG Maximum amplitude (mm)	59 ± 2.5	63 ± 3.3	60 ± 3.1	62 ± 2.8	60 ± 3.4	59 ± 2.1	60 ± 2.1

P-285 | Development of the optimal formulation of platelet lysate for wound healing

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Background: The use of autologous and allogeneic blood products, rich in growth factors to promote wound healing and tissue repair, is gaining increasing attention. Examples include platelet rich plasma (PRP) and platelet lysate (PL). Serum eye drops can promote corneal wound healing in patients suffering from dry eye disease. However, AB serum donors are scarce and the product is relatively expensive. As such, we are looking at growth factor-rich alternatives such as PRP and PL.

Aims: To compare the growth-promoting effect of the PL formulations. PL was serum-converted and separately heat-inactivated from platelet concentrate (PC) in PAS-E® (65% PAS-E®, 35% plasma) and from PC in plasma, using HUVEC proliferation as a model.

Methods: PL was made from platelets pooled in PAS-E® (n=3) and from platelets pooled in plasma (n=3). Serum conversion ($CaCl_2$, final concentration 23 mmol/L) was performed, to prevent coagulation of the product and separately heat inactivation (1 hour, 56°C) to prevent antibody-mediated complement deposition and lysis. For the degree of wound healing induced by the four PL products, the proliferation was measured on confluent cell layers of HUVEC. PL was added to growth medium without FCS at 3 concentrations: 20%, 10% and 2%. AB serum and FCS were included as controls. Proliferation was measured on days 1, 2, 3 and 4 after seeding of the cells. After staining with 0.5% Crystal Violet, the attached cells were lysed with 2% Sodium Dodecyl Sulphate. Absorbance was measured at 590 nm.

Results: Results were reproducible and in most cases the highest proliferation was induced with 20% addition of the respective product. Serum-converted PL and AB serum resulted in HUVEC proliferation slightly superior or comparable to the positive FCS control. For heat-inactivated PL, proliferation was significantly lower when suspended in PAS-E®, i.e. between 25% - 40% of the positive FCS control. When suspended in plasma, heat-inactivated PL resulted in equally well to superior proliferation rates as compared to FCS.

Summary/Conclusions: Using this assay, we identified significant proliferation differences in response to the various PL formulations. Heat-inactivation clearly negatively affected proliferation.

P-286 | A case of severe hemolytic disease of the fetus and newborn (HDFN) caused by anti-KPA

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Background: The Kp^a antigen, or KEL3, was identified in 1957 and initially named Penny, but renamed when it was understood that the antigen belongs to the Kell blood group system. Approximately 2% in the Caucasian population are Kp^a positive and less than 0.01% in black population.

Anti-Kp^a is most often a benign IgG antibody that seldom causes hemolytic transfusion reactions (HTR). Very rarely, it may be the cause of HDFN: to our knowledge, two cases have earlier been described that led to intrauterine transfusions (IUTs) and four cases that led to treatment post-partum.

Anti-Kp^a is a common antibody in Sweden and during the last decade (2011-2020), we have identified 38 pregnancies at our center with maternal anti.Kp^a.

Aims: We describe a case of severe HDFN due to anti-Kp^a.

Methods: At pregnancy week 11, an anti-Kp^a was detected and identified in an AB RhD positive 1-para woman of Swedish origin, pregnant with the same partner as before. In the previous pregnancy, the antibody screening test was negative in the first trimester as well as at delivery. The partner is Kp(a+). In the current pregnancy titers were followed, and a monocyte monolayer assay (MMA) was performed in pregnancy week 29. Fetal ultrasonography of the middle cerebral

artery peak systolic velocity (MCA PSV) was performed nine times from gestational week 29 to 35 and multiple of median (MoM) calculated. A MoM <1.5 correlates to fetal anemia.

Results: Titers performed in Ortho Vision varied between 256 and 512. The MMA was negative. The first MCA PSV showed, unexpectedly, signs of fetal anemia (MoM 1.57) and was confirmed three days later at pregnancy week 29+6 (MoM 1.70). An intrauterine transfusion (IUT) was performed; 70 mL of O RhD positive Kp(a-) red blood cells (RBCs) increased the hemoglobin from 3.9 g/dL and the hematocrit from 11.7% to 30.6%. In pregnancy week 31+1 a second IUT was performed; 45 mL of RBCs increased the hemoglobin from 10.5 g/dL to 13.4 g/dL and the hematocrit from 30.2% to 38.4%. The fetus was A RhD positive but no Kp(a) typing was performed, because of a weak positive direct antiglobulin test (DAT) and a very weak anti-Kp^a was eluted from the RBCs. The fetus was thereafter monitored by weekly ultrasound, with no further signs of anemia (MoM <1.5).

A boy was delivered by caesarian section (patient request) in pregnancy week 36+6 with Apgar 9+10+10. A few hours after birth the hemoglobin was 9.9 g/dL and the baby received one exchange transfusion and was treated with phototherapy for two days. Bilirubin had the highest peak on day 4 (242 μmol/L). He received three top-up transfusions, the first one 35 days after birth. We were then not able to detect any RBCs with blood group A. He received the other two top-up transfusions at the age of two months and three months.

Summary/Conclusions: Anti-Kp^a is most often a benign antibody that seldom causes HTR or HDFN, but in rare cases it may be a dangerous antibody for the fetuses and newborns. In our case, the fetal hemoglobin was 39.9 g/dL in gestational week 29+6. The mechanisms causing fetal anemia is not clear, but possibly similar to anti-K antibodies, by suppression of fetal erythropoiesis.

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AUTHOR INDEX

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