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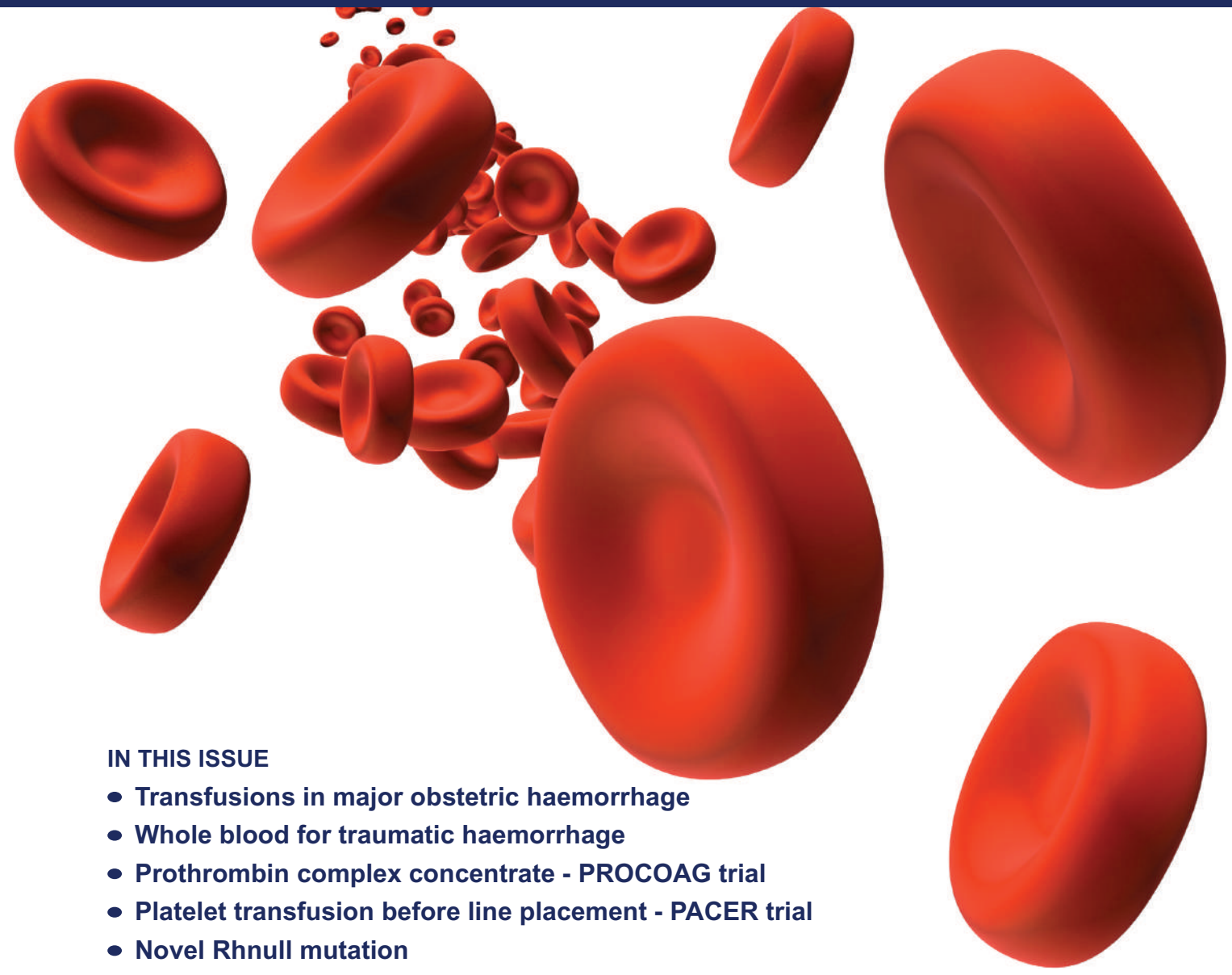

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An international journal published for the British Blood Transfusion Society

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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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TRANSFUSION MEDICINE WILEY

COMMENTARY

Evidence cornered: Transfusion evidence summary—efficacy and safety of early administration of 4-factor prothrombin complex concentrate in patients with trauma at risk of massive transfusion—the PROCOAG randomised clinical trial (JAMA)

Dear Editor,

A 33-year-old Caucasian woman was referred at 32^{+5} weeks of gestation after the finding of severe fetal intracranial haemorrhage (ICH) at routine ultrasound. The woman had two previous uneventful pregnancies (singleton and twins). Fetal ultrasound and magnetic resonance imaging confirmed multiple ICHs especially located in the left hemisphere, wide areas of periventricular leukomalacia, obstructive hydrocephalus and macrocrania. Fetal-neonatal alloimmune thrombocytopenia (FNAIT) was investigated. Maternal blood group was A RhD positive, paternal O RhD positive. The maternal sample was screened for platelet-reactive antibodies using solid phase technology for the detection of IgG anti-HLA class I and anti-HPA antibodies (SPRCA Capture P Ready Screen, Immucor, Italy) with and without chloroquine treatment to remove HLA antigen interference. The results were positive and negative respectively. No antibodies attached to maternal platelets were found (Capture-P, Immucor, Italy). ELISA and Luminex based platforms were used to identify the specificity of the detected antibodies (Pak-Lx Luminex and ELISA Pak plus, Immucor, Italy and Luminex MoAb, Lagitre, Italy). The assays only recognised the presence of anti-HLA A02 and anti-HLA B51 at high titre, greater than 8000 and 20 000 MFI (average fluorescence intensity) respectively, in association with different cross-reactions. Cross-match testing (Capture-P, Immucor, Italy) using maternal serum against paternal platelets tested reactive with both chloroquine-untreated and treated platelets. Additional cross-match testing was performed using platelets. Additional cross-match testing was performed using maternal serum against 14 random donor platelet samples. Eight donors were compatible and six were not. Two non-compatible donors were HLA class I A*02 and A*02 B*51 respectively. The remaining four non-compatible donors were not typed for HLA I antigen. All compatible donors were typed for the main HPA antigens but comparison of the typings did not allow to quickly exclude HPA 4b, 6b, 7b, 8b, 9b, and 11b antigen immunisation. A male newborn was delivered by caesarean section at 36 weeks of gestation after spontaneous onset of labour. At birth, platelet count was $4x10^3/\mu l$ with normal white and red blood cell count. An urgent transfusion with a platelet blood component not tested with maternal serum increased platelets to $116 \times 10^3/\mu l$; intravenous immunoglobulins were also infused. Another two transfusions were administered on days 4 and 13 due to a drop in the number of platelets ($28 \times 10^3/\mu l$ and $48 \times 10^3/\mu l$ respectively): the platelet pools were obtained from cross-match between maternal serum and sample platelets of random donors. Normal values were reached on day 17.

The newborn blood group was 0 Rh D positive. Capture-P Ready Screen aimed to detect anti-platelet antibodies was non-reactive. Cross-match testing using newborn blood was performed twice. At birth, the neonatal sample was cross-matched against paternal and maternal platelet samples: results were positive and negative, respectively. After 14 days, cross-matching against paternal platelets was repeated with and without chloroquine treatment; both resulted non-reactive.

Results of parental and neonatal HLA I and HPA genotyping performed using polymerase chain reaction (PCR) with sequence-specific oligonucleotides (PCR-SSO) and HPA BeadChip (Immucor, Italy), are shown in Table 1. The mismatches identified prompted further testing in the mother. Cross-match testing against 6 HPA-9b antigen negative donors resulted in two non-compatible and four compatible donors. The same two non-compatible donors were all compatible when

The same two non-compatible donors were all compatible when cross-matching was performed with chloroquine. Cross-match testing against two donors expressing the HPA9b antigen was reactive with and without chloroquine. Cross-match testing against paternal and neonatal platelets with and without chloroquine was equally reactive. Cross-match testing was performed between maternal serum and 107 different donors in order to have available and compatible blood components available for any neonatal transfusions.

FNAIT is a cause of severe thrombocytopenia and ICH in both the fetus and newborn.[1] FNAIT-related ICH is estimated to occur in at least 10:100 000 neonates. It mainly occurs in the third trimester of pregnancy and is associated with severe neurological sequelae and mortality. In most cases, FNAIT is caused by an alloimmune response against human platelet antigens (HPAs). In the Caucasian population HPA-1a antigen accounts for up to 80% cases,[2] followed by HPA-5b (8–15% of cases) and to a lesser extent HPA-3a/5a/15b.[3] More rarely, FNAIT is associated with low-frequency human platelet antigens (LFHPAs) or to HLA class I antigens, especially when related to locus A and B and with a highly expressed titre.[4] Among LFHPAs, HPA-9b is emerging as a significative trigger for FNAIT.[5] Almost two-thirds of apparent cases of FNAIT are not resolved by laboratory confirmation of maternal immunisation against HPA antigens. When other causes of thrombocytopenia are not identified, a possible explanation may be involvement of HLA antibodies or limitations of laboratory studies.

In the current case, parental ABO compatibility excluded ABO-mediated thrombocytopenia. The detection of maternal HLA class I antibodies, identified as HLA A02 and HLA B51 antibodies, and paternal HLA I genotype (HLA A*02; B*35*51), were consistent with the clinical suspicion of FNAIT. However, an additional factor was likely to be involved, presumably related to the HPA system: genotyping showed a parental mismatch in the HPA 9 locus (mother HPA- 9a/a, father HPA 9a/b) and neonatal inheritance of the HPA-9b antigen from the father. Search for HPA antibodies (Pak-Lx Luminex and ELISA Pak plus) was inconclusive because no reactivity was detected against the glycoproteins GPIIb/IIIa, GPIa/IIa, GPIb/IX and GPIV. This can be explained by the limitations of the GP assay used which was not able to recognise the rare specificity HPA-9b. Moreover, it was not possible to find readily available source platelets from local donors carrying the target antigen because only a limited number of them had been typed for HPA and the expected frequency of HPA 4b, 6b, 7b, 8b, 9b, 11b in the population is extremely low (<1%).[3] Cross-match testing between maternal serum and both paternal and neonatal platelets was reactive after chloroquine treatment. This finding was supported by cross-match performed against HPA9b positive donors. This allowed to attribute the FNAIT to the presence of the paternally inherited HPA9b antigen on the son's platelets. It was not possible to identify any antibody specificity in neonatal serum (Capture—P Ready Screening method) presumably due to the extremely low platelet count as a result of the adhesion of the maternal alloantibodies to the neonatal platelets with consequent uptake and elimination.

Since the first report of a HPA-9b related FNAIT in 1995,[6] a total of 15 cases have been reported[5,7,8] and increasing evidence suggests that its prevalence in the population and among fathers of unresolved cases of FNAIT might be greater than previously reported.[6,9] The severity of thrombocytopenia and clinical presentation in our case is consistent with the argument that HPA-9b might be more immunogenic than others HPAs.[5] Our case supports the need to investigate alloimmunisation to HPA-9b and other rare specificities when routine screening for the most common antigens is negative or inconsistent with the laboratory and clinical findings.[3,5] We experienced diagnostic limitations mainly due to the fact that the Ag panel used (Pak-Lx Luminex Immunocor and Elisa Pak Plus Immunocor) does not identify HPA-9b. Difficulties with the detection of antibodies against HPA-9b antibodies have been described by some authors who urged further studies to fully understand the issue.[5,7] Nonetheless, even if FNAIT was strongly suspected both on clinical grounds and after the finding of anti-HLA I antibodies, the mother could not be offered intrauterine therapy of proven efficacy to begin at that gestational age.[1,9]

CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are not openly available due to sensitivity reasons and are available from the corresponding author upon reasonable request.

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SHORT COMMUNICATION

TRANSFUSION MEDICINE WILEY

Evidence Cornered: Transfusion Evidence Summary – Platelet transfusion before CVC placement in patients with thrombocytopenia (PACER trial)

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KEYWORDS: bleeding risk, central venous catheter, platelet transfusion

Clinical question:
In haematology and intensive care (ICU) patients with a platelet count of 10–50 × 10^9/L, does withholding platelet transfusion prior to central venous catheter (CVC) insertion increase the incidence of bleeding in comparison to transfusing one unit of platelets?

Evidence from study:
Withholding platelet transfusion did not meet the criteria for non-inferiority and resulted in more CVC-related bleeding.

1 | INTRODUCTION

A lack of good evidence for the safe platelet count for invasive procedures in patients with thrombocytopenia has led to conflicting recommendations of platelet targets between 20 and 50 × 10^9/L.[1–6] The routine use of ultrasound in CVC insertion has reduced bleeding complications,[7] and some studies have shown a harmful effect of platelet transfusions.[8] This study aims to see whether, in this context, omission of prophylactic platelet transfusion increases the risk of CVC-related bleeding in patients with platelet counts 10–50 × 10^9/L.

Evidence Box

Study design: Single-blinded, randomised controlled non-inferiority trial.

Study years: 2016–2022.

Countries: Netherlands.

Setting: ICU and haematology wards.

No. of patients: 411 CVC placements randomised. 373 included in the per-protocol analysis.

Baseline characteristics: 56%–57% haematology patients. Otherwise broadly similar – age, gender, median platelet count, INR, APTT, CVC type.

Inclusion criteria:

- ICU or haematology patient.
- Platelet count 10–50 × 10^9/L within 24 h of the procedure.
- CVC due to be in place for >24 h.

Exclusion criteria:

Use of therapeutic anticoagulation, history of acquired/congenital bleeding disorder, INR >3 (changed from INR >1.5 mid-trial), <24 h from previous CVC placement.

Comparison:

1:1 randomisation – receive a one-unit platelet transfusion before CVC placement or no platelet transfusion. Any

[Correction added on 28 June 2024, after first online publication: The article title was corrected in this version.]

type of CVC was permitted. The non-inferiority margin was set at 2.5% in the no transfusion group.

Primary outcome: Grade 2–4 catheter-related bleeding within 24 h of CVC placement assessed according to this bleeding scale.

Secondary outcomes: Grade 1 or >Grade 3 bleeding, Platelet/red cell transfusions within 24 h, Hb/platelet count at 1 + 24 h, allergic transfusion reactions, TRALI, length of ICU/hospital stay, inpatient mortality, financial costs.

