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Cryopreserved platelets compared with liquid-stored platelets for the treatment of surgical bleeding: protocol for two multicentre randomised controlled blinded non-inferiority trials (the CLIP-II and CLIPNZ-II trials)

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ABSTRACT

Introduction Cryopreservation at −80°C in dimethylsulphoxide extends platelet shelf-life from 7 days to 2 years. Only limited comparative trial data supports the safety and effectiveness of cryopreserved platelets as a treatment for surgical bleeding. Cryopreserved platelets are not currently registered for civilian use in most countries.

Methods and analysis CLIP-II and CLIPNZ-II are harmonised, blinded, multicentre, randomised, controlled clinical non-inferiority trials comparing bleeding, transfusion, safety and cost outcomes associated with cryopreserved platelets versus conventional liquid platelets as treatment for bleeding in cardiac surgery. CLIP-II is planning to enrol patients in 12 tertiary hospitals in Australia; CLIPNZ-II will recruit in five tertiary hospitals in New Zealand. The trials use near-identical protocols aside from details of cryopreserved platelet preparation. Patients identified preoperatively as being at high risk of requiring a platelet transfusion receive up to three units of study platelets if their treating doctor considers platelet transfusion is indicated. The primary endpoint is blood loss through the surgical drains in the 24 hours following intensive care unit (ICU) admission after surgery. Other endpoints are blood loss at other time points, potential complications, adverse reactions, transfusion and fluid requirement, requirement for procoagulant treatments, time to commencement of postoperative anticoagulants, delay between platelet order and commencement of infusion, need for reoperation, laboratory and point-of-care clotting indices, cost, length of mechanical ventilation, ICU and hospital stay, and mortality. Transfusing 202 (CLIP-II) or 228 (CLIPNZ-II) patients with study platelets will provide 90% power to exclude the possibility of greater than 20% inferiority in the primary endpoint. If cryopreserved platelets are not inferior to liquid-stored platelets, the advantages of longer shelf-life would justify rapid change in clinical practice. Cost-effectiveness analyses will be incorporated into each study such that, should clinical non-inferiority compared with standard care be demonstrated, the hospitals in each country that would benefit most from changing to a cryopreserved platelet blood bank will be known.

Ethics and dissemination CLIP-II was approved by the Austin Health Human Research Ethics Committee (HREC/54406/Austin-2019) and by the Australian Red Cross Lifeblood Ethics Committee (2019#23). CLIPNZ-II was approved by the New Zealand Southern Health and Disability Ethics Committee (21/STH/66). Eligible patients are approached for informed consent at least 1 day prior to surgery. There is no provision for consent provided by a substitute decision-maker. The results of the two trials will be submitted separately for publication in peer-reviewed journals.

Trial registration numbers NCT03991481 and ACTRN12621000271808.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ A blinded, randomised design minimises bias.
⇒ The primary outcome, blood loss through the surgical drains in the 24 hours from the time of intensive care unit admission after surgery, can be quantified objectively.
⇒ The two trials, using slightly different methods of manufacturing cryopreserved platelets, are harmonised, which will facilitate an individual patient data meta-analysis.
⇒ While the trials are adequately powered for important safety outcomes, minor adverse events are common after cardiac surgery and causation might be difficult to attribute to study platelet transfusion.
⇒ In some study hospitals, staffing limitations mean cryopreserved platelets can only be reconstituted during normal working hours, meaning not all randomised patients will receive the intervention as intended.

INTRODUCTION

Platelets are essential for haemostasis, adhering to sites of vascular injury to form a mechanical scaffold for blood clots and

catalysing the generation of thrombin by activation of the coagulation cascade on their phosphatidylserine-rich membrane surface. Cardiac surgical patients form the largest single patient group transfused platelets to treat bleeding.[1]

After donation, liquid-stored platelets are kept at room temperature to prolong circulation time in recipients. Potential bacterial growth limits shelf-life to 7 days in Australia and New Zealand.[2] Short shelf-life leads to wastage: studies have reported 25%–33% of platelet units are discarded because they reach their expiry date,[1 3] although this can be reduced to 7%–10% with modern inventory management (D. Marks, Australian Red Cross Lifeblood, unpublished data). To limit wastage, platelets are only routinely stored in larger hospitals where use is high and predictable. In centres where platelets are not readily available, patients with life-threatening bleeding can wait many hours for platelets to be delivered from central blood banks, or do not receive any at all.

The short shelf-life of liquid-stored platelets usually limits the ability of military field hospitals to provide platelet transfusion. In response, the Netherlands Military Blood Bank modified US Navy research protocols to freeze, store and reconstitute platelets.[4] Within 24 hours of donation, platelets are frozen as 10–20 mL concentrates using dimethylsulphoxide (DMSO) as a cryoprotectant, then stored at −80°C for up to 2–4 years. The platelets can be reconstituted within 30 min,[4 5] a process requiring minimal equipment and training. Experience with 1143 cryopreserved platelet components transfused into 349 military casualties suggested both safety and effectiveness,[6] although no comparisons with liquid-stored platelets or whole blood were made.

Cryopreserved platelets have also been used in limited civilian contexts. For example, >1600 cryopreserved platelet units were transfused to chemotherapy patients at the University of Maryland, 1976–1981.[7] When liquid-stored platelets are unavailable, 11 000–13 000 cryopreserved platelet units are transfused annually in Poland.[8] France maintains a supply of cryopreserved platelets for use in alloimmunised haematology patients requiring HLA-matched units,[8] and the military university hospital in Prague, Czech Republic uses cryopreserved platelets for heavily bleeding trauma patients.[9] However, evidence of comparative safety and effectiveness is very limited.

Extensive preclinical assessments of cryopreserved platelets have been published.[5 10] Only 54% of thawed platelets are present in the circulation 2 hours after transfusion,[11] but these have a higher capacity to bind factor V[12] and produce more thromboxane A2 after ADP stimulation.[13] Platelet-derived microparticles, more abundant after cryopreservation, are also haemostatically active.[14] A phase I human trial found lower 24-hour platelet recovery compared with liquid-stored platelets, but with survival times exceeding US Food and Drug Administration requirements.[15] A randomised dose-escalation phase IIa study found bleeding haematology patients had dose-dependent increases in platelet counts, and all patients had stabilisation of their bleeding.[16] In summary, human cryopreserved platelets have reduced circulating time that might reflect a 'preactivated' phenotype, haemostatic efficacy and no evidence of harm.

