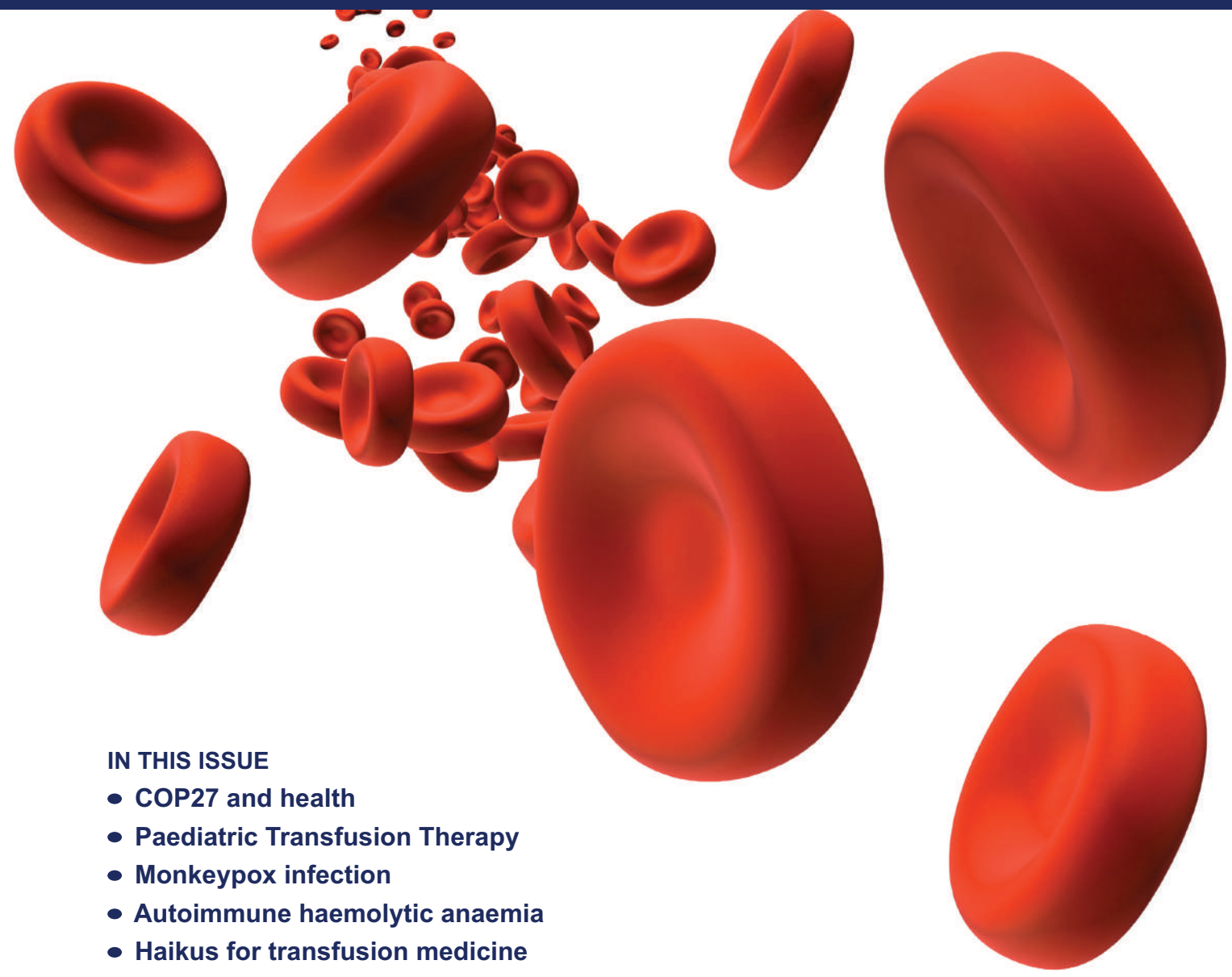
Volume 32 Number 6 • December 2022

TRANSFUSION MEDICINE

Official Journal of the British Blood Transfusion Society and the Network for the Advancement of Patient Blood Management, Haemostasis and Thrombosis



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Transfusion Medicine will publish articles on transfusion medicine in its widest context, including blood transfusion practice (blood procurement, pharmaceutical, clinical, scientific, computing and documentary aspects), immunohaematology, immunogenetics, histocompatibility, medico-legal applications, and related molecular biology and biotechnology.
In addition to original articles, which may include brief communications and case reports, the journal will contain a regular educational section (based on invited reviews and state-of-the-art reports), technical section (including quality assurance and current practice guidelines), leading articles, letters to the editor, occasional historical articles and signed book reviews. Some lectures from Society meetings that are likely to be of general interest to readers of the Journal may be published at the discretion of the Editor and subject to the availability of space in the Journal.

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Periodical ID Statement

Transfusion Medicine (ISSN 0958-7578), is published bimonthly. US mailing agent: Mercury Media Processing, LLC 1850 Elizabeth Avenue, Suite #C, Rahway, NJ 07065 USA. Periodical postage paid at Rahway, NJ. Postmaster: Send all address changes to *Transfusion Medicine*, John Wiley & Sons Inc., C/O The Sheridan Press, PO Box 465, Hanover, PA 17331.

Publisher

Transfusion Medicine is published by John Wiley & Sons Ltd, 9600 Garsington Road, Oxford, OX4 2DQ, UK. Tel: +44 1865 776868; Fax: +44 1865 714591.
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The Journal is indexed by Current Contents Life Sciences, Index Medicus, Medline and Science Citation Index.

ISSN 0958-7578 (Print)
ISSN 1365-3148 (Online)

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Transfusion Medicine

Volume 32, Number 6 December 2022

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This journal is available online at *Wiley Online Library*.
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and register for table of contents e-mail alerts.

Received: 15 October 2021 | Accepted: 15 November 2021

DOI: 10.1111/tme.12744

EDITORIAL

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A difficult year… so keep on keeping on

Although Rh immune globulin (RhIG) has been in use clinically for over 50 years, pregnant patients continue to become alloimmunised to the Rh D antigen due to inadequate perinatal care or RhIG failure.[1-3] In order to identify patients at risk for Haemolytic Disease of the Fetus and Newborn (HDFN), blood banks generally screen for anti-D using a qualitative assay. Once identified, further tests are used to quantify anti-D, with levels used to estimate the risk for HDFN. Although most blood banks throughout the world use the saline indirect antiglobulin test (SIAT; also known as tube test) to determine anti-D titre levels, the British Society of Haematology (BSH) revised guidelines recommend using continuous flow analysis (CFA), which yields a concentration of anti-D measured in international units/mL (IU/mL).

The level of anti-D is critical as it is used to guide patient care. Patients with levels above an accepted threshold are considered to be at high risk for HDFN and must be closely monitored by the obstetric service. Conversely, very low concentrations of anti-D can be categorised as low risk for HDFN, although correlation with clinical history is needed to distinguish between alloimmunisation, which carries the potential for HDFN, and passive antibodies from RhIG treatment. As a result, the test used to quantify antibody levels should be easy to use, allow for reasonable turnaround time and be reproducible.

Based on these requirements, the CFA and tube test both have critical drawbacks. The CFA test requires expensive equipment and specially trained technicians. As a result, the majority of labs in the United Kingdom must send their samples to a reference lab, creating potential delays in critical antenatal care. Also, significant inter-laboratory variability has been reported for the CFA.[4] Conversely, titre levels obtained by tube testing are inexpensive but are time intensive and prone to variability. A third alternative uses automated platforms to run column agglutination technology (CAT) or solid phase technology (SPT). CAT and SPT are affordable test options with decreased variability in methodology and interpretation, as recently demonstrated in evaluation of isohaemagglutinins.[5,6]

Automated platforms are commonplace within modern hospital laboratories, enabling improved workflow for blood typing and antibody screening. Automated platforms may also improve consistency of results, though this has not been extensively demonstrated. However, assessment of antibody levels by automated titration has lagged behind tube testing, as clinically actionable anti-D levels were previously defined by manual tube methods.[7] As studies have shown increased sensitivity in CAT and solid phase when compared to tube, there is concern that these modalities may result in relatively higher titres, which may lead to unwarranted testing as well as undue stress for the patient.

Prior evaluation by Mikesell et al showed that gel testing for RhIG with CAT was more sensitive than SIAT but less sensitive than when using SPT.[3] This group also showed that passive D reactivity can persist for up to 3.5 to 4.5 months after administration with expected variation among different commercially available formulations.[3] As most half-lives range between 20 and 30 days, with more sensitive testing, persistence of antibodies can become problematic as 5 to 6 half-lives are required for drug clearance.[8-11] As RhIG may be detected for long periods of time after prenatal administration, it is critical to delineate the true nature of an antibody and categorise it as passive and benign vs immunogenic with a concomitant risk of HDFN.

As such, it is with great interest that we read Evans and colleagues' work evaluating antibody titre scores by automated CAT vs CFA in the assessment of immune and passive anti-D antibodies. Herein, they describe their experience using the ORTHO VISION automated CAT platform to evaluate nearly 200 anti-D samples in five separate UK hospital transfusion laboratories. This study builds on the work of Bruce et al that initially compared anti-c and anti-D titre scores by manual CAT vs CFA, showing increasing manual CAT titre scores with higher concentrations by CFA.[4] A titre score is a value assigned to assess an antibody's level and avidity. It is calculated using the strength of reactivity at each titration with levels of reactivity assigned with scores (4+ 12, 3+ 10, 2+ 8, 1+ 5, ± 3, 0 0).[4] Evans et al expand on this work with a larger cohort and application of the titre score in conjunction with clinical history of RhIG administration.

The group shows automated CAT testing can effectively distinguish between high and low antibody levels. These low levels defined by titre scores align with currently in use definitions of high and low by CFA (low likely passive<0.4 IU/mL < high likely immune). This would make automated CAT testing an appropriate screening test for the identification of true immune anti-D antibodies vs passive antibodies not requiring CFA. Likewise, they suggest a testing algorithm in which patients could be screened out using titre score. Based on this schema, in which indeterminate results would be reflexed to CFA, all patients would have received appropriate testing in their study.

As current UK standards dictate quantification of anti-D to rule out alloimmunisation, availability of testing is a key factor.[12] This pilot study shows promising results and may represent a solution to the problem of anti-D level assessment. Moreover, this important work helps establish a correlation between automated CAT titre scores and absolute levels by CFA. Though no linear correlation was

demonstrated, understanding this correlation is key for the management of HDFN and ensuring appropriate perinatal care.

Anti-D antibody titre scores are a reasonable starting point for assessment of automated CAT as an antenatal testing modality, as Rh-alloimmunisation represents the prototypic cause of HDFN. Evaluation of maternal antibodies to other blood group systems is a key area of future investigation and is necessary for generalisation of results. This is a pilot study and the group intend to continue their work adding additional clinical correlation and interlaboratory comparison in further studies. Whether labs will internationally adopt this testing is unclear; however, the results point to automated CAT testing as an attractive possibility. Currently, automated CAT titre scores represent a practical screening test for passive anti-D antibody identification.

CONFLICT OF INTEREST

The authors declare no competing interests.

Brian D. Adkins[1]
Claudia S. Cohn[2]

[1]*Department of Pathology, University of Virginia Health System, Charlottesville, Virginia*
[2]*Department of Laboratory Medicine and Pathology, University of Minnesota, Minneapolis, Minnesota*

Correspondence
Claudia S. Cohn, Department of Laboratory Medicine and Pathology, University of Minnesota, Minneapolis, MN.
Email: cscohn@umn.edu

ORCID

Brian D. Adkins https://orcid.org/0000-0002-9480-2762
Claudia S. Cohn https://orcid.org/0000-0001-9847-0470

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DOI: 10.1111/tme.12922

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EDITORIAL

COP27 Climate Change Conference: Urgent action needed for Africa and the world

Wealthy nations must step up support for Africa and vulnerable countries in addressing past, present and future impacts of climate change

The 2022 report of the Intergovernmental Panel on Climate Change (IPCC) paints a dark picture of the future of life on earth, characterised by ecosystem collapse, species extinction, and climate hazards such as heatwaves and floods.[1] These are all linked to physical and mental health problems, with direct and indirect consequences of increased morbidity and mortality. To avoid these catastrophic health effects across all regions of the globe, there is broad agreement—as 231 health journals argued together in 2021—that the rise in global temperature must be limited to less than 1.5°C compared with pre-industrial levels.

While the Paris Agreement of 2015 outlines a global action framework that incorporates providing climate finance to developing countries, this support has yet to materialise.[2] COP27 is the fifth Conference of the Parties (COP) to be organised in Africa since its inception in 1995. Ahead of this meeting, we—as health journal editors from across the continent—call for urgent action to ensure it is the COP that finally delivers climate justice for Africa and vulnerable countries. This is essential not just for the health of those countries, but for the health of the whole world.

AFRICA HAS SUFFERED DISPROPORTIONATELY ALTHOUGH IT HAS DONE LITTLE TO CAUSE THE CRISIS

The climate crisis has had an impact on the environmental and social determinants of health across Africa, leading to devastating health effects.[3] Impacts on health can result directly from environmental shocks and indirectly through socially mediated effects.[4] Climate change-related risks in Africa include flooding, drought, heatwaves, reduced food production, and reduced labour productivity.[5]

Droughts in sub-Saharan Africa have tripled between 1970–1979 and 2010–2019.[6] In 2018, devastating cyclones impacted 2.2 million people in Malawi, Mozambique and Zimbabwe.[6] In west and central Africa, severe flooding resulted in mortality and forced migration from loss of shelter, cultivated land, and livestock.[7] Changes in vector ecology brought about by floods and damage to environmental hygiene have led to increases in diseases across sub-Saharan Africa, with rises in malaria, dengue fever, Lassa fever, Rift Valley fever, Lyme disease, Ebola virus, West Nile virus and other infections.[8,9] Rising sea levels reduce water quality, leading to water-borne diseases, including diarrhoeal diseases, a leading cause of mortality in Africa.[8] Extreme weather damages water and food supply, increasing food insecurity and malnutrition, which causes 1.7 million deaths annually in Africa.[10] According to the Food and Agriculture Organization of the United Nations, malnutrition has increased by almost 50% since 2012, owing to the central role agriculture plays in African economies.[11] Environmental shocks and their knock-on effects also cause severe harm to mental health.[12] In all, it is estimated that the climate crisis has destroyed a fifth of the gross domestic product (GDP) of the countries most vulnerable to climate shocks.[13]

The damage to Africa should be of supreme concern to all nations. This is partly for moral reasons. It is highly unjust that the most impacted nations have contributed the least to global cumulative emissions, which are driving the climate crisis and its increasingly severe effects. North America and Europe have contributed 62% of carbon dioxide emissions since the Industrial Revolution, whereas Africa has contributed only 3%.[14]

THE FIGHT AGAINST THE CLIMATE CRISIS NEEDS ALL HANDS ON DECK

Yet it is not just for moral reasons that all nations should be concerned for Africa. The acute and chronic impacts of the climate crisis create problems like poverty, infectious disease, forced migration, and conflict that spread through globalised systems.[6,15] These knock-on impacts affect all nations. COVID-19 served as a wake-up call to these global dynamics and it is no coincidence that health professionals have been active in identifying and responding to the consequences of

This Comment is being published simultaneously in multiple journals. For the full list of journals see: https://www.bmj.com/content/full-list-authors-and-signatories-climate-emergency-editorial-october-2022

growing systemic risks to health. But the lessons of the COVID-19 pandemic should not be limited to pandemic risk.[16,17] Instead, it is imperative that the suffering of frontline nations, including those in Africa, be the core consideration at COP27: in an interconnected world, leaving countries to the mercy of environmental shocks creates instability that has severe consequences for all nations.

The primary focus of climate summits remains to rapidly reduce emissions so that global temperature rises are kept to below 1.5°C. This will limit the harm. But, for Africa and other vulnerable regions, this harm is already severe. Achieving the promised target of providing $100 bn of climate finance a year is now globally critical if we are to forestall the systemic risks of leaving societies in crisis. This can be done by ensuring these resources focus on increasing resilience to the existing and inevitable future impacts of the climate crisis, as well as on supporting vulnerable nations to reduce their greenhouse gas emissions: a parity of esteem between adaptation and mitigation. These resources should come through grants not loans, and be urgently scaled up before the current review period of 2025. They must put health system resilience at the forefront, as the compounding crises caused by the climate crisis often manifest in acute health problems. Financing adaptation will be more cost-effective than relying on disaster relief.

Some progress has been made on adaptation in Africa and around the world, including early warning systems and infrastructure to defend against extremes. But frontline nations are not compensated for impacts from a crisis they did not cause. This is not only unfair, but also drives the spiral of global destabilisation, as nations pour money into responding to disasters, but can no longer afford to pay for greater resilience or to reduce the root problem through emissions reductions. A financing facility for loss and damage must now be introduced, providing additional resources beyond those given for mitigation and adaptation. This must go beyond the failures of COP26 where the suggestion of such a facility was downgraded to "a dialogue".[18]

The climate crisis is a product of global inaction, and comes at great cost not only to disproportionately impacted African countries, but to the whole world. Africa is united with other frontline regions in urging wealthy nations to finally step up, if for no other reason than that the crises in Africa will sooner rather than later spread and engulf all corners of the globe, by which time it may be too late to effectively respond. If so far they have failed to be persuaded by moral arguments, then hopefully their self-interest will now prevail.