Bleeding Grade	Definition
0	Nil
1	Oozing, haematoma, <20 min compression
2	Requiring minor intervention/>20 min compression
3	Radiological or elective intervention required, or RBC transfusion
4	Haemodynamic instability, increased red cell transfusion or death.

1.1 | Summary of study findings

Grade 2–4 bleeding occurred in 4.8% of the transfusion group, and 11.9% of the non-transfusion group. Most bleeding was Grade 2, with no Grade 4 events. In subgroup analysis, the bleeding risk was higher for haematology inpatients than on ICU, and for subclavian CVCs (Table 1).

1.2 | Limitations

- The authors state that 18% of inpatients in the Netherlands have a CVC inserted, which is higher than in the UK.[9] Subclavian CVCs accounted for 37% of lines inserted. The increased bleeding risk associated with these represented the majority of excess bleeding in this study, so findings may not be generalisable to countries where this site is not commonly used.
- The majority of bleeding in this study was Grade 2, which settled with conservative measures. It is unclear whether this is clinically significant and offsets the risks and financial costs of transfusion.
- The authors conclude that they would consider giving platelet transfusions prior to CVC insertion for patients with platelets <30 $\times$ 10^9/L. It is unclear how this threshold was selected, given the numbers in the subgroup analysis were small and the effect of withholding transfusion in patients with platelet counts 10–20 $\times$ 10^9/L was not significant. They also suggest platelet transfusions should be considered for tunnelled line insertion, despite there being no evidence of a reduction in bleeding risk in this context.
- The overall rate of bleeding was higher than in other studies, likely due to its prospective nature and the site of CVC insertion.
- Further limitations of this study are discussed in this letter.[10]

1.3 | Evidence in context

This is the first RCT of platelet transfusions prior to invasive procedures.

TABLE 1 Results.

Outcome	Transfusion Events/total (%)	No transfusion Events/total (%)	Relative risk (95% CI)
Primary outcome			
Grade 2–4 catheter-associated bleeding.	9/188 (4.9)	22/185 (11.9)	2.45 (1.27–4.70)
Subgroup analysis			
Type of catheter			
Tunnelled	3/20 (15)	3/18 (16.7)	1.26 (0.25–6.34)
Non-tunnelled	6/168 (3.6)	18/167 (10.8)	3.01 (1.2–7.55)
Line insertion site			
Internal Jugular Vein	7/93 (7.5)	6/93 (6.5)	0.93 (0.31–2.7)
Subclavian Vein	2/71	13/70	6.19 (1.39–27.64)
Femoral Vein	0/24	2/22 (9.1)	3.72 (0.38–36.52)
Hospital department			
ICU	3/80 (3.8)	4/81 (4.9)	1.36 (0.30–6.11)
Haematology	6/108 (5.6)	18/104 (17.3)	2.99 (1.19–7.54)
Platelet count			
10–19 $\times$ 10^9/L	7/45 (15.6)	9/41 (22)	1.30 (0.48–3.55)
20–29 $\times$ 10^9/L	0/46	8/51 (15.7)	7.53 (0.91–62.5)
30–39 $\times$ 10^9/L	1/59 (1.7)	3/51 (5.9)	3.9 (0.41–37.05)
40–50 $\times$ 10^9/L	1/38 (2.6)	2/42 (4.8)	1.68 (0.15–18.68)

1.4 | Implications for future research

The authors suggest further research is needed to explore whether we should consider giving multiple platelet transfusions in patients with a platelet count <20 × 10^9/L.

An alternative study design, such as the ongoing T4P study (IRAS ID: 312405) may be required to explore what a safe platelet count is for invasive procedures.

1.5 | Implications for practice

This study applies only to CVC insertion, not to other invasive procedures.

The authors conclude that haematology patients with platelet counts <30 × 10^9/L should have a platelet transfusion pre-CVC insertion, and that ICU patients having non-tunnelled lines may be able to omit platelet transfusion.

I consider that this study confirms that thrombocytopenia is a risk factor for bleeding post-CVC insertion, and that platelet transfusions may help to mitigate this in some patients, but it does not tell us what the threshold should be for platelet transfusion in order to prevent bleeding. Ultrasound use and patient, operator and clinical site selection remain vital for safe CVC insertion.

CONFLICT OF INTEREST STATEMENT

The author has no competing interests.

DATA AVAILABILITY STATEMENT

None.

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ORIGINAL ARTICLE

TRANSFUSION MEDICINE WILEY

Cost analysis of care and blood transfusions in patients with Major Obstetric Haemorrhage in Ireland

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Abstract

Background and Objectives: Obstetric haemorrhage is the leading cause of maternal morbidity and mortality worldwide. We aimed to estimate the economic cost of Major Obstetric Haemorrhage (MOH) and the cost of therapeutic blood components used in the management of MOH in Ireland.

Materials and Methods: We performed a nationwide cross-sectional study utilising top-down and bottom-up costing methods on women who experienced MOH during the years 2011–2013. Women with MOH were allocated to Diagnostic Related Groups (DRGs) based on the approach to MOH management (MOH group). The total number of blood components used for MOH treatment and the corresponding costs were recorded. A control group representative of a MOH-free maternity population was designed with predicted costs. All costs were expressed in Euro (€) using 2022 prices and the incremental cost of MOH to maternity costs was calculated. Cost contributions are expressed as percentages from the estimated total cost.

Results: A total of 447 MOH cases were suitable for sorting into DRGs. The estimated total cost of managing women who experienced MOH is approximately €3.2 million. The incremental cost of MOH is estimated as €1.87 million. The estimated total cost of blood components used in MOH management was €1.08 million and was based on an estimated total of 3997 products transfused. Red blood cell transfusions accounted for the highest contribution (20.22%) to MOH total cost estimates compared to other blood components.

Conclusions: The total cost of caring for women with MOH in Ireland was approximately €3.2 million with blood component transfusions accounting for between one third and one half of the cost.

KEYWORDS

cost analysis, obstetric haemorrhage, severe maternal morbidity, transfusion

1 | INTRODUCTION

Obstetric haemorrhage is a global health challenge with Major or Massive Obstetric Haemorrhage (MOH) causing significant maternal morbidity and mortality.[1] In the Republic of Ireland, MOH is defined as: blood loss of at least 2500 mL or transfusion of five or more units of blood or documented treatment for coagulopathy.[2] MOH is the largest contributor to Severe Maternal Morbidity (SMM) in Ireland.[2] A recent study has demonstrated that the rate of MOH in Ireland has increased from 2.4 to 3.7 cases per 1000 deliveries between the years 2011 and 2018.[3] It is difficult to compare worldwide trends of MOH due to differences in MOH definitions; but, studies have reported incidences up to 13.3 per 1000 births.[4]

Blood and its components are utilised in the management of obstetric haemorrhage; however, they are a scarce health resource.[5,6] The reduced availability of therapeutic blood components can be attributed to a decline in suitable donor pools and increasing demands from many different clinical services.[6] Furthermore, the cost of producing allogenous blood components is rising due to cost escalations in blood collection, safe processing, storage and distribution.[7] Though the majority of blood transfusions are safe, they also carry the risk of reactions, infection and alloimmunization which may impact future pregnancies.[8] Therefore, interventions aimed at reducing the rates of maternal haemorrhage and blood transfusions are of primary concern.

With rising healthcare costs, costing studies can be useful in guiding clinical and policy decision.[9] To date, the health economic burden of MOH in Ireland is unknown. Identifying the current economic impact of MOH in Ireland would provide a useful baseline for future cost-effectiveness studies on obstetric haemorrhage prevention and care strategies. This study aims to estimate the cost of MOH in Ireland and to estimate the cost contribution of blood components in MOH management.

2 | METHODS

2.1 | Study design

A top-down costing approach using Diagnostic Related Groups (DRGs) was performed to estimate the cost of MOH management in Ireland in conjunction with a study on MOH incidence and management in Irish maternity units.[3] All costs are expressed in Euro (€) according to the year 2022. The study assumes the perspective of the healthcare system and only costs to the Health Service Executive (HSE) were included. The DRG system categorises patients into clinically similar groups that are expected to consume a similar level of resources. Currently, the HSE uses the Australian Redefined DRG system (version 8) to determine hospital activity and guide hospital funding. The DRGs cost estimates vary according to hospital stay duration and inpatient inlier prices represent the cost estimate based on the average length of hospital stay. The DRGs costs were obtained from the Healthcare Pricing Office (HPO) admitted patient price list for the year 2022. All DRGs used in this study and their corresponding prices are listed on Table 1.

2.2 | Data collection

Relevant information on the management and care of women with MOH were obtained from the SMM database, for the years 2011 to 2013 inclusive, from the National Perinatal Epidemiology Centre (NPEC) in Ireland. Women with MOH (MOH group) were sorted in to DRGs according to the management of MOH (Figure 1A). Women with missing MOH management details were excluded from DRG allocation. A control group was designed to represent a MOH-free maternity population based on the published incidence rates of miscarriage hospitalisation (63.0 per 1000 maternities), ectopic pregnancy (14.6 per 1000 maternities) and Caesarean delivery (36.6% of deliveries) in Ireland (Figure 1B).[10,11] For the purpose of comparison, the size of the control group equalled the total

TABLE 1 Inpatient inlier costs according to Diagnostic Related Groups (DRG) for Major Obstetric Haemorrhage (MOH) and control groups.

Study group	DRG code	DRG description	Cost (€)[a]
MOH	O01A	Caesarean delivery, major complexity	11 158
	O01B	Caesarean delivery, intermediate complexity	5353
	O02A	Vaginal delivery in operating theatre, major complexity	8055
	O03A	Ectopic pregnancy, major complexity	5977
	O04A	Postpartum and post abortion in operating theatre, major complexity	10 534
	O04B	Postpartum and post abortion in operating theatre, minor complexity	3560
	O60A	Vaginal delivery, major complexity	5276
Control	O01C	Caesarean delivery, minor complexity	3934
	O03B	Ectopic pregnancy, minor complexity	3561
	O60C	Vaginal delivery, minor complexity	2584
	O61B	Postpartum and post abortion, minor complexity	1373

[a]Healthcare Pricing Office activity-based funding admitted patient price list, 2022.

number of MOH group. In the control group, the early pregnancy cases were excluded when calculating the number of vaginal and Caesarean deliveries. All control group cases were sorted into their appropriate DRGs (Figure 1B). All costs were based on inpatient inlier prices as patient hospital stay durations were not included in the SMM database produced by NPEC for the years studied. The estimated incremental cost of MOH was calculated by subtracting the total cost of the control group from the total cost of the MOH group.

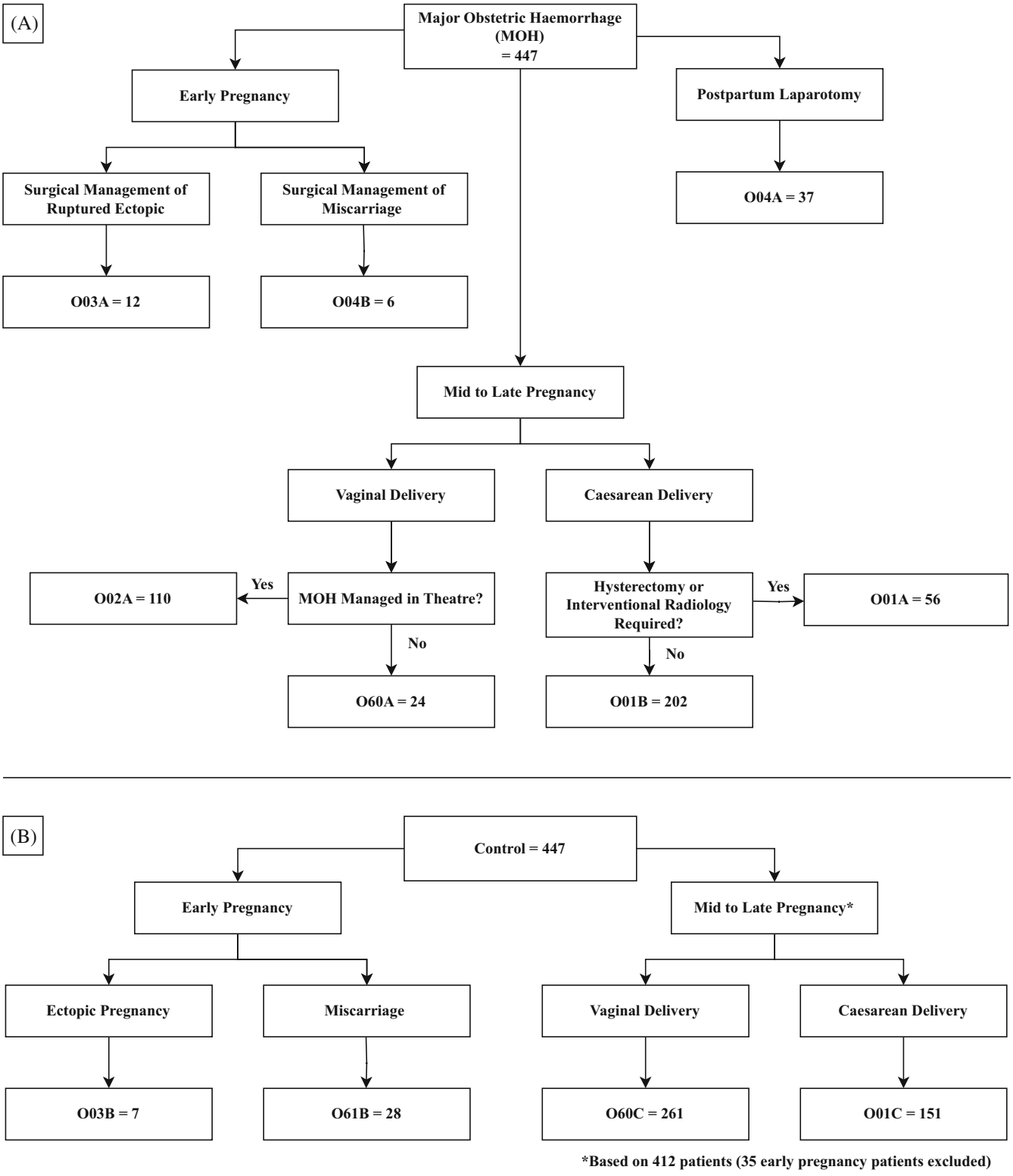

FIGURE 1 Patient sorting algorithm according to Diagnostic Related Groups (DRGs) for Major Obstetric Haemorrhage (MOH) patients (A) and control groups (B).

