Reade Michael (Orcid ID: 0000-0003-1570-0707)
Johnson Lacey (Orcid ID: 0000-0003-3303-4437)

A randomized, controlled pilot clinical trial of cryopreserved platelets for perioperative surgical bleeding. The CLIP-I trial.

Michael C. Reade [1,2], Denese C. Marks [3], Rinaldo Bellomo [4], Renae Deans [2], Daniel J. Faulke [5], John F. Fraser [5], David J. Gattas [6], Anthony D. Holley [2], David O. Irving [3], **Lacey Johnson [3],** Bronwyn L. Pearse [5], Alistair G. Royse [7], Janet Wong [3], for the Cryopreserved vs Liquid Platelet (CLIP) Investigators*, the Australian and New Zealand College of Anaesthetists Clinical Trials Network and the Australian and New Zealand Intensive Care Society Clinical Trials Group. *The CLIP investigators at each of the participating trial sites are listed as authors at the end of the manuscript

[1] Joint Health Command, Australian Defence Force, Canberra, ACT, Australia. [2] University of Queensland, Brisbane, QLD, Australia. [3] Australian Red Cross Blood Service, Sydney, NSW, Australia. [4] Austin Hospital, Melbourne, VIC, Australia. [5] The Prince Charles Hospital, Brisbane, QLD, Australia. [6] Royal Prince Alfred Hospital, Sydney, NSW, Australia. [7] Royal Melbourne Hospital, Melbourne, VIC, Australia

Address correspondence to:

Professor Michael Reade, Level 9, University of Queensland Health Sciences Building, Royal Brisbane and Women's Hospital Queensland 4029 Australia. Tel +61 7 3365 5114 Fax +61 7 3365 5192. m.reade@uq.edu.au

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ABSTRACT AND KEY WORDS

(Abstract 336 words)

BACKGROUND Cryopreservation extends platelet shelf-life from 5-7 days to 2-4 years. However, only 73 patients have been transfused cryopreserved platelets in published randomized controlled trials (RCTs), making safety data insufficient for regulatory approval.

STUDY DESIGN AND METHODS The Cryopreserved vs. Liquid Platelet (CLIP) study was a double-blind, pilot, multicenter RCT involving high-risk cardiothoracic surgical patients in four Australian hospitals. The objective was to test, as the primary outcome, the feasibility and safety of the protocol. Patients were allocated to study group by permuted block randomization, with patients and clinicians blinded by use of an opaque shroud placed over each study platelet unit. Up to three units of cryopreserved or liquid-stored platelets were administered per patient. No other aspect of patient care was affected. Adverse events were actively sought.

RESULTS One hundred and twenty-one patients were randomized, of whom 23 received cryopreserved platelets and 18 liquid-stored platelets. There were no differences in blood

loss (median 715 vs. 805 ml at 24hr; difference between groups 90ml (95% CI -343.8 – 163.8ml), p=0.41), but the Bleeding Academic Research Consortium criterion for significant postoperative hemorrhage in cardiac surgery composite bleeding endpoint occurred in nearly twice as many patients in the liquid-stored group (55.6% vs. 30.4%, p=0.10). Red blood cell transfusion requirements were a median of 3 in the cryopreserved group vs. 4 units with liquid stored platelets [difference between groups 1 unit (95% CI -3.1 to 1.1 unit); p=0.23]. Patients in the cryopreserved group were more likely to be transfused FFP (78.3% vs. 27.8%, p=0.002) and received more study platelet units (median 2 units vs. 1 unit, difference between groups 1 unit (95% CI -0.03 to 2.0 unit) p=0.012). There were no between-group differences in potential harms including DVT, myocardial infarction, respiratory function, infection, renal function. No patient had died at 28 days, and postoperative length of stay was similar in each group..

CONCLUSION In this pilot RCT, compared to liquid-stored platelets, cryopreserved platelets were associated with no evidence of harm. A definitive study testing safety and hemostatic effectiveness is warranted.

KEY WORDS

Cryopreservation; blood platelets; platelet transfusion; hemorrhage; cardiac surgical procedures

INTRODUCTION

After donation, platelets are stored at 20-24°C with agitation to prolong circulating time after transfusion. This limits their shelf-life to 5-7 days[1] but despite this, platelets cause 84% of transfusion-transmitted bacterial infections.[2] Short shelf-life also leads to high levels of wastage: 1/4 – 1/3 of units are discarded due to expiry.[3,4] To limit wastage, platelets are only kept routinely in larger hospitals where use is high and predictable. Bleeding patients in smaller hospitals are more likely to be denied this potentially life-saving treatment.