CONFLICT OF INTERESTS

In the interest of transparency the authors wish to declare the following roles and relationships: James Kigera is the Ex-Officio, President and Secretary of the Kenya Orthopedic Association; Paul Yonga been paid to speak or participate at events by Novartis, bioMerieux and Pfizer; Chris Zielinski is a paid consultant for the UK Health Alliance on Climate Change; Joy Muhia is an unpaid board member of the International Working Group for Health systems strengthening; David Ofori-Adjei has a relationship with GLICO Healthcare Ltd. The authors declare no further conflicts of interest beyond those inherent in the editorial roles listed above.

Lukoye Atwoli[1]
Gregory E. Erhabor[2]
Aiah A. Gbakima[3]
Abraham Haileamlak[4]
Jean-Marie Kayembe Ntumba[5]
James Kigera[6]
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Paul Yonga[1]
Lilia Zakhama[18]
Chris Zielinski[19]

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Correspondence
Email: chris.zielinski@ukhealthalliance.org

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Received: 10 June 2022 | Revised: 17 August 2022 | Accepted: 11 September 2022

DOI: 10.1111/tme.12918

REVIEW

TRANSFUSION MEDICINE WILEY

Evaluating the risk of transfusion and transplant-transmitted monkeypox infections

Heli Harvala[1] | Peter Simmonds[2]

[1]Microbiology Services, NHS Blood and Transplant, London, UK

[2]Nuffield Department of Medicine, University of Oxford, Oxford, UK

Correspondence
Nuffield Department of Medicine, University of Oxford, Peter Medawar Building, South Parks Road, Oxford, OX1 3SY, UK.
Email: peter.simmonds@ndm.ox.ac.uk

Funding information
National Institute of Health Research, Grant/Award Number: NIHR203338

Abstract

The recent emergence of monkeypox virus (MPXV) in the UK and elsewhere is of urgent public health concern. Several aspects of MPXV epidemiology and pathogenesis, including its systemic spread and viraemia during acute infection, furthermore represent an important potential threat to the safety of blood transfusion and organ transplantation. Reported infections in the UK have been exponentially increasing over the last 2 months, with 1552 reported cases in the UK by 7th July 2022. This is likely to be considerable underestimate given current limitations in diagnostic capacity and clinical diagnoses hampered by its similar disease presentations to other causes of rash and genitourinary disease. While MPXV infections are currently most widespread in gay, bisexual or other men who have sex with men, wider spread of MPXV outside defined risk groups for infection may prevent identification of infection risk in donors. While typically mild disease outcomes have been reported in UK cases, case fatality rates ranging from 1% to over 10% are reported for different MPXV strains in its source area in sub-Saharan Africa. Recipients of blood components and organs transplant, especially those who are immunosuppressed, may reproduce the greater systemic spread and morbidity of those infected through percutaneous routes. There is a potential risk of MPXV transmission and severe disease outcomes in blood and transplant recipients. In addition to current risk assessments performed in the UK and exclusion of donors with recent MPXV exposure, determining viraemia frequencies in donors and directly evaluating transmission risk would be of considerable value in assessing whether MPXV nucleic acid screening should be implemented.

KEYWORDS
blood transfusion, monkeypox, monkeypox virus, nucleic acid testing, poxviridae, transmission, transplant

1 | INTRODUCTION

There have been a multitude of reports of monkeypox (MPX) in the United Kingdom, several countries in Europe, North America and increasingly worldwide over the last 2 months (Figure 1). Reported cases, initially described in the UK at the beginning of May have dramatically increased in number with a cumulative total of 1552 at the time of writing (10th July), and an estimated doubling time of 15 days (95% confidence interval: 10–18 days).[1] Exponentially increasing number of cases have also been reported in several European countries, in North America and elsewhere (Figure 1). MPX virus (MPXV) infections have been preferentially detected in males (all

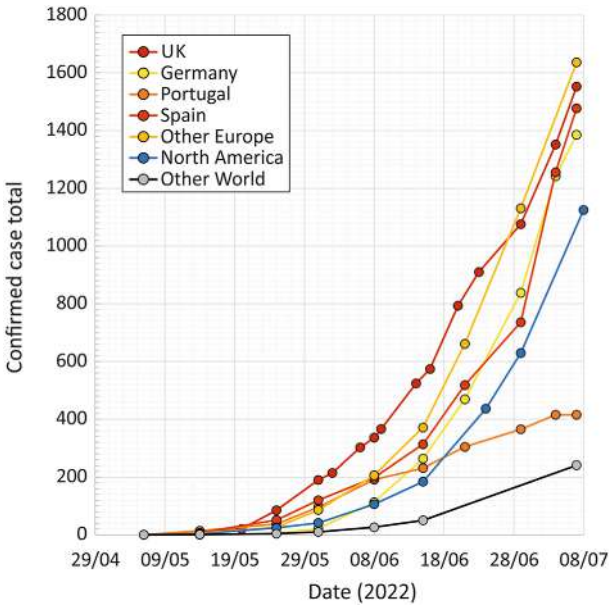

FIGURE 1 Totals of confirmed cases of MPXV in UK, selected European countries and totals of other regions since its emergence in early May. Data sources: ECDC, UKHSA, CDC, (https://www.ecdc.europa.eu/en/monkeypox-outbreak; https://www.gov.uk/government/publications/monkeypox-outbreak-epidemiological-overview; https://www.cdc.gov/poxvirus/monkeypox/response/2022/world-map.html)

but 4 of currently reported UK cases), primarily in those aged between 20 and 49, and preferentially infecting gay, bisexual, or other men who have sex with men (GBMSM).

Interim case definitions for MPX has been proposed by the UK Health Security Agency (UKHSA) and European Centre for Disease Prevention and Control (ECDC),[2,3] but MPXV infections remain problematic to diagnose clinically, often presenting with a single lesion only or with lesions that may be mistaken for varicella zoster or where affecting genital areas, those caused by sexually transmitted diseases such as syphilis. Access to diagnostic testing for MPXV DNA is highly limited at the time of writing and may delay definitive diagnoses from being made in many cases. Although relevant professional associations and sexual health clinics across Europe have been contacted with information about MPXV infections and presenting symptoms, an informed clinical diagnosis is difficult as MPXV may present clinically similarly to other sexually transmitted infections. Finally, infections may be mild, present with atypical symptoms or be entirely asymptomatic and hence remain undiagnosed. Collectively, it is almost certain that the extent of MPXV spread is currently substantially under-estimated across Europe.

MPXV infections are associated with systemic spread and a prolonged period of viraemia in pre-symptomatic and symptomatic stages of infection.[4] MPXV may therefore potentially be transmitted by blood components and organ transplantation. In this review, we evaluate current transmission risk in a transfusion or transplant setting, determine what steps are required to evaluate the extent of its emergence and how transmissions may be prevented in the short term.

2 | BACKGROUND

MPXV is one of 12 species of poxviruses classified in the *Orthopoxvirus* genus of the virus family, *Poxviridae*.[5] Poxviruses form large rectangular virions (250–450 nm) that show a complex non-isometric symmetry. They possess large DNA genomes that are replicated in membranous structures in the cytoplasm and encode several structural, replicative and moreover an extremely large and highly variable complement of "accessory" genes that enable the virus to evade cellular and adaptive immune responses to infection.[6] Poxviruses are typically exquisitely adapted to their hosts, partly through the specificity of their evasion mechanisms but with variable relationships between transmissibility and pathogenicity, smallpox of humans representing one end of a continuum that extends to clinically inapparent infections with vaccinia virus (buffalopox) at the other.

MPXV was first recognised as a separate entity from smallpox and other poxviruses in the late 1950ss/early 1960s[7,8] and currently represents the most widely distributed orthopoxvirus to infect humans following the eradication of smallpox by vaccination.[9–11] MPXV has been detected in the wild in the rope squirrel, the tree squirrel and the Gambian pouched rat in central Africa[12–14] with serological evidence for a wider distribution in sub-Saharan rodents.[15] Rodents may represent a source of zoonotic infections of humans and other primates in Central and West Africa.[16,17] The route of zoonotic transmission to humans is not well characterised, although MPXV is not generally considered to be easily transmitted between humans.[18] The outbreak of MPXV infection in the USA in 2003[19–21] is illustrative of the typically zoonotic spread of the virus—81 human infections occurred after contact with imported infected rodents and subsequently infected pet prairie dogs but without evidence of subsequent human to human transmission.[22,23]

Discontinuation of smallpox vaccination worldwide from the 1980s, which partially protects from infection with the antigenically related MPXV, may have contributed to recent reports of MPXV outbreaks in Central and West Africa,[10,24–26] including 188 suspected or confirmed cases in Nigeria in 2017[27,28] where human-to-human transmission in this and other outbreaks was proposed.[29–31] More recently, often ongoing and larger outbreaks of MPXV have been documented in Central and West African countries particularly Cameroon, Central African Republic, and the Congo, with several infection-related fatalities.[9,27,32]

Human travel-related importations of MPXV to non-African countries such as the UK have been occasionally reported.[33,34] However, the scale of the current outbreak of MPXV raises the possibility of potentially wider and sustained spread of MPXV outside of its African source area. Its suspected capability for human-to-human transmission in UK and European outbreaks in 2022 makes for the alarming possibility that MPXV infections may become indigenously established in human populations worldwide. An ability of MPXV to adapt to and efficiently transmit between humans in this outbreak situation is a critical determining factor for this possibility.

3 | MPXV-ASSOCIATED HUMAN DISEASE

MPXV infections are initially manifested by a non-specific prodrome of fever, myalgia, lymphadenopathy, fatigue and headache 1–2 weeks after exposure, accompanied by the development of a characteristic centrifugal rash often becoming disseminated and affecting palms and soles of the feet.[35,36] The rash is initially maculopapular that proceeds to the development of vesicles and scabbing over a period of 12 days. Infectious virus is shed throughout the period of rash and in the prodromal period of systemic infection. Disease severity and the extent of rash formation is highly variable between individuals although more severe in those co-infected with HIV-1.[29] Incubation and disease severity has been reported to vary by route of transmission; infection from percutaneous routes, such as bites and scratches from infected prairie dogs, showed greater severity of systemic symptoms, higher likelihood of hospitalisation and shorter incubations periods compared to those infected through close contact or respiratory routes.[37] Greater systemic disease severity may be of relevance for those infected by viraemic blood component transfusions or transplant, where relatively large virus amounts may be introduced systemically.

There is little or no information on disease severity in highly immunocompromised individuals, although these may follow the severe and often potentially fatal outcomes of other transfusion-transmitted infections of human cytomegalovirus, and hepatitis B and E viruses in these patients. This should be considered for transplant and transfusion safety given the high proportion of immunocompromised recipients of blood, platelets and organs. The existence of residual immune protection from vaccinia vaccination may, however, ameliorate disease severity in patients over 50.

Detailed clinical and virological characterisation of MPXV infection in seven young individuals without comorbidities demonstrated a highly variable course of infection and infection markers,[4] in terms of the occurrence of rash and observational period relative to time from exposure. However, six from seven showed often prolonged periods of viraemia (up to 30 days) and excretion of MPXV in respiratory samples and urine.

4 | DIAGNOSIS AND CASE DEFINITIONS

Clinical diagnosis of MPXV infections may be compounded by marked differences in severity and extents of spread of rash. The European Centre for Disease Control (ECDC) has proposed case definitions of "Confirmed" on the basis of a positive result in a PCR specific for MXPX or for orthopoxviruses and sequence confirmation.[3] "Probable" cases are defined as those with rash, one from a range of associated disease features typical of MPXV and epidemiological links to diagnosed MPXV cases, travel to endemic countries, being an MSM, having multiple sexual partners or positivity in an orthopoxvirus-specific PCR without demonstration that it is MPXV. Case definitions for possible, probable and confirmed MPX published by UKHSA are comparable,[2] with probable cases defined as "a person with an unexplained rash on any part of their body plus one or more classical symptom or symptoms of monkeypox infection since March 15, 2022 and either: has an epidemiological link to a confirmed or probable case of monkeypox in the 21 days before symptom onset; or reported a travel history to west or central Africa in the 21 days before symptom onset; or is a gay, bisexual or other man who has sex with men." The "probable" case definition clearly lacks sensitivity and many laboratory-confirmed cases of MPX would fall outside this description particularly with increasing transmission within the community.[38]

While nucleic acid tests (NATs) for MPXV DNA provide a definitive diagnosis of MPXV infection, there are currently no commercially available MPXV molecular assays in Europe although the GenXpert PCR-based assay has been widely used in Africa.[39] Testing in Europe currently requires a laboratory to perform in-house PCRs and access to positive and negative control materials for assay validation. Alternative methods such as electron-microscopy, isolation and tissue immunocytochemistry for MPXV virus detection are less sensitive[40] and impractical for large-scale routine diagnostic purposes. Networks of collaborating laboratories for screening and reference testing for MPXV are established in many European countries and by UKHSA, but testing is currently on far too small a scale to enable routine diagnosis of infection and operates at a fraction of the scale required to monitor transfusion and transplant safety (discussed below). Limited testing capacity, and difficulties with clinical ascertainment of cases makes substantial underestimation of the scale of the current outbreaks in Africa and Europe more than likely.

5 | EMERGENCE OF MPXV IN THE UK AND EUROPE

A travel-related infection (from Lagos, Nigeria) was reported by UKHSA on the 7th May 2022, but six subsequent confirmed cases and one probable case were reported on the 13th–15th May in individuals without travel histories to Africa and no evident links to imported cases.[41] Four of these were MSMs as were a further two diagnosed on the 18th May, and a further 11 on the 20th May. At time of writing (10th July, 2022) the latest available UK total stands at 1552 confirmed cases[1] (Figure 1). Reporting from elsewhere in Europe between the in early July described 4913 cases, primarily in GBMSMs. Rapidly increasing numbers of cases have also been reported in in Canada and the US (1125 cases on the 8th July). MPXV infections remain strongly associated with GBMSMs with shared risk factors for HIV-1 infections and other sexually transmitted infections (STIs). An enhanced surveillance questionnaire by UKHSA[1] identified 96% of reported MPX cases in the UK to be in GBMSMs, 54% with a history of STI in the previous year and use of pre-exposure prophylaxis for HIV-1 in nearly 80% of those uninfected with HIV-1. A comparison of the epidemiological and clinical features of pre- and post-outbreak MPXV infections is summarised in Table 1.

All MPXV strains genetically characterised in the current outbreak belong to clade 3, part of the "West African" clade found in Cameroon and Sierra Leone.[42] Importantly, this clade shows a lower (<1%) case-fatality ratio (CFR) than clade 1 viruses found in Central

TABLE 1 Reported cases of MPXV infections associated with the current outbreak

	Pre-outbreak MPX	Outbreak MPX
Source	Suspected wide distribution in rodents in sub-Saharan Africa	Unknown
Human Infections	Sporadic infections in Central / West Africa, travel-related introductions into Europe, USA and elsewhere	Infections primarily among GBMSMs in UK, elsewhere in Europe and North America
Transmission	Inefficient between humans; infections primarily zoonotic	Sustained chains of human-to-human transmission by close or sexual contact
Disease presentations	Prodromal systemic symptoms, including lymphadenopathy, pyrexia, myalgia. Centrifugal maculopapular rash proceeding to vesicle formation	Prodromal systemic symptoms common, perianal or rectal lesions often resembling other STIs.
Morbidity and Mortality	High—10-15% mortality in cases in Central Africa (clade 1 MPXV); around 1% mortality for clades 2, 3, (West Africa)	Low—10% hospitalisation rate primarily for symptom control. No MPX-associated deaths recorded in Europe.
Demographics	All ages, male, female	Primarily males; age range 20–55
Risk factors for infection	Travel from endemic area (Central, West Africa), contact with infected rodents	Close or sexual contact with GBMSMs; shared risk factors for HIV-1 infection
Virus genetics	MPXV strains genetically diverse—Clade 1 (central Africa), clades 2, 3 (West Africa)	Minimal genetic variability—point source for all cases to date; multiple potential APOBEC related mutations.

Africa with a CFR of 10% or greater.[43,44] Consistent with their genetic affiliation, infections of MPXV in the current outbreak have been mild, with no deaths from MPXV infection in the UK and 3 from 6027 cases reported worldwide from the current outbreak.[1] Approximately 10% of UK cases have been hospitalised although some instances are for containment reasons.