In order to determine the contribution of blood products to total cost we used bottom-up costing. We recorded the total number of therapeutic blood components for red blood cells, plasma, platelets, fibrinogen and Octaplas (Octapharma, Anderlecht, Belgium) used for MOH management. The cost of therapeutic blood components were obtained from the Irish Blood Transfusion Service on September 2022.

2.3 | Data analysis

Cost contributions are expressed as percentages from the total estimated cost for the 3 years. Data was recorded using Microsoft Excel 2019 (Microsoft Corporation, Redmond, WA, USA). Descriptive statistics were performed using SPSS 24 (IBM Corporation, Armonk, NY, USA). We utilised a median imputation method when blood component information was missing in the provided NPEC dataset (i.e., we replaced missing blood product quantities with the median number of blood products of the available data).

TABLE 2 Cost estimates (€) according to Diagnostic Related Groups (DRGs) of Major Obstetric Haemorrhage (MOH) and control groups.

Study Group	DRGs	Frequency (*n*)	DRG Size (%)	DRG cost (€)	DRG cost contribution (%)
MOH	01A	56	11.7	624 848	19.5
	01B	202	42.3	1 081 306	33.8
	02A	110	23.1	886 050	27.7
	03A	12	2.5	71 724	2.2
	04A	37	7.8	389 758	12.2
	04B	6	1.3	21 360	0.7
	60A	24	5.0	126 624	4.0
MOH totals		447	100.0	3 201 670	100.0
Control	O01C	151	33.8	594 034	44.6
	O03B	7	1.6	24 927	1.9
	O60C	261	58.4	674 424	50.6
	O61B	28	6.3	38 444	2.9
Control totals		447	100.0	1 331 829	100.0

3 | RESULTS

A total of 455 MOH cases were registered in the NPEC database of SSM for the years 2011 to 2013. Only 447 women were suitable for sorting into DRGs as eight women had missing MOH management information. The largest DRG identified was O01B (42.3%, Caesarean delivery without hysterectomy/interventional radiology) followed by O02A (23.1%, vaginal delivery in operating theatre) and O01A (11.7%, Caesarean delivery with hysterectomy/interventional radiology). The smallest DRG found was O04B (1.3%, surgical management of early pregnancy miscarriage).

The estimated total cost of care of the MOH group for the years studied is €3 201 670 (Table 2). The total cost was €1 602 737 in 2011; €1 054 519 in 2012 and €1 084 414 in 2013 based on 2022 prices. The largest DRG contributing to the total cost was O01B (33.8%, Caesarean delivery without hysterectomy/interventional radiology), followed by O02A (27.7%, vaginal delivery in operating theatre) and O01A (19.5%, Caesarean delivery with hysterectomy/interventional radiology). The DRG that contributed the least to the total MOH cost was O04B (0.7%, surgical management of early pregnancy miscarriage).

We projected that a control group of 447 women would include 28 miscarriages; 7 ectopic pregnancies; 151 Caesarean deliveries and 261 vaginal births based on published Irish incidence rates. Assuming no MOH occurs in this group, the total estimated cost of care would amount to €1 331 829. Therefore, the incremental cost of MOH is estimated to be €1 869 851.

An estimated total of 3997 units of therapeutic blood components were administered during the 3 years (Table 3). The estimated

TABLE 3 Costs (€) of therapeutic blood components utilised for the management of Major Obstetric Haemorrhage (MOH).

Blood component	Component code/description	Product cost (€)	Total number transfused (*n*) [with missing data imputation]	Total cost (€) [with missing data imputation]	Cost contribution (%) [with missing data imputation]
Red blood cells	E7429V00	295	2196 [2446]	647 820 [721570]	20.22 [22.54]
Fresh frozen plasma	E4048V00	133.28	373 [937]	49713.44 [124883.36]	1.55 [3.9]
Platelets	X000200	640	207 [474]	132 480 [303360]	4.14 [9.48]
Fibrinogen	Riastap 1g	532.40	466 [908]	248098.4 [483419.20]	7.75 [15.1]
Octoplas	X000200	0.58	755 [1406]	437.9 [815.48]	0.01 [0.03]
Total			3997 [6171]	1078549.74 [1634048.04]	33.66 [51.04]

total cost of blood components used for the years studied was €1078549.74 which amounts to one third (33.66%) of the estimated total cost of care for women with MOH. Red blood cell transfusions accounted for the highest contribution (20.22%) to MOH total cost estimates compared to other blood components. However, approximately 10% of women had missing information on red blood cell transfusions and between 45% and 60% of women had missing details on other blood components utilised. Based on the observed data, we calculated a median of five units (range 1–24) of red blood cells, two units of plasma (range 0–16), one unit of platelets (range 0–8), two units of fibrinogen (range 2–10) and 3 units of Octoplas (range 0–23). By replacing corresponding missing blood component data with median values, we predict that missing data could contribute an additional 2174 units or an additional €555498.3 to the observed total cost of blood components. Through this model, overall blood product costs represent approximately half of MOH total cost estimates (51.04%), and red blood cell transfusions remain the highest contributor to MOH total cost estimates compared with other blood components (22.54%).

4 | DISCUSSION

4.1 | Main findings

Micro-costing is a methodology which uses unit cost data and information on resources utilised to generate precise cost estimates.[12] It can be particularly useful for estimating the cost of healthcare in scenarios where there is large variability across patients or providers and for estimating the cost to the health system and to society.[12] We estimated using DRGs the total cost of care of women with MOH, and calculated the total price to be €3.2 million for the years 2011–2013. We estimated that MOH contributed an additional €1.87 million or 140% to maternity care. We also approximate that one third, and potentially up to half, of the costs are attributed to blood component transfusions. While further studies are needed to identify reasons for the increase in MOH rates in Ireland, we discuss possible MOH cost reduction strategies.

4.2 | Implications

There is an association between MOH and *emergency* Caesarean section deliveries.[3,4] MOH occurred twice as common in emergency Caesarean sections than elective Caesarean sections in the Irish MOH case review.[3] A review of 199 cases of Caesarean sections at full dilatation identified 50% of women experiencing blood loss >500 mL with seven women having blood loss greater than 1500mL.[13] Another review of 184 cases of blood loss greater than 2000 mL identified the majority of women (55%) delivered by emergency Caesarean section; and the study found no difference in the maternal characteristics (including age, ethnicity, parity, body mass index) and obstetric practices in women with 2000–3000 mL and greater than 3000 mL blood loss.[14] A nationwide cohort study in the Netherlands assessing laparotomy in women with SMM identified that the main proportion (68.4%) of postpartum laparotomies were due to obstetric haemorrhage and that the risk of postpartum laparotomy was 16 times higher in Caesarean births than vaginal births.[15] The absolute risk of postpartum laparotomy was higher in emergency Caesarean deliveries than planned Caesarean deliveries (39.5 and 19.1 per 10 000 births respectively).[15] In our study, 64% of MOH cases had Caesarean delivery (codes O01A and O01B), but the DRG sorting did not consider delivery urgency. Postpartum laparotomy (code O04A) was the fourth highest (12.2%) contributor to MOH costs in our study. It is unclear whether it is the procedure itself or the indication for emergency Caesarean delivery that contributes to MOH. However, extra care and situational awareness should be considered by delivery teams dealing with emergency Caesarean births and that routine senior clinician presence at these cases may have beneficial cost implications.

In Canada, the incidence of severe maternal haemorrhage have declined although the cause is unclear.[16] Possible explanations could include the increased use of tranexamic acid, an antifibrinolytic.[16] The negotiated cost of tranexamic acid for intravenous administration in our hospital is €2.23 per 1 g ampule and we predict this cost to be similar across Ireland. The TRAAP2 clinical trial comparing tranexamic acid with placebo in women undergoing Caesarean section found 26.7% of women in tranexamic acid group and 31.6% of women in the placebo group experience blood loss greater 1000 mL or receive a red cell transfusion.[17] A cost-effectiveness analysis on tranexamic acid demonstrated that it is a cost effective method in reducing maternal morbidity and mortality due to haemorrhage.[18] The E-MOTIVE trial incorporated tranexamic acid as part of a first response bundle for treating haemorrhage following vaginal delivery which resulted in a 60% reduction in a composite primary outcome of blood loss greater than 1000 mL, laparotomy for bleeding and maternal death from bleeding when compared to usual care.[19] Therefore, its use should be encouraged in early first-line treatment rather than a late adjunct treatment of suspected obstetric haemorrhage.

MOH is also associated with abnormal placentation like placenta previa or placenta accreta spectrum (PAS).[20] There is an increase in the incidence of PAS thought to be the result of a similar increase in the rate of Caesarean deliveries.[21] A review of 77 PAS cases identified a mean blood loss of 3000 mL with 10 women having blood loss exceeding 10000 mL.[22] A 22-year review on massive blood transfusions identified that women with PAS are at 41-fold increased risk of receiving 10 or more units of red blood cells.[23] Furthermore, women with PAS and prior Caesarean delivery are at 4-fold increased risk of massive blood transfusion.[23] Management of women with PAS should be reserved to centres of excellence with an experienced multidisciplinary team.[24] Antenatally diagnosed women with PAS managed in centres of excellence experience lower emergency surgery rates, are less likely to need massive blood transfusion and suffer lower bleeding complications compared with women managed without specific protocols.[24,25]

Quality improvement programmes in Wales have reduced the incidence of MOH from 6.4 to 4.9 per 1000 maternities.[26] These

initiatives included obstetric blood loss risk assessment, accurate blood loss quantification, prompt escalation to senior clinicians and use of point-of-care testing for targeted blood component therapy.[26] In addition, intraoperative cell salvage is effective in reducing donor blood transfusion during Caesarean section; however, its routine use in all Caesarean deliveries is not cost-effective and should be reserved for cases at high risk of obstetric haemorrhage.[27,28]

4.3 | Strengths and limitations

This study estimates the cost of MOH management in Ireland from a healthcare perspective and identifies the distribution costs of by analysing MOH subgroups. We designed a control group to best reflect a maternity population free of MOH in Ireland and used published incidence rates to mitigate selection bias within the control group. This approach allows for the incremental cost of MOH to be estimated relative to a maternity population rather than a specific pregnancy outcome.

The SMM database relied on the voluntary participation of maternity units, which might have affected how thoroughly cases were identified and recorded. The accuracy of our cost estimates is limited by missing information from the SMM database (1) on antenatal care such as antenatal admissions for antepartum haemorrhage, (2) maternal morbidities like pre-eclampsia or bleeding disorders (3) and hospital stay durations. Our control group also presumed no other maternal morbidities. This affects case complexities and sorting into DRGs and subsequent cost estimates. Another limitation is missing blood component information which would influence the total cost attributed to blood component transfusions. We used median imputation as it was uncertain whether data was missing at random or due to a systematic reason (i.e., data missing because the blood product was not given, possibly evidenced by the significant missing data for other blood products—they might not have been used so no data was inputted). Furthermore, transfusion practices could vary between clinicians and maternity units in Ireland. Therefore, our initial blood component cost estimates without imputation are likely to be conservative. Future studies would also incorporate estimates of the long term health service costs following MOH and study the economic impact of novel MOH management strategies to the government.

5 | CONCLUSION

This was the first study to describe the economic burden of MOH and provides information on reducing the cost of MOH and maternal blood transfusions in Ireland. The total cost of care of women with MOH during the years 2011 to 2013 was approximately €3.2 million with blood component transfusions accounting for between one third and one half of the cost. Care plans targeting at risk groups like emergency Caesarean section and women with PAS may result in reduced MOH incidence and blood transfusions and provide cost saving.

AUTHOR CONTRIBUTIONS

All authors have contributed equally to the development of the work.

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CONFLICT OF INTEREST STATEMENT

AL is a doctoral candidate at University College Cork. Data presented in this work will be submitted in partial fulfilment of the doctorate. All other authors have no relevant conflicts of interest to declare.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the National Perinatal Epidemiology Centre (NPEC) but restrictions apply to the availability of these data, which were used under licence for the current study, and so are not publicly available. Data are however available from the authors upon reasonable request and with permission of National Perinatal Epidemiology Centre (NPEC), University College Cork, Cork, Ireland.

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ORIGINAL ARTICLE

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Lipidomic changes occurring in platelets during extended cold storage

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Abstract

Objectives: Cold storage is being implemented as an alternative to conventional room-temperature storage for extending the shelf-life of platelet components beyond 5–7 days. The aim of this study was to characterise the lipid profile of platelets stored under standard room-temperature or cold (refrigerated) conditions.

Methods: Matched apheresis derived platelet components in 60% PAS-E/40% plasma ($n = 8$) were stored at room-temperature (20–24°C with agitation) or in the cold (2–6°C without agitation). Platelets were sampled on day 1, 5 and 14. The lipidome was assessed by ultra-pressure liquid chromatography ion mobility quadrupole time of flight mass spectrometry (UPLC IMS QToF). Changes in bioactive lipid mediators were measured by ELISA.

Results: The total phospholipid and sphingolipid content of the platelets and supernatant were 44 544 ± 2915 μg/mL and 38 990 ± 10 880 μg/mL, respectively, and was similar over 14 days, regardless of storage temperature. The proportion of the procoagulant lipids, phosphatidylserine (PS) and phosphatidylethanolamine (PE), increased by 2.7% and 12.2%, respectively, during extended cold storage. Cold storage for 14 days increased sphingomyelin (SM) by 4.1% and decreased ceramide by 1.6% compared to day 1. Further, lysophosphatidylcholine (LPC) species remained unchanged during cold storage for 14 days. The concentration of 12- and 15-hydroxyeicosatetraenoic acid (HETE) were lower in the supernatant of cold-stored platelets than room-temperature controls stored for 14 days.

Conclusion: The lipid profile of platelets was relatively unchanged during storage for 5 days, regardless of temperature. However, during extended cold storage (14 days) the proportion of the procoagulant lipids, PS and PE, increased, while LPC and bioactive lipids were stable.