The single-controlled phase IIb clinical trial of thawed cryopreserved platelets randomised 73 patients to cryopreserved or liquid-stored platelets for treatment of bleeding after cardiac surgery.[17] No adverse effects were observed in the 24 patients who received cryopreserved platelets. Blood loss in the patients who received cryopreserved platelets was significantly less, despite lower post-transfusion platelet increments and a tendency towards decreased platelet survival. While encouraging, several questions remain. The sample size was underpowered to assess safety, and outcomes beyond the immediate perioperative period were not assessed. If the apparent hypercoagulable state induced by cryopreserved platelets led to more graft occlusion or thromboembolic disease, or if the platelet microparticles predisposed to acute respiratory distress syndrome (ARDS), this might not have been observed.

From 2015 to 2017, 41 patients were randomised into the CLIP-I pilot study of cryopreserved platelets prepared using a protocol developed by Australian Red Cross Lifeblood.[18] A similar pilot trial was conducted in New Zealand involving 23 patients.[19] Different methods of cryopreserved platelet manufacturing and reconstitution necessitated separate pilot trials: Australian Red Cross Lifeblood prepares the platelets as a small-volume concentrate that is resuspended in thawed fresh-frozen plasma from a different donor immediately before transfusion, whereas the NZ Blood service prepares the platelets and plasma in a single transfusion bag, separated prior to freezing using a temporary occlusion device. During thawing in a 30°C–32°C water bath, this larger volume results in a different rate of platelet rewarming, potentially affecting in vivo platelet function. Both trials demonstrated protocol feasibility and the utility of pursuing a non-inferiority 24-hour bleeding primary outcome, which would ensure sufficient power to detect differences in major prespecified safety endpoints. Accordingly, we now present the protocol for harmonised phase III trials (CLIP-II and CLIPNZ-II) of cryopreserved platelets, written to conform to the Standard Protocol Items: Recommendations for Interventional Trials 2013 statement.[20] As these trials continue to employ the different methods of cryopreserved platelet preparation tested in their pilot phases, they are being conducted as separate studies. Harmonisation of all other aspects of the trial protocols will facilitate comparison of the effect of method of cryopreserved platelet preparation in an individual patient data meta-analysis.

Objectives

The aim of the CLIP-II and CLIPNZ-II trials is to assess the effectiveness, safety and cost-effectiveness of cryopreserved platelets, compared with conventional liquid-stored platelets, to treat active bleeding due to surgery.

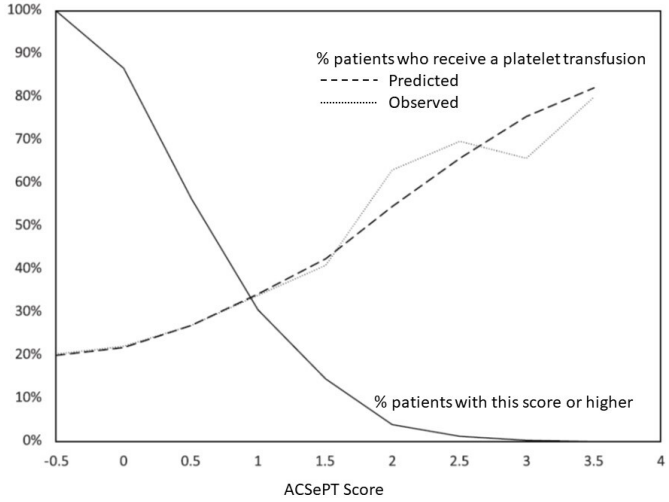

Figure 1 Observed and predicted probabilities of platelet transfusion at or above each value of the Adult Cardiac Surgery Platelet Transfusion (ACSePT) score[22] and the corresponding percentage of patients with at least each ACSePT score. No patients with ACSePT scores of 4 were observed or predicted to have platelet transfusions.

METHODS AND ANALYSIS

Trial design

Blinded, multicentre, randomised, controlled clinical non-inferiority trials comparing bleeding, transfusion, safety and cost outcomes associated with cryopreserved platelets versus conventional liquid platelets as treatment for bleeding as a result of cardiac surgery, deemed by treating doctors to require a platelet transfusion.

Protocol version

This document has been written based on information contained in the study protocol version 4, dated 29 June 2020 and registered on ClinicalTrials.gov on 19 October 2020 (CLIP-II) and study protocol version 3.1 dated 17 March 2021 registered in the Australian New Zealand Clinical Trials Registry on 11 March 2021 (CLIPNZ-II—ACTRN12621000271808p).

Study setting

Operating theatres and intensive care units (ICUs) in 12 Australian and 5 New Zealand tertiary hospitals undertaking cardiac surgery. The first patients were enrolled in August 2021 (CLIP-II) and March 2022 (CLIPNZ-II). If enrolment continues at initial rates, recruiting will be completed in October 2023 (CLIP-II) and April 2024 (CLIPNZ-II).

Eligibility criteria

Approximately 35% of patients randomised in CLIP-I were transfused platelets. The inclusion criteria for CLIP-I were based on the Transfusion Risk Understanding Scoring Tool (TRUST) criteria, which predict the need for red cell transfusion.[21] However, several TRUST criteria (eg, haemoglobin concentration) are red cell-specific. Consequently, for CLIP-II and CLIPNZ-II, a platelet-specific transfusion risk score (the Adult Cardiac Surgery Platelet Transfusion (ACSePT) score)[22] was developed (table 1). Balancing the desire for a high proportion of patients randomised to receive a platelet transfusion with the requirement to randomise sufficient patients (figure 1), patients are included in the trials if they have an ACSePT score ≥1, which corresponds to a >30% probability of receiving a platelet transfusion. Noting cryopreserved platelets are unlikely to be used only in cardiac surgery patients with an ACSePT score ≥1 if the trials show non-inferiority, patients can also be enrolled if their clinicians believe they are at a 'high risk' of a platelet transfusion. Exclusion criteria are listed in table 2.