Notwithstanding the difficulties in diagnosis and likely substantial under-reporting of cases, the current global MPXV outbreak is unprecedented. Its spread may be facilitated by greater travel in the COVID-19 post-pandemic period and through a growing reduction in population immunity following the cessation of vaccinia vaccination for smallpox. Alternatively, there may be genetic adaptations among currently spreading strains of MPXV that facilitate its human-to-human spread. The latter hypothesis is supported by current (and still preliminary and incomplete) genetic analysis of currently sequenced strains of MPXV. While the estimated substitution rate of the related vaccinia virus is of the order of 1–2 mutations across a genome of over 150 000 base pairs per year, current outbreak strains are genetically highly homogeneous, consistent with a very recent point source origin for UK strains and subsequent spread into other European countries and North America.[42,45]

Intriguingly, outbreak associated MPXV sequences also contain a large number of C->U or G->A mutations,[42,45] thought to be introduced by the action of nucleic acid editing proteins (APOBECs) on positive or negative DNA strands of the MPXV genome. This cellular defence mechanism reduces the replication of susceptible viruses through the introduction of mutations into the virus genome during its replication.[46] Such mutational biases are not typically observed among MPXV strains infecting rodents, other mammals or indeed human infection directly derived from reservoir sources and it appears that they may originate early in human-to-human transmission chains before the reported emergence of MPXV. Its adaptation for efficient human-to-human transmission may ultimately require the evolution of more effective defences against this and potentially many other host-specific defence pathways. Given the scale of the current outbreak and the rapid increase in reported cases in the UK and elsewhere (Figure 1), perhaps this has already been achieved.

6 | EVALUATION OF THE RISK OF TRANSFUSION- AND TRANSPLANT-ASSOCIATED TRANSMISSION

The risk of transmission of MPXV by substances of human origin (SOHO) for recipients in the EU/EEA area has been evaluated by the European Center for Disease Control,[3,47] While it recognises that monkeypox virus is likely to be transmissible through SOHOs, the overall risk is estimated to be low, for reasons of a historical absence of documented transmission of this virus from such sources, uncertainty about the duration of viremia and a lack of data on the duration and viral loads of MPXV in asymptomatic patients. In the UK, the UK Standing Advisory Committee on Transfusion Transmitted Infections (SACTTI) has produced a position statement on MPXV infection in donors and its potential transmissibility. The Joint United Kingdom (UK) Blood Transfusion and Tissue Transplantation Services Professional Advisory Committee (JPAC) have subsequently issued guidance on donor selection, specifically that donors with recently diagnosed MPXV infections cannot donate within 28 days nor can close contacts of individuals with MPXV infection within the last 21 days.[48] Questions to ascertain MPXV exposure have been added to donor interview in the UK and elsewhere.

Our brief review of available information about the current MPXV outbreak and has highlighted several areas to be considered in an assessment of the possibility of transfusion and transplant-associated transmission of the virus from donors with undiagnosed MPXV infections. Reduction of virus transmission risk relies on donor selection to

avoid high-risk sexual or other behaviours associated with HIV and other blood-borne infections and through highly sensitive screening for defined transfusion-transmissible pathogens (including HIV, hepatitis B, C and E viruses). In the absence of specific screening for MPXV, the most important factors contributing to likelihood of transmission are therefore the frequency of actively infected donors, the amount of infectious virus in blood or organs, retained virus infectivity during component manufacture and storage, the overlap of risk behaviours in donors associated with MPXV infections with those of other pathogens and the susceptibility of blood, platelet and transplant donations to severe MPXV-induced disease.

Information on these variables is currently incomplete and often speculative. However, several factors contribute to the authors' concern about the risk that MPXV poses for transfusion and transplant safety:

a. While the current number of report of MPXV infections is relatively low, they are rapidly increasing at the time of writing[1,49] and showing a maintained doubling time of around 2 weeks in the UK and elsewhere (Figure 1). Given the lag between infection, diagnosis and case reporting, the frequency of active infections in the wider community in the UK and elsewhere in Europe may be considerably higher than currently reported.
b. Current investigations of infection outcomes of MPXV are only described for those presenting with symptomatic infection (and then only in the latter stages of the infection cycle). The extent of virus replication and potential transmissibility in prodromal stages of infection or those with asymptomatic or undiagnosed infections,[50] collectively representing those most likely to donate, are unknown. For many other viruses such as HIV-1, hepatitis B, and C viruses, however, viral loads are highest before symptom or disease onset while even those with asymptomatic infections may display prolonged viraemia throughout what is a relatively long period of virus replication (up to 30 days). Combined, these factors suggest that that blood component and organ donations from donors with entirely inapparent MPXV infections may nevertheless be viraemic.
c. What dose of MPXV might be infectious by transfusion or transplant is unknown. For example, MPXV virions may be immune-complexed and potentially neutralised by anti-MPXV antibodies in blood or tissues, although this might typically reduce infectivity only in later stages of infection post-seroconversion for antibodies. As described, donors are more likely to donate when in an early prodromal stage of infection where viraemia levels may be actually higher than recorded in those with symptomatic infections (see above).
d. Under the recently introduced FAIR guidelines for donor selection, those who disclose performing anal sex within the previous 3 months with a new or multiple partners cannot donate, and this would therefore exclude many individuals currently identified in these MPXV outbreak investigations in the UK. A travel history to Central or West Africa would similarly defer donors for four or more months from donating. While these donor selection criteria will substantially reduce transmission risk, ongoing spread of MPXV outside the currently identified main risk groups for infection may hamper the future identification of risk factors for infection (as encapsulated by the likely relatively insensitive criteria used by UKHSA and ECDC to detect "possible" or "probable" infections). These include men who do not identify as MSM as they are less likely to be in contact with genitourinary medicine services.
e. If MPXV adapts to humans and become more transmissible, it may then become endemic; current associations with defined risk behaviours will progressively weaken making identification of infected donors problematic and increasingly non-specific. Based on current data, MPXV may already be substantially adapted for human-to-human transmission, in which case, the current case numbers will be largely underestimated.
f. Although some MPXV strains in Central Africa show case fatality rates of greater than 10%,[44,51] infections in the UK and elsewhere associated with the current outbreak have been so far typically mild and spontaneously resolving, with no MPX-associated deaths to date and a low hospitalisation rate.[1] There is, however, a likelihood of much more severe disease outcomes in recipients of blood components and organ transplant. Firstly current data indicates greater disease severity in individuals infected through percutaneous routes.[37] Secondly, blood component and transplant recipients are likely to be generally much more susceptible to severe disease from immunosuppression and severe intercurrent disease.

NHSBT and other UK blood services subscribe to the ABO Risk-Based Decision-Making Framework for Blood Safety and recognise that blood transfusion cannot be zero-risk. In this context, broader strategies that inform the Department of Health and Social Security are provided by the Advisory Committee on the Safety of Blood, Tissues and Organs for blood services in the UK. However, we believe that the currently unquantifiable but likely real risk of MPXV transmission and the greater susceptibility of infections in blood and transplant recipients should prompt further urgent consideration. An important element in evaluating risk could be establishing a mechanism to rapidly determine viraemia frequencies in donors. This would provide much more direct data on transmission risk that would complement evaluations of projected infection incidences, risk behaviour and donor selection criteria that are essential parts of current risk assessments.

7 | STRATEGIES FOR PREVENTION OF TRANSMISSION OF EMERGING PATHOGENS

We consider that a second testing channel within the blood services premises that can be activated at short notice for testing for additional agents (in this case MPXV) would be highly advantageous and be of major longer term strategic value for blood safety. Activation of such testing might be triggered through initial unlinked anonymised testing of several tens of thousands of donation samples to establish viraemia frequencies. The data might then justify taking no action

with reassurance, implementing selective screening or alternatively urgent adoption of universal screening and exclusion of donations positive for the new agent. The rapid identification, recall and clinical assessment of donors identified in this way would be of medical value to those identified, and have substantial importance for national surveillance, not least in providing the means to identify donors most at risk for infection.

Identification of infected individuals would furthermore allow investigation of secondary transmission, wider contact tracing and ultimately prevention. Establishing a second channel testing capability within the blood services infrastructure would have to resourced nationally although it would be assisted considerably by recently advances in automated extraction methods, robotics for PCR setup and associated laboratory information management systems. Putting this into a wider context, such testing would be dwarfed in scale compared to the testing implemented for SARS-CoV-2 in UK government testing laboratories during the COVID-19 pandemic. Even universal screening of blood and platelet donors in England would entail testing of five to six hundred pooled samples per week compared to up to 100 000 samples per day for SARS-CoV-2.

While the extent of MPXV emergence is currently limited and may ultimately subside, the expansion of the testing infrastructure so described would function as an important element in pandemic preparedness; SARS-CoV-2 was not in the end a blood-borne pathogen but the next one may be and an inability to implement a short-term response testing framework may have severe consequences for blood safety in the future.

AUTHOR CONTRIBUTIONS

PS and HH conceived the reveiw and both contributed to the literature review, writing, review and journal submission.

ACKNOWLEDGEMENT

This work was funded in part by a National Institutes for Health Research grant to PS and HH (NIHR203338). We are grateful to Professor Su Brailsford for useful comments and discussion.

CONFLICT OF INTEREST

The authors declare no competing interests.

ORCID

Heli Harvala https://orcid.org/0000-0001-9154-4190
Peter Simmonds https://orcid.org/0000-0002-7964-4700

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How to cite this article: Harvala H, Simmonds P. Evaluating the risk of transfusion and transplant-transmitted monkeypox infections. *Transfusion Medicine*. 2022;32(6):460-466. doi:10.1111/tme.12918

Received: 4 February 2022 | Revised: 6 September 2022 | Accepted: 29 September 2022

DOI: 10.1111/tme.12925

CONFERENCE REPORT

TRANSFUSION MEDICINE WILEY

Congress report: Online workshop on assessment of technical files for blood screening in vitro diagnostics for sub-Sahara African countries

Herbert A. Mbunkah[1,2] | Chancelar Kafere[1] | Washington Samukange[1] | Micha Nübling[1] | Jens Reinhardt[3]

[1]Division G3 – International Coordination, Regulatory Service, Paul-Ehrlich-Institut, Langen, Germany

[2]Department of Biological Sciences, Faculty of Science, University of Bamenda, Bambili, Cameroon

[3]Division of Haematology and Transfusion Medicine, Paul-Ehrlich-Institut, Langen, Germany

Correspondence
Jens Reinhardt, Division of Haematology and Transfusion Medicine, Paul-Ehrlich-Institut, Paul-Ehrlich-Str. 51-59, Langen 63225, Germany.
Email: jens.reinhardt@pei.de

Funding information
Bundesministerium für Gesundheit

Abstract

Objectives: The online workshop on IVD regulation was performed to broaden the understanding of the technical documentation needed for IVD licensing and the strategies to asses it.

Background: Testing of blood donors and donations significantly reduces the risk of transmitting transfusion-transmissible infections. Many test systems are commercially available, but not all meet the recommended sensitivity and specificity standards. Many African countries either lack functional structures for the regulation of IVDs this poses a threat to the quality of the blood supply.

Materialsand Methods: The Paul-Ehrlich-Institut BloodTrain organised an online workshop in September 2021 to introduce staff from several National Regulatory Authorities (NRAs) in Africa to the regulation of IVD and the technical information that need to be provided by the manufacturers of blood screening IVD. Their evaluation was trained in practical exercises.

Results: This online workshop brought together over hundred participants from NRAs of 12 African countries. Speakers from PEI, Blood Train, WHO and academia, with experience in IVD regulation trained participants in the various topics addressed during this workshop.

Conclusions: This workshop presented a great starting point for most participating NRAs to set up and/or strengthen their regulatory structures for IVDs.

KEYWORDS
blood safety, blood transfusion, in vitro diagnostics, online workshop, technical requirements

1 | INTRODUCTION

Suitable testing of all blood donations is critical for the provision of safe blood for transfusion. Screening donated blood for transfusion-transmissible infections (TTIs) such as HIV, HBV, HCV and syphilis significantly reduces the risk of transmission of these infections to recipients to very low levels. Similarly, blood group testing such as ABO and Rh typing and screening for red cell antibodies ensures transfusion of compatible blood, hence improves safety. Different systems, techniques and equipment are available for both TTI testing and blood group serology ranging from manual, semi-automated to fully automated. To guarantee a safe and quality supply of blood and blood products in any country, there is need for thorough regulation of these testing systems by National Regulatory Authorities (NRAs). In

the European Union (EU) for instance, in vitro diagnostics (IVDs) like those for blood screening belonging to higher risk categories undergo a conformity assessment procedure by a Notified Body that audits the quality management system and assesses the technical documentation of the device.[1] Other requirements are confirmed during performance evaluation of the device. Only then can a European certificate (CE mark) be issued to confirm compliance with the EU requirements. The WHO though not a regulatory body, assists her Member States with prequalification assessments for medicinal products including IVDs.[1]

The Global Health Protection Programme (GHPP) was launched by the German Ministry of Health to contribute to international initiatives to strengthen infrastructure of health systems in low and middle income countries.[2] One of the projects of the GHPP being implemented by the Paul-Ehrlich-Institut (PEI) is the BloodTrain, which is aimed at ensuring availability, safety and quality of blood and blood products in Africa, through strengthening of capacities of national regulatory systems for blood.[3] Regulatory oversight of associated substances and medical devices including IVDs used in blood product preparation processes is one of the key regulatory functions for which capacity building is essential.

Although many test systems for the detection of infectious diseases are also commercially available in Africa, not all meet the recommended sensitivity and specificity standards for the reliable detection of TTIs. By virtue of them having a significant impact on the safety, quality and efficacy of blood and blood products, associated substances and medical devices including IVDs need to be thoroughly regulated. In 2012 a survey of East African Community partner states found that regulation of IVDs was a neglected area among member states.[4] In most of the states, a legal framework for regulating medical products was present. However, their capacity to regulate IVDs was limited. A benchmarking exercise conducted by the PEI BloodTrain in 2018[5] revealed that only three out of ten benchmarked NRAs in Africa have established functional structures for the regulation of medical devices and IVDs. In a 2020 survey (unpublished data) involving NRAs in Africa, conducted by the African Medical Devices Forum, only approximately 30% of participating NRAs had implemented basic level controls for the regulation of IVDs as prescribed in the WHO Global Model Regulatory Framework for Medical Devices including IVD medical devices.[6]

An IVD technical file embodies all the information that is held by a manufacturer in relation to a particular IVD product. These are detailed under the different subheadings of the technical file (see Box 1). The documentation is normally an output of the manufacturer's quality management system, and includes information generated throughout the design, development, production and monitoring phases of the IVD device.[7] Conformity to the essential principles of safety and performance for an IVD device is often demonstrated in various sections of the technical dossier. Assessment of a technical file for an IVD device is one of the crucial regulatory functions that every sound medical device regulatory body must perform before approving the device for use in the country. Consequently, this workshop presented a starting point for the participating NRAs to set up and/or strengthen their regulatory structures for IVD assessment and licensing. The workshop was designed to address all subheadings of the technical file to give the participants a good introduction of the content of the dossier that will need to be assessed.

BOX 1 Content of the technical documentation for in vitro diagnostics/medical devices

The technical documentation for IVDs/MDs

- General and administrative information
- Device description
- Design and manufacturing information
- Quality management system
- Commercial and regulatory history
- Essential principles of safety and performance, and evidence of conformity
- Risk management
- Product performance evaluation: analytical and diagnostic studies
- Stability and robustness
- Labels and instructions for use
- Planned post-marketing surveillance

Due to the Covid-19 pandemic, this meeting had to be convened as a virtual training. This allowed the BloodTrain increase the number of participants to also include those from additional African countries that are presently not partner countries, where the BloodTrain activities usually focus.

2 | THE WORKSHOP AND PARTICIPANTS

This online workshop organised by the PEI-GHPP BloodTrain from 27–30 September 2021 brought together over a hundred participants—mostly regulators of medical devices and IVDs from NRAs of 12 African countries. These countries included Ethiopia, Ghana, Kenya, Liberia, Malawi, Nigeria, Rwanda, South Africa, Tanzania, Uganda, Zambia and Zimbabwe. The exact number cannot be evaluated, since some participants did not log onto the system and some used the system together with only one log-in. Five of these countries (Ghana, Nigeria, Tanzania, Zambia and Zimbabwe) are BloodTrain partner countries while the rest are countries where benchmarking of blood regulatory systems was previously conducted by the BloodTrain. A variety of speakers from PEI, BloodTrain, WHO and academia, with experience in IVD regulatory matters were invited to school participants in the various topics addressed during this 4-day workshop (see Appendix for the programme).

3 | WORKSHOP OBJECTIVES AND PROGRAMME

The overall objective of this workshop was to establish and strengthen competencies in reviewing and assessing blood screening IVD technical files in the participating countries' NRAs. Specifically, we sought to:

- Familiarise participants with the fundamental principles of regulating IVDs,
- Develop and improve skills and competences in IVD technical file assessment,
- Share best practices in the regulation of blood screening IVDs,
- Discuss reliance as a practice in the regulation of IVDs with related existing platforms and systems for implementation,
- Familiarise participants with emergency use authorization/approval procedures for diagnostics.