KEYWORDS
lipid, platelet, transfusion

1 | INTRODUCTION

Conventionally, platelet concentrates are stored at room-temperature (RT) with constant agitation for up to 7 days.[1] The shelf-life is imposed in an effort to reduce the risk of bacterial proliferation and the effects of the platelet storage lesion (PSL).[2] Despite this, platelets still undergo a degree of storage associated activation. Current storage conditions also present issues for maintaining the supply of platelets in remote locations.

There is renewed interest in cold-stored (refrigeration at 2–6°C) platelets, as the increased shelf-life and improved haemostatic potential afforded by cold storage may outweigh the reduction in circulation time,[3,4] particularly if reserved for the treatment of active bleeding.[5] Cold-stored platelet components can currently be stored for up to 14 days due to the reduced risk of bacterial proliferation and reduction in metabolic rate.[6–10] During this time, cold storage induces changes to the membrane, including glycoprotein clustering, extracellular vesicle (EV) formation and phosphatidylserine (PS) externalisation.[11–13] Despite a growing body of evidence describing the impact of cold storage on platelet components, only a few historical studies have examined the lipidome.[14,15]

Previous lipidomic studies have examined platelet components stored in 100% plasma at both RT and cold temperatures.[14–18] However, it is necessary to provide an updated perspective that reflects current transfusion practices, including the use of platelet additive solution and storage beyond 7 days. Storage for 5–7 days at RT in 100% plasma resulted in a decrease in the total platelet lipid content and altered the overall composition of phospholipid, sphingolipid and cholesterol classes.[16,17] These lipid classes are the major constituents of the platelet lipid membrane and are the substrates for the formation of many bioactive lipid mediators, including lysophosphatidylcholine (LPC), ceramide (Cer), sphingosine 1-phosphate (S1P) and arachidonic acid derived lipid eicosanoids (5-hydroxyeicosatetraenoic acid (HETE), 12-HETE and 15-HETE).[19,20] As such, an assessment of the lipidomic changes resulting from cold storage was undertaken with a specific focus on the phospholipid and sphingolipid profile and their derived bioactive lipid mediators.

2 | METHODS

Ethics approval was obtained from the Australian Red Cross Lifeblood Research Ethics Committee prior to the commencement of this study. All donations were obtained from eligible, voluntary, non-remunerated blood donors.

2.1 | Preparation of platelet concentrates

Double-dose, leukoreduced apheresis platelet components ($n = 8$) were collected using a Trima Accel apheresis system (TerumoBCT, Lakewood, CO, USA), and stored in 40% plasma and 60% PAS-E (SSP +, Macopharma, Tourcoing, France). The platelet components were split into single dose equivalents, and the matched components were randomly assigned for storage at either RT (20–24°C) with constant agitation (Helmer Inc. Noblesville, IN, USA) or refrigerated (2–6°C) without agitation. The platelet components were aseptically sampled on day 1 (prior to allocation to storage temperature), 5 and 14 post collection, by docking on a sample pouch with a sterile-welding device. Prior to sampling, the cold-stored components were placed on an agitator at RT for 10 min to ensure that any platelets that had settled during storage were homogenously mixed.

2.2 | Analysis of platelet concentrates

The platelet count was determined using a haematology analyser (CELL DYN Ruby, Abbott Laboratories, Chicago, IL, USA). All platelet components were assessed for the presence of swirl and macroaggregates by visual inspection.[13]

The externalisation of phosphatidylserine was assessed by staining platelets with annexin-V and the percentage of positive cells were determined by flow cytometry (FACSCanto II, Becton Dickson, Franklin Lakes, NJ, USA).[11]

2.3 | Preparation of platelet and supernatant fractions

A standard number of platelets (500×10^6 platelets) were transferred to a 1.5 mL tube and centrifuged (Eppendorf 5415D; Eppendorf, Germany) at $1500 \times g$ for 15 min at RT to isolate the platelets. The supernatant was transferred to a new tube and the platelet pellet was stored at −80°C until analysis. The supernatant was then subjected to multiple, sequential rounds of centrifugation ($1500 \times g$ for 15 min at RT, $14000 \times g$ for 2 min at 4°C and $16\,000 \times g$ for 30 min at 4°C), with the supernatant being transferred to a new 1.5 mL tube between spins. The final supernatant was transferred to a new 1.5 mL tube and frozen and stored at −80°C until analysis.

2.4 | Lipid extraction and mass spectrometry

Lipids were extracted by methods adapted from Matyash et al.[21] A detailed description is presented in Supplementary Materials 1, but briefly, lipids were extracted from platelets (500×10^6) and supernatant (50 µL) by resuspension in methanol and addition of methyl tert-butyl ether (MTBE). Phase separation was induced by the addition of ddH_2O and the upper organic phase, containing lipids, was transferred to clean glass auto sampler vials. Global lipidomic analysis was conducted using an ACQUITY™ ultra-pressure liquid chromatography™(UPLC) I-Class system (Waters Corporation, Milford, MA, USA) coupled to a Vion Ion Mobility Spectrometry (IMS) Quadrupole Time-of-Flight (QToF) Mass Spectrometer (MS) (Waters Corporation, Milford, MA, USA) in an untargeted data-independent acquisition (DIA) manner. A detailed description of the chromatographic conditions and UPLC IMS QToF settings are presented in Supplementary Materials 1. Mass chromatograms were deconvoluted and data was normalised

using Progenesis QI software version 2.3 (Nonlinear Dynamics, a Waters Company, Newcastle upon Tyne, UK), according to manufacturer's workflow. Lipids were reported as normalised relative abundance, determined by the comparison of ion intensities of the internal standard, EquiSPLASH LIPIDOMIX Quantitative Mass Spectrometry Internal Standard (Avanti Polar Lipids, Alabaster, AL, USA), with a known concentration of lipid (μg/mL), or normalised to sum of ion content within a respective class (percentage %). Data are presented at the lipid species level as described by Liebisch et al.[22]

2.5 | Bioinformatics methodology for pathway analysis

Lipid pathway analysis was performed using the open access web-based tool, Bioinformatics Methodology For Pathway Analysis (BioPAN).[23] LC–MS/MS data was loaded into the platform and analysed according to the developer's workflow. BioPAN calculates a Z-score and determines if a given reaction is significant ($p < 0.05$). Further, a reaction is determined to be activated ($Z > 1.645$) or suppressed ($Z < -1.645$) depending on the direction of the change. The following options were selected; type: lipid, status: active, level: lipid subclass, subset of lipid data: reactions, p-value: 0.05, paired data: yes.

2.6 | Enzyme linked immunosorbent assays

The concentration of arachidonic acid (LSBio, Seattle, WA, USA), 5-HETE (LSBio), 12(S)-HETE (Abcam, Cambridge, UK), 15(S)-HETE (Abcam) and S1P (Echelon Bioscience, Inc., Salt Lake City, UT, USA) in the supernatant was determined using commercially available enzyme linked immunosorbent assay (ELISA) kits. All samples were tested in duplicate with the concentration determined from a standard curve.

2.7 | Statistical analysis

Results are expressed as mean ± standard deviation (SD). Data were analysed using GraphPad Prism (GraphPad Software Inc., Version 9, La Jolla, CA, USA). The effect of storage was assessed using a two-way repeated measures analysis of variance (ANOVA). Post hoc Bonferroni's multiple comparisons tests were performed to determine the differences between storage temperature (RT vs. cold-stored at day 5 and day 14) and time (day 1 vs. day 5 and day 1 vs. day 14). A p-value of less than 0.05 was considered statistically significant.

3 | RESULTS

The mean platelet concentration on day 1 was $1052 \pm 117 \times 10^9$/L. Although the platelet concentration decreased slightly during 14 days of storage to $953 \pm 123 \times 10^9$/L and $966 \pm 93 \times 10^9$/L under RT and cold storage conditions, respectively, there was no significant difference between the storage conditions ($p = 0.99$). Platelet swirl was apparent in all RT-stored components but not in cold-stored components. Macroscopic aggregates were not observed in any component.

3.1 | Characterisation of the phospholipid and sphingolipid profile of platelets

The effect of storage time and temperature on the lipid profile of platelets were assessed by UPLC IMS QToF. The total phospholipid and sphingolipid content of the day 1 platelets was 44 544 ± 2915 μg/mL and was similar over 14 days, regardless of whether the platelet components were stored at RT (48 101 ± 9704 μg/mL) or in the cold (44 405 ± 3908 μg/mL, $p = 0.99$).

Phosphatidylcholine (PC) is a major constituent of platelet lipid membranes and is the precursor for the bioactive lipid mediator, LPC, which has been associated with adverse transfusion reactions.[24–26] Compared to day 1, the percentage of PC was significantly lower in platelets after 14 days of cold storage, while the percentage of LPC was significantly lower in RT platelets at the same time point (Figure 1A). There were some shifts in PC species profile (Figure 1B), primarily at day 14 of cold storage. PC(34:2) and PC(36:3) were significantly higher in cold-stored components at day 14 compared to day 1, and compared to RT platelets at the same time point. In contrast, PC(36:1), PC(38:1) and PC(38:2) were significantly lower after 14 days of cold storage, compared to day 1. Only PC(38:3) increased during RT storage, at day 14. The LPC species profile was relatively stable during cold storage, with the exception of LPC(18:0), which was decreased at day 14, compared to day 1 (Figure 1C). In contrast, storage at RT resulted in a decrease in LPC(18:0), LPC(18:2), LPC(20:4) and LPC(22:4) by day 14. Conversely, LPC(20:0) and LPC(O-20:0) increased during RT storage.

The aminophospholipids, phosphatidylethanolamine (PE) and PS are structural membrane lipids that become externalised on the membrane surface to mediate coagulation, but also act a substrates for bioactive mediators and signalling molecules.[27] A significant increase (~4-fold; $p = 0.0051$) in the percentage of annexin-V positive platelets was observed at day 5 in the cold-stored platelets, indicative of increased phosphatidylserine externalisation (Figure 2A). However, by day 14 approximately 20% of platelets were annexin-V positive in both the cold and RT groups. Overall, the PS and PE lipid classes were significantly increased by cold storage at day 14, compared to day 1 (Figure 2B). In contrast, PEP was significantly decreased in platelets after 14 days of cold storage ($p = 0.0001$). In terms of specific species shifts, the most dramatic change was seen with PS(38:3), which increased significantly during cold storage, with a concomitant reduction during RT storage (Figure 2C). The proportion of PS(38:1) increased during storage in both groups, but the increase was greater during RT storage. Conversely, a time-related reduction in PS(40:1) and PS(40:3) was observed, to a similar degree in both the cold and RT platelets. Multiple differences were found in the PE species as a result of storage time and temperature (Figure 2D). In general, PE (34:2), PE(36:1), PE(36:2) and PE(40:4) increased during storage, but to a significantly higher extent in cold-stored components at day 14.

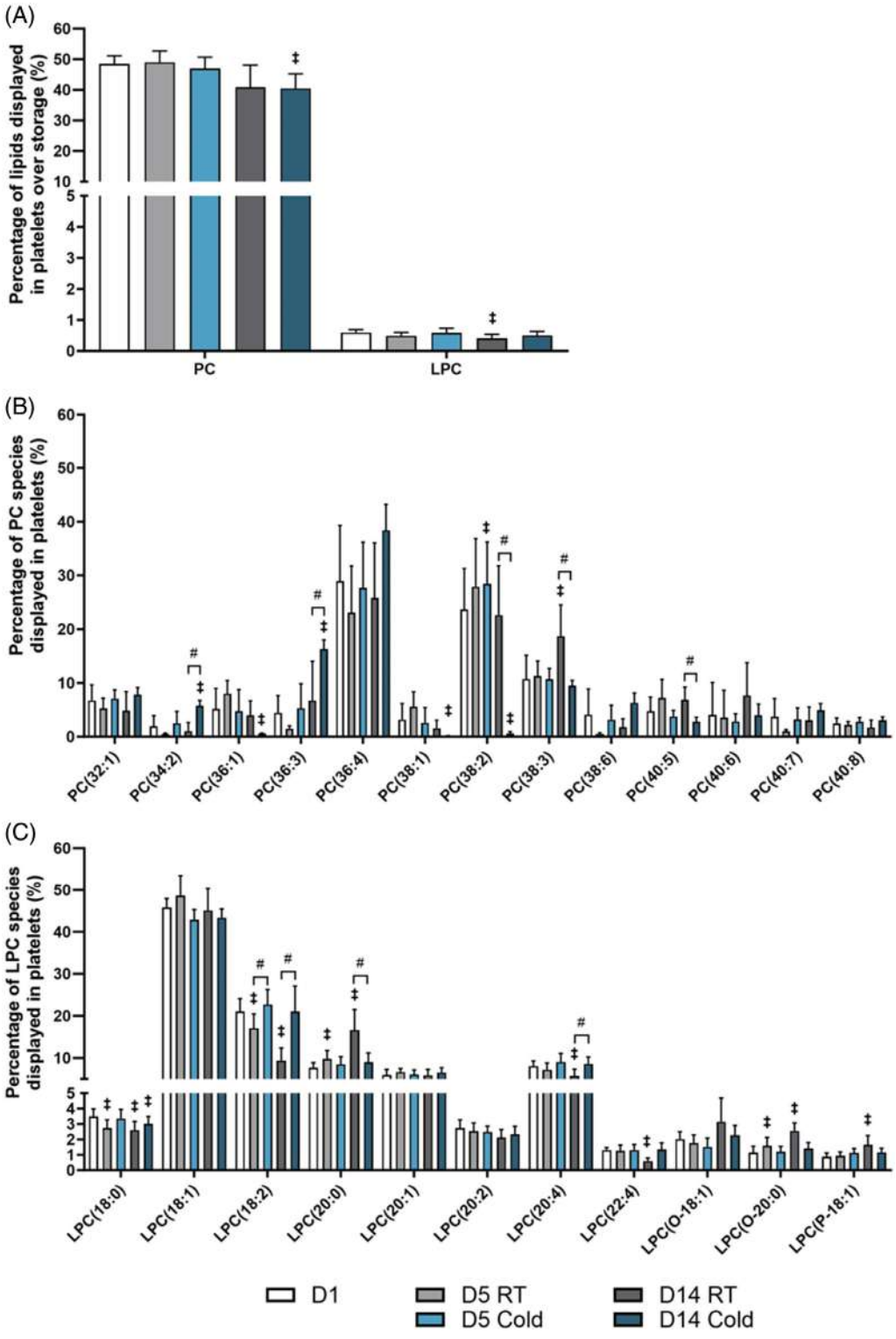


FIGURE 1 The effect of cold storage on phosphatidylcholine and lysophosphatidylcholine. Platelet components were stored at room-temperature (RT; 20–24°C) or cold-stored (cold; 2–6°C). The platelets were isolated by differential centrifugation, lipids were extracted and measured by UPLC IMS QToF. The percentage composition of (A) phosphatidylcholine (PC) and lysophosphatidylcholine (LPC) classes and (B) PC and (C) LPC lipid species are displayed. The data represents mean (bars) ± SD (error bars); $n = 8$. ‡$p < 0.05$ compared to day 1. # indicates $p < 0.05$ compared to RT at the same time point.