In response to these problems, the Netherlands Military Blood Bank built on US Navy research[5,6] to operationalize a process to freeze (using dimethyl sulfoxide (DMSO) as a cryopreservative), store (at -80°C for up to 2-4 years) and reconstitute platelets.[7] Reconstitution takes < 30 minutes[7,8] and requires minimal equipment and training. Dutch and Australian military experience in Afghanistan with 1,143 cryopreserved platelet units transfused into 349 patients suggested they were both safe and effective, [9,10] although no comparisons with liquid-stored platelets or whole blood were made. Extensive preclinical assessments [7,8,11,12 13-15] have found that, compared to liquid-stored platelets, cryopreserved platelets have increased procoagulant activity, with alterations in membrane receptors and higher microparticle concentrations.[16,17] A phase I human study found lower 24-hr platelet recovery in comparison to liquid-stored platelets, but with survival times exceeding US Food and Drug Administration requirements, and with no associated adverse events.[18] Three

small phase II trials have been performed in bleeding hematology,[19] cardiac surgery,[20] and a mixed population of mostly trauma patients,[21,22] all of which suggested at least comparable hemostatic efficacy and no evidence of adverse effects. However, the total number of patients transfused cryopreserved platelets in these three trials is only 73. Safety outcomes were not reported in detail, and efficacy outcomes were too variably assessed to allow data pooling. Safety is a particular concern: for example if cryopreserved platelets induce a hypercoagulable state, this could lead to coronary artery occlusion or thromboembolic disease, but this might not have been observed.

In the absence of sufficient comparative trial evidence, cryopreserved platelets are not registered for routine clinical use in most countries, despite clear hypothesized advantages that include: a. potentially greater hemostatic activity; b. reduced risk of infectious disease transmission; c. reduced wastage; d. not having to transport liquid platelets urgently to hospitals unable to hold an adequate supply; e. providing platelets in hospitals where this currently is not possible; and f. prolonged storage of rare platelet units for use in alloimmunized patients.[23] Accordingly, we embarked on a program of research designed to generate sufficient evidence for regulatory approval and widespread introduction into clinical practice. As part of this program we performed a pilot randomized controlled trial in cardiac surgery patients. The objective was to test, as the primary outcome, the feasibility

and safety of the protocol. Secondary outcomes were to assess measures of safety and effectiveness that might be used in any subsequent, definitive trial.

MATERIALS AND METHODS

The Cryopreserved vs. Liquid Platelets (CLIP) study was a double-blind, parallel-group, standard-care controlled multicenter pilot trial conducted in four Australian tertiary academic hospitals. The protocol was approved by the Human Research Ethics Committees of the Royal Brisbane and Women's Hospital and the Australian Red Cross Blood Service. Consent was obtained from patients during the preoperative visit with their cardiac surgeon or anesthesiologist. A Data Safety Monitoring Committee was appointed to review adverse events as required, but no interim analysis was planned. The protocol was prospectively registered (ACTRN12612001261808).

Study platelets Cryopreserved group O (RhD positive) platelets were prepared by the Australian Red Cross Blood Service (ARCBS) using a method based on that of the Netherlands Military Blood Bank,[7] as previously described.[8] Briefly, 27% DMSO in 0.9% NaCl was infused into apheresis platelet concentrates to give a final concentration of 5-6% DMSO. Platelets were concentrated by centrifugation, leaving 20-30 mL platelet concentrate with approximately 506±37 x 10^9 platelets/unit. These were frozen at -80°C, transported to the hospital blood banks of participating sites, and stored for 11.7±3.6 months. In the

hospital blood bank, cryopreserved platelets were thawed in a water bath at 37°C and reconstituted using AB or group-specific plasma either thawed specifically for the purpose or maintained pre-thawed for emergencies. Plasma was transferred to the platelet bag using sterile tubing inserted into the access port. Platelets could be transfused up to 4 hours after reconstitution.

Patients randomized to receive liquid-stored platelets were transfused pooled buffy coat platelets or (if a suitable buffy-coat platelet unit was unavailable) apheresis platelets prepared by the ARCBS and distributed to hospital blood banks according to routine practice. The hospital blood bank selected the platelet ABO group to be infused according to its standard practice.