Table 1 The Australian Cardiac Surgery Platelet Transfusion (ACSePT) risk prediction tool[22]

Category	Points
A: Age 70 years or above	0.5
B: Preoperative dialysis	0.5
C: Diagnosis of infective endocarditis	0.5
D: Cardiogenic shock at time of procedure	0.5
E: Clopidogrel within 7 days prior to surgery	0.5
F: Previous cardiac valve surgery	0.5
G: Emergency or salvage surgery	1
H: 'Coronary artery bypass and valve surgery' or 'Other cardiac surgery, aortic or non-cardiac surgery'	1
I: BMI ≥30 kg/m2	−0.5
ACSePT score=A+B+C+D+E+F+G+H+I	

Setting the entry criterion using an ACSePT score of ≥1 identifies the 30% of patients who have a predicted risk of platelet transfusion of approximately 30%.
BMI, body mass index.

Interventions and participant timeline

In both trials, patients are randomised 1:1 to receive either cryopreserved platelets or conventional liquid-stored platelets. In CLIP-II, randomisation occurs preoperatively, while in CLIPNZ-II this is performed at the time of the first request for platelet transfusion. Patients only receive study platelets if their treating clinicians decide a platelet transfusion is indicated. As the primary outcome is assessed 24 hours after ICU admission, the first study platelet unit must be commenced either intraoperatively or in the first 24 hours after ICU admission. Up to three units of study platelets can be given, after which open-label platelets are administered if more are indicated. The second and third study platelet unit can be commenced any time intraoperatively until the end of the ICU stay (figure 2). If the first platelet transfusion the patient requires is later than 24 hours after ICU admission, open-label standard issue platelets are transfused.

Table 2 Exclusion criteria

1.	**Aged less than 18 years (CLIP-II) or 16 years (CLIPNZ-II)**
2.	Females of childbearing age (18–55 years) who are RhD-negative or whose RhD status is unknown
3.	Receipt of platelet transfusion during this hospital admission
4.	Deep venous thrombosis or pulmonary embolus first diagnosed within the preceding 6 months
5.	More than one lifetime episode of deep venous thrombosis or pulmonary embolism
6.	Known inherited or acquired bleeding disorder (eg, haemophilia, von Willebrand disease, idiopathic thrombocytopenic purpura, aplastic anaemia, haematological malignancy, chronic liver disease) or any undiagnosed bleeding condition, if (and only if) such a disorder or condition is associated with a significant laboratory abnormality at the time of preoperative screening, that is, ► Preoperative platelet count <50 000 x 10^9/L ► INR >2 ► aPTT >2 × upper limit of normal
7.	Treatment with warfarin, intravenous heparin or low-molecular-weight heparin at 'full' therapeutic anticoagulant doses, or other anticoagulant or antiplatelet medications (with the exception of aspirin) such as factor Xa inhibitors (rivaroxaban, apixaban); factor II inhibitors (dabigatran); ADP receptor inhibitors (clopidogrel, prasugrel, ticagrelor, ticlopidine); glycoprotein IIB/IIIA inhibitors (abciximab, eptifibatide, tirofiban); phosphodiesterase inhibitors (cilostazol) or adenosine reuptake inhibitors (dipyridamole) unless this medication has been discontinued in advance of surgery and its effect allowed to dissipate (guidance on specific time intervals for all commonly used medications is provided to trial sites)
8.	Known allergy to dimethylsulphoxide
9.	Planned presence of an arterial line and central venous catheter for less than 12 hours postoperatively
10.	Known objection to receipt of human blood components
11.	The treating physician believes it is not in the best interest of the patient to be randomised in this trial
12.	Previous enrolment during this admission in a clinical trial of a medication or technique thought to influence bleeding, with the exception of any trial of aspirin (ie, trials involving aspirin are permitted), or previous enrolment in a clinical trial with a protocol that affects the transfusion of blood components
13.	Previous enrolment in this study

ADP, Adenosine Diphosphate; aPTT, Activated Partial Thromboplastin Time; INR, International Normalised Ratio.

Cryopreserved platelets

In both trials, cryopreserved group O platelets are prepared using a method based on that of the Netherlands Military Blood Bank,[4] as previously described.[5] This involves collection of apheresis platelet concentrates with storage at 22°C for <48 hours. Approximately 80 mL 27% DMSO in 0.9% NaCl is infused into the agitating platelet bag over 10 min. After transfer to a freezing bag, the platelets are centrifuged at 1350 x g for 10 min. The supernatant is discarded, leaving 20–30 mL platelet concentrate with 325±41 × 10^9 platelets/unit. This is frozen to −80°C at 3°C–5 °C/min then stored for up to 2 years.

The method of platelet reconstitution differs between CLIP-II and CLIPNZ-II. In CLIP-II, the platelet unit is warmed to 25°C–35°C over 5 min in a water bath, then resuspended in approximately 300 mL 25°C–35°C ABO-compatible (group-matched) fresh frozen plasma that has been thawed in a separate process, then joined to the platelet unit using a double-spike connector. In CLIPNZ-II, the platelets and plasma are prepared in one transfusion bag separated by a temporary occlusion device before freezing (figure 3). The temporary occlusion device is removed prior to storage, as the plasma and platelets cannot mix while frozen. To prepare for

Figure 2 Study timeline including window for administration of study platelets. The first unit of study platelets (★) can be administered in the operating theatre (OT) or begin up to the end of the first 24 hours in the ICU. The second and subsequent unit of study platelets (to a maximum of 3) can be administered in the OT or any time up to the time of index ICU discharge. Non-study platelets (☆) can be given at other times. Three patients have been presented as examples. ICU, intensive care unit.