Since the workshop was held virtually, it was decided to limit each day to a maximum of 3 h. From earlier experience, longer virtual meetings are less effective, as it is challenging for the participants to stay focused. The 4 days were thematically divided into a general part, where the role of IVDs and the elements of their successful regulation were presented (day 1), a part in which the technical file and its assessment was discussed in detail (days 2 and 3), and a last part in which experts from WHO explained their approaches to support the global availability of IVDs (day 4).

3.1 | Day 1—Overview of regulation for blood screening IVDs

The workshop started with a brief welcome and introductory note from Dr. Jens Reinhardt (deputy project lead of the BloodTrain) on the background and mission of the BloodTrain and the objectives of the workshop. Dr. Micha Nübling from PEI proceeded with a detailed overview of the state-of-the-art of IVD and their role of diagnostics in the blood supply. He pointed out that the prevention of infections as a hallmark of blood safety is achieved by multiple strategies, including the physical donor examination, the donor questionnaire and the testing of each donation for markers of infection. He also presented the history of blood testing and the correlated evolution of molecular testing methods. Starting in the 1970s, where the liver marker ALAT was used to exclude donors with a high probability of hepatitis B and C, to nowadays available multiplex PCR testing. This talk also brought to light the different levels of sensitivity of test systems and addressed the aspect, which level of sensitivity can be accepted by regulators. Dr Nübling also discussed regional differences in virus genotypes, which is also relevant for the selectivity of the assay and needs to be taken into account when deciding about the utility of an IVD kit in a specific area. By pointing towards the guidance documents and standard preparations that WHO provides, he concluded his presentation.

Ms. Agnes Kijo from WHO gave the next talk on the Elements of Regulatory Systems for Diagnostics based on the WHO Global Model Regulatory Framework for medical devices including IVDs. This framework document was published in 2017 and provides strategies to establish a regulatory control system starting from a basic level and expanding it. This talk created awareness on the basic level controls from pre-market to post-market, which countries in the early stages of regulating IVDs must strive to enforce. Enabling conditions for effective regulation of medical devices and IVDs were also discussed.

3.2 | A snapshot of IVD regulation in different African countries

An overview of the IVD regulatory situation in four African countries was presented by Ms. Jeniva Rugaiza from TMDA, Mr. Frank Laban from ZAMRA, Dr. Edwin Nkansah from the Ghana FDA and Mr. Aina Olugbenga Stephen from NAFDAC. These NRAs have enforced some of the basic level regulatory controls from pre-market to post-market as recommended in the WHO Global Model Regulatory Framework for Medical Devices including IVDs. In all countries, the responsibility to control IVDs is defined by law and the NRAs are the responsible organisation tasked with this duty. The speakers presented the recent developments and the current challenges within the IVD regulation.

The day ended with a polling exercise led by Mr. Washington Samukange to gather feedback from participants. This exercise started with an introduction into the concept of WHO Global Benchmarking Tool Plus (GBT+) Blood, which allows to perform a globally standardised self-assessment of NRAs and to identify gaps in their capability to stringently regulate medicines including blood and blood products. While at that time, medical device regulation was not implemented into the GBT+ Blood, the IVDs for blood screening are already in the focus of the tool. Subsequently, an anonymous online WebEx poll for all participants to indicate their level of implementation of IVD regulatory controls in their countries was performed.

3.3 | Day 2—Assessment of the IVD technical file: Part 1

The second and the third day covered most of the subheadings of the technical file (see Box). Day 2 focused on a detailed understanding of the content of the technical file and approaches to the evaluation of the content. After a summary talk by Dr. Herbert Mbunkah (BloodTrain) about the composition of a typical IVD dossier, shortly summarising all subheadings, a more detailed insight into the subheadings were provided in the following talks. First Prof. Willy Urassa, an independent IVD consultant, trained participants on the *Essential Principles of Safety and Performance* for medical devices and IVDs. The presentation pointed to the conformity assessment that has to be provided by the manufacturer to prove to regulators that the IVD (or any other medical device) is safe and performs as intended. This conformity assessment uses systematic examination of the IVD to generate scientific evidence of the performance, for example the sensitivity and selectivity of the assay, the stability of the components and the robustness in the hand of the end user. It was further explained, how the risk management approach should be described in the technical file. This presentation helped to understand what kind of information need to be assessed and also pointed to some international standards for it, for example, those from the International Organisation for Standardisation (ISO) and the International Medical Devise Regulators Forum.

Dr. Mbunkah then discussed the *Design and Manufacturing Information* for an IVD that is required for assessment by the regulator.

The design and/or manufacture includes all aspects from specification development, production, fabrication, assembly, processing, packaging, repackaging, labelling, relabelling, sterilisation, installation or remanufacturing of a medical/IVD device.[8] All the information must comply with the aforementioned Essential Principles of Safety and Performance on one hand, and with the current Good Manufacturing Practices and the Quality System Regulation on the other. The manufacturer of the IVD needs to provide sufficient data on all these aspects. The presentation gave a detailed view on which information needs to be included under the different headers. The way to assess this information by the NRA was exemplified by the relevant parts of the checklist developed and routinely used by the WHO-Prequalification Team, as discussed by Dr. Mbunkah. Dr. Joey Gouws (WHO Prequalification Programme) presented a holistic approach to addressing pertinent issues pertaining to the assessment of the *Quality Management System* for IVD products. This talk also highlighted the work of inspectorates during the IVD licensing process exemplified by the work of the WHO inspectorate during the process of Prequalification of IVDs. It also gave insights into the areas where non-compliance deficiencies are most commonly found during inspections.

Dr. Nübling concluded the session with a presentation on *Risk Management* for IVD manufacturers. Starting from a standardised definition of risk according to ISO EN 14971, whereby risk is the probability of occurrence of harm combined with its severity, the manufacturer has to deal systematically with the risk of the individual IVD and provide this risk management as part of the quality management system as laid down in ISO 13485. This talk detailed the different steps of the risk management process, and explained the Failure Mode and Effects Analysis as one possible tool of risk management. The day ended with a question and answer session, allowing the participants to clarify some outstanding issues.

3.4 | Day 3—Assessment of the IVD technical file: Part 2

This day started with *Product Performance Evaluations* and associated validation and verification studies for an IVD product. Two kinds of product performance studies are needed for the characterisation if IVDs, the analytical performance studies, which evaluate the ability of an IVD to detect or measure a particular analyte, that is a biomarker of interest, like a viral antigen; and the clinical performance studies, presented in the first talk by Prof. Rosanna Peeling (London School of Hygiene and Tropical Medicine) in the talk on evaluating clinical performances of diagnostics for blood screening. The presentation explained the relevance of donor screening to ensure a safe blood supply and then laid out how to evaluate the clinical performance of diagnostics for blood screening. *Clinical Performance Evaluation* is defined as a set of ongoing activities that use scientifically sound methods for the assessment and analysis of clinical data to verify the safety, clinical performance and/or effectiveness of the medical device when used as intended by the manufacturer.[9] The focus here was on the aspects of reference standards and blinding, the source of clinical samples, the sample size necessary to evaluate the performance, and data management and analysis as important building block for a good evaluation of the clinical performance.

The talk of Dr Heiner Scheiblauer (IVD Testing Laboratory at PEI) elaborated on *Analytical Performance Evaluation*, which analyses the ability of an IVD to detect or measure a particular analyte, that is a biomarker of interest, like a viral antigen. The analytical data are generated using well-characterised samples, for which the expected outcome is already known. This is in contrast to the aforementioned clinical performance studies that focus on the performance on clinical samples from patients for which the diagnostic tool is intended. By using these highly predictable samples, parameters like specimen type suitability, accuracy, trueness, traceability, precision, analytical sensitivity (limit of detection), analytical specificity (interferences and cross-reactivity), assay measuring range, validation of assay cut-off, robustness and high-dose hook effect can be studied to characterise the performance of the assay. All the parameters were presented in detail and their relevance for the evaluation of IVDs was explained.

Mr. Washington Samukange proceeded with Stability and Robustness studies for blood screening diagnostics. This talk focused on the review of stability data for IVD products, regulatory requirements for stability, basic elements for stability testing, as well as sample stability. Participants got an opportunity to see the key points to look out for when reviewing stability data, and what to look out for. More importantly, they got a tour of the reference documents they can use as part of their review process. The session was drawn to a close with a talk given by Mr. Chancelar Kafere of the BloodTrain on *Labelling and Instructions for Use* (IFU) requirements. It is important to point out here that medical device labelling comprises not only the label on the device or its box, but also the Instructions For Use, the Information intended for the User and/or Patient, and all other information that come with the device.[10] This presentation educated participants on the elements of medical device labelling, the available standards guiding labelling requirements and some general requirements for labels and the IFU. These requirements include, for example, the location of the information, the format and the required information on the label, as well as in the IFU. In general, the information provided should be readily understood by the intended user (professional or lay person) and be sufficiently comprehensive to allow the user a clear understanding, for what the IVD is intended, how long it can be used, how it is to be stored and so on. The presentation ended with a summary of common labelling non-conformances to provide the participants with some practical examples, for example an incomplete claim for the intended use or the exclusion of limitations associated with the IVD.

3.5 | Day 4—Global regulatory harmonisation for IVDs and WHO tools to facilitate access to IVDs

On this day the first talk from Ms. Anita Sands ended the series on the subheadings of the technical file by presenting *Post-Market Surveillance* of blood screening IVDs. This was based on the WHO's

guidance document for post-market surveillance and market surveillance of medical devices including IVDs.[11] The concept of planned Post-Market Surveillance allows the manufacturer of an IVD to learn about problems on the market in order to ameliorate the product, so that all potential problems with the product can be sufficiently addressed in a timely manner. Post-Marketing surveillance concepts always include information of the regulatory authorities to allow a re-evaluation of the benefit/risk analysis of the IVD in cases of for example, malfunctions or unforeseen side-effects. The presentation also detailed the roles of the user, the manufacturer and the regulator in the process of Post-Marketing surveillance and discussed the relevance of lot testing of IVDs on the market.

Subsequently, several presentations were made by colleagues from the WHO to edify participants on several IVD-related activities on-going at the WHO. Dr Ana Aceves Capri introduced participants to the WHO Model List of Essential IVDs—a policy document based on scientific evidence, consisting of a list of categories of essential IVD tests and recommended assay formats for those tests. The goal is to provide evidence-based guidance to countries for creating or updating their national essential IVD list and offer a reference to countries for prioritising the IVDs that should be available at different levels of the health-care system. Ms. Marie Valentin presented the WHO Good Reliance Practices (GRelP). This presentation informed on the principles of GRelP and buttressed the importance of international cooperation to ensure the safety, quality and efficacy/performance of locally used medical products. Reliance makes best use of available resources and expertise, avoiding duplication and concentrating regulatory efforts and resources where it is most needed.

Dr Ute Ströher talked about the WHO Emergency Use Listing (EUL) procedure, citing examples of IVD for Ebola, Zika and SARS-CoV-2. The EUL procedure was set up as an extraordinary process intended to provide guidance to interested United Nations procurement agencies and NRAs on the quality, safety and performance of IVDs. It is a risk-based approach to expedite the availability of IVDs needed in public health emergency situations. The EUL IVD assessment procedure was outlined in this presentation and includes assessment of a technical documentation relating to safety and performance (product information, product performance specifications and labelling), a review of manufacturing documentation and an on-site inspection based on ISO 13485, and finally an independent laboratory evaluation coordinated by the WHO. Ms. Agnes Kijo concluded the session with a presentation on the facilitated regulatory pathways for medical products and the WHO Collaborative Procedure (CRP). This talk emphasised on the need for globalisation in the regulation of medical products, facilitated pathways to 'transfer' regulatory information and knowledge from trusted sources to facilitate in-country approval, and how exactly the CRPs work. The example and lessons learned from a CRP that was used as a pilot to facilitate registration of IVDs in four NRAs (Ethiopia, Nigeria, Tanzania Mainland and Ghana) was presented. Following a very informative plenary discussion, Dr. Jens Reinhardt ended the workshop with closing remarks and a vote of thanks.

4 | WORKSHOP OUTCOMES AND OUTLOOK/CONCLUSION

The participants of the virtual workshop obtained a systematic training on the composition and principles for assessing IVD technical files for blood screening IVDs, following the structure of the dossier. This information can be easily adapted and expanded to include further IVDs with intended uses being not for blood screening. Participants also grasped current information on the general regulatory requirements for IVDs. An awareness on the best practices in IVD regulation was created. In addition, an overview of the current situation of IVD regulation in four countries was provided to allow participants to profit from the experiences gathered by the countries and the challenges facing the establishment of effective IVD regulation. Participants also took home a vivid understanding of how to implement reliance models for their specific country situations. The concept of EUL or authorization for diagnostics and other health products was also introduced to participating NRAs to better prepare them for emergencies. We strongly believe that all vital information obtained in this workshop will help to enable countries implanting a good foundation as they begin the basics of IVD regulation. In 2022, BloodTrain is planning several face-to-face on-site meetings with the individual partner NRAs in Africa, to instil the practicalities and technical skills involved with assessing dossiers by also including detailed training with case studies. This will help the assessors to be more effective in critically reviewing and assessing IVD technical files submitted to their respective jurisdictions. Feedback obtained from the workshop participants was overall very positive, with many of them testifying to the relevance and timeliness of such a workshop, and looking forward to more workshops on IVDs.

The online workshop rounded off smoothly with almost no technical hitches thanks to the e-learning platform of the PEI-GHPP projects through which the workshop was hosted.

ACKNOWLEDGEMENTS

The Global Health Protection Programme is supported by the Federal Ministry of Health on the basis of a decision by the German Bundestag.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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How to cite this article: Mbunkah HA, Kafere C, Samukange W, Nübling M, Reinhardt J. Congress report: Online workshop on assessment of technical files for blood screening in vitro diagnostics for sub-Sahara African countries. *Transfusion Medicine*. 2022;32(6):467-474. doi:10.1111/tme.12925

APPENDIX A

GHP Programme

Paul-Ehrlich-Institut

AGENDA

PEI-GHPP BloodTrain Online Workshop

Assessment of Technical Files for Blood Screening IVDs

(27 - 30 September 2021)

Time zone: CEST – Central European Summer Time

Time	Topic	Speaker
	DAY 1: 27 September 2021 *Chair: Dr. Mbunkah & C. Kafere*	
10:00 – 10:15	Welcome remarks	PEI/WHO/NEPAD/AMDF
10:15 – 10:30	Introduction and workshop objectives	J. Reinhardt
10:30 – 11:20	Role of diagnostics in blood supply: state-of-the-art diagnostics for blood screening	M. Nübling
11:20 – 11:50	Elements of Regulatory Systems for Diagnostics / GBT Medical Devices/Global regulatory framework for MDs	A. Kijo
11:50 – 12:20	IVD regulatory situation in TMDA, ZAMRA, FDA and NAFDAC	J. Rugaiza/D. Mwakyoma (TMDA), F. Laban (ZAMRA), E. Nkansah (FDA), O. Aina (NAFDAC)
12:20 – 12:50	Exercise: GBT+ Blood – Regulatory Oversight of associated substances and medical devices including in vitro diagnostics	W. Samukange
12:50 – 13:00	Discussion	All
	End of Day 1	
	DAY 2: 28 September 2021 *Chair: W. Samukange*	
10:00 – 10:30	The IVD Technical File (Dossier): summary of the technical documentation	H. Mbunkah
10:30 – 11:00	Essential Principles of Safety and Performance	W. Urassa
11:00 – 11:30	Design and Manufacturing Information	H. Mbunkah
11:30 – 12:00	Quality Management System Assessment	Joey Gouws
12:00 – 12:30	Risk Assessment: risk analysis and control	M. Nübling
12:30 – 13:00	Discussion	All
	End of Day 2	
	DAY 3: 29 September 2021 *Chair: Dr. Heinrich*	
10:00 – 10:45	Evaluation of Clinical Performance: Diagnostic Sensitivity and Diagnostic Specificity	R. Peeling
10:45 – 11:25	Analytical Studies: Analytical performance characteristics	H. Scheiblauer

APPENDIX A (Continued)

GHP: Programme

Paul-Ehrlich-Institut

11:25 – 12:05	Robustness and Stability studies: Robustness, Claimed shelf-life, In-use stability, Shipping stability	W. Samukange
12:05 – 12:35	Labeling and Instructions for Use (IFU)	C. Kafere
12:35 – 13:00	Discussion	All
	End of Day 3	
DAY 4: 30 September 2021 *Chair: Ms. J. Atemnkeng*		
10:00 – 10:25	Regulatory History and Planned Post-Market Surveillance Activities	A. Sands
10:25 – 10:50	WHO list of essential IVDs	A. Aceves
10:50 – 11:15	Reliance models (WHO Good reliance practices in regulatory decision-making for medical products)	M. Valentin
11:15 – 11:20	*Short Break*	
11:20 – 11:45	WHO Emergency Use Listing e.g. SARS-CoV-2 IVDs	U. Ströher
11:45 – 12:10	WHO Collaborative Procedure with country examples	A. Kijo
12:10 – 12:50	Discussion	All
12:50 - 13:00	Closing remarks and end of workshop	J. Reinhardt

Received: 21 February 2022 | Revised: 29 August 2022 | Accepted: 11 September 2022

DOI: 10.1111/tme.12919

TRANSFUSION MEDICINE WILEY

ORIGINAL ARTICLE

Splanchnic-cerebral oxygenation ratio associated with packed red blood cell transfusion in preterm infants

Kiran Kumar Balegar V[1] | Madhuka Jayawardhana[2] | Philip de Chazal[3] | Ralph Kay Heinrich Nanan[4]

[1]Department of Neonatology, Nepean Hospital, Sydney Medical School Nepean, The University of Sydney, Sydney, Australia

[2]School of Electrical Engineering, Charles Perkins Center, The University of Sydney, Sydney, Australia

[3]School of Biomedical Engineering, Charles Perkins Center, The University of Sydney, Sydney, Australia

[4]Charles Perkins Center Nepean, The University of Sydney, Sydney, Australia

Correspondence
Kiran Kumar Balegar V, Department of Neonatology, Nepean Hospital, Derby Street, Kingwood, NSW 2747, Australia.
Email: kiran.balegarv@health.nsw.gov.au

Funding information
Australian Women and Children's Research Foundation, Grant/Award Number: 12/191202

Abstract

Background: Splanchnic-cerebral oxygenation ratio (SCOR), the ratio of splanchnic tissue oxygen (StO_2s) to simultaneously measured cerebral tissue oxygen (StO_2c), has been described as a surrogate to detect impaired splanchnic oxygenation associated with hypoperfusion status such as necrotizing enterocolitis. This concept is based on the presumption that any change in SCOR indicates a corresponding change in splanchnic tissue oxygenation as the numerator, whereas cerebral tissue oxygenation as the denominator remains stable. However, it is questionable to utilise this concept to detect splanchnic oxygenation changes in the context of packed red blood cell transfusion (PRBCT).