Inclusion and exclusion criteria Adult patients (aged ≥ 18 years) were eligible for the study if they were scheduled to undergo cardiac surgery and at least three of the TRUST criteria[24] (which predict a high risk of red cell transfusion) were present: preoperative hemoglobin < 13.5 g/dl; female sex; redo surgery; preoperative creatinine >120 μmol/l; non-elective surgery; age > 65 years; body weight <77kg; and non-isolated surgery. No published validated score predicts platelet transfusion in cardiac surgery. Patients with ≥3 TRUST criteria have a >65% chance of requiring a red blood cell transfusion. The incidence of

platelet transfusion is 44% that of red cell transfusion,[25] so a patient with ≥3/8 TRUST criteria has a 44% x 65% = 29% chance of receiving platelets.

Patients were excluded if they had received a platelet transfusion earlier during the same hospital admission; were women of childbearing age (18-55 years); death was deemed inevitable in <24hrs; had been previously enrolled in this study or a clinical trial of a medication (with the exception of aspirin) or technique thought to influence bleeding; had a known bleeding diathesis (for example, hemophilia or Von Willebrand Disease) or hematological malignancy associated with abnormal clotting on blood investigations taken in the immediate preoperative period (i.e. platelet count <100 000, INR>1.5, aPTT > 1.5 x upper limit of normal); had a known allergy to DMSO; had a known objection to receipt of human blood products; had intellectual impairment such that they were unable to consent for surgery themselves; or if their treating physician believed it was not in their best interest to participate in the trial.

Trial procedures Patients were randomized 1:1, stratified by site, using computer-generated permuted block randomization. Randomization allocation was recorded in each hospital blood bank. Patients could receive up to three units of study platelets (cryopreserved or liquid-stored) if their treating clinicians decided they required a platelet transfusion either intraoperatively or during their postoperative stay in the intensive care unit (ICU). Clinicians

could use any clinical or laboratory indications to judge the requirement for platelet transfusion. The protocol highlighted contemporary Australian guidelines,[26] which only advised that 'the prophylactic use of platelets after cardiac surgery is not supported' and that 'in general, patients with a platelet count ≥50 × 10^9/L can undergo invasive procedures without any serious bleeding; however, lower platelet counts may be tolerated'. Laboratory and point of care (thromboelastometry (TEG) or thromboelastography (ROTEM)) tests were encouraged as good clinical practice immediately prior to and following platelet transfusion. These were recorded whenever they were measured. If, having received three units of study platelets, more were required, open-label platelets were administered. Each unit of study platelets was infused over the time considered to be clinically indicated. No other aspect of patient care was affected by the trial protocol.

When a platelet transfusion was ordered, an unblinded blood bank scientist supplied study platelet units to operating room or ward staff. Cryopreserved platelets were suspended in approx. 300 mL of thawed fresh-frozen plasma (FFP) as part of the process of reconstitution, making their appearance similar to that of liquid-stored platelets. However, regulatory constraints required cryopreserved platelets to be supplied with the donor numbers of both the platelet and plasma units, unblinding bag labels. Study platelets were therefore supplied with an opaque shroud that obscured the storage method. Two clinicians not responsible

for transfusion decisions performed mandatory identity checks at the bedside before handing the platelet unit, inside the shroud, to clinical staff for transfusion.

The only investigation performed in addition to routine clinical care was a lower limb duplex venous ultrasound, performed between 48 and 96 hours after surgery.

Cardiac surgical patients, particularly those with postoperative bleeding, commonly develop postoperative complications. Many of these are similar to the possible complications of cryopreserved platelets. When reporting attributable adverse events, treating clinicians were required to judge if an adverse event was related to study platelets or to the expected complications of surgery This judgement was guided by temporal association with the study platelet transfusion and the presence of other possible explanations. DMSO toxicity was deemed by the protocol to be unlikely more than six hours after the last study platelet infusion, but no further specific guidance was provided by the trial protocol on the timing of other possible complications. Rather, clinicians required to utilize their understanding of the possible pathophysiological effects of cryopreserved platelets, as outlined in the study Investigator Brochure. Highlighted possible adverse reactions included: signs of DMSO toxicity (neurological or renal impairment; systemic vasoconstriction; hypertension; marked tachy- or brady- cardia), which were marked as present or absent every two hours until six hours after the last study platelet transfusion; venous thromboembolic disease; arterial