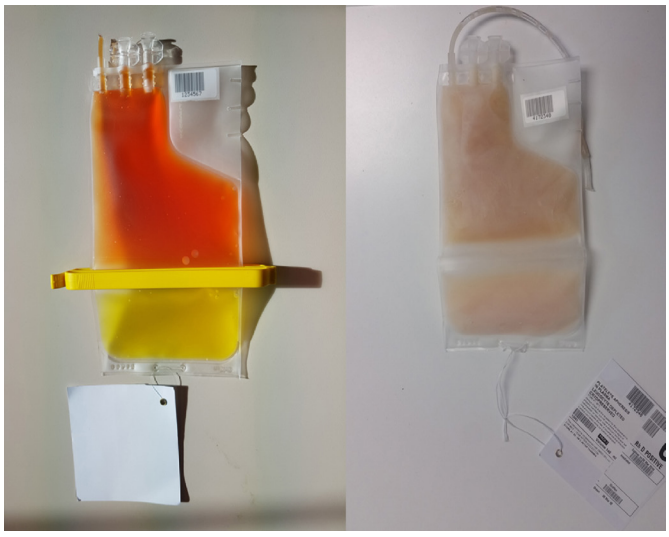

Figure 3 Study platelets used in the CLIPNZ-II trial prior to freezing, with the temporary occlusion device separating plasma and platelet components, and after freezing, with the temporary occlusion device removed.

transfusion, the bag is thawed in a 30°C–32°C water bath, allowing the plasma and platelets to mix.

Thawed reconstituted platelets can be kept at room temperature (20°C–24°C) for up to 4 hours before transfusion.

Conventional liquid-stored platelets

Patients receive conventional liquid pooled buffy coat platelets or apheresis platelets according to routine practice. Pooled buffy-coat platelets are produced from four whole blood donations, a process that results in a component with a similar platelet count (263±36× 10^9/unit) to that of a single platelet apheresis unit (274±31× 10^9/unit).[23] Pooled buffy coat platelets are suspended in 336±15 mL SSP+™ platelet additive solution (Macopharma, Mouvaux, France), 30% of which is plasma. Apheresis platelets are suspended in a similar solution (40% plasma), with on average approximately two-thirds of the total volume (198±11 mL) but a greater range (100–400 mL) than pooled buffy coat platelets. No clinical trial evidence suggests these two types of conventional platelet transfusions produce different clinical effects.

As conventional liquid-stored platelet volume is approximately 200–340 mL per bag, only 30%–40% of which is plasma (compared with cryopreserved platelet volume of 300 mL of which ≥90% is plasma), there is consequently a difference in the total volume and volume of plasma between study groups. However, this is likely to be clinically insignificant, and moreover reflects actual practice should cryopreserved platelets be introduced into routine practice. The hospital blood bank is required to select the liquid-stored platelet ABO group to be infused (ie, either O or ABO-matched) according to its standard practice.

Each unit of study platelets is infused over the time considered to be clinically indicated. Typically, this is 15–60 min, but this decision is left to treating clinicians.

After three units of study platelets, if the clinician orders further platelet transfusions, these are open-label, non-study liquid-stored platelets, conforming to routine hospital practice. Patients receive open-label liquid-stored platelets if any of the following occur:

- Their first platelet transfusion is required >24 hours after ICU admission.
- A total of three packs of study platelets have been transfused.
- After patients are first discharged from the ICU, that is, after ICU discharge on the ward and on readmission to ICU.
- Any of the 'withholding criteria' are met (see the 'harms' section).

Rhesus D considerations

Most study cryopreserved platelets are collected from Rhesus D (RhD)-positive donors, and RhD compatibility does not influence study platelet unit selection. Platelets do not express RhD antigens, but sensitisation can occur due to residual red cells in platelet concentrates. In routine practice, RhD-negative recipients sometimes receive RhD-positive platelets. Some clinicians provide prophylactic RhD immunoglobulin. In Australia and NZ, this practice is not universal and is usually restricted to women of childbearing potential. Women of childbearing age are excluded from enrolment in these trials for this reason. Anti-D immunoglobulin is not mandated for RhD-negative patients who receive RhD-positive cryopreserved platelets, but treating clinicians may provide anti-D if they assess it is warranted for a particular patient.

ABO matching considerations

ABO-matched or donor-recipient compatible platelet transfusions improve post transfusion platelet increment to a small degree,[24] although this practice is not universal in standard care. When comparing platelet increments between the two arms as an outcome in the trial, some patients receiving cryopreserved platelets (all type O) may be at a disadvantage if a higher proportion of standard-care patients receive ABO-matched or donor-recipient compatible platelets. Accordingly, a prespecified sensitivity analysis using only type-compatible recipients from each study group will be performed.

Outcomes

The trial outcome measures are listed in tables 3 and 4. The US National Library of Medicine clinicaltrials.gov registry does not allow 'tertiary' outcomes, only a primary outcome, secondary outcomes and 'other prespecified outcome measures'. Consequently, the tertiary outcome measures listed in the table below are listed as 'other prespecified outcome measures' in this registry. The Australian and New Zealand

Table 3 Trial primary and secondary outcome measures

Primary	Time frame
Volume of postsurgical chest drain bleeding	The first 24 hours from the time of ICU admission
Secondary	
Total volume of postsurgical chest drain bleeding	From time of ICU admission up to removal of drains, death or day 28, whichever occurs first
Composite bleeding outcome using the Bleeding Academic Research Consortium (BARC4) criteria (intracranial bleeding within 48 hours; reoperation after closure of sternotomy; transfusion of ≥5 units whole blood or RBCs (red blood cells) within the 48 hour intraoperative or postoperative period (excluding cell saver blood); chest tube output ≥2 L within a 24-hour period)[32]	Up to ICU discharge, death or day 90, whichever occurs first
No of units of RBC transfused in the first 24 hours after admission to ICU	The first 24 hours from the time of ICU admission.
Total no of units of red blood cells transfused by the time of ICU discharge, including intraoperative transfusion	From operation commencement up to ICU discharge, death or day 7, whichever occurs first.
Occurrence of any one of the following specified adverse events/potential adverse events: ► Venous thromboembolism ► Arterial occlusion ► Acute coronary syndrome ► Acute respiratory distress syndrome (ARDS) at any point of the ICU stay, graded as mild, moderate or severe according to the 2012 Berlin definition of ARDS.[30]	From operation commencement up to hospital discharge or death.

ICU, intensive care unit.

Clinical Trials Registry (ANZCTR) does not permit anything other than primary and secondary outcomes to be registered. Consequently, the tertiary outcome measures in the table below are listed as secondary outcomes in this registry.