Aim: The current study examines the contribution of both cerebral and splanchnic oxygenation components to PRBCT-associated SCOR changes in preterm infants.

Design: Prospective cohort study.

Setting: Neonatal intensive care.

Patients: Hemodynamically stable infants: Gestation <32 weeks; birth weight <1500 g; postmenstrual age <37 weeks: tolerating ≥120 ml/kg/day feed volume.

Interventions: PRBCT at 15 ml/kg, over 4 h.

Main Outcome Measures: Transfusion-associated changes were determined by performing mixed models for repeated measures analysis between the 4-h mean pre-transfusion values (SCOR 0, StO_2s 0, and StO_2c 0) and the post-transfusion hourly mean values for the next 28 h (SCOR 1–28, StO_2s 1–28, and StO_2c 1–28). Dunnett's method was used to adjust for the multiplicity of the *p* value.

Results: Of 30 enrolled infants 14 [46.7%] male; median [IQR] birth weight, 923 [655–1064] g; gestation, 26.4 [25.5–28.1] weeks; enrolment weight, 1549 [1113–1882] g; and postmenstrual age, 33.6 [32.4–35.0] weeks, one infant was excluded because of corrupted NIRS data. With the commencement of PRBCT, SCOR demonstrated a downward trend throughout the study period. This drift was associated with an increasing StO_2c trend, while StO_2s remained unchanged throughout the study period.

Conclusions and Relevance: PRBCT-associated SCOR decrease suggests improvement in cerebral oxygenation rather than worsening splanchnic oxygenation. Our study underlines that it is necessary to determine individual components of SCOR, namely cerebral and splanchnic StO_2 to understand SCOR changes in the context of PRBCT.

KEYWORDS
cerebral tissue oxygenation, necrotizing enterocolitis, neonate, near-infrared spectroscopy, packed red blood cell transfusion, preterm, splanchnic-cerebral oxygenation ratio, splanchnic tissue oxygenation

1 | INTRODUCTION AND OBJECTIVES

Cerebral autoregulation is well-established in adults.[1] Under hypotensive conditions, the blood supply is diverted from less essential organs, such as the gastrointestinal tract, to more essential organs, such as the brain. Autoregulation is also demonstrated, albeit less rigorously, in newborns.[2] The term splanchnic-cerebral oxygenation ratio (SCOR) has been coined to compare splanchnic with simultaneously measured cerebral tissue oxygen.[3] SCOR is computed using near-infrared spectroscopy[3–6] (NIRS), a continuous, noninvasive, and portable technique that measures oxygen saturation in the tissue bed, about 1–2 cm beneath the sensors placed on the abdomen/head, and displays it as regional tissue oxygen saturation values (StO_2).

The utility of SCOR is based on the principle that cerebral tissue oxygenation remains stable during hypoperfusion at the cost of splanchnic tissue oxygenation through hierarchical autoregulatory processes. While cerebral autoregulation maintains cerebral perfusion at a constant level despite changes in systemic perfusion, splanchnic perfusion changes much more readily in response to triggers such as feeds or stress.[7] A decrease in SCOR is, therefore, conceptualised as an indicator of splanchnic hypoperfusion. The studies that propose using SCOR have stated that comparing splanchnic to cerebral StO_2 can make splanchnic oxygenation changes more interpretable and inter- and intrapatient comparable.[8]

In a cohort of 40 infants, Fortune et al.[3] demonstrated that a decrease in SCOR had a 90% sensitivity to detect surgically proven splanchnic ischemia such as necrotizing enterocolitis (NEC). More recent studies, including meta-analysis, have evaluated low SCOR as a diagnostic tool for NEC in preterm infants and suggested that decreasing SCOR trends rather than specific cut-off values are more meaningful.[8–10] A recent systematic review[11] exploring normative values of regional tissue saturation measured by NIRS indicated that the published values lack quality or adequacy of sample size. It is, therefore, unclear if SCOR can be used for clinical decision-making.

The concept of decreasing SCOR in NEC has been extrapolated to other settings such as feeding[4,12] and intrauterine growth restriction[13] using SCOR as a general measure of splanchnic oxygenation. Similarly, a handful of studies[5,14,15] utilises SCOR to evaluate splanchnic tissue oxygen changes associated with packed red blood cell transfusion (PRBCT) in preterm infants. A more extended NIRS monitoring period, high sampling frequency, and short data averaging time have been recommended to overcome errors involved in SCOR measurement due to fluctuations in splanchnic oxygenation readings owing to peristaltic changes in the sampled tissue.[16–18] Unfortunately, the existing studies are fraught with episodic/shorter duration of measurements/lower sampling frequency/longer data averaging time.

This study aimed to examine the contribution of both cerebral and splanchnic tissue oxygenation components to SCOR changes associated with PRBCT in preterm infants.

2 | MATERIALS AND METHODS

This prospective observational study was conducted in the tertiary neonatal intensive care at Nepean Hospital, Sydney, Australia, from September 2014 to November 2016 and analysed between August 2017 and October 2018. Eligible participants included: gestation <32 weeks; birth weight <1500 g; postmenstrual age <37 weeks; tolerating enteral feed volume at least 120 ml/kg/day; hemodynamically stable and receiving elective PRBCT to treat anaemia of prematurity. Infants with NEC (current or previous); feed intolerance (defined as the treating clinical team's decision to withhold feeds/withhold grading up of feeds for at least 12 h); sepsis (defined as the treating clinical team's decision to commence antibiotics); PRBCT in the previous 72 h; PDA or its treatment with Ibuprofen/Indomethacin/surgery in the previous 72 h and/or congenital gastrointestinal, complex cardiac/lethal anomalies were excluded. An infant was enrolled only once, even when receiving multiple transfusions. For pragmatic reasons, enrolment occurred when PRBCT was only initiated during regular business hours (weekdays: 8:00 AM–5:00 PM). Consecutive babies satisfying the above criteria were enrolled after written informed parental consent by the chief investigator/one of the designated officers. The study protocol, including parental consent, was approved by the human research ethics committee, Nepean Blue Mountain Local Health Committee (Approval number: Study 12/67 - HREC/12/NEPEAN/148).

2.1 | Study protocol

Once a clinical decision was made for transfusion, the study period extended from 4 h before the PRBCT (15 ml/kg over 4 h, without Furosemide) to 24 h after its completion. The decision to give PRBCT

was made by the attending neonatologist independent of the study. No formal protocol existed regarding the Hb threshold at which infants received a PRBCT. As a general principle, PRBCT administration was considered when Hb was 100–120 g/L in critically unwell preterm infants on invasive ventilation/inotropes; 80–100 g/L in infants on noninvasive ventilation (CPAP/HFNC); <80 g/L for infants with no breathing support. An individual clinician could overrule this based on a given clinical scenario and by taking into account other clinical factors. The decision to transfuse was made based on a combination of clinical factors in addition to low Hb (e.g., respiratory support, increasing desaturations, poor growth, gestation, reticulocyte response). As per the unit protocol, feeds were not withheld during PRBCT. Babies on second hourly feeds received feeds via a syringe pump at a constant rate of 120 ml/h for the purpose of standardisation. Those who did not tolerate bolus feeds received continuous feeds. Nature and volume of feeding and total fluid intake remained unaltered during the entire study period. A detailed protocol has also been published elsewhere.[19–21]

2.2 | Determination of SCOR

Data including SpO_2, StO_2c, and StO_2s were prospectively recorded continuously. The data recording extended from at least 4 h before the beginning of PRBCT until 24 h after its completion. SpO_2 was used along with StO_2 to calculate fractional tissue oxygen extraction for other related publications from the same study.[19–21] SCOR (also called CSOR, cerebro-splanchnic oxygenation ratio[3]; MCOR, mesenteric-cerebral oxygenation ratio[15]) was calculated by a continuous prospective evaluation of StO_2c and StO_2s. StO_2 was evaluated using a four-wavelength NIRS cerebral and splanchnic tissue monitor (FORE-SIGHT® absolute cerebral oximeter, CASMED, Branford, Connecticut, United States). StO_2c was recorded using a neonatal sensor placed over the temporal region of the head. A second sensor was placed on the lower quadrant of the abdomen just below the umbilicus[4] to obtain simultaneous StO_2s. An increase in StO_2 represents an increase in oxygen level in the tissue bed located 1–2 cm beneath the sensor and vice versa. SCOR was calculated as a ratio of concurrently measured StO_2s/StO_2c. As cerebral StO_2 is hypothetically unchanged, any decrease in SCOR is perceived to indicate decreased splanchnic oxygenation.

Skin integrity was closely monitored by lifting the sensors and inspecting the skin every 6 h at the time of 'cares' (handling of neonates for a nappy change, eye care, change of posture, etc.). The start and end times of the study, as well as various events (transfusion, feeds, and cares), were electronically annotated in real-time. Other demographic parameters (Table 1) were also collected.

2.3 | Data processing

StO_2 and SpO_2 data were downloaded in real-time as an analog output at a sampling rate of 1000 Hz and aligned along the time axis in LabChart reader format (.adicht files) using a PowerLab data acquisition system (PowerLab®, ADInstruments, Sydney, Australia). The .adicht files were converted into .mat file format using a simple Python script (Python™ version 3.7.3) and resampled at 1 Hz for faster processing. Data that could not be physiologically explained (e.g., the absence of variability,[22] a 30% step change in StO_2 between two subsequent data points for StO_2[23]) were tagged. Data during the period of 'cares' were tagged as they were presumed to be artefactual for two reasons: NIRS sensors were lifted for inspection of underlying skin during this period; babies underwent a nappy change, oral care, change of position, and so forth during this period that would have caused significant movement artefacts. The tagged data were replaced with 'NaN' or 'Not a Number' which is recognised by Matlab (MATLAB 9.3, The MathWorks, Inc., Massachusetts, United States), and ignored for all subsequent processing while maintaining the correct time point of the StO_2 signals.

Average SCOR was derived from the real-time StO_2 data for each baby. Baseline SCOR during anaemia was determined by computing the mean pre-transfusion SCOR values over 4 h before the beginning of PRBCT (SCOR 0). To determine transfusion-associated changes, SCOR levels were binned into hourly means during the 4 h of the transfusion period (SCOR 1–4) and 24 h after the completion of PRBCT (SCOR 5–28). The hourly intervals were arbitrarily chosen a priori as pragmatic time points to capture SCOR changes along the time course after the commencement of PRBCT.

2.4 | Sample size

A pragmatic sample size of 30 was selected to facilitate a reasonable enrolment over 2 years based on the number of potential eligible infants typically admitted to the Neonatal Intensive Care Unit (NICU) and the transfusion rate.

2.5 | Statistical analysis

A mixed models for repeated measures (MMRM) analysis was applied to the oxygen kinetic data (StO_2C, StO_2s, and SCOR) from all 29 infants. Timepoint (i.e., baseline and post-transfusion hours 1–28) was fitted as a fixed effect, and the infant was fitted as a random effect. The MMRM was used to perform paired comparisons between the baseline pre-transfusion mean value and the post-baseline hourly mean values (e.g., SCOR 0 vs. each of SCOR 1–28). Due to their ability to consider both fixed and random effects, mixed models are ideally suited in this setting to assess the trajectory of the outcome. Unlike other methods, MMRM has an additional advantage of accommodating unbalanced data patterns due to random missing of data and is associated with lesser inflation of type 1 error.[24] Dunnett's method was used to adjust for multiplicity. All p values were two-sided, and a value less than 0.05 were considered statistically significant.

TABLE 1 Main clinical characteristics of studied patients ($N = 30$)

Characteristics	
Gestation (weeks)	
Mean (SD)	26.9 (1.9)
Median (IQR)	26.4 (25.5–28.1)
Range (Min–Max)	24–30.4
Birth weight, grams	
Mean (SD)	919 (298)
Median (IQR)	923 (655–1064)
Range (Min-Max)	507–1530
Male gender, *N* (%)	14 (46.7%)
Restricted umbilical arterial Doppler, *N* (%)	5 (16.7%)
Small for gestation, *N* (%)	6 (20%)
Enrolment characteristics	
Postmenstrual age, weeks	
Mean (SD)	33.2 (2.5)
Median (IQR)	33.6 (32.4–35)
Range (Min-Max)	26.1–36.6
Postnatal age, days	
Mean (SD)	44 (19)
Median (IQR)	43 (27–59)
Range (Min–Max)	10–80
Weight, grams	
Mean (SD)	1530 (456)
Median (IQR)	1549 (1113–1882)
Range (Min–Max)	714–2513
Breathing support, *N* (%)	
None	5 (16.7%)
Continuous positive airway pressure	15 (50%)
High flow nasal cannula	8 (26.7%)
Low flow oxygen	2 (6.7%)
Daily feed intake, ml/kg/day	
Mean (SD)	157 (11)
Median (IQR)	155 (150–160)
Range (Min–Max)	130–180
Formula milk, *N* (%)	10 (33.3%)
Caffeine, *N* (%)	25 (83.3%)
Patent ductus arteriosus, *N* (%)	0 (0%)
Transfusion characteristics	
Pre transfusion Hb, (g/L)	
Mean (SD)	93 (11)
Median (IQR)	93.5 (87.8–102.3)
Range (Min–Max)	68–109
Hb of packed red cells, (g/L)	
Mean (SD)	197 (13)
Median (IQR)	198 (189–203)
Range (Min–Max)	164–236
Age of packed red cell transfusion (days)	
Mean (SD)	14 (10)
Median (IQR)	13 (4–24)
Range (Min–Max)	2–31
Total number of previous transfusions	
Mean (SD)	3 (3)
Median (IQR)	2 (0–5)
Range (Min–Max)	0–8
Most recent transfusion (days)	
Mean (SD)	19 (11)
Median (IQR)	16 (9–30)
Range (Min–Max)	4–35
Necrotizing enterocolitis prior to discharge	None
Feed intolerance during the study period	None

3 | RESULTS

3.1 | Demographic features

Characteristics of potentially eligible, excluded and analysed babies are depicted in Figure 1. Of 91 infants who received PRBCT during the study period, 30 infants were enrolled in the study. Others did not meet the eligibility criteria for various reasons described in Figure 1. Twenty-nine out of thirty enrolled infants were included in the SCOR analysis, as one baby had corrupted NIRS data that could not be downloaded.

The demography of the study cohort is presented in Table 1. Median (IQR) gestation and postmenstrual ages were 26.4 (25.5–28.1) and 33.6 (32.4–35) weeks, respectively. Median (IQR) birth weight and weight at enrolment were 923 (655–1064) g and 1549 (1113–1882) g, respectively. Fourteen babies (46.7%) were males; 6 (20%) were small for gestation (SGA), and 5 (16.7%) had restricted umbilical artery flows on antenatal Doppler studies. At enrolment, five babies (16.7%) spontaneously ventilated in room air without any respiratory support; 2 (6.7%) needed low flow oxygen; 8 (26.7%) needed high flow nasal cannula, and 15 (50%) received continuous positive airway pressure support. None had PDA; 25 (83.3%) received caffeine, and 10 (33.3%) babies received formula milk during the study period. Median (IQR) daily feed intake was 155 (150–160) ml/kg/day; pre-transfusion Hb was 93.5 (87.8–102.3) g/L, and the age of the transfused blood pack was 13 (4–24) days. None of the enrolled infants developed feed intolerance or NEC within 72 h after PRBCT.