A time related increase in PE(38:4) was observed, with both temperature groups showing a significant increase at day 14, compared to day 1. PE(38:1) and PE(40:2) showed the opposite trend, with significant reductions observed at day 14, compared to day 1. In terms of PEP species, PE(P-40:1) represented the most abundant species, and only minor shifts in low abundance species were observed (Figure 2E).

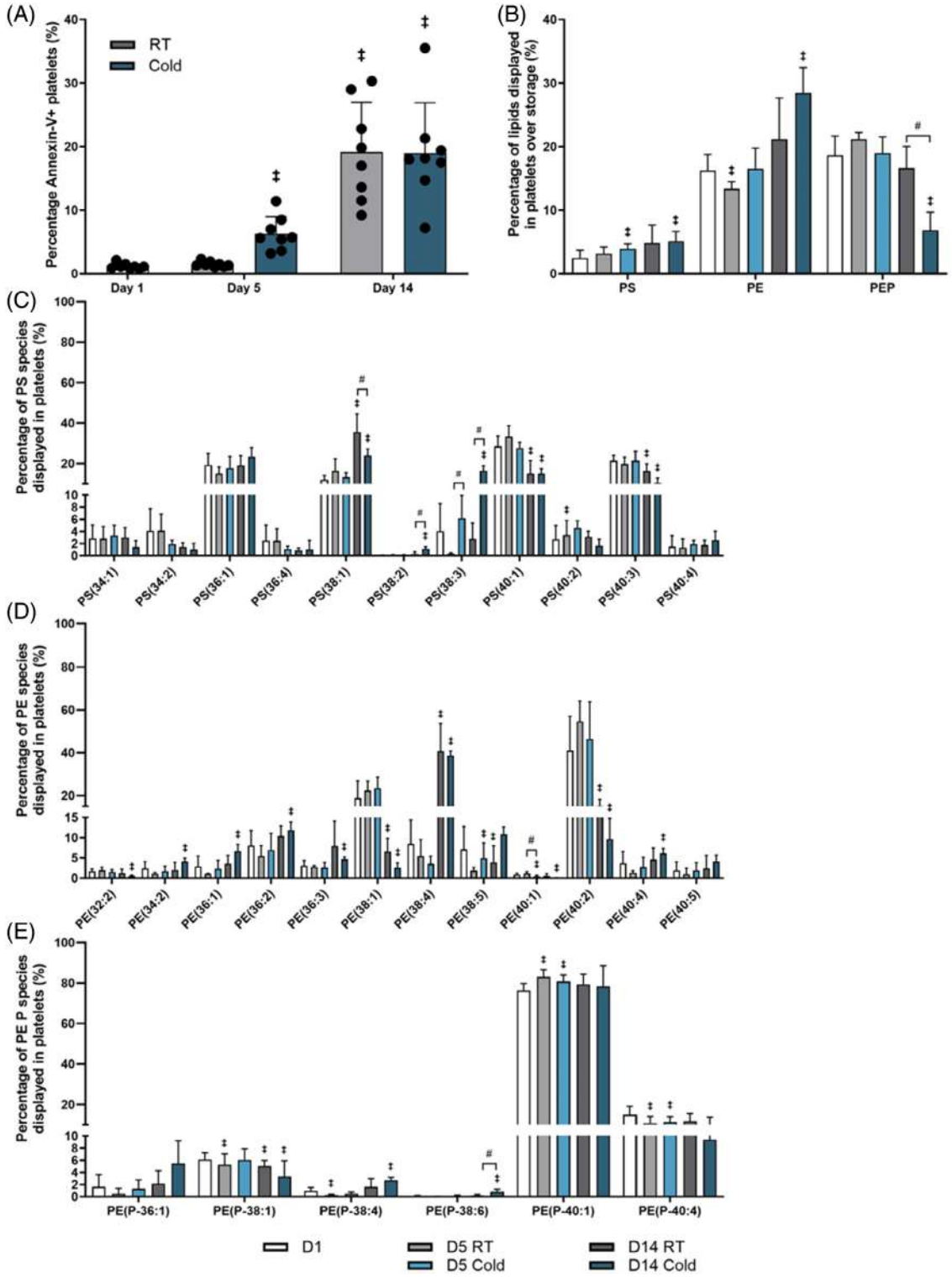


FIGURE 2 The effect of cold storage on phosphatidylserine, phosphatidylethanolamine and ether linked phosphatidylethanolamine. Platelet components were stored at room-temperature (RT; 20–24°C) or cold-stored (cold; 2–6°C). (A) Phosphatidylserine (PS) externalisation was measured by flow cytometric analysis of annexin-V (FITC) positive platelets. The platelets were isolated by differential centrifugation, lipids were extracted and measured by UPLC IMS QToF. The percentage composition of (B) PS, phosphatidylethanolamine (PE) and ether linked phosphatidylethanolamine (PEP) and lipid species of (C) PS, (D) PE and (E) PEP are displayed. The data represents individual data points, mean (bars) ± SD (error bars); $n = 8$. ‡$p < 0.05$ compared to day 1. # indicates $p < 0.05$ compared to RT at the same time point.

Sphingomyelin (SM) is a precursor to the second messengers and bioactive lipid mediators, ceramide, sphingosine and S1P.[28] SM was significantly increased during cold storage, compared to day 1, and to RT-stored platelets at day 14 (Figure 3A). In contrast, Cer was significantly decreased in cold-stored platelets after 14 days ($p = 0.0016$), while being significantly higher in platelets stored at RT. The

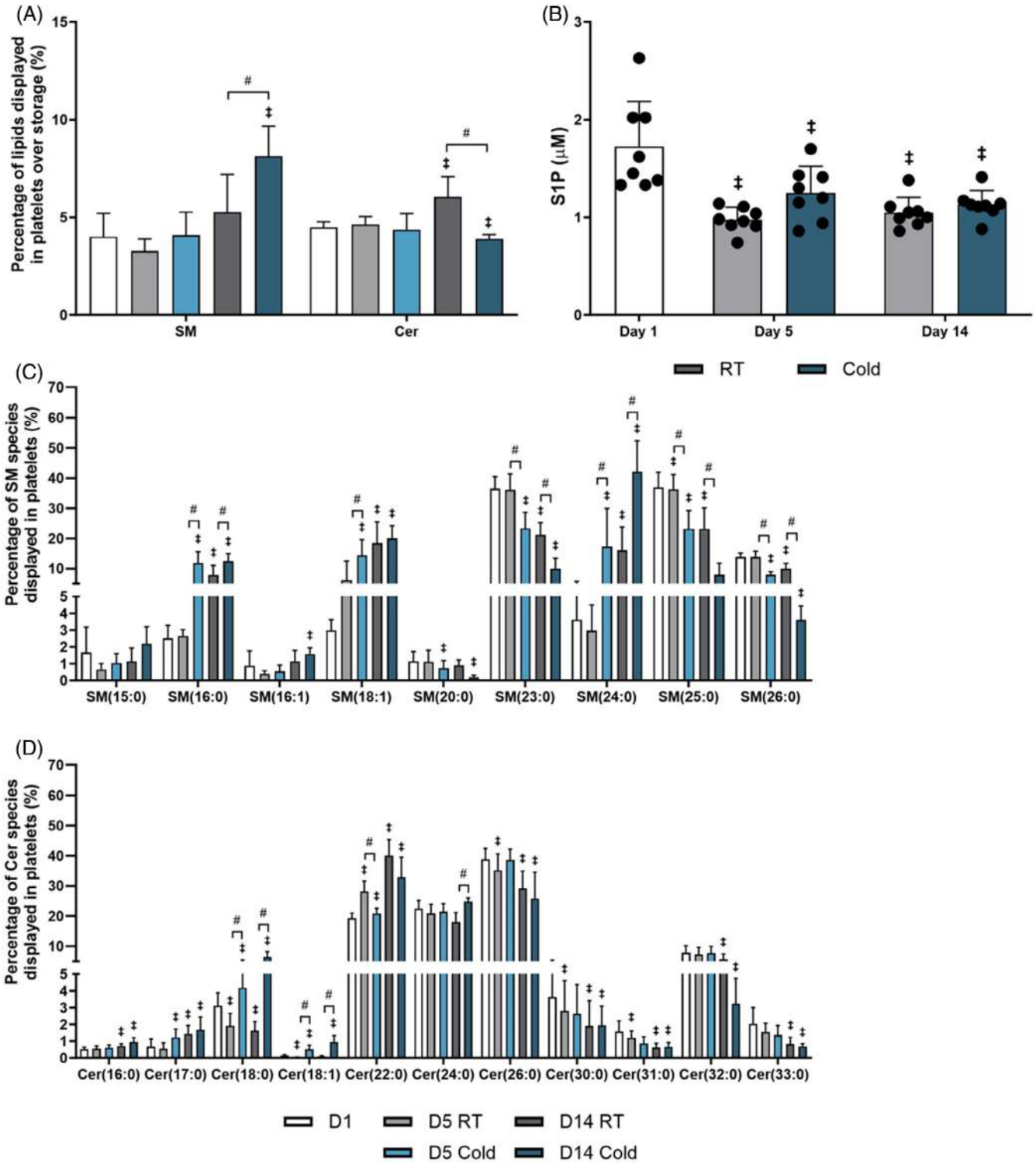


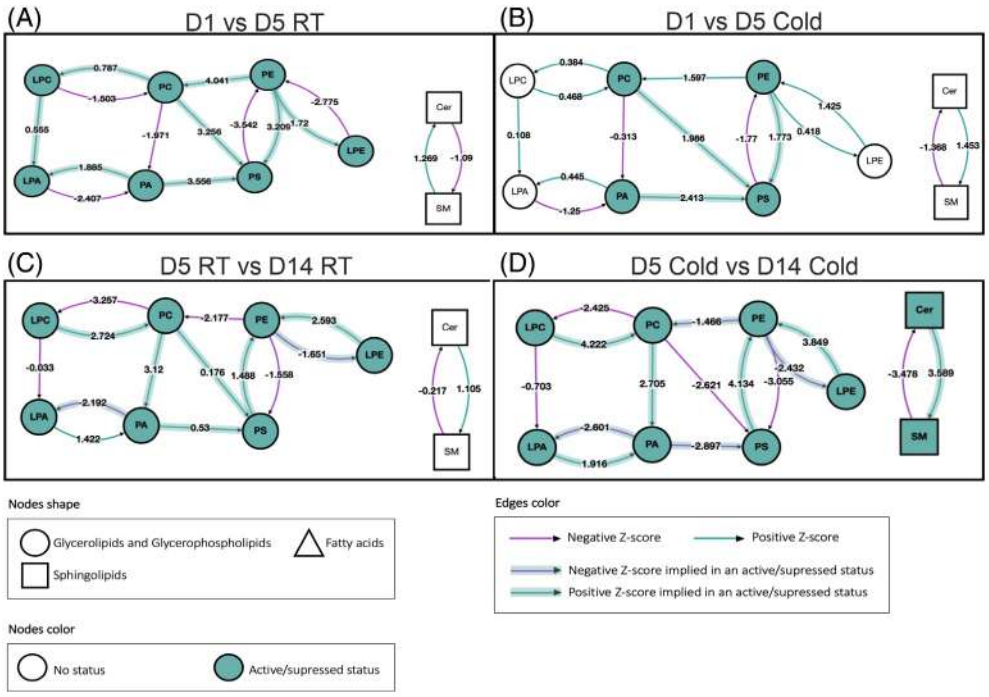
FIGURE 3 The effect of cold storage on sphingomyelin and ceramide. Platelet components were stored at room-temperature (RT; 20–24°C) or cold-stored (cold; 2–6°C). The platelets were isolated by differential centrifugation, lipids were extracted and measured by UPLC IMS QToF. The (A) percentage composition of sphingomyelin (SM) and ceramide (Cer) are displayed. The concentration of (B) sphingosine 1-phosphate (S1P) was determined by ELISA. The percentage composition of (C) SM and (D) Cer lipid species are displayed. The data represents mean (bars) + SD (error bars); $n = 8$. ‡$p < 0.05$ compared to day 1. # indicates $p < 0.05$ compared to RT at the same time point.

concentration of S1P decreased over storage, but was similar between the storage temperatures (Figure 3B). In terms of species changes, SM(16:0), SM(18:1) and SM(24:0) increased during storage, and cold storage amplified this effect (Figure 3C). Conversely, the opposite effect was seen with SM(23:0), SM(25:0) and SM(26:0), which decreased during storage at both temperatures, with a greater

loss of these species in cold-stored platelets. Regarding ceramide species, there was a trend for short chain ceramide species to increase over storage (Figure 3D). More specifically, Cer(18:0) and Cer(18:1) increased in cold-stored samples, with a concomitant decrease in RT-stored platelets. Cer(22:0) was significantly increased at day 5 and day 14 in both groups, and the increase was more pronounced in RT stored samples. Cer(24:0) was stable during early storage, but a significant difference between RT and cold platelets was observed after 14 days of storage. Interestingly, the long chain ceramide species tended to decrease with storage time regardless of temperature.