occlusion (including coronary vessel occlusion identified on continuous ECG monitoring); or acute respiratory distress syndrome (according to the 'Berlin definition').[27] Venous thromboembolic disease was diagnosed as present if a deep venous thrombosis (DVT) was identified on the mandated lower limb duplex ultrasound performed between 48-96 hours after surgery, or a DVT or pulmonary embolus (PE) was identified on any other investigation (duplex ultrasound of any limb, echocardiography, computerized tomography – pulmonary angiography or nuclear medicine ventilation – perfusion scan) performed for clinical indications up until the time of hospital discharge. Irrespective of judgements of attribution to the study intervention, several other postoperative safety indices were compared between groups, including: core temperature; ratio of partial pressure of arterial oxygen tension to fraction of inspired oxygen (PaO_2 / FiO_2 ratio); hospital length of stay; 28-day mortality; peak postoperative creatinine and requirement for postoperative renal replacement therapy. The incidence of wound and systemic infection (defined as requiring treatment with other than prophylactic antibiotics) was also compared. If withdrawn from the trial after randomization, the patient was retained in the intention to treat analysis according to their allocated study group. Patients allocated to cryopreserved platelets who received open-label platelets for any reason were excluded from a treatment-received sensitivity analysis.

Statistics The primary outcome of this pilot study was the feasibility and safety of the protocol. Secondary endpoints, assessed for possible use in a subsequent phase III trial, included volume of post-surgical bleeding and requirement for postoperative blood products. Consequently, no a priori sample size calculation targeting sufficient statistical power to demonstrate between-group differences in any quantitative outcome was performed. Rather, it was decided that recruiting 20 patients / site would be sufficient to demonstrate feasibility. Trial recruitment was terminated when a stable recruitment rate had been attained and when 20 patients had been recruited at one site.

Categorical outcomes were compared using $\chi 2$ or Fisher exact tests, and continuous outcomes were compared using either Student's t tests or Mann-Whitney U tests depending on whether the data appeared normally distributed. The statistical significance of differences in select outcomes was adjusted for random imbalances in baseline characteristics of the two study groups using linear or logistic regression. In all cases, a 2-sided p value of < 0.05 was considered significant. No adjustment was made for multiple comparisons. As there was <5% missing data for all reported outcomes (except where specified), no imputation for missing data has been made. Analyses were performed using Stata version 12.1 (StataCorp, College Station, Texas, USA).

There were no changes to the trial protocol after commencement of patient recruitment.

RESULTS

Between 03 July 2015 and 31 December 2017, 121 patients were randomized, of whom 41 required a platelet transfusion (figure 1). Three patients received a unit of open-label platelets before they received all three units of study platelets; in each case this was because the clinician considered a study platelet unit might take too long to arrive, and conventional liquid-stored platelets were ordered instead. This was permitted by the protocol under the 'loss of equipoise' clause. As these three patients all received at least one unit of cryopreserved platelets, they were retained in the primary intention-to-treat analysis, but a secondary sensitivity analysis is presented with them excluded. All study platelets (both liquid-stored or cryopreserved) that were ordered from the hospital blood banks were subsequently transfused to their intended recipients; none were wasted.

Study groups were generally well-balanced at baseline (Table 1). Most patients were >70 years old, male, with a high prevalence of comorbidities. Cardiopulmonary bypass times were approximately three hours, consistent with complex surgery, and comparable between study groups. Most patients (72.2-87.0%) received their first platelet transfusion in the operating room (table 2).

There were no differences in any measure of intra- or post- operative blood loss (table 3 and figure 2). The Bleeding Academic Research Consortium (BARC) defines significant bleeding following cardiac surgery as that with one or more of: intracranial hemorrhage <48 hours postoperatively; reoperation after sternotomy closure for the purpose of controlling bleeding; transfusion of ≥ 5 units whole blood or red blood cells within 48 hours postoperatively; or chest tube output ≥ 2L within 24 hours postoperatively.[24] Nearly twice as many patients in the liquid-stored group met these criteria (55.6% vs. 30.4%; p=0.10). Clinicians typically commence aspirin and heparin postoperatively only when concern over ongoing hemorrhage has passed; these medications were most commonly commenced on day 2, with no difference between study groups.

There were several differences in quantities of blood products transfused between the groups (table 4). Whole blood was not available for use during the study. Red cell transfusion was not different, although numerically fewer patients in the cryopreserved group were transfused in the operating room (OR), and were transfused one less unit overall. Patients in the cryopreserved group were significantly more likely to be transfused FFP, and overall a greater median volume of FFP was transfused to this group. However, among only those patients transfused FFP, the median number of FFP units was not different. Patients in the cryopreserved group received a median of one more unit of study

platelets. Very few patients received non-study open-label platelet transfusions, and this did not differ between groups.