3.2 | Changes in SCOR, StO_2c, and StO_2s in association with PRBCT

Figure 2 shows the mean values of SCOR, StO_2c, and StO_2s during the entire study period and the trend lines. Each measurement

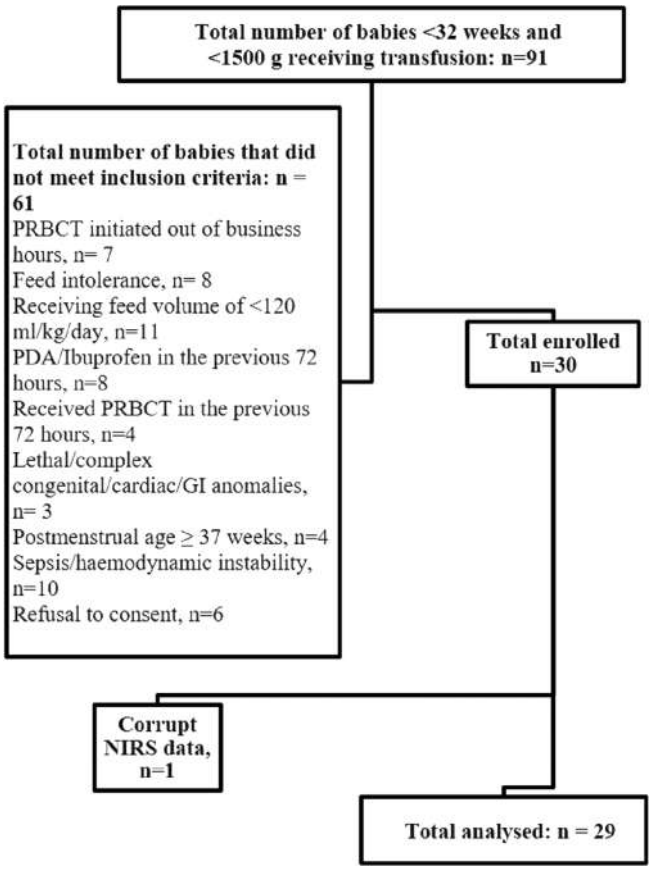

FIGURE 1 Flow diagram showing participants. Enrolled and excluded participants are shown in the flow diagram. GI, gastrointestinal; NIRS, near-infrared spectroscopy; PDA, patent ductus arteriosus; PRBCT, packed red blood cell transfusion

represents the average value obtained from all 29 patients. The measurement at time point 0 represents the baseline pre-transfusion value; time points 1–4 represent hourly values during the transfusion, and 5–28 represent hourly values after the completion of transfusion. Compared to the pre-transfusion value, post-transfusion SCOR showed a declining trend, reaching statistical significance at several time points. Evaluating the components of SCOR indicated that the post-transfusion StO_2c showed an increasing trend, reaching statistical significance at several time points. On the other hand, post-transfusion StO_2s remained unaltered with no definitive trend throughout the study period (Table 2, Figure 2).

4 | DISCUSSION

4.1 | PRBCT was associated with decreasing SCOR, with unchanged StO_2s numerators and increased StO_2c denominators

StO_2c showed a decreasing trend associated with PRBCT, reaching statistical significance at multiple points during the post-transfusion period. It is logical to assume that StO_2c increases due to an improvement in the oxygen content of the blood due to PRBCT. The ability of cerebral circulation to swiftly increase its tissue oxygen levels following PRBCT underscores its dynamic autoregulatory capability.[25,26] Studies in preterm and term infants, as well as adult patients, indicate that cerebral autoregulation in the setting of anaemia and transfusion is controlled by the oxygen content, viscosity of blood, and/or proportion of fetal haemoglobin.[25,27] The result of our study is in concordance with other studies.[25,26,28–30]

In contrast, our study showed that StO_2s surprisingly did not increase despite improvement in blood oxygen content from PRBCT. One physiologically plausible explanation is an impaired ability of splanchnic circulation to swiftly increase tissue oxygen levels following PRBCT, underscoring delayed splanchnic autoregulatory capabilities. The autoregulation of splanchnic blood flow is complex, controlled by intrinsic and neuro-humoral mechanisms aiming to buffer cerebral oxygenation.[31] Splanchnic tissue has a low oxygen consumption and tolerates large fluctuations in oxygen delivery, safeguarding cerebral oxygenation.[32]

We speculate that failure to improve splanchnic tissue oxygenation reflects this buffering mechanism, prioritising restoration of cerebral tissue oxygenation following PRBCT.

Our findings align with White et al.[33] who showed improvement in post-transfusion splanchnic oxygenation only after 36 h. Although we followed our cohort for 28 h from the commencement of PRBCT, it is reasonable to assume that splanchnic oxygenation may eventually improve beyond 28 h. Other studies demonstrated variable results, some showing improvement[15,30,34,35] and others showing deterioration.[36,37] Underlying methodological discrepancies characterised by episodic splanchnic NIRS monitoring for 20 min or less, with a duration as short as 15–60 min before PRBCT to 15–180 min after its completion, might explain the discrepancies.[30] Furthermore, the inclusion of unstable babies (e.g. septic)[30]; discrepancies in feeding status[15,30,34] might also account for discordant findings. Splanchnic oxygenation is known to have greater variability[6,16,18] most likely due to variable segments of the intestine interrogated, changing gas-fluid-faecal interfaces, momentary NIRS signal losses, and periods of very low readings resulting from peristalsis.[6,38] Differentiating real changes in tissue bed oxygenation from the variability due to these mechanical factors may be difficult with episodic and short duration of NIRS monitoring.[39] As a result, splanchnic tissue oxygenation is meaningful only if monitored continuously and over longer periods. Mintzer et al.[16] recommended utilising relatively short (5–15 min) epochs of data averaging intervals and trending these epochs over longer periods. Our splanchnic tissue oxygenation data was very robust as we sampled at the rate of 1/s, monitored continuously over 32 h, and used shorter data averaging periods. We excluded a mean 2.4% of the data (equivalent to about 46 min out of 32 h of data per baby) during the entire study period due to reasons including lifting of sensors and the data deemed to be physiologically unexplained (e.g., absence of variability).

This paper compares the hourly changes in splanchnic oxygenation in the context of PRBCT. All of our infants received feeds throughout the study period. As the babies were fed at different times

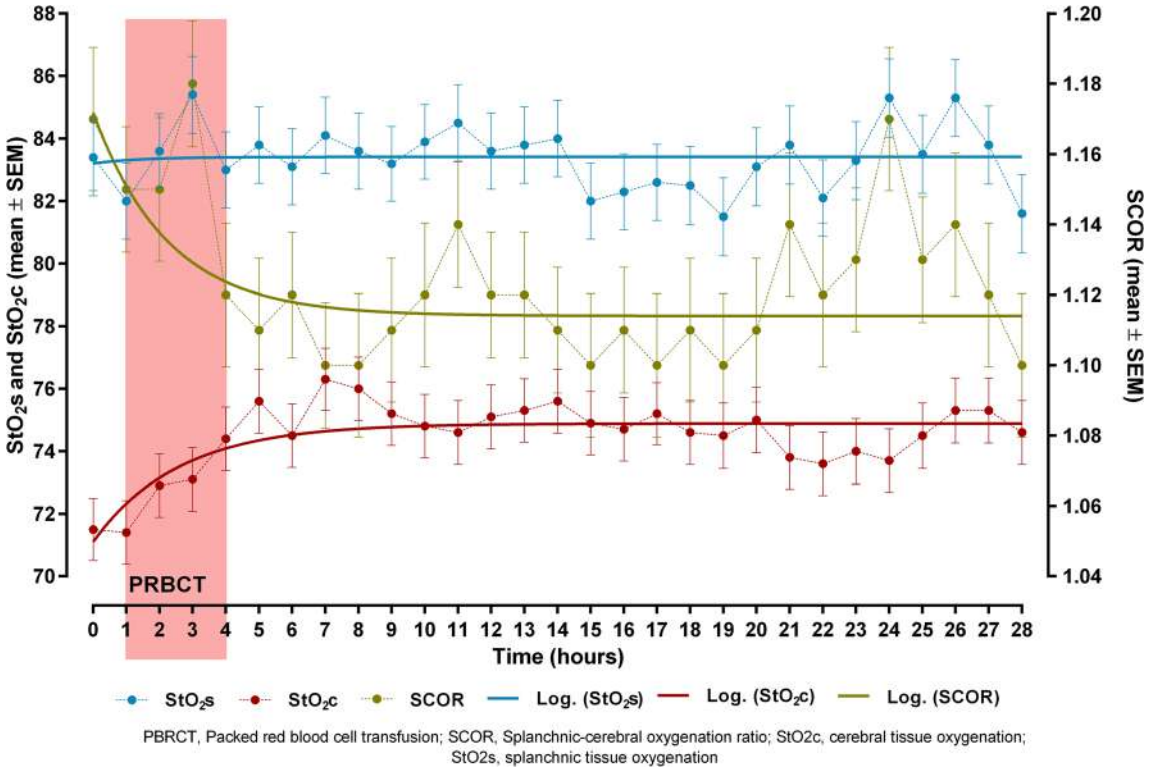

FIGURE 2 Transfusion-associated changes in SCOR, StO_2c, and StO_2s during the study period. PRBCT, packed red blood cell transfusion; SCOR, splanchnic-cerebral oxygenation ratio; StO_2c, cerebral tissue oxygenation; StO_2s, splanchnic tissue oxygenation

in relation to PRBCT, the feeding cycles did not necessarily coincide with the beginning and end of PRBCT. As a result, there was a variable and random overlap of hourly SCOR values with feed cycles. In other words, the hourly SCOR values possibly incorporated different components of the feeding cycle throughout the study period. Moreover, the frequency, volume, and nature of feeds remained unchanged before versus after PRBCT. Because of these reasons, our analysis very likely excludes the confounding effect of feeding on splanchnic oxygen values in relation to PRBCT. In a separate publication that includes 24 out of 29 infants from the current cohort, we have specifically analysed the response of splanchnic and cerebral oxygenation to feeding.[19]

SCOR mirrors the splanchnic oxygenation status associated with acute abdomen, feeding, and IUGR status.[3,4,12,13,40–43] based on the principle that cerebral StO_2 remains unchanged due to cerebral autoregulation at the cost of variable splanchnic StO_2. Accordingly, a decrease in SCOR indicates splanchnic ischemia (e.g., acute abdomen,[3] IUGR[13,40]), whereas an increase in SCOR suggests splanchnic hyperemia (e.g., post-feeds[4,12,41–43]) However, our findings suggested that in association with PRBCT, a decrease in SCOR suggests improvement in cerebral tissue oxygenation rather than deterioration in splanchnic tissue oxygenation. A few other studies evaluated SCOR changes associated with PRBCT[5,14,15,44,29] but differed from our results, most likely due to longer data averaging periods, episodic, or shorter duration of monitoring. The strength of our study is the continuous 32 h of SCOR monitoring at the high sampling frequency, rigorous methodology, shorter data averaging with voluminous data points, and robust statistical analysis.

We acknowledge several limitations. The study involved stable premature babies with postmenstrual age 33.6 (32.4–35.0) weeks, and as such, caution must be exercised in inferring similar findings in extremely premature infants, those with cardiorespiratory instability, term infants, or adults. Likewise, caution must be exercised in interpreting the clinical relevance of the statistical difference. This is especially in the background of the lack of normative values that allow us to define cerebral and splanchnic hypoxia.[11,45,46]

As this is an observational study involving PRBCT, it is not possible to distinguish between an increase in oxygenation secondary to PRBCT and the effect of the increase in volume. A randomised controlled study of PRBCT versus saline in such a setting is ethically not permissible. In addition, the effect of change in volume is unlikely to last for 28 h. Moreover, the effect should be reflected in both cerebral and splanchnic tissue. On the other hand, a rapid improvement in cerebral oxygenation probably underscores the autoregulatory capability of cerebral circulation to improve its oxygen levels after a PRBCT. Failure to improve splanchnic oxygenation in the immediate post-transfusion period may indicate a delay in triggering splanchnic autoregulation in an attempt to buffer cerebral oxygenation. Hence it is physiologically logical to assume that the changes reflect an increase in oxygenation rather than a volume change.

For pragmatic reasons, we chose to monitor for 24 h after the completion of PRBCT. However, it would have been interesting to

TABLE 2 Exploratory analysis of SCOR, StO_2c, and StO_2s in association with packed red blood cell transfusion—Mixed model for repeated measures ($n = 29$)

Label		Estimated difference from StO_2s 0 (95% CI)	*p* Value[a]	Estimated difference from StO_2c 0 (95% CI)	*p* Value[a]	Estimated difference from SCOR 0 (95% CI)	*p* Value[a]
Pre-PRBCT	0						
PRBCT	1	−1.4 (−5.3 to 2.5)	1.0	−0.1 (−2.7 to 2.6)	1.00	−0.02 (−0.08 to 0.04)	1.00
	2	0.2 (−3.7 to 4.1)	1.0	1.5 (−1.2 to 4.2)	0.72	−0.02 (−0.08 to 0.04)	1.00
	3	2.0 (−1.9 to 5.9)	0.8	1.6 (−1.1 to 4.3)	0.61	0.01 (−0.05 to 0.06)	1.00
	4	−0.4 (−4.3 to 3.5)	1.0	3.0 (0.3 to 5.6)	0.02	−0.05 (−0.11 to 0.01)	0.11
Post-PRBCT	5	0.4 (−3.5 to 4.3)	1.0	4.1 (1.4 to 6.8)	0.0001	−0.06 (−0.12 to 0.00)	0.049
	6	−0.3 (−4.2 to 3.6)	1.0	3.1 (0.4 to 5.7)	0.01	−0.05 (−0.11 to 0.00)	0.10
	7	0.7 (−3.2 to 4.6)	1.0	4.9 (2.2 to 7.6)	<0.0001	−0.07 (−0.13 to −0.01)	0.01
	8	0.2 (−3.7 to 4.1)	1.0	4.5 (1.9 to 7.2)	<0.0001	−0.07 (−0.13 to −0.01)	0.008
	9	−0.1 (−4.1 to 3.8)	1.0	3.8 (1.1 to 6.5)	0.0006	−0.06 (−0.12 to 0.00)	0.03
	10	0.5 (−3.4 to 4.4)	1.0	3.3 (0.7 to 6.0)	0.004	−0.05 (−0.11 to 0.01)	0.18
	11	1.1 (−2.8 to 5.0)	1.0	3.1 (0.4 to 5.8)	0.01	−0.03 (−0.09 to 0.02)	0.67
	12	0.2 (−3.7 to 4.2)	1.0	3.7 (1.0 to 6.4)	0.0009	−0.06 (−0.12 to 0.00)	0.08
	13	0.4 (−3.5 to 4.3)	1.0	3.9 (1.2 to 6.5)	0.0004	−0.06 (−0.12 to 0.00)	0.08
	14	0.6 (−3.3 to 4.5)	1.0	4.2 (1.5 to 6.9)	<0.0001	−0.06 (−0.12 to 0.00)	0.1
	15	−1.4 (−5.3 to 2.5)	1.0	3.4 (0.8 to 6.1)	0.003	−0.07 (−0.13 to −0.01)	0.006
	16	−1.1 (−5.0 to 2.9)	1.0	3.2 (0.5 to 5.9)	0.007	−0.06 (−0.12 to −0.01)	0.02
	17	−0.8 (−4.7 to 3.1)	1.0	3.8 (1.1 to 6.5)	0.0006	−0.07 (−0.13 to −0.01)	0.009
	18	−0.8 (−4.8 to 3.2)	1.0	3.1 (0.4 to 5.9)	0.01	−0.06 (−0.12 to 0.00)	0.049
	19	−1.9 (−5.8 to 2.1)	0.9	3.1 (0.4 to 5.8)	0.01	−0.07 (−0.13 to −0.01)	0.009
	20	−0.3 (−4.3 to 3.7)	1.0	3.6 (0.9 to 6.3)	0.002	−0.06 (−0.12 to 0.00)	0.06
	21	0.4 (−3.5 to 4.4)	1.0	2.3 (−0.4 to 5.1)	0.15	−0.03 (−0.09 to 0.03)	0.75
	22	−1.3 (−5.3 to 2.7)	1.0	2.1 (−0.6 to 4.9)	0.24	−0.05 (−0.11 to 0.01)	0.16
	23	0.0 (−4.0 to 4.0)	1.0	2.5 (−0.2 to 5.2)	0.09	−0.04 (−0.10 to 0.02)	0.39
	24	1.9 (−2.1 to 5.9)	0.9	2.3 (−0.5 to 5.0)	0.18	0.00 (−0.06 to 0.06)	1.00
	25	0.1 (−3.9 to 4.1)	1.0	3.0 (0.3 to 5.7)	0.02	−0.05 (−0.11 to 0.02)	0.30
	26	1.9 (−2.1 to 5.9)	0.9	3.9 (1.2 to 6.6)	0.0005	−0.03 (−0.09 to 0.03)	0.73
	27	0.5 (−3.5 to 4.5)	1.0	3.8 (1.1 to 6.5)	0.0007	−0.05 (−0.11 to 0.01)	0.12
	28	−1.7 (−5.7 to 2.3)	0.9	3.1 (0.4 to 5.9)	0.01	−0.07 (−0.13 to −0.01)	0.006

Abbreviations: PRBCT, packed red blood cell transfusion; SCOR, splanchnic-cerebral oxygenation ratio; StO_2c, cerebral tissue oxygen; StO_2s, splanchnic tissue oxygen.