3.2 | Assessment of lipid dynamics and lipid pathways

The metabolic pathways involved in converting the lipids in platelets are regulated by well characterised enzymatic catalytic conversions.[18,19] As an integral part of platelet function, it is not only important to understand quantitative changes but also to understand how the metabolic pathways are affected. BioPAN is an open access web-based tool that provides a statistical score for possible lipid metabolism pathways.[23] Lipid networks were computed, comparing the storage time (day 1 vs. day 5 and day 5 vs. day 14), for RT and cold-stored



(E)

	D1vsD5 (RT)	D5vsD14 (RT)	D1vsD5 (Cold)	D5vsD14 (Cold)
LPC->LPA	0.555	-0.033	0.108	-0.703
LPC->PC	-1.503	2.724 ‡	0.468	4.222 ‡
PC->LPC	0.787	-3.257‡	0.384	-2.425‡
LPA->PA	-2.407 ‡	1.422	-1.250	1.916 ‡
PA->LPA	1.885 ‡	-2.192‡	0.445	-2.601‡
LPE->PE	-2.775 ‡	2.593 ‡	1.425	3.849 ‡
PE->LPE	1.720 ‡	-1.651‡	0.418	-2.432‡
PA->PS	3.556 ‡	0.530	2.413 ‡	-2.897‡
PC->PS	3.256 ‡	0.176	1.986‡	-2.621‡
PE->PS	3.209 ‡	-1.558	1.773‡	-3.055‡
PS->PE	-3.542‡	1.488	-1.770‡	4.134 ‡
PE->PC	4.041 ‡	-2.177‡	1.597	-1.466
PC->PA	-1.971 ‡	3.120‡	-0.313	2.705 ‡
Cer->SM	-1.090	1.105	1.453	3.589 ‡
SM->Cer	1.269	-0.217	-1.368	-3.478‡

RT Cold

FIGURE 4 The effect of cold storage on lipid metabolism as predicted using BioPAN. Lipid networks were generated from BioPAN using data acquired by UPLC IMS QToF. The lipid networks comparing samples at day 1 (D1) with (A) day 5 room temperature (D5 RT) or (B) day 5 cold (D5 Cold) and (C) room temperature samples at day 5 (D5 RT) versus day 14 (D14 RT) or (D) cold samples at day 5 (D5 Cold) versus day 14 (D14 Cold). (E) The Z-scores from the lipid network summarised and depicted as a heat map. Values shown as Z-score of the given reaction, whereby a Z-score >0 represents an active reaction (blue) and a Z-score <0 represents a suppressed reaction (grey). A paired t-test was performed by BioPAN to determine differences between indicated groups. ‡ indicates $p < 0.05$ a significant reaction (corresponding to a Z-score >1.645 or <−1.645).

platelets (Figure 4A–D). The BioPan lipid network showed distinct differences in lipid pathways depending on storage conditions, as summarised in the Heat Map (Figure 4E). During RT storage, pathway activity was greater between day 1 and day 5. Conversely, during cold storage the greatest activity in lipid pathways occurred between day 5 and day 14. PS formation by phosphatidic acid (PA), PC, and PE was downregulated by cold storage between day 5 and day 14, while the catabolism of PS to PE was an active pathway during prolonged cold storage. Further, cold storage drastically altered sphingolipid metabolism. Specifically, between day 5 and day 14 of cold storage the formation of SM from Cer was a highly active pathway.

3.3 | Characterisation of the phospholipid and sphingolipid profile of the supernatant

Recipients of platelet transfusions will receive both the platelets and the contents of the storage solution (plasma, platelet additive solution, biological response modifiers and EVs). The lipidome of platelets and the platelet component is dynamic and lipids are released or exchanged with the surrounding environment, or transformed by enzymatic reactions.[20] Thus it is important to consider the storage solution and its contribution to the overall lipid profile of the platelet component. The phospholipid and sphingolipid content of the supernatant fraction was assessed by UPLC IMS QToF. The mean concentration on day 1 was 38 990 ± 10 880 μg/mL, which remained similar over 14 days, regardless of whether the platelet components were stored at RT (33 541 ± 5661 μg/mL) or in the cold (36 727 ± 10 880 μg/mL, $p = 0.99$ when comparing RT and cold-stored at day 14).

In terms of lipid classes, numerous changes were apparent in the supernatant. Lipid changes in the supernatant were observed at day 5 when platelet components were stored at RT (Figure 5A), while lipids in the supernatant of cold-stored platelets components were relatively stable throughout 14 days of storage. There was a decrease in PC, PEP and Cer in both RT and cold storage, compared to day 1, with a trend for a larger decrease in the RT group at day 5. Conversely, lysophosphatidic acid (LPA), lysophosphatidylethanolamine (LPE), PE, phosphatidylinositol (PI) and PS were significantly increased during RT storage, compared to day 1. Similarly, PI and PS were significantly increased during cold storage, compared to day 1. Despite these changes, only small and subtle changes were observed in the lipid species of most classes (PC, PS, PE, PEP SM and Cer) (Supplementary Materials 2; Figures S1, S2 and S3).

While the overall proportion of the LPC class did not differ as a result of storage time or temperature, differences at the species level were of interest due to their association with transfusion outcomes.[26] LPC(18:1) increased during storage at both temperatures, although the increase was greater during RT storage. Concomitantly, LPC(18:2) decreased over storage at RT (Figure 5B). Overall, with the exception of LPC(18:1), the LPC species profile was relatively unchanged by cold storage until day 14.

The concentration of several bioactive lipid mediators in the supernatant was measured by ELISA. The concentration of arachidonic acid and 5-HETE remained relatively constant over storage, regardless of storage temperature (Figure 5C,D). However, the concentration of 12(S)-HETE and 15(S)-HETE increased during storage, with a larger increase during RT storage (Figure 5E,F).

4 | DISCUSSION

A paucity of recent data examining the lipidome of cold-stored platelets exists, particularly regarding extended storage in platelet additive solution. This study assessed the lipidomic changes occurring in platelets stored for the current (5–7 days) and feasible (14 days) shelf-life of RT and refrigerated components, respectively. The overall phospholipid and sphingolipid profile of platelets stored in additive solution at day 1 was similar to that previously reported for platelets in plasma,[16,17] and cold storage had little impact during early storage (5 days). However, the proportion of PE, PS, and SM classes increased during extended (14 days) cold storage, while PC, PEP and Cer decreased, compared to day 1.

Differences in phospholipid content between RT and cold-stored platelets may arise due to altered lipid metabolism or a change in the kinetics of incorporation from plasma lipoproteins; both of which are temperature dependent.[29] Further, supplementation with platelet additive solution, which is now standard practice in many blood collection centres, may reduce the concentration of plasma enzymes responsible for much of the lipid metabolism occurring during platelet storage.[18] Thus, differences between the changes in the lipid profile over storage between this and other studies[14–17] are likely due to differences in the storage solution.

The externalisation of aminophospholipids, PS and PE, is essential to support normal haemostatic function.[27] Further, long chain fatty acid species, such as arachidonic acid (20:4), provide better support for coagulation compared to short chain species.[30] While there was no change in arachidonic acid containing PS species, an increase in PE and PEP species, in which one of their fatty acid tails could be arachidonic acid (20:4), such as PE(38:4), PE(40:4) and PE(P-38:4) was observed. These subtle shifts may contribute to the increased haemostatic profile of cold-stored platelets.[13,31]

Recently cold storage has been shown to alter the immunomodulatory potential of platelets, evidenced by changes in the abundance of toll-like receptors on the platelet surface and release of immunomodulatory bioactive response modifiers during storage.[32] While certain LPC species, including 16:0, 18:0, 18:1, 18:2, 20:4 and 22:0, have been associated with inflammatory processes,[33,34] their exact roles remain unclear due to limited investigations. Further, transfusion of components containing high concentrations of polar (LPC and LPC derivatives) and non-polar (12-HETE and 15-HETE) bioactive lipid mediators are associated with TRALI.[24–26,35] During RT storage, LPC (18:1) increased, while LPC(18:2) decreased in the supernatant, which may have opposing effects. Similarly, the concentration of 12-HETE and 15-HETE increased to a greater degree in platelets stored at RT. Overall, cold storage resulted in less accumulation of these mediators, which may be a result of the temperature dependence of enzymatic reactions, decreased metabolic activity of the platelets and lower granule release.[13,36,37] Taken together, cold storage may be beneficial in reducing the incidence of transfusion reactions.

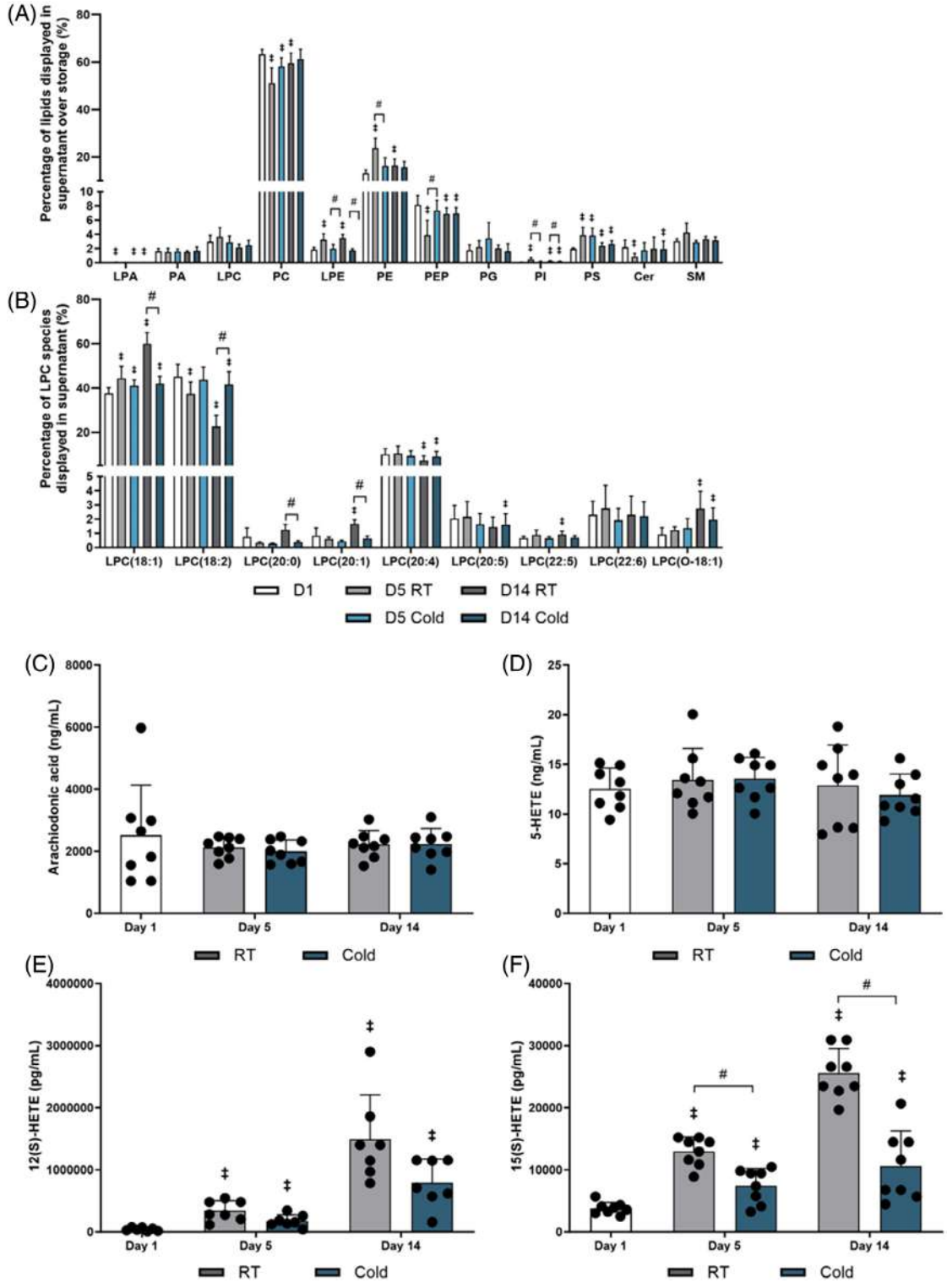


FIGURE 5 The effect of cold storage on the supernatant lipid profile. Platelet components were stored at room-temperature (RT; 20–24°C) or cold-stored (cold; 2–6°C). The supernatant was isolated by differential centrifugation, lipids were extracted and measured by UPLC IMS QToF. The percentage composition of (A) lipid classes and (B) lysophosphatidylcholine (LPC) species in the supernatant are displayed. The concentration of (C) arachidonic acid, (D) 5- hydroxyeicosatetraenoic acid (HETE), (E) 12(S)-HETE and (F) 15(S)-HETE were determined by ELISA. The data represents individual data points, and mean (bars) ± SD (error bars); $n = 8$. ‡$p < 0.05$ compared to day 1. # indicates $p < 0.05$ compared to RT at the same time point.

Sphingolipid metabolism is unique in platelets as they lack the enzymes necessary for de novo synthesis and S1P catabolism.[38] The catabolism of SM is considered to be an essential pathway by which potent bioactive lipid mediators, such as Cer and S1P, are formed.[38] Pienimaeki-Roemer et al., report that during conventional RT storage, S1P catabolism results in ceramide formation though the

transmembrane cycling and salvage pathway.[16,39] In this study, RT stored platelets followed a similar trend, where decreased S1P was associated with an increase in Cer, although no change in SM was observed. In contrast, while a decrease in S1P was also observed in cold-stored platelets, this was associated with a significant decrease in Cer, while SM significantly increased. Taken together, these results suggest that platelet storage temperature influences sphingolipid metabolism.

Ceramide has received considerable attention as a key regulator of apoptosis in nucleated cells,[40,41] although this has not been investigated in platelets. The elevated ceramide levels and low S1P observed in platelets stored at RT for 14 days suggests that the sphingolipid rheostat is tipped towards a cell death fate. This aligns with previous in vitro data, whereby RT platelets exhibit increased apoptotic signalling during prolonged storage (beyond 7 days), while the progression of apoptosis is delayed by cold storage.[7,42] It remains to be determined whether specific ceramide species (e.g., Cer 16:0, Cer 18:0 and Cer 22:0) are involved in mediating apoptosis in platelets, as they have been implicated in other cell types.[40,41] This is of particular interest as many ceramide species were found to be differentially modulated by RT and cold storage.