Formal cost-effectiveness analyses will be incorporated into each trial. Separate analyses are required in order to take account of the different costs of cryopreserved platelet production, cost of providing liquid-stored platelets (including transport costs), dispersion of the population and challenges of platelet supply to hospitals of various sizes in each country. A goal of these analyses will be to define the characteristics of hospital in each country in which introduction of cryopreserved platelets would be cost-effective.

Sample size

In the CLIP-I trial,[18] the mean (SD) 24-hour blood loss after ICU admission in the two study groups combined was 943 (426) mL. Setting the non-inferiority bleeding limit at 20% of the mean 24-hour bleeding observed (ie, 188 mL) if there is truly no difference between standard and experimental treatments, 176 patients (88 per group) will be required to be 90% sure that the lower limit of a one-sided 95% CI is above the non-inferiority limit (Stata V.9.2). Equivalent calculations based on the New Zealand pilot trial[19] result in a sample size of 99 patients per group. In both cases, the sample size estimate was increased by 15% to account for the possibility of requiring non-parametric analyses,[25] resulting in a requirement for 101 patients (CLIP-II) and 114 patients (CLIPNZ-II) transfused per group.

This sample size will provide sufficient power to detect non-inferiority in clinically important differences in safety outcomes, based on data assessed in the CLIP-I pilot trial.[18] For example, no patients suffered an acute myocardial infarction in the CLIP-I pilot trial. If the true incidence of this outcome is 1% in the control group, 68 transfused patients in each group are required to be 90% sure that the upper bound of the 95% CI around the point estimate in the cryopreserved group excludes a relative difference in incidence of more than 5%. More common outcomes require more patients. DVT was more common, occurring in 4.9% of pilot trial patients. Eighty transfused patients in each group are required to be 90% sure that the upper bound of the 95% CI around the point estimate in the cryopreserved group excludes a relative difference in incidence of more than 10%. For continuous outcomes such as partial pressure of oxygen in arterial blood / fraction of inspired oxygen (PaO_2/FiO_2) ratio 3 hours after transfusion, 65 patients in each group are required to be 90% sure that the 95% CI does not cross a 20% non-inferiority limit.

In the CLIP-I pilot study, 43/119 (36%) of the high-risk patients randomised were actually transfused platelets. Unpublished Australian audit data 2005–2011 found the median proportion of all cardiac surgical patients transfused platelets was 25% (IQR 17%–28%). An ACSePT score of ≥1 identifies the 30% of patients who have a predicted risk of platelet transfusion of ≥30%. If

Table 4 Trial tertiary outcome measures

Tertiary	
Volume of postsurgical chest drain bleeding (analysed as landmark data and as trajectory time-series data)	The first 6, 12, 18, 48 hours, beginning from the time of ICU admission
Individual elements of the Bleeding Academic Research Consortium (BARC4) composite bleeding outcome[32]	Up to ICU discharge, death or day 90, whichever occurs first
No of units of blood components (red cells, plasma, cryoprecipitate, open-label platelets, fibrinogen concentrate, recombinant factor VIIa, prothrombin complex concentrate, whole blood) transfused (analysed as landmark data and as trajectory time-series data)	Intraoperatively, in the first 6, 12, 18, 24, 48 hours* and at ICU discharge or death or day seven whichever occurs first
Delay between platelet order and commencement of first study platelet infusion	From time of order to time of infusion commencement
Volume of blood in chest drains at the time of ICU admission	From operation commencement up to ICU admission, death or 24 hours, whichever occurs first
Time to commencement of postoperative aspirin and prophylactic heparin	From ICU admission up to commencement of aspirin and prophylactic heparin, death or day seven whichever occurs first
Volume of fluid resuscitation recorded on the anaesthetic chart intraoperatively (analysed as landmark data and as trajectory time-series data)	Following ICU admission in the first 6, 12, 18, 24, 48 hours and at ICU discharge or death, whichever occurs first
Viscoelastic coagulation test indices before first and after last study platelet transfusion (where performed)	Before first and after last study platelet transfusion
Haemoglobin concentration, platelet count, fibrinogen concentration, INR and aPTT	Most abnormal value on postoperative day one and on the last measurement prior to ICU discharge, death or day 28, whichever occurs first
Adverse events or potential adverse events; specifically:	
► Venous thromboembolism	Up to hospital discharge
► Arterial occlusion	Up to hospital discharge
► Acute coronary syndrome	Up to hospital discharge
► Acute respiratory distress syndrome.[30]	At any point of the ICU stay
► Incidence of DMSO toxicity (suggested by neurological, cardiac and renal effects)	Within 6 hours of study platelet transfusion
► Local wound infection	
► Systemic infection	Up to hospital discharge
► Fever (incidence of temperature >39°C)	Up to hospital discharge
► Need for surgical intervention	At any point of the ICU stay Up to hospital discharge
Duration of mechanical ventilation	In the first 90 postoperative days for the index admission
Length of postoperative stay in ICU and in hospital	NA
Total estimated healthcare cost, incorporating the cost of provision of cryopreserved or liquid-stored platelets	Up to hospital discharge, death or day 90, whichever occurs first
ICU, hospital and 90-day mortality	NA

*Noting red cell transfusion at 24 hours and total red cell transfusion are secondary outcomes.
aPTT, Activated Partial Thromboplastin Time; DMSO, dimethylsulphoxide; ICU, intensive care unit; INR, International Normalised Ratio.

in reality only 25% of randomised patients are transfused, 808 patients would need to be randomised. However, each trial need only continue randomising patients until 202 (CLIP-II) or 228 (CLIPNZ-II) patients have been transfused.