[a]The Dunnett method was used to correct *p* values and 95% CIs for the pairwise tests.

find out the time point when the expected increase in splanchnic oxygenation might have occurred.

Many factors influence regional tissue oxygenation, including the nature of the NIRS device, the type of NIRS sensor (adult vs. paediatric), the site of measurement, and the demography of the study population.[11,47–49] Cerebral and splanchnic oxygenation vary with gestation, postnatal age, small for gestational age, haemoglobin, gender, and hemodynamically significant PDA.[6,22,23,46,50] Results of association of splanchnic oxygenation with feeding are also conflicting.[4,12,19,41–43,51] These findings imply that it is prudent to account for these confounding factors in regional tissue oxygenation studies. Although our study involved a small sample size, so the clinical significance of SCOR changes must be elucidated by studying a larger sample; the study design utilised longitudinal comparisons with each baby acting as its control (intra-individual comparison) before versus after PRBCT, thereby removing multiple confounders that would have required a very large sample size.

Nevertheless, our research underscores the importance of examining the individual components of the SCOR ratio rather than interpreting the ratio itself.

In summary, our study showed that a decrease in SCOR associated with PRBCT is due to improvement in cerebral rather than worsening of splanchnic oxygenation. Our study underlines that it is necessary to determine cerebral and splanchnic StO_2 to understand changes in SCOR. This explorative study includes a small number of stable preterm infants to understand changes in cerebral and

splanchnic oxygenation contributing to SCOR changes. Future methodologically uniform studies involving a larger sample size are warranted before SCOR becomes a routine assessment tool in neonatal clinical practice.

ACKNOWLEDGMENT

Kiran Kumar Balegar V had full access to all the data in the study, and he takes responsibility for the integrity of the data and the accuracy of the data analysis. Open access publishing facilitated by The University of Sydney, as part of the Wiley - The University of Sydney agreement via the Council of Australian University Librarians.

AUTHOR CONTRIBUTIONS

Kiran Kumar Balegar V initiated the research idea. Kiran Kumar Balegar V and Ralph KH Nanan contributed to study concept and design. Kiran Kumar Balegar V, Madhuka Jayawardhana, and Philip de Chazal performed data acquisition and analysis. Kiran Kumar Balegar V contributed to data interpretation, manuscript preparation, and review. All authors approved the final version of this manuscript and agree to be accountable for all aspects of this work.

FUNDING INFORMATION

We acknowledge the Australian Women and Children's Research Foundation (OZWAC) for providing funding to cover the expenses of purchasing NIRS sensors. OZWAC had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

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How to cite this article: Balegar V KK, Jayawardhana M, de Chazal P, Nanan RKH. Splanchnic-cerebral oxygenation ratio associated with packed red blood cell transfusion in preterm infants. *Transfusion Medicine*. 2022;32(6):475-483. doi:10.1111/tme.12919

Received: 16 September 2021 | Revised: 7 July 2022 | Accepted: 18 August 2022

DOI: 10.1111/tme.12913

SHORT COMMUNICATION

TRANSFUSION MEDICINE WILEY

Pathogen inactivation treatment of triple-dose apheresis platelets with amotosalen and ultraviolet a light

Laura Infanti[1,2] | Vildana Pehlic[1] | Sandra Mitrovic[3] | Andreas Holbro[1,2] | Silke Andresen[4] | Jean-Marc Payrat[4] | Jin-Sying Lin[5] | Andreas Buser[1,2]

[1]Regional Blood Transfusion Service, Swiss Red Cross, Division of Hematology, University Hospital, Basel, Switzerland

[2]University of Basel, Basel, Switzerland

[3]Clinical Chemistry, Department of Laboratory Medicine, University Hospital Basel, Basel, Switzerland

[4]Cerus Europe B.V., Amersfoort, The Netherlands

[5]Cerus Corporation, Concord, California, USA

Correspondence
Laura Infanti, MD, Regional Blood Transfusion Service, Swiss Red Cross, Basel, and Hematology, University Hospital Basel, University of Basel, Basel 4031, Switzerland.
Email: laura.infanti@usb.ch

Funding information
Cerus Corporation

Abstract

Background: A triple storage (TS) set allows for pathogen inactivation (PI) treatment of triple-dose apheresis platelet products with amotosalen + UVA. We evaluated the quality and metabolic parameters of platelet concentrates (PCs) pathogen inactivated and stored for 7 days.

Materials and methods: Twelve triple-dose products collected with two different apheresis platforms were treated with amotosalen+UVA. Products were split into three single-dose units. Testing was made pretreatment, after splitting, at days 5 and 7 of storage.

Results: Single-dose PI PCs had a mean platelet content of 2.89 ± 0.35 x 10^{11}. From baseline to day 7, pH remained stable (7.1 ± 0.1 vs. 7.0 ± 0.1), pO_2 increased (11.3 ± 2.4 vs. 18.3 ± 3.5 kPa) as did LDH (201 ± 119 vs. 324 ± 203 U/L) and lactate (3.6 ± 1.7 vs. 12.1 ± 1.5 mmol/L) (all $p < 0.01$); pCO_2 decreased (4.1 ± 0.8 vs. 1.5 ± 0.7 mmHg; $p < 0.01$) and so did bicarbonate (6.6 ± 1.1 vs. 2.5 ± 1.4 mmol/L), glucose (5.6 ± 1.2 vs. 0.4 ± 0.4 mmol/L) and ATP (3.4 ± 0.9 vs. 2.5 ± 1.4 nmol/10^8 platelets) (all $p < 0.05$).

Conclusion: Triple-dose PCs processed with the TS sets fulfilled the quality requirements and displayed metabolic changes of expected extent during 7-day storage.

KEYWORDS
pathogen inactivation, triple-dose apheresis platelet components

1 | INTRODUCTION

The INTERCEPT® blood system for platelets was introduced for pathogen inactivation (PI) treatment of platelet concentrates (PCs) by the Swiss Red Cross transfusion services in November 2011.[1] At the Regional Blood Transfusion Service in Basel, which was involved in the validation process of the method in Switzerland, PI for PCs was implemented by January 2011. The large amount of data collected in more than 8 years of routine use in Basel demonstrates the safety and the clinical efficacy of PI-treated PCs for various clinical indications.[2]

Besides the benefits in safety of PCs, adoption of PI also comes with some cost increase and logistical challenges. To address these issues, possible strategies are the collection of large dose (double- or triple-dose) platelet products by apheresis and the manufacturing of double-dose PC by pooling of whole blood derived buffy coats.[3] The economic advantages of this approach have recently been evaluated.[4]

In our institute, prior to the introduction of PI, approximately 70% of the platelet apheresis products were of double-dose and 20% of triple-dose size with minimum 2.4 x 10^{11} platelets per single unit. PCs were prepared in plasma/platelet additive solution (PAS) and gamma-irradiated. After the implementation of PI, the proportion of double-dose collections

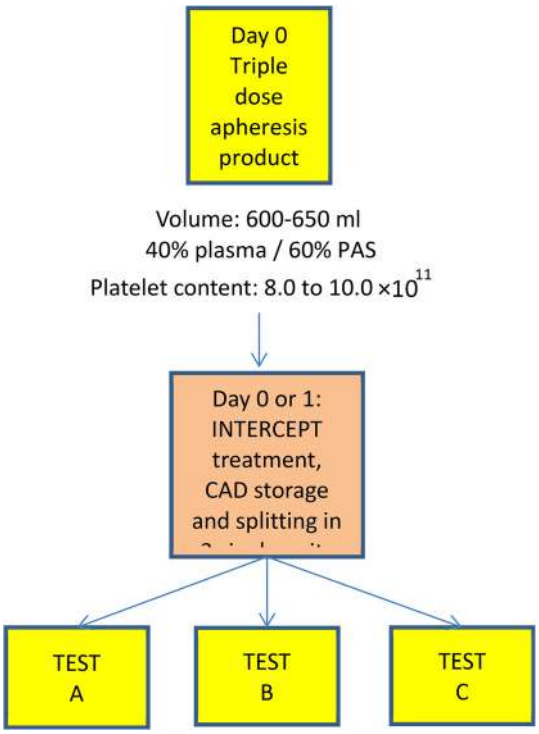

FIGURE 1 Design of the study. Day 1–7: testing for volume and platelet content (units A, B, C). Testing for storage parameters (unit A on day 1, unit B on day 5, unit C on day 7).

rose to 90%, because triple-dose apheresis were no longer performed. This was because the available sets for PI only allowed the treatment of products up to a maximum volume of 420 ml with a maximum platelet content of up to 7.0 x 10^{11} (large volume set) and 8.0 x 10^{11} (dual storage set). Thus, PI of triple-dose products was not possible in a closed system.

The INTERCEPT platelet processing set with triple storage containers (TS set) was designed to treat buffy coat and apheresis derived platelet products with a platelet content of 5–12 x 10^{11} in a volume of 420–650 ml in 32%–47% plasma and PAS. As additional improvement, the TS sets are manufactured with a plastic composition of containers which improves gas transfer properties.[5] The di(2-ethylhexyl) phthalate (DEHP) plasticiser is present in isolated components of the kit that have limited exposure to the product during processing. The TS set received the CE mark in January 2018.

We conducted a study to evaluate platelet standard quality parameters and metabolism in PCs obtained from triple-dose apheresis collections, pathogen inactivated and stored in TS sets during 7 days. Hereby we present the results of this analysis.

2 | MATERIALS AND METHODS

2.1 | Study design and platelet collection

Twelve triple-dose apheresis products were collected to obtain a total of 36 single-dose PCs. Experienced blood donors who had donated triple-dose platelets by apheresis in the past were asked to take part in the study. Donors had to comply with the eligibility criteria for blood donation and were tested for infectious disease markers according to the national regulations. Blood donors provided written informed consent before their participation in the study. The trial protocol was approved by the local Scientific Ethic Committee (trial number 2017–00516).

Figure 1 illustrates the general design of the study. Platelets were collected on the Amicus (AM, Fresenius Kabi, Frankfurt, Germany; $n = 6$) and Trima (TR, Terumo BCT, Lakewood, Colorado, USA; $n = 6$) apheresis devices and suspended in 40% plasma/60% PAS (SSP+; Macopharma, Tourcoing, France). Targeting a minimal amount of 2.4 x 10^{11} platelets per single-dose unit, and anticipating a loss of about 10% of platelets due to processing and PI treatment, the goal was to collect a dose of 8.0–10.0 x 10^{11} in a volume of 600–650 ml with each procedure. Specifically, the target yield was set at 9 x 10^{11} platelets for both AM and TR. After collection and before PI treatment, apheresis products were stored on a flatbed platelet agitator (~60 cpm) in an incubator at 22 ± 2°C for at least 2 h.

2.2 | Processing and PI treatment

The TS set consists of the following sequentially integrated components: 6 mM amotosalen solution in a 15 ml container, a 1L illumination container, a 1.3L container with dual compound adsorption device (CAD) wafers, an in-line filter, and three 1.3L storage containers (Figure 2). For processing, the PC unit was passed from the collection bag through the amotosalen container into the illumination container and was then exposed to UVA light (3.0 J/cm^2) in an INT100 Illuminator (Cerus Europe B.V., Amersfoort, The Netherlands). Following illumination, platelets mixed with amotosalen were transferred into the CAD container, which was then placed in an incubator at 22 ± 2°C on a flatbed platelet agitator for the removal of amotosalen and photoproducts. Six apheresis products (AM $n = 4$; TR $n = 2$) were treated on day 0 (day of collection) and stored in the CAD for a period of about 16 h (maximum CAD time); six products (AM $n = 2$; TR $n = 4$) were treated on day 1 (day after collection) and kept in the CAD for about 4 h (minimum CAD time). After CAD incubation, platelets were transferred evenly into the three final storage containers. The units were labelled (test unit A, test unit B, and test unit C, respectively) and stored at 22 ± 2°C on a flatbed platelet agitator for 7 days post-donation. PCs were used for study purposes only.

2.3 | Quality parameters and in vitro analysis of platelet metabolism

The acceptability criteria for PI treated PCs were those of the Council of Europe Recommendation No. R(95) 15[6] and those of the Swiss Regulations (Vorschriften BSD SRK Kap. 18B) that require a dose of ≥2.4 x 10^{11} platelets/unit in at least 90% of units tested.

On day 0 or day 1, before INTERCEPT treatment, volume, platelet concentration, residual red blood cells, and residual leukocytes were

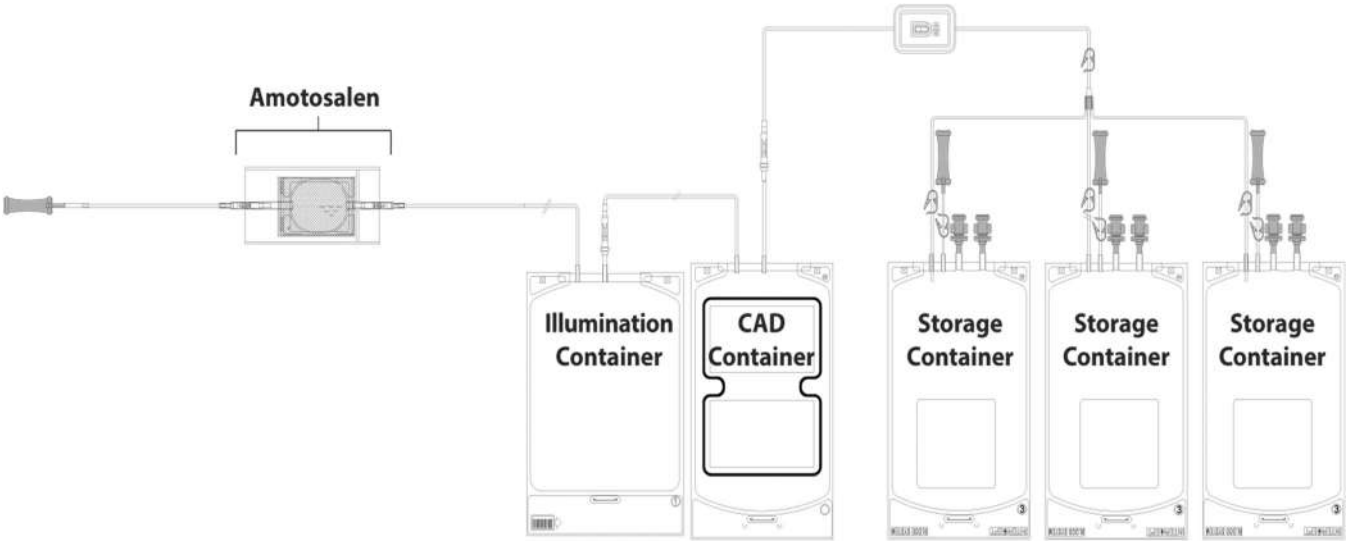

FIGURE 2 Schematic view of the INTERCEPT platelet processing set with triple storage containers. The required ranges of platelet content and volume in apheresis products in plasma/PAS (32%–47% plasma content) are 5–12 x 10^{11} and 420–650 ml, respectively.

measured in each triple-dose apheresis product. Visual inspection for swirling (present or absent) and platelet aggregates were also performed. Volume was calculated based on the weight before and after sampling (volume in ml = weight in g/1.01), Platelet concentrations were measured on a blood cell counter (Sysmex K-4500, Sysmex Digitana AG, Horgen, Switzerland) for the calculation of the platelet yield per unit. Residual leukocytes were measured with a flow cytometer (Attune™ NxT, Thermo Fisher, Life Technologies, Carlsbad, CA, USA) and the red blood cell contamination by microscopy with a Neubauer counting chamber.

On day 1 after PI treatment, volume and platelet content of Test units A, B, and C were measured in order to calculate the percentage of recovery after processing.

Samples (10 ml) for in vitro quality assessment with single measurements were collected on day 1 (immediately following transfer from the CAD), day 5, and day 7 from test units A, B, and C, respectively.

Parameters of extracellular metabolic environment (pO_2, pCO_2, bicarbonate, glucose, and lactate) were measured on a blood gas analyser (ABL 800 Flex, Radiometer GmbH, Copenhagen, Denmark) immediately after sampling, and pH at 22°C with a pH metre (Seven Compact S210-Basic, Mettler-Toledo, Albstadt, Switzerland). Lactate dehydrogenase (LDH) activity was measured with a UV-based spectrophotometric test (Cobas 8000 c702 Roche, Roche Diagnostics Gmbh Rotkreuz Schweiz). ATP was extracted from platelet samples with the addition of strong trichloracetic acid followed by pH neutralisation of the supernatant, then stored below −25°C until the ATP content was analysed using a firefly luciferase bioluminescence assay with ENLITEN® ATP Assay System (Promega, Fitchburg, WI, USA).