The only difference in laboratory parameters the day after surgery was the platelet count, which was significantly lower in the cryopreserved platelet group (table 5).

There were no between-group differences in any safety outcome (table 6) or measure of postoperative physiological instability or requirement for organ support (table S1). Only one adverse event was reported: a non-occlusive thrombus, present *prior* to administration of trial platelets, was identified in the soleal vein of a patient who had received cryopreserved platelets. As this was not temporally related to the administration of platelets, it was ruled to be unrelated to the protocol.

Only approximately one third of patients in each study group had a ROTEM test performed immediately before and after study platelet transfusion (table S2). There were no significant between-group differences in the improvements made by study platelet transfusion. While patient numbers are too small to draw any conclusions from these data, more patients in the cryopreserved group had improvements in their EXTEM Clotting Time and Alpha angle, and these improvements were of a greater magnitude, than in the liquid-stored group.

A sensitivity analysis that excluded the three patients in the cryopreserved group who received open-label platelets prior to three study platelet units found no qualitative differences in any of the outcomes reported above. The difference in total red blood cell units transfused increased (median (IQR) cryopreserved 2 (1-4) vs. liquid 4 (3-5), p=0.07), as did the blood in the drains at 24 hours (672.5 (520-795) vs. 805 (591-1080), p=0.12) and 48 hours (925 (675-1127.5) vs. 1075 (810-1540), p=0.15).

Study group was not a statistically significant predictor of 24-hour blood loss or total postoperative red blood cell transfusion when all of the baseline variables listed in table 1 were entered into backwards stepwise linear regression models using $p<0.1$ for retention. Backwards stepwise logistic regression with the Bleeding Academic Research Consortium criterion for significant postoperative hemorrhage in cardiac surgery (BARC4) bleeding outcome as the dependent variable was unable to fit a satisfactory model with these predictors, so forward regression was used instead. The optimal model found that liquid platelets, having been an inpatient >24 hours prior to surgery, chronic liver disease, chronic renal disease and malignancy all significantly increased the odds of the BARC4 outcome, whereas diabetes and recent myocardial infarction reduced these odds. Final models are shown in tables S3-S5 of the electronic Supporting Information.

DISCUSSION

In this double-blind randomized controlled trial involving bleeding cardiac surgical patients, transfusion of up to three units of cryopreserved platelets, compared to conventional liquid-stored platelets, was feasible and not associated with any difference in adverse outcomes. Patients in the cryopreserved platelet group were transfused more FFP and platelets, and had a lower platelet count on postoperative day 1. Cryopreserved platelets were associated with small, non-statistically significant benefits in some indices of bleeding and red cell transfusion.

The results of this study are largely consistent with those of the first and largest randomized controlled trial of cryopreserved platelets in bleeding surgical patients.[20] In that trial, cryopreserved platelets were prepared using a slightly different method that involved freezing without prior removal of DMSO, which consequently had to be removed in a washing step after thawing prior to resuspension in plasma. Platelets prepared in this manner had a lower proportion of platelet recovery, higher GPIb receptor expression, and lower annexin V binding than those from which most DMSO had been removed prior to freezing and which were resuspended in saline after thawing.[6] However, how many of these differences were attributable to resuspension in saline is unclear. Blinded transfusion of cryopreserved platelets in this study was associated with significantly less blood loss (median (IQR) cryopreserved 1721 (1118) mL vs. liquid-stored 2298 (1639) mL ($p = 0.007$)),

and significantly less transfusion requirement (total volume of all blood products, mean ± SD 1933 ± 1042 mL vs. 3426 ± 1963 mL, p = 0.0012). Platelet increment after cryopreserved platelet transfusion was significantly lower than after liquid platelets, but unlike our trial, the day 1 platelet count was not different between trial groups. The volume of cryopreserved platelets transfused was significantly less, presumably an artefact of reconstituting cryopreserved platelets in only 30 mL plasma. A major criticism of this trial is post-randomization withdrawal of several participants. Two of the 73 patients randomized into the study died, one who had been transfused cryopreserved platelets and one who had received liquid platelets; however, both were excluded from the analysis as their causes of death were judged 'unrelated' to these transfusions. A further 3 patients (2 cryopreserved and 1 liquid-stored) were excluded because they received ε-aminocaproic acid, arbitrarily prohibited by the trial, and 6 more were excluded (3 cryopreserved and 3 liquid-stored) because their bleeding had a 'surgical' cause rather than a diffuse ooze. The incidence of adverse reactions was not reported, with the paper noting only that 'thromboembolic complications and infections (wound infection, pneumonia) did not differ statistically between the 2 groups'.