The registry entries for these trials differ due to different definitions each uses. ClinicalTrials.gov requires the number of patients 'enrolled', defined as 'agreement to participate in a clinical study following completion of the informed consent process'. By this definition, the number of patients 'enrolled' in CLIP-II is 808. The registry entry further states 'It is estimated to require 808 high-risk cardiac surgical patients to be recruited, to obtain 202 patients who receive transfused study platelets for surgical bleeding', thereby removing any confusion over the number of patients that will be included in the analysis. In contrast, the ANZCTR requires statement of the 'target sample size'. The NZ protocol does not randomise patients until the decision to transfuse platelets is made, whereas the Australian protocol randomises all enrolled patients. Using the different definition of the ANZCTR, the CLIPNZ-II record does not include patients who have given consent

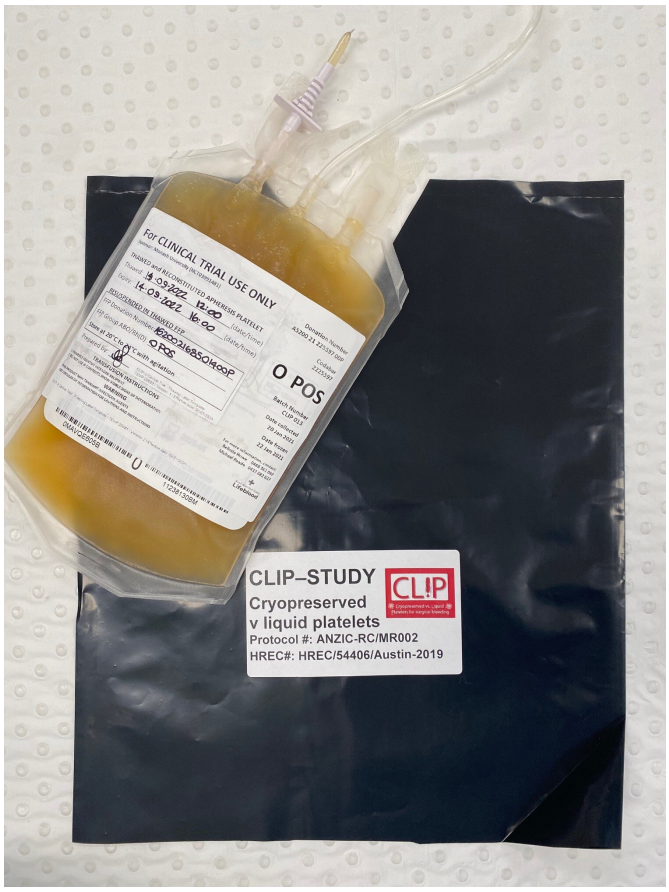

Figure 4 Cryopreserved platelet unit resuspended in plasma alongside the opaque bag used to blind clinical staff.

to be randomised into the study but who are never randomised.

Allocation sequence generation

Randomisation is performed using a password-protected website available at all times, stratified by hospital.

Allocation concealment mechanism

Treatment allocation is determined using a computer-generated permuted block randomisation schedule with block sizes of 2–4.

Blinding

Study group allocation is concealed from the participating patients, the nurse caring for the patient and medical staff involved in transfusion decisions. Regulatory requirements specify cryopreserved platelets must be labelled with both plasma and platelet donor numbers, meaning unit labels must be unblinded. Study platelets are therefore supplied with an opaque cover (figure 4) obscuring their method of storage, but that retains the original donation identification number, ABO and Rh group, and expiry date. This information is checked in the usual manner by bedside clinical staff not responsible for transfusion decisions for that patient.

If an adverse event (AE) occurs that is thought to be related to the study, the site investigator or treating clinician must decide whether knowledge of treatment allocation is required for treatment. Unblinding only occurs if it will affect decisions regarding patient management other than whether to continue to transfuse the patient with study platelets, as transfusion should be discontinued regardless.

Data collection methods

All data are collected by Good Clinical Practice-trained staff at each study site and entered into an online database designed and maintained by the trial coordinating centres. Case report forms are available in online supplemental file 1. Randomised patients are followed to death or 90 days postrandomisation, whichever occurs first. To facilitate 90-day follow-up, patients are asked to provide two possible points of contact (home and close family contact details) prior to discharge.

Data management

Several procedures ensure data quality and protocol compliance, including:

- Study training and initiation visits for participating sites held prior to study commencement.
- A detailed data dictionary defines all data to be collected.
- Early onsite monitoring visit and continuing visits as required.
- Automated validity checking of data entry to the case report forms, timely validation of data, queries and corrections.

Statistical methods

Analysis and reporting of the results will follow the Consolidated Standards of Reporting Trials guidelines.[26] The primary analysis will compare all patients who received any number of study platelet units (cryopreserved vs liquid stored) (figure 5). Enrolled patients never transfused any study platelets will not be analysed. In the CLIP-II study (more than CLIPNZ-II), this planned approach is likely to exclude a substantial number of patients post-randomisation. As allocation to study arm could not influence the likelihood that patients would be transfused platelets, this is one of the few circumstances (termed 'premature randomisation') in which this is preferable to including all randomised patients.[27] Clinicians might come to lack equipoise for an individual patient, despite overall equipoise for the trial, as in many hospitals the time to prepare and deliver cryopreserved platelets (approximately 30–40 min) will be longer than the 10–15 min for liquid-stored platelets. Further, in some study hospitals, blood bank staffing limitations mean cryopreserved platelets can only be reconstituted during normal working hours. A number of patients unable to receive study platelets for each of these reasons will be reported as an implementation feasibility outcome and compared between the two trials.