The differential modulation of ceramide in stored platelets may also have implications for EVs, as ceramide promotes EV formation.[43] Previous studies have shown that cold-stored platelets release more EVs into the supernatant,[13,36,44] which is interesting given that this study shows ceramide did not increase in cold-stored platelets over storage. It is speculated that perhaps the apparent lack of ceramide accumulation in cold-stored platelets may arise as a result of the platelet ceramide being packaged into EVs. Targeted investigations of the lipid content of EVs in stored platelets are required to specifically address this.

Opportunities for further study arise from the limitations of our study. Our data was obtained from a relatively small number of apheresis donors ($n = 8$), so donor variability may limit the generalisability of the findings.[45] Further, linking the identified lipid changes to the functional capacity of the platelets, as has recently been done with metabolomics,[46] could improve our understanding of certain aspects of platelet biology and storage. As mentioned above, while the lipid content of EVs generated during cold storage was not interrogated in this study, this information would be valuable given that EVs can serve as shuttles, transferring their lipid content to other cells, to mediate inflammatory processes such as lung injury.[16,43]

In summary, this work provides novel insight into changes in the lipid profile of platelet components during extended storage at both RT and cold storage. We demonstrate that the sphingolipid and phospholipid profile of platelets and the supernatant were differentially altered by storage time and temperature. It is apparent that storage for 5 days does not drastically affect the lipid profile. However, as storage progresses, differences in lipid classes and species associated with coagulation, apoptosis and inflammation become apparent. These changes may have functional effects once the components are transfused, warranting further studies to understand the clinical consequences of these changes.

AUTHOR CONTRIBUTIONS

Lacey Johnson, Matthew P. Padula, and Denese C. Marks designed the study. Tyren M. Dodgen and Amani Batarseh provided intellectual input on experimental design. Sarah M. Green, Lacey Johnson and Matthew P. Padula performed the research. Sarah M. Green and Lacey Johnson analysed the data, prepared the figures, and drafted the manuscript. All authors contributed to writing, critically revised, and approved the final manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors have no competing interests.

DATA AVAILABILITY STATEMENT

The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request. Reprints will not be made available from the author.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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TRANSFUSION MEDICINE WILEY

CASE STUDY

Frequency of red blood cell phenotypes from genotyped Australian blood donors

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Abstract

Background: Australian Red Cross Lifeblood (Lifeblood) performs human erythrocyte antigen (HEA) genotyping for a subset of repeat whole-blood donors through preferential selection which aims to maximise variation of results and possibility of identifying donors lacking high frequency red cell antigens.

Materials and Methods: The HEA Molecular Bead chip™ assay is used by Lifeblood for donor genotyping. A review of all donor HEA genotype data from March 2019 to May 2022 (3 years) was conducted.

Results: HEA genotyping was performed for 20,185donors. Due to selective genotyping of donors, a higher frequency of R1R1 predicted phenotype was identified. However, frequencies of other red cell phenotypes were relatively similar to previous reported in the Australian population. A small number of donors with rare red cell phenotypes was identified.

Conclusion: Genotyping of blood donors provides an available pool of extended matched red blood cell products for matching to recipients. Additionally genotyping can improve the identification of donors with rare phenotypes. Whilst limitations exist, genotyping may reduce the need for labour intensive serotyping, improve blood inventory management, and may be useful in donor recruitment and retention.

KEYWORDS
blood donor, genotype, red blood cell

Red blood cell (RBC) products are routinely matched to patients' ABO and Rh antigens to minimise the risk of sensitisation and haemolytic transfusion reactions.[1] Serologic methods have traditionally been used to determine donor and patient phenotypes when transfusion of blood products is required. However, as an alternative, genotyping, which uses DNA variation to predict phenotype, has been developed.[2,3]

Genotyping is beneficial when commercial reagents are unavailable, when serologic results are unreliable, or following recent blood transfusion.[3,4] Additionally, genotyping can produce predictions for multiple antigens simultaneously, and predict weak and variant antigens.[3,5,6] Extended RBC genotyping of both donor and transfusion recipient has the potential to reduce alloimmunisation through improved antigen matching and assist blood collection agencies with optimisation of their inventories.[5,6] However, the high cost of genotyping technology currently makes it economically infeasible to be performed on all Australian blood donors and recipients. Hence, Lifeblood performs genotyping for a subset of repeat whole-blood donors (<1% annually) through preferential selection which aims to maximise variation of results and possibility of identifying donors lacking high-frequency antigens.

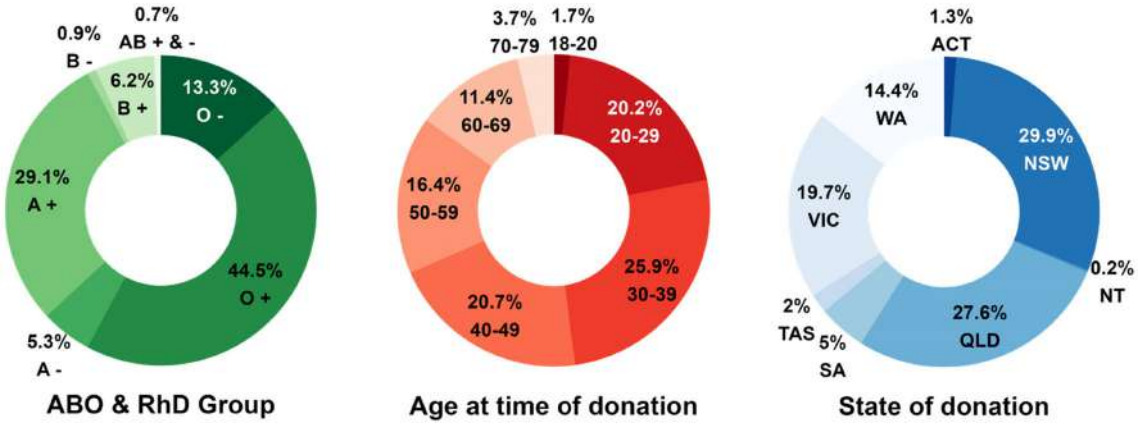

FIGURE 1 Donor demographics.

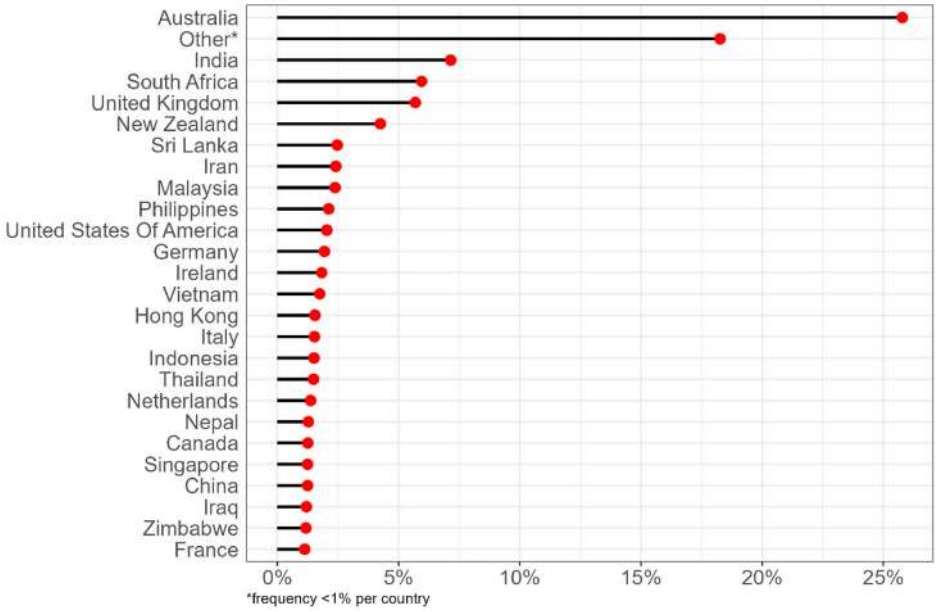

FIGURE 2 Donor's country of birth.

A retrospective observational review of Australian Red Cross Lifeblood's (Lifeblood) de-identified donor RBC genotype results from March 2019 to May 2022 (3 years, 2 months) was conducted. Lifeblood uses the Human Erythrocyte Antigen (HEA) Molecular Beadchip™ assay, which performs in vitro analysis of 24 known polymorphisms associated with 35 HEAs and 3 phenotypic variants to determine allelic variants that predict phenotypes.[7] The analysed data are comprised of results from donor's prioritised by O and A blood group (Figure 1), Rh phenotypes R_1R_1, R_2R_2, R_0r or rr and certain self-reported ethnic backgrounds or countries of birth. Additionally, genotyping may be performed to resolve discrepancies in donor serology. Donor's gender, age at time of donation and location of donation data were also collected.

HEA genotyping was performed for 20,185 donors. Of these, 11,458 (56.8%) were male, majority (66.8%) were aged between 20 and 50 years and over half (57.5%) were from either New South Wales or Queensland (Figure 1). Country of birth varied widely (Figure 2), while ethnicity was only reported by <10% of genotyped donors ($n = 1658$). Of those that did report their ethnicity, European (58.1%) and Asian (32.1%) ethnicities were the most prevalent. Serologic results identified the majority of donors as group O or A and RhD positive (Figure 1).

The frequency of predicted RBC phenotypes from our genotyped donor cohort are listed in *table* 1. Weak Fy^b was detected in 252 donors (236 Fya+, 16 Fya−) and the GATA silencing mutation of Fy^b was identified for 431 donors (169 Fya+, 262 Fya−). One donor lacked the high prevalence U antigen (M-N + S-s-) and another four were found to have a U variant. Clinically significant high prevalence antigens lacking in the genotyped donors included 41 k-, 24 Co(a−), 15 Lu(b−), 4 Jo(a−), 3 Kp(b−) and Js(b−), respectively, and 2 Di(b−). Twenty (0.1%) genotyped donors were identified to have possible (C)ce^s haplotype. No donors were identified to be Kell null, Kidd null, or Sc1 negative. Finally, 18 (0.09%) donors were identified to be heterozygous for a polymorphism in the beta-globin gene which has been shown to be concordant with sickle cell trait clinically.[3]

The selective genotyping of donors at Lifeblood aims to maximise the availability of blood products for the largest number of

TABLE 1 Genotyped donors predicted phenotypes.

Predicted phenotype	% Frequency in genotyped donors	Predicted phenotype	% Frequency in genotyped donors	Predicted phenotype	% Frequency in genotyped donors
MNS antigen group		**Rh antigen group**		**Kell antigen group**	
MMSS	5.4	R_1r	34.6	K−k+	96.1
MMss	13.6	R_1R_1	27.5	K + k−	0.2
MMSs	14.1	R_1R_2	4.4	K + k+	3.7
MNSS	3.0	R_2r	10.0	Kp(a + b−)	0.01
MNss	26.3	R_2R_2	1.5	Kp(a−b+)	98.6
MNSs	18.7	R_0r	2.1	Kp(a + b+)	1.4
NNSS	0.4	rr	18.7	Js(a + b−)	0.01
NNss	14.4	r′r	0.5	Js(a−b+)	99.7
NNSs	4.1	r″r	0.3	Js(a + b+)	0.3
Lutheran antigen group		V+	1.1	**Kidd antigen group**	
Lu(a + b−)	0.07	VS+	1.2	Jk(a + b−)	28.2
Lu(a−b+)	95.8			Jk(a−b+)	23.4
Lu(a + b+)	4.2	**Duffy antigen group**		Jk(a + b+)	48.4
Lu(a−b−)	0	Fy(a + b−)	32.7		
		Fy(a−b+)	24.4	**Dombrock antigen group**	
Diego antigen group		Fy(a + b+)	40.4	Do(a + b−)	13.0
Di(a + b−)	0.01	Fy(a + b + w)	1.2	Do(a−b+)	43.8
Di(a−b+)	99.4	Fy(a−b−)	1.3	Do(a + b+)	43.2
Di(a + b+)	0.6				
		Scianna antigen group		**Landsteiner-Weiner Group**	
Colton antigen group		Sc:1, −2	99.4	LW(a + b−)	99.6
Co(a + b−)	94.8	Sc:−1, 2	0	LW(a−b+)	0
Co(a−b+)	0.1	Sc:1, 2	0.6	LW(a + b+)	0.4
Co(a + b+)	5.0				

recipients and identify rare donors who lack clinically significant high prevalence antigens. This study showed Lifeblood's selection method has resulted in a blood product inventory with a wide variety of available phenotypes, including for blood groups (Rh, Kell, Duffy, Kidd and MNS) which antigen matched RBC products are most commonly requested.[4] Additionally, rare phenotypes k-Fy(a−b−), Kp(b−), U^{var}Fy (a−b−), U-Fy(a−b−) and Jo(a−)Fy(a−b−) were identified for 2, 3, 4, 1 and 4 donors, respectively. Frequencies of other phenotypes were relatively similar to previous reported in the Australian population.[8] However, a higher than expected number of donors were found to be Fy(b−) or Do(a−) while a lower than expected number of donors were Co(b+) or LW(b−). The Fy(a−b−) predicted phenotypes for our genotyped donor cohort were also exclusively due to GATA silencing mutations of the *FYB* gene. These individuals represent a population who as transfusion recipients will not form antibodies to Fy^b, due to the presence of the antigen on non-erythroid cells.[5] Furthermore, in the context of Fy^b being a poor immunogen, if our genotyping data is representative of the broader Australian population this suggests that Fy^b antigen matching is likely to rarely be required for RBC products.[5,9,10] This is also further reinforced by the low reports of anti-Fy^b, anti-Fy3 or anti-Fy5 antibodies in the Australian population.[11]

Currently, in Australia, extended antigen-matched transfusions in the absence of a formed antibody is not specifically recommended, rather clinical situation and blood product inventory are used to determine if such extended matching is appropriate.[1] Yet, there is a potential that as high throughput technologies evolve, extended antigen-matching may become the norm for non-emergency red cell transfusions, with confirmatory serotyping only when recipients are known to have alloantibodies. Additionally, genotyping of transfusion-dependent patients is likely to increase which may increase requests for prophylactic matching where recipients are negative for high prevalence antigens, especially antigens known to have high immunogenicity and clinically significant alloantibodies. This will likely further drive the need for Australia's blood donor panel to be suitably diverse with readily available blood products, including those with rare RBC phenotypes. Further highlighting the benefit of the high throughput testing by genotyping over serologic testing, as both a higher number of donors and antigens can be screened simultaneously, therefore improving resource use.