The only other controlled study of bleeding non-hematology patients compared clinical and laboratory parameters of 25 patients (mostly with life-threatening bleeding due to trauma) transfused a total of 81 units of cryopreserved platelets with those of 21 patients transfused

67 units of liquid-stored apheresis platelets. The method of cryopreserved platelet preparation reported in this study appears essentially identical to that used in the CLIP trial. As in our trial, patients transfused cryopreserved platelets had a lower post-transfusion platelet count compared to those given liquid-stored platelets (41 500/μL vs. 97 000/μL; p=0.02). An earlier account of this study published in the Czech language reported cryopreserved platelet transfusion produced TEG traces with a shorter time to clot initiation but reduced maximum amplitude, consistent with the general pattern of the ROTEM results observed in our trial.[22] No other post-transfusion laboratory parameters or vital signs were different between the groups, which also had comparable durations of hospital stay, adverse events and survival, although these outcomes were not reported in detail.

There has been one other comparative trial of cryopreserved platelets, of a markedly different design.[19] Twenty-eight adult hematology-oncology patients hospitalized with thrombocytopenia and bleeding were randomized to receive liquid-stored apheresis platelets or 0.5, 1, 2, or 3 units of thawed cryopreserved platelets reconstituted in saline, with allocation unblinded to clinicians and investigators. There were no thrombotic adverse events, and each of the reported 11 serious adverse events was related to infection (explicable in the context of the underlying disease) or the known effect of an intercurrent medication. Most patients (58%) given cryopreserved platelets had improvement in their bleeding, some with dramatic resolution of symptoms; however, this was not clearly related

to initial degree of hemorrhage or dose of platelets, and this was no different to the observed effect of liquid-stored platelets. Notably, cryopreserved platelets caused a substantially smaller increment in post-transfusion platelet count than liquid-stored platelets.

More cryopreserved platelet patients in our trial were transfused FFP. This contrasts with the results of the Khuri et al. trial, which found cryopreserved platelets associated with significantly less volume of FFP transfused (approximate mean ± SD 350±150 vs. 700±150 mL, p=0.03, although exact values are not reported).[20] Why cryopreserved platelets might increase the perceived requirement for FFP is unclear. If clinicians were waiting longer for cryopreserved platelets to be delivered, they might have transfused FFP instead; however, the delay in cryopreserved platelet preparation is thawing FFP, so this is an unlikely explanation. Cryopreserved platelets are reconstituted in FFP, raising the concern that some trial sites might have erroneously recorded the platelet FFP as a separate FFP unit. However, in all but one patient (whose record was corrected) this was not the case; individual charts showed different start/stop times for each transfusion. If clinicians were observing a lesser clot amplitude on TEG or ROTEM trace with cryopreserved platelets, this might have been treated with FFP (or cryoprecipitate or clotting factor concentrates). While this could be a plausible explanation, only a minority of patients had viscoelastic coagulation testing before

and after platelet transfusion, and FFP would typically be used to treat a prolonged Clotting Time, not an abnormal clot amplitude.

This study has several strengths in comparison to earlier trials. All primary analyses were by intention-to-treat without patient exclusions, although supplementary analyses tested the hypothesis that receipt of open-label platelets in the cryopreserved group might have influenced the results, and multivariable analysis was used to adjust for the random imbalances in baseline characteristics inherent in such a small pilot study. Adverse events potentially related to cryopreserved platelets, in particular arterial or venous thrombosis, acute respiratory distress due to pulmonary deposition of microparticles, and effects of DMSO such as renal dysfunction, hypertension, or neurological dysfunction, were all actively monitored by clinical staff who had to report their presence or absence. Duplex ultrasound screening for DVT and continuous ECG monitoring for myocardial ischemia were both mandated. The incidence of asymptomatic DVT observed in our trial (4.3-5.6%) was below the median 14.8% reported in the literature in similar populations.[28] Nonetheless, having actively monitored for adverse effects of cryopreserved platelets, we are confident that absence of any suggestion of harm indicates the protocol is sufficiently safe to proceed to a phase III study. Our trial was pragmatic, allowing clinicians to decide on indications for platelet and other transfusions, and not placing restrictions on other aspects of ongoing care. While this is likely to have introduced 'noise' to any 'signal' showing different

hemostatic activity, it shows our trial protocol can be implemented in the context of 'real-world' clinical practice making it suitable for use with a larger number of patients and ensuring broad applicability. We estimated that 29% of randomized patients would be transfused, finding the actual figure to be 34%, validating the feasibility of our risk prediction technique. Several of our reported clinical outcomes would have been expected on the basis of preclinical and other clinical studies: for example, the lower day 1 platelet count after transfusion with cryopreserved platelets (also observed in other clinical studies), the greater number of platelet units that were transfused in the cryopreserved platelet group (most likely explained by clinicians transfusing to a target platelet increment, with a smaller increment caused by cryopreserved platelets), and the observed trends in the ROTEM results.