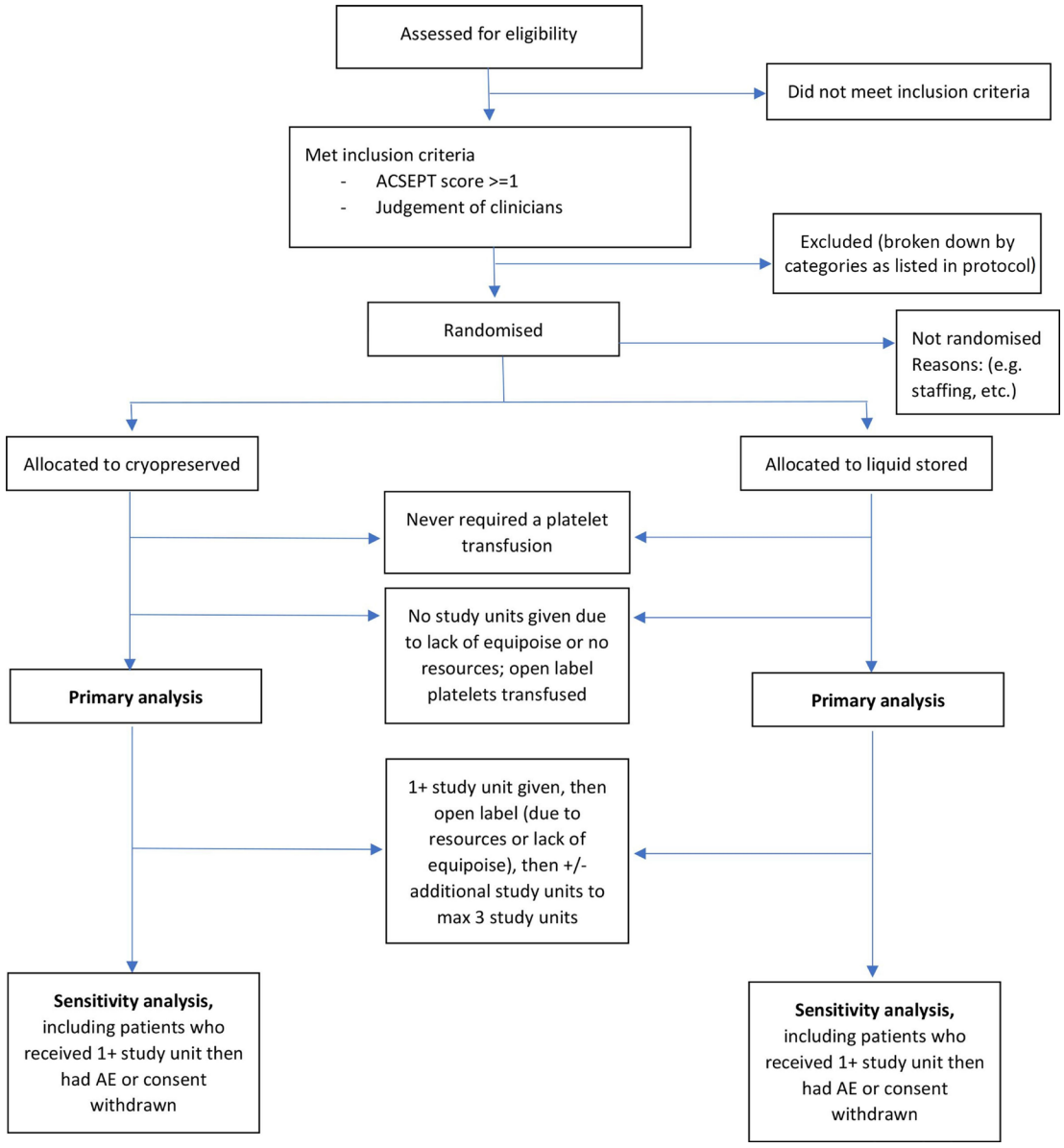

Figure 5 Trial analysis groups taking account of post-randomisation exclusions. Primary analysis: comparing all patients who received any number of cryopreserved platelet units with all patients who received any number of liquid-stored study platelet units. Sensitivity analysis: comparing patients who received up to three units of study cryopreserved or liquid-stored study platelets as intended, along with those who did not receive the full intended number of study platelet units because of an adverse event or because consent was withdrawn.

Some patients will be transfused at least one unit of study platelets but will not then continue to receive the total of three study units as per protocol. International guidelines[28] recommend assessing potential bias under such circumstances. The primary means to do this will be a planned sensitivity analysis (figure 5) excluding patients who received at least one unit of study platelets, but (prior to receiving three study platelets units) who then received open-label platelets. Additionally, baseline characteristics of patients transfused at least one unit of trial platelets, then open-label platelets due to lack of equipoise or resources, will be compared with the overall trial groups, to investigate whether they differed in any measurable way.

The primary outcome will be reported as the mean or median difference between groups with 95% CIs and significance tested using a t-test or Mann-Whitney test as appropriate. Other outcomes will be assessed as differences in proportion (with 95% CI), mean/median difference between groups, or comparison of time-series data using trajectory analysis. To account for any effect of site and for baseline imbalances, regression models for the primary and secondary outcomes will be built with patients nested within site, site treated as a random effect and the elements of the EuroSCORE II[29] used to adjust for patient risk.

Heterogeneity of treatment effect across prespecified subgroups will assess the effect of ABO-compatible versus

non-compatible platelet transfusion, location of the first study platelet transfusion (intraoperative vs postoperative), main indication for surgery, and whether aspirin had been given within 7 days prior to surgery.

If the proportion of patients missing the primary outcome exceeds 5%, multiple imputation will be used to construct imputation models separately for each treatment arm using relevant baseline and postbaseline variables.

All statistical analyses for both studies will be conducted by a qualified professional biostatistician based at the study co-ordinating centre.

Data and safety monitoring

An independent data and safety monitoring committee (DSMC) overseeing both trials was established before patient enrolment began. The DSMC is notified within 24 hours of each individual serious AE and suspected unexpected serious adverse reaction. The DSMC will conduct one interim analysis after approximately half the target total number of patients have been transfused study platelets and reached day 90 outcomes in each trial. The DSMC Charter is available in online supplemental file 2.

Harms

In the intraoperative and immediate postoperative period, cardiac surgical patients experience many aberrations in laboratory values and many abnormal signs and symptoms due to the nature of the underlying disease and the impact of standard therapies. These do not necessarily constitute an AE unless they are considered to be, in the judgement of the site principal investigator, related to transfusion of study platelets.

AEs are reported on for the duration of the hospital admission. Any diagnosis of pulmonary embolus or deep venous thrombosis is reported as an AE or Serious AE. AEs decided to be possibly causally related to study platelets are recorded in a safety database reviewed by the DSMC on a regular basis. Serious AEs are reported to the coordinating centres within 24 hours. The coordinating centres report each Serious AE to the chair of the DSMC, who makes recommendations as appropriate.

Study platelets might be required to be withheld if, at any time during or up to 6 hours after the infusion of study platelets, the patient develops a medical condition that suggests DMSO toxicity, including neurological effects not consistent with the expected degree of postoperative confusion, unexplained severe renal impairment, marked systemic vasoconstriction not explicable on other grounds, marked tachycardia or bradycardia, or moderate or severe ARDS.[30] If study platelets are withheld, all subsequent platelet transfusions must be open-label liquid-stored platelets. The decision on whether to complete a study platelet unit transfusion that has already commenced once an AE is detected is left to the treating clinician in consultation with the site principal investigator

A running study platelet transfusion must be immediately discontinued, and the patient must not receive further study platelets, if at any time during or after the infusion of study platelets, the patient develops venous thromboembolic disease or arterial occlusion (including coronary vessel occlusion as evidenced by significant new ischaemia/infarction on a 12-lead ECG), or the patient suffers a suspected unexplained serious AE thought possibly related to study platelets.