The concentration of residual amotosalen was measured in post-CAD samples with a reverse-phase high-performance liquid chromatographic method using a Zorbax SB-CN column (Agilent, Santa Clara, CA, USA).

2.4 | Statistical analysis

Data generated from this study was analysed by basic statistical methods. Mean and standard deviation of amotosalen concentrations and in vitro data at each sampling interval were calculated. When applicable, the *p*-value from a paired *t*-test was calculated to evaluate whether the mean change from baseline to each testing period was different from zero as indicated by a *p* value of <0.05. Results between the two apheresis platforms (AM vs. TR) were also compared and *p*-values from 2-sample *t*-test were calculated. Statistical analyses were performed using SAS® software (Version 9.4).

3 | RESULTS

The 12 triple-dose apheresis collections were performed without technical problems or donor adverse events.

3.1 | Platelet preparation parameters

Table 1 summarises the platelet preparation data of products before and after PI treatment. Before PI processing, all apheresis products exhibited swirling upon visual inspection and met the PI process guard bands (volume 420–650 ml, platelet content 5–12 x 10^{11}, plasma content 32%–47%, red blood cell count <4 x 10^6/ml).

The average volume of products before PI treatment was 617 ± 24 ml. After PI treatment and separation into the three storage containers, the mean volume of the final single-dose units was 188 ± 9 ml. The volume loss after processing and PI treatment, including 15 ml amotosalen, was 52 ± 5 ml on average, corresponding to 8% ± 1% and to a volume recovery of about 92%.

TABLE 1 Data of platelet products preparation and quality before and after PI treatment

	AM ($n = 6$)	TR ($n = 6$)	Combined ($n = 12$)
Volume before PI (ml)[a]	600 ± 25	634 ± 5	617 ± 24
Volume after PI (ml)[a]	183 ± 8	194 ± 7	188 ± 9
Volume Loss (ml)	52 ± 6	53 ± 5	52 ± 5
Volume recovery (%)	91 ± 1	92 ± 1	92 ± 1
PLT content before PI (10^{11}/unit)[a]	8.58 ± 2.30	9.87 ± 0.76	9.23 ± 1.77
PLT content after PI (10^{11}/unit)[b]	2.83 ± 0.41	2.96 ± 0.26	2.89 ± 0.35
PLT recovery (%)[c]	92 ± 5	90 ± 2	91 ± 3
CAD incubation (h)	11.8 ± 5.9	7.9 ± 5.9	9.8 ± 6.0
Residual Amotosalen (μM)	0.17 ± 0.07	0.39 ± 0.26	0.28 ± 0.21

Note: Results are expressed as mean ± SD.
[a]Values refer to the final single-dose platelet units ($n = 18$ for AM and TR; combined $n = 36$).
[b]Two values above 100% for Amicus (AM) were not taken into account.
[c]Values refer to the triple-dose apheresis products ($n = 12$).

The mean platelet yield of triple-dose products after collection was 9.23 ± 1.77 x 10^{11}. After PI treatment and splitting, the mean final platelet concentration was 2.89 ± 0.35 x10^{11}/unit. Overall, the platelet recovery post-PI processing was 91%.

Both the mean red blood cell and white cell count before PI treatment were below 4 x 10^6/ml and 1 x 10^6/unit, respectively.

After CAD incubation residual amotosalen was 0.28 ± 0.21 μM on average, complying with the value below 2.0 μM required in the current Swiss regulations.

3.2 | Parameters of platelet metabolism during storage

Storage parameters of platelet products are presented in Table 2. Since no difference was observed for any evaluated parameters at any day of storage between PCs incubated with the minimum and then maximum CAD time, results are presented per collection platform (AM and TR) and as combined values.

Significant changes from baseline (day 0/1) during storage included a decrease of platelet concentration, pCO2, bicarbonate, glucose and ATP from day 1 to day 7, and an increase of MPV, pO_2, and lactate also from day 1 to the end of storage, and of LDH on day 5 and day 7.

Comparing products from AM and TR in PCs collected with the AM lower values of pH were measured on day 5 and of pCO2 and bicarbonate on days 5 and 7, whilst pO_2 at days 5 and 7 was higher compared to that in PCs collected with the TR.

Platelet concentration decreased significantly from days 0/1 to 7 with statistical significance for TR from day 1 and for both platforms combined only at day 7. The relatively large standard deviation of the baseline platelet concentration for AM may explain the non-significance of the apparent decrease.

Mean platelet volume (MPV) increased continuously starting from day 1 for products collected by AM and when combining results Values of pH at 22°C remained quite stable from baseline to day 7 (average level varying from 7.1 ± 0.1 to 7.0 ± 0.1). Values of pH were on average 0.2 point higher for TR at days 5 and 7.

Values of pO_2 increased during storage with platelets from both platforms, and were slightly but significantly lower for TR products from day 5. Values of pCO_2 were significantly lower at days 5 and 7 with AM and also bicarbonate and glucose decreased between the pretreatment baseline and days 1, 5, and 7 more rapidly with AM. Similarly, ATP decreased with PI processing and storage, with values being significantly higher for TR products before processing and at every evaluation post-processing. LDH levels tended to increase significantly from day 5 and with lower values throughout storage for TR.

4 | DISCUSSION

In this study, we evaluated standard quality and metabolic parameters of triple-dose PCs collected on two apheresis platforms, suspended in plasma/PAS and treated with amotosalen + UVA with the TS set using two collection platforms. All products met both the EDQM and the Swiss requirements of platelet content, volume and pH values. During a 7-days storage, PCs collected with the AM and TR displayed some statistical differences in metabolic activity, as observed in another recent study.[7]

The procurement and manufacturing of PCs has become increasingly efficient over the years due to multidose platelet collections and the achievement of double-dose products from pooled buffy-coats.[4] At the same time, the safety of components was greatly improved by the use of PAS[8,9] and of PI methods.[1,10] Moreover, many regulatory authorities allow storage for up to 7 days of pathogen inactivated PCs, which leads to a significant reduction of product wastage.[2,4] A more recent development is the use of storage bags and sets with most components manufactured with DEHP-free plastic that allow for improved gas exchange.[11,12]

In Basel, multidose apheresis PCs in plasma/PAS have been produced for more than 20 years. Triple-dose collections amounted to

TABLE 2 Data of quality and in vitro metabolic parameters as evaluated over 7 days of storage

		Pretreatment day 0/day 1	Day 1	Day 5	Day 7
PLT concentration[a] (10^3/μl)	AM	1424 ± 353	1544 ± 179	1366 ± 154	1258 ± 150
	TR	1557 ± 120	1529 ± 128*	1462 ± 148*	1440 ± 165*
	Combined	1491 ± 261	1536 ± 148	1414 ± 152	1349 ± 178*
PLT MPV (fL)[b]	AM	7.3 ± 0.5	7.5 ± 0.6*	7.9 ± 0.5*	8.1 ± 0.5*
	TR	7.9 ± 1.0	7.9 ± 1.1	8.0 ± 1.0	8.1 ± 1.0
	Combined	7.6 ± 0.9	7.7 ± 0.8*	7.9 ± 0.7*	8.1 ± 0.7*
pH (22°C)[b]	AM	7.1 ± 0.1	7.0 ± 0.1*	6.9 ± 0.0*	6.9 ± 0.1
	TR	7.1 ± 0.1	7.0 ± 0.1	7.1 ± 0.1#	7.1 ± 0.1
	Combined	7.1 ± 0.1	7.0 ± 0.1*	7.0 ± 0.1	7.0 ± 0.1
pO_2 (kPa)[b]	AM	12.0 ± 2.2	17.2 ± 1.6*	20.8 ± 0.7*	20.6 ± 1.8*
	TR	10.6 ± 2.6	16.1 ± 1.3*	16.7 ± 1.4*#	15.9 ± 3.1*#
	Combined	11.3 ± 2.4	16.6 ± 1.5*	18.7 ± 2.3*	18.3 ± 3.5*
pCO_2 (kPa)[b]	AM	4.2 ± 0.7	4.7 ± 0.9	1.2 ± 0.3*	0.9 ± 0.2*
	TR	4.0 ± 1.0	4.5 ± 1.0*	2.1 ± 0.3*#	2.1 ± 0.3*#
	Combined	4.1 ± 0.8	4.6 ± 0.9*	1.7 ± 0.6*	1.5 ± 0.7*
Bicarbonate (mmol/L)[b]	AM	6.9 ± 1.0	5.5 ± 1.0*	1.4 ± 0.5*	1.2 ± 0.5*
	TR	6.3 ± 1.1	5.6 ± 0.9*	4.1 ± 0.2*#	3.6 ± 0.7*#
	Combined	6.6 ± 1.1	5.6 ± 0.9*	2.8 ± 1.5*	2.5 ± 1.4*
Glucose (mmol/L) [b]	AM	5.2 ± 1.2	4.2 ± 1.3*	0.5 ± 0.8*	0.1 ± 0.0*
	TR	6.1 ± 1.1	5.2 ± 1.2	2.7 ± 1.1*#	0.7 ± 0.5*#
	Combined	5.6 ± 1.2	4.7 ± 1.3*	1.6 ± 1.5*	0.4 ± 0.4*
Lactate (mmol/L)[b]	AM	3.6 ± 1.9	5.4 ± 1.8*	11.7 ± 1.8*	12.1 ± 1.7*
	TR	3.6 ± 1.7	5.0 ± 2.6	9.6 ± 1.7*	12.2 ± 1.3*
	Combined	3.6 ± 1.7	5.2 ± 2.1*	10.6 ± 2.0*	12.1 ± 1.5*
ATP (nmol/10^8 PLTs)[b]	AM	2.7 ± 0.4	2.5 ± 0.4	1.8 ± 0.6*	1.3 ± 0.6*
	TR	4.1 ± 0.7#	3.9 ± 0.7*#	3.9 ± 0.7*#	3.7 ± 0.7*#
	Combined	3.4 ± 0.9	3.2 ± 0.9*	2.8 ± 1.3*	2.5 ± 1.4*
LDH (U/L)	AM	292 ± 73	237 ± 78	417 ± 133*	508 ± 99*
	TR	87 ± 5#	98 ± 9#	137 ± 20*#	141 ± 11*#
	Combined	201 ± 119	167 ± 90	277 ± 172*	324 ± 203*

Note: Results are expressed as mean ± SD.

#*p*-values <0.05 indicate significant difference for Amicus (AM) versus Trima (TR).

**p*-values <0.05 indicate significant difference compared to the baseline. [a]Values refer to the triple-dose apheresis products ($n = 12$).

[b]Values refer to the final single-dose platelet units tested during storage (A on day 1, B on day 5, and C on day 7) for both the minimum and the maximum CAD incubation.

approximately 20% of procedures until the INTERCEPT® blood system for platelets was implemented in January 2011. Due to the unavailability of sets suitable for PI treatment of products with such large volume and high platelet content, triple-dose apheresis was no longer performed. At the same time, the demand of PCs from the clinic increased steadily[2] and the procurement of sufficient numbers of units became challenging at times. Thus, the new TS set is of particular interest for our centre, since it allows for the re-introduction of the manufacturing of PCs from triple-dose collections in compliance with the current quality and safety standards.

Overall, data on the quality of triple-dose PCs are limited. Triple-dose products stored for 7 days in PAS 5, PAS 5/plasma or in 100% plasma collected with the AM and the TR were equivalent for in vitro storage parameters.[13] To date, only one other study assessed the quality of PCs manufactured with comparable methods as described in our study. Lotens et al.[7] analysed standard quality and metabolic parameters up to day 7 in PCs treated with amotosalen + UVA using large volume sets or TS sets and prepared with three different platforms (AM, TR, and pooled buffy-coats). In that study, PCs stored in TS sets had a higher volume and platelet recovery and higher residual amotosalen (although within the required limits), and displayed less platelet activation during storage compared to PCs processed with large volume sets. Differences in metabolic parameters were observed both between AM and TR and between apheresis-derived and buffy

coat derived components. Compared to the results of Lotens et al. on apheresis-derived PCs treated with the TS set (the study products with the same characteristics as ours), platelet content, pH at 22°C, glucose and bicarbonate of our products remained higher throughout storage and lactate was lower. On day 7, pO_2 was higher and pCO_2 lower, whilst ATP levels were comparable to the day 7 values reported by Lotens et al. for the units collected with AM. Thus, we observed an overall slightly better metabolic profile of stored platelets from apheresis, but with differences between AM and TR along the same line.

Platelet loss after treatment (%) was in line with the volume loss. Quality parameters were not different in PCs treated with the minimum versus the maximum CAD time (results not shown) recommended in the instructions for use. This observation does not indicate a substantial influence of different CAD times on platelet metabolism after treatment with amotosalen + UVA, as also reported by others.[14,15]

Storage containers made of DEHP-free plastics, as in the TS sets, display improved gas permeability. Platelets of double-dose buffy coat derived PCs treated with amotosalen + UVA and stored in the same type of containers had reduced glucose and bicarbonate consumption and less lactate production.[5] DEHP-free containers are particularly interesting for the storage of multidose, highly concentrated PCs, since the improved gas exchange can reduce the negative effects of a relative O_2 deprivation on platelet metabolism and energy supply.[16]

Effects on platelet quality were described for highly concentrated products,[16,17] for PI,[18,19] and for prolonged storage,[20] which are also the manufacturing conditions of our study components.

High platelet concentrations were associated with decreased swirling and glucose content and increased lactate in double-dose, pathogen inactivated PCs from apheresis.[17]

Because anaerobic metabolites accumulate in these components, Feys et al. suggested that the upper guard band ranges for amotosalen + UVA treatment of such apheresis products be revalidated,[17] but in that study products were stored in one or two containers post-PI treatment, whilst they were split into three storage containers in our study, leading to a lower platelet content per bag. Indeed, despite a higher platelet concentration, we did not observe a significant increase in anaerobic metabolisms in our PCs.

We observed in all products after collection. Glucose, lactate and pH at 22°C values and the changes of the other parameters from day 1 to day 7 of storage were overall comparable to those reported previously for apheresis-derived double-dose PCs (having a lower platelet concentration).[21]

High concentrations of platelets, like those of multidose products, and further processing of PCs impact in vitro platelet biology and function to a different extent,[22–24] even if standard quality parameters comply with regulations. However, changes in platelet function are in part reversible after transfusion to the patient, and overall do not seem to impair transfusion efficacy in vivo.[2,25]

A 7 days storage of pathogen inactivated PCs from triple-dose apheresis collections would further improve cost efficiency of PC manufacturing, as described for double-dose buffy-coat derived products.[4] Besides reducing material consumption and workload, and allowing for a rapid replenishment of stores, this strategy could facilitate the procurement of PCs for patients with particular requirements (e.g., HLA-matched PCs or platelets negative for specific HPA antigens).

Limitations of the present study include the small number of products evaluated and that no assessment of biologic platelet functions (e.g., aggregation tests) was performed. Further studies to analyse the in vivo transfusion efficacy would also be required.

In conclusion, our study demonstrates apheresis-derived triple-dose PCs treated with amotosalen + UVA with the TS sets fulfilled the quality requirements and exhibited metabolic activity during 7-day storage. Some differences were observed with metabolic parameters when comparing both platforms, but not large enough to expect differences in platelet quality. The INTERCEPT TS sets would both allow further savings in materials and labour time for PI treatment of PCs and facilitate the procurement of platelet products.

AUTHOR CONTRIBUTIONS

LI and JMP designed the study. LI and VP recruited the study donors. SM performed the laboratory tests. SA and JSL performed the data analysis. LI and JMP wrote the manuscript. AH and AB revised the manuscript.

ACKNOWLEDGMENT

Open access funding provided by Universitat Basel.

FUNDING INFORMATION

The study was partly financially supported by Cerus Corporation under a service agreement.

CONFLICT OF INTEREST

Silke Andresen, Jean-Marc Payrat, and Jin-Sying Lin are employees of Cerus Corporation.

ORCID

Laura Infanti https://orcid.org/0000-0001-7241-426X

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How to cite this article: Infanti L, Pehlic V, Mitrovic S, et al. Pathogen inactivation treatment of triple-dose apheresis platelets with amotosalen and ultraviolet a light. *Transfusion Medicine*. 2022;32(6):505-511. doi:10.1111/tme.12913

Accepted: 11 October 2022

DOI: 10.1111/tme.12931

TRANSFUSION MEDICINE WILEY

CORRIGENDUM

In the published article,[1] the authors' names in the author by line were corrected to:

Gordana Tomac, Koraljka Gojčeta, Ruža Grizelj, Desiree Coen Herak, Sanja Baršić Ostojić, Branka Golubić Ćepulić

The author apologizes for this error. The original article in the online version has been updated.

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