This study also has several limitations. While no patient suffered coronary graft occlusion or acute myocardial infarction for any other reason, just under half the trial patients underwent coronary revascularization surgery. There is no evidence from any trial that myocardial ischemia is a risk, but a subsequent definitive trial will nonetheless need to account for this possibility during its conduct. Our trial was blinded, but regulatory requirements meant that blinding could be circumvented if clinicians looked under the covering shroud. There would be no benefit to clinicians, investigators or patients from unblinding the study, and our trial network has previously used this technique in a 5000-

patient trial of red blood cell storage duration,[29] so we are confident this is a minor concern. We studied only bleeding cardiac surgical patients. There is little to suggest these patients would not be representative of all bleeding surgical patients. However, medical (generally hematology / oncology) patients are mostly (86%) transfused platelets prophylactically.[3] Such patients would need to be evaluated in a different clinical trial. It is possible that the dose of platelets received in each study group, and within the liquid-stored group by those who received buffy coat (whole-blood derived) vs. apheresis platelets, might not have been comparable. However, the mean ± SD platelet counts in apheresis platelet units (280 ± 34 x 10^9/unit) and buffy coat units (284 ± 40 x 10^9/unit) produced by the ARCBS are almost identical, and while there is some uncertainty due to the lack of randomized trials, the clinical effectiveness of buffy coat and apheresis platelet units is considered essentially equivalent.[30] Cryopreserved platelets reconstituted in thawed FFP are similar in volume and platelet concentration to liquid-stored buffy coat and apheresis platelets.[8] In any case, if cryopreserved platelets were to replace liquid-stored platelets in routine clinical practice, the unit of administration would be that used in the trial. If more units are required because of any lack of dose equivalence, this is essential pharmaco-economic information.

Cryopreserved platelets take only 8-10 minutes to reconstitute if thawed plasma is available. However, some trial sites did not routinely keep thawed FFP. FFP takes 30-40 minutes to thaw. Anticipated delay in receiving platelets for transfusion was the reason that

3 of the 23 patients who received cryopreserved platelets received a unit of open-label liquid-stored platelets prior to their third unit of study platelets. The clinical impact of delay in cryopreserved platelet administration would presumably be quantified best by indices of bleeding or transfusion requirement, which in this pilot trial (other than for FFP) were not significantly different between study groups. Rather than consider differences in time to availability a source of bias in the trial results, it is important to understand that like differences in platelet composition, this is important information for the application of the trial to 'real-world' practice. Cryopreserved platelets must be able to produce at least comparable clinical results *despite* any extra delay required during their preparation. Whether clinicians perceive this delay is acceptable impacts the feasibility of any subsequent definitive trial.

Current guidelines recommend transfusing ABO-matched platelets wherever possible to optimize platelet increments and reduce hemolysis.[31,32] In our trial, liquid-stored platelet groups were chosen according to standard Australian practice,[32] meaning these were largely ABO-matched. However, cryopreserved platelets were all group O. Liquid-stored group O platelets are not suitable as a 'universal donor', as their plasma can contain high concentrations of hemolyzing antibodies.[31] However, the cryopreservation process removes almost all the donor plasma. The plasma in which the platelets were re-suspended was recipient group-specific or AB, removing this risk.

Cryopreservation of platelets would offer substantial advantages over current liquid storage, even if clinical results of transfusion to individual patients are no different. However, this study, even when taken together with other published evidence, does not yet provide sufficient evidence to warrant changing blood bank practice. Our findings support the conduct of a non-inferiority trial with bleeding and safety outcomes.

In summary, this pilot RCT has demonstrated that a larger, definitive clinical trial of cryopreserved platelets vs. liquid-stored platelets in cardiac surgical patients would be feasible, safe, and justified on both clinical and pharmaco-economic grounds.