Monitoring

The study project manager will conduct an on-site 100% source data verification of study consent forms, AE reports and the primary outcome, and selective source data verification applying the principles of risk-based monitoring to other elements of individual case report forms. Each site will be monitored at least twice; once near the commencement of recruitment and once following completion of recruitment, with additional monitoring visits determined by perceived risk.

Patient and public involvement

Patients were not involved in the development of the study research question and outcome measures. Patients are given the opportunity to receive any of the results of their diagnostic tests that have been obtained in the course of the study, and a summary of the results when the research is completed.

ETHICS AND DISSEMINATION

Research ethics approval

CLIP-II was approved by the Austin Health Human Research Ethics Committee (HREC/54406/Austin-2019) on 23 September 2019 under the terms of National Mutual Acceptance in Australian human research, and by the Australian Red Cross Lifeblood Ethics Committee (2019#23) on 5 December 2019. CLIPNZ-II was approved by the New Zealand Southern Health and Disability Ethics Committee (21/STH/66) on 30 April 2021.

Consent

Patients listed for surgery and identified as meeting the inclusion criteria are provided with information about the study at least 1 day prior to their planned procedure, either by written information posted to them or by discussion with a research team member. On the day of surgery, a study team member provides any further information necessary and confirms written informed consent. The study patient consent forms are in online supplemental files 3; 4. Patients are deemed competent to consent to the study if they are able to provide their own consent for cardiac surgery. There is no provision for consent provided by a substitute decision maker. Consent for participation may be withdrawn by the patient, or in the case that they are unable to make such a decision (if, eg, they are sedated postoperatively in the ICU), by their nominated surrogate decision maker at any time.

Confidentiality

The enrolment log which contains identifiable information does not leave the locked research office at the study site. Deidentified study data are entered into a secure, password-protected database. Identifying details of the patient and their family (including name, address and telephone numbers) that allow the site investigators to conduct telephone follow-up at 90 days do not leave the study site.

Access to data

Designated site study staff have access to identified participant data at each site to the extent necessary to complete the requirements of the study protocol. Coordinating centre staff, including the coordinating principal investigator, project manager, data managers, study statistician, members of the management committee and members of the DSMC, have access to coded data. At the completion of the study, at the discretion of the trial management committee and Monash University, an extract of the trial data without any patient identifiers may be made available on a case-by-case basis to investigators from reputable research organisations with a defined protocol and analysis plan, using the principles to protect patient anonymity described by the UK Medical Research Council.[31] Data availability will begin 9 months after publication of the individual patient data meta-analysis combining both trial results, and end 36 months after this publication. In accordance with the ANZICS CTG Terms of Reference for an endorsed trial, the CTG chair must approve sharing of data with any third party, manuscripts derived from shared data must be submitted for CTG endorsement prior to publication and must acknowledge the role of the CTG in the original study, and a copy of the published manuscript must be provided to the CTG office.

At study completion, all data and study documents will be retained for 15 years in compliance with the study ethical approval. Retention will be on Monash University and Medical Research Institute of New Zealand (MRINZ) network storage for all data and electronic files. Paper documents will be stored in the Monash University and MRINZ archives. At the end of this retention period, all electronic data and files will be erased permanently and paper documents will be destroyed securely.

Ancillary and post-trial care

Study patients receive all elements of standard care apart from open-label liquid-stored platelets, which may only be given after three bags of study platelets have been administered. Specifically, as part of routine perioperative care, patients may receive aspirin or subcutaneous heparin at prophylactic doses. Patients may also receive postoperative warfarin, intravenous heparin or low-molecular-weight heparin at 'full' therapeutic anticoagulant doses, or other anticoagulant or antiplatelet medications. Such medications include but are not limited to: factor Xa inhibitors, factor II inhibitors, ADP receptor inhibitors, glycoprotein IIB/IIIA inhibitors, phosphodiesterase inhibitors or adenosine reuptake inhibitors.

Dissemination policy

The CLIP-II and CLIPNZ-II trials will be submitted separately for publication in peer-reviewed journals. Following publication, an individual patient data meta-analysis with a prespecified statistical analysis plan with hypotheses informed by the results of the constituent trials will be published. This may, for example, use the greater power of the combined studies to explore the effect of platelet dose, method of platelet preparation or possible differences in adverse effects.

The CLIP-II and CLIPNZ-II investigators will comprise the management committee and the principal and associate investigators from the participating sites of each trial. The full list of CLIP-II and CLIPNZ-II investigators will be listed as authors for the purpose of indexing in PubMed, etc in an appendix to the publication.

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Contributors MCR and DCM conceived the program of research and with ZM and LJ secured Australian funding from the National Health and Medical Research Council for CLIP-II. SM along with RP, RC and MCR secured New Zealand funding from the Health Research Council of New Zealand for CLIPNZ-II. MCR drafted the protocol for both trials, and SM led the adaptation of the protocol in CLIPNZ-II. DCM and LJ developed the platelet manufacturing protocol in CLIP-II, and RC led the development of this protocol in CLIPNZ-II. BH is the Project manager for CLIP-II, with substantial input to the protocol design, and oversight of the initiation and maintenance of patient recruitment and data collection at all sites. LN is the Project Manager for CLIPNZ-II, undertaking similar responsibilities in that study. All authors contributed to the design of the protocols and have approved the final manuscript.

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Competing interests MCR is a serving officer in the Australian Defence Force, which intends to operationalise cryopreserved platelets if the trial shows favourable

results. DCM and LJare employed by Australian Red Cross Lifeblood, and RC is employed by the NZ Blood Service, which could both adopt cryopreserved platelets if the trial shows cost effectiveness. Other authors declare no conflicts of interest.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Not applicable.

Provenance and peer review Not commissioned; peer reviewed for ethical and funding approval prior to submission.

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