Limitations of the Beadchip™ assay include unreliability of the Kidd null phenotype results and risks of unreliable results in the context of unique mutations and variants.[7] The most common variant (*JK*IVS5–1G > A*) responsible for the Jk(a−b−) phenotype in the Oceania region is

unfortunately not detected by the Beadchip™ assay. Hence, the lack of Kidd null phenotype identified in these results cannot be relied upon. Hence, serology is superior for determining these results. However, given this phenotype is reported to occur exclusively in less than 1% in Polynesian individuals[9] a large proportion of donors would likely need to be serotyped to identify one Kidd null phenotype. With regard to false results, mutations affecting the assays probe binding may result in false negative results, whereas those affecting antigen expression may result in false positives.[7] While false positives in the donor population are unlikely to cause harm to recipients, false negative results could pose a risk for antibody formation in antigen negative transfusion recipients if prophylactic antigen-negative matching occurs. Finally, the Beadchip™ assay is limited by the number of antigens included (35 HEAs) and the time required to complete testing and provide results. Hence, genotyping is unsuitable when transfusion recipients have antibodies to antigens not identified by the assay or as an urgent test for antigen status for donors or recipients.

Limitations of our data include the generalisability to the broader donor and Australian population. This is due to the selective nature of Lifeblood's genotype testing which limits costs but aims to ensure increased suitability for matching to a higher proportion of recipients through targeting certain ABO and Rh antigens. However, the results from the genotyped donors were relatively heterogenous with a broad range of ages, ABO and RhD group, and location within Australia. The last of which is limited by the location of Lifeblood donation and testing centres.

The self-reported ethnicity data was also significantly lacking, likely due to voluntary reporting of ethnicity only recently being introduced during Lifeblood's donation processes. This information is significantly valuable for donor genotyping as identifying donors who lack very high-frequency antigens is complex. Both global frequency data and ethnicity information are often used when selecting donors to be screened to increase the probability of identifying rare antigen profiles. Hence, the use of Country of birth is likely to have been a less specific surrogate than ethnicity for increasing identification of rare phenotypes in our donor population.

As genotyping technology improves and is more commonly available, its use could also assist with donor recruitment and retention.[6] The provision of information about blood types has previously been shown to increase donor return rates.[6] Hence, the personalised information produced from genotyping could be used in donor retention campaigns and for recruiting and retaining blood donors of diverse ethnic backgrounds.

This review of the results Lifeblood's genotyped donor's RBC antigens provides an updated phenotype frequency within Australia's blood donor population. This is important for matching of blood products to recipients and improves the identification of donors with rare RBC phenotypes. While limitations exist, genotyping, due to its high throughput and simultaneous antigen typing, may reduce the need for labour intensive serotyping and improve blood inventory management. As genotyping technology improves and is more widely adopted the results could also be used for donor recruitment and retention by providing personalised results to emphasise the importance of an individual's donation.

AUTHOR CONTRIBUTIONS

G.J. drafted manuscript and analyzed data; T.P. proposed project, prepared data, and reviewed and edited manuscript; J.D. supervised research, and reviewed and edited manuscript. The manuscript has been seen and approved by all authors.

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CONFLICT OF INTEREST STATEMENT

The authors have no competing interests.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study may be available from the corresponding author, upon reasonable request and approval from a governing human research ethics committee.

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TRANSFUSION MEDICINE WILEY

LETTER TO THE EDITOR

Optimizing blood utilization: Experience of blood redistribution policy from a tertiary care hospital based blood centre

Dear Editor,

A 33-year-old Caucasian woman was referred at 32^{+5} weeks of gestation after the finding of severe fetal intracranial haemorrhage (ICH) at routine ultrasound. The woman had two previous uneventful pregnancies (singleton and twins). Fetal ultrasound and magnetic resonance imaging confirmed multiple ICHs especially located in the left hemisphere, wide areas of periventricular leukomalacia, obstructive hydrocephalus and macrocrania. Fetal-neonatal alloimmune thrombocytopenia (FNAIT) was investigated. Maternal blood group was A RhD positive, paternal O RhD positive. The maternal sample was screened for platelet-reactive antibodies using solid phase technology for the detection of IgG anti-HLA class I and anti-HPA antibodies (SPRCA Capture P Ready Screen, Immucor, Italy) with and without chloroquine treatment to remove HLA antigen interference. The results were positive and negative respectively. No antibodies attached to maternal platelets were found (Capture-P, Immucor, Italy). ELISA and Luminex based platforms were used to identify the specificity of the detected antibodies (Pak-Lx Luminex and ELISA Pak plus, Immucor, Italy and Luminex MoAb, Lagitre, Italy). The assays only recognised the presence of anti-HLA A02 and anti-HLA B51 at high titre, greater than 8000 and 20 000 MFI (average fluorescence intensity) respectively, in association with different cross-reactions. Cross-match testing (Capture-P, Immucor, Italy) using maternal serum against paternal platelets tested reactive with both chloroquine-untreated and treated platelets. Additional cross-match testing was performed using platelets. Additional cross-match testing was performed using maternal serum against 14 random donor platelet samples. Eight donors were compatible and six were not. Two non-compatible donors were HLA class I A*02 and A*02 B*51 respectively. The remaining four non-compatible donors were not typed for HLA I antigen. All compatible donors were typed for the main HPA antigens but comparison of the typings did not allow to quickly exclude HPA 4b, 6b, 7b, 8b, 9b, and 11b antigen immunisation. A male newborn was delivered by caesarean section at 36 weeks of gestation after spontaneous onset of labour. At birth, platelet count was $4x10^3/\mu l$ with normal white and red blood cell count. An urgent transfusion with a platelet blood component not tested with maternal serum increased platelets to $116 \times 10^3/\mu l$; intravenous immunoglobulins were also infused. Another two transfusions were administered on days 4 and 13 due to a drop in the number of platelets ($28 \times 10^3/\mu l$ and $48 \times 10^3/\mu l$ respectively): the platelet pools were obtained from cross-match between maternal serum and sample platelets of random donors. Normal values were reached on day 17.

The newborn blood group was 0 Rh D positive. Capture-P Ready Screen aimed to detect anti-platelet antibodies was non-reactive. Cross-match testing using newborn blood was performed twice. At birth, the neonatal sample was cross-matched against paternal and maternal platelet samples: results were positive and negative, respectively. After 14 days, cross-matching against paternal platelets was repeated with and without chloroquine treatment; both resulted non-reactive.

Results of parental and neonatal HLA I and HPA genotyping performed using polymerase chain reaction (PCR) with sequence-specific oligonucleotides (PCR-SSO) and HPA BeadChip (Immucor, Italy), are shown in Table 1. The mismatches identified prompted further testing in the mother. Cross-match testing against 6 HPA-9b antigen negative donors resulted in two non-compatible and four compatible donors. The same two non-compatible donors were all compatible when The same two non-compatible donors were all compatible when cross-matching was performed with chloroquine. Cross-match testing against two donors expressing the HPA9b antigen was reactive with and without chloroquine. Cross-match testing against paternal and neonatal platelets with and without chloroquine was equally reactive. Cross-match testing was performed between maternal serum and 107 different donors in order to have available and compatible blood components available for any neonatal transfusions.

FNAIT is a cause of severe thrombocytopenia and ICH in both the fetus and newborn.[1] FNAIT-related ICH is estimated to occur in at least 10:100 000 neonates. It mainly occurs in the third trimester of pregnancy and is associated with severe neurological sequelae and mortality. In most cases, FNAIT is caused by an alloimmune response against human platelet antigens (HPAs). In the Caucasian population HPA-1a antigen accounts for up to 80% cases,[2] followed by HPA-5b (8–15% of cases) and to a lesser extent HPA-3a/5a/15b.[3] More rarely, FNAIT is associated with low-frequency human platelet antigens (LFHPAs) or to HLA class I antigens, especially when related to locus A and B and with a highly expressed titre.[4] Among LFHPAs, HPA-9b is emerging as a significative trigger for FNAIT.[5] Almost two-thirds of apparent cases of FNAIT are not resolved by laboratory confirmation of maternal immunisation against HPA antigens. When other causes of thrombocytopenia are not identified, a possible explanation may be involvement of HLA antibodies or limitations of laboratory studies.

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Since the first report of a HPA-9b related FNAIT in 1995,[6] a total of 15 cases have been reported[5,7,8] and increasing evidence suggests that its prevalence in the population and among fathers of unresolved cases of FNAIT might be greater than previously reported.[6,9] The severity of thrombocytopenia and clinical presentation in our case is consistent with the argument that HPA-9b might be more immunogenic than others HPAs.[5] Our case supports the need to investigate alloimmunisation to HPA-9b and other rare specificities when routine screening for the most common antigens is negative or inconsistent with the laboratory and clinical findings.[3,5] We experienced diagnostic limitations mainly due to the fact that the Ag panel used (Pak-Lx Luminex Immunocor and Elisa Pak Plus Immunocor) does not identify HPA-9b. Difficulties with the detection of antibodies against HPA-9b antibodies have been described by some authors who urged further studies to fully understand the issue.[5,7] Nonetheless, even if FNAIT was strongly suspected both on clinical grounds and after the finding of anti-HLA I antibodies, the mother could not be offered intrauterine therapy of proven efficacy to begin at that gestational age.[1,9]

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The authors declare no conflict of interest.

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The data that support the findings of this study are not openly available due to sensitivity reasons and are available from the corresponding author upon reasonable request.

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TRANSFUSION MEDICINE WILEY

LETTER TO THE EDITOR

Efficacy and safety of BNT162b2 mRNA vaccine in a cohort of 90 transfusion dependent thalassemia patients

Dear Editor,

A 33-year-old Caucasian woman was referred at 32^{+5} weeks of gestation after the finding of severe fetal intracranial haemorrhage (ICH) at routine ultrasound. The woman had two previous uneventful pregnancies (singleton and twins). Fetal ultrasound and magnetic resonance imaging confirmed multiple ICHs especially located in the left hemisphere, wide areas of periventricular leukomalacia, obstructive hydrocephalus and macrocrania. Fetal-neonatal alloimmune thrombocytopenia (FNAIT) was investigated. Maternal blood group was A RhD positive, paternal O RhD positive. The maternal sample was screened for platelet-reactive antibodies using solid phase technology for the detection of IgG anti-HLA class I and anti-HPA antibodies (SPRCA Capture P Ready Screen, Immucor, Italy) with and without chloroquine treatment to remove HLA antigen interference. The results were positive and negative respectively. No antibodies attached to maternal platelets were found (Capture-P, Immucor, Italy). ELISA and Luminex based platforms were used to identify the specificity of the detected antibodies (Pak-Lx Luminex and ELISA Pak plus, Immucor, Italy and Luminex MoAb, Lagitre, Italy). The assays only recognised the presence of anti-HLA A02 and anti-HLA B51 at high titre, greater than 8000 and 20 000 MFI (average fluorescence intensity) respectively, in association with different cross-reactions. Cross-match testing (Capture-P, Immucor, Italy) using maternal serum against paternal platelets tested reactive with both chloroquine-untreated and treated platelets. Additional cross-match testing was performed using platelets. Additional cross-match testing was performed using maternal serum against 14 random donor platelet samples. Eight donors were compatible and six were not. Two non-compatible donors were HLA class I A*02 and A*02 B*51 respectively. The remaining four non-compatible donors were not typed for HLA I antigen. All compatible donors were typed for the main HPA antigens but comparison of the typings did not allow to quickly exclude HPA 4b, 6b, 7b, 8b, 9b, and 11b antigen immunisation. A male newborn was delivered by caesarean section at 36 weeks of gestation after spontaneous onset of labour. At birth, platelet count was $4x10^3/\mu l$ with normal white and red blood cell count. An urgent transfusion with a platelet blood component not tested with maternal serum increased platelets to $116 \times 10^3/\mu l$; intravenous immunoglobulins were also infused. Another two transfusions were administered on days 4 and 13 due to a drop in the number of platelets ($28 \times 10^3/\mu l$ and $48 \times 10^3/\mu l$ respectively): the platelet pools were obtained from cross-match between maternal serum and sample platelets of random donors. Normal values were reached on day 17.

The newborn blood group was 0 Rh D positive. Capture-P Ready Screen aimed to detect anti-platelet antibodies was non-reactive. Cross-match testing using newborn blood was performed twice. At birth, the neonatal sample was cross-matched against paternal and maternal platelet samples: results were positive and negative, respectively. After 14 days, cross-matching against paternal platelets was repeated with and without chloroquine treatment; both resulted non-reactive.

Results of parental and neonatal HLA I and HPA genotyping performed using polymerase chain reaction (PCR) with sequence-specific oligonucleotides (PCR-SSO) and HPA BeadChip (Immucor, Italy), are shown in Table 1. The mismatches identified prompted further testing in the mother. Cross-match testing against 6 HPA-9b antigen negative donors resulted in two non-compatible and four compatible donors. The same two non-compatible donors were all compatible when The same two non-compatible donors were all compatible when cross-matching was performed with chloroquine. Cross-match testing against two donors expressing the HPA9b antigen was reactive with and without chloroquine. Cross-match testing against paternal and neonatal platelets with and without chloroquine was equally reactive. Cross-match testing was performed between maternal serum and 107 different donors in order to have available and compatible blood components available for any neonatal transfusions.

TABLE 1 Trio HLA and platelet genotyping. Newborn genes not present in the mother are in bold

	Mother	Father	Newborn
HLA class I genotype	A*01*69 B*35*37 C*06*12	**A*02** **B*35*51** C*04*16	A*01***02** B*37***51**
HPA genotype	1a/a, 2a/a, 3a/**b**, 4a/a, 5a/a, 6a/a, 7a/a, 8a/a, **9a/a**, 11a/a, 15a/b	1a/a, 2a/a, 3b/b, 4a/a, 5a/a, 6a/a, 7a/a, 8a/a, **9a/b**, 11a/a, 15a/b	1a/a, 2a/a, 3a/b, 4a/a, 5a/a, 6a/a, 7a/a, 8a/a, **9a/b**, 11a/a, 15b/**b**

Abbreviations: HLA, human leukocyte antigen; HPA, human platelet antigen.

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