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FULL AUTHOR LIST

The CLIP Investigators comprise: Rinaldo Bellomo, Laurence Weinberg, Glenn Eastwood, Leah Peck, Helen Young, Sofia Sidiropoulos, Sarah Baulch, Amanda Dalyell, Diana Kolar, Tony Martinelli, Yvonne Reidy, Natalie Caldwell (Austin Hospital, Melbourne); Alistair Royse, Lynda Tivendale, Maria Bisignano, Michael Hausler, Zelda Williams, Natasha Dong (Royal Melbourne Hospital); David J. Gattas, Heidi Buhr, Paul Bannon, Bruce Cartwright, Lisa Turner, John Gibson, Bernadette Blayney, Lorna Beattie, Debra Hutch, James Wun Jennifer Coles (Royal Prince Alfred Hospital, Sydney); Bronwyn Pearse, Daniel Faulke, Marc Zeigenfuss, Peter Tesar, John Fraser, Joanne Perel, Cheryl Kahn, Billy Vincent, Donalee

O'Brien (Prince Charles Hospital, Brisbane); Michael C. Reade, Renae Deans, Anthony Holley (University of Queensland); Denese C. Marks, Lacey Johnson, Janet Wong and David Irving (Australian Red Cross Blood Service).

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FIGURE LEGENDS

Figure 1. Patient flow diagram

Figure 2. Blood volume in chest drains during the first 48 hours after ICU admission. Data points represent medians; error bars represent interquartile ranges. n=23 (cryopreserved platelets), 18 (liquid-stored platelets)

TABLES

Table 1. Baseline characteristics

	Cryopreserved (n=23)	Liquid (n=18)
Sex, male	16 (70)	12 (67)
Age, years, median (IQR)	71 (66-77)	70 (65-75)
Blood group		
O	11 (47.8)	8 (44.4)
A	6 (26.1)	6 (33.3)
B	3 (13.0)	4 (22.2)
AB	3 (13.0)	0 (0)
Rhesus D positive	22 (95.7)	14 (77.8)
Weight, kilograms, median (IQR)	70 (64-86.1)	74.5 (70-90)
Hospital where transfusion occurred		
A	11 (47.8)	9 (50.0)
B	4 (17.4)	4 (22.2)
C	2 (8.7)	1 (5.6)
D	6 (26.1)	4 (22.2)
Comorbidity		
Previous myocardial infarction	5 (21.7)	7 (38.9)
Congestive heart failure	6 (26.1)	6 (33.3)
Peripheral vascular disease	2 (8.7)	3 (11.1)
Chronic lung disease	2 (8.7)	5 (27.8)
Chronic liver disease	1 (4.3)	3 (16.7)

Chronic renal impairment	3 (13.0)	1 (5.6)
Diabetes	5 (21.7)	7 (38.9)
Malignancy	5 (21.7)	0 (0)
Pulmonary hypertension identified preoperatively	4 (17.4)	2 (11.1)
Main indication for surgery		
Aortic valve	12 (52.2)	7 (38.9)
Congestive heart failure	0 (0)	4 (22.2)
Endocarditis	2 (8.7)	0 (0)
Mitral / Tricuspid disease	6 (26.1)	3 (16.7)
Stable angina	1 (4.3)	1 (5.6)
Unstable angina	2 (8.7)	3 (16.7)
Coronary artery bypass graft	9 (39.1)	10 (55.6)
Inpatient for >24hrs prior to surgery	9 (39.1)	9 (50.0)
Urgent surgery	5 (21.7)	7 (38.9)
Redo surgery	0 (0)	2 (11.1)
Aspirin given within 7 days of surgery	12 (52.2)	9 (50.0)

Data are expressed as number (%) unless otherwise indicated.

Abbreviations: IQR, interquartile range.

Table 2. Indices of intraoperative complexity

	Cryopreserved (n=23)	Liquid (n=18)	Difference between groups (95% CI)	p
OR duration, minutes, median (IQR)	365 (317-485)	368.5 (280-465)	-3.5 (-108.6 – 101.6)	0.70
Required 2nd cardiopulmonary bypass run	4 (17.4)	1 (5.6)	11.8 (-0.7-0.3)	0.36
Total cardiopulmonary bypass time, minutes, median (IQR)	182 (118-220)	169 (116-204)	13.0 (-46.8 – 72.8)	0.67
Deep hypothermic arrest	1 (4.3)	1 (5.6)	-1.208(-14.67-12.16)	1.0
Location 1st platelet transfusion, intraoperative	20 (87.0)	13 (72.2)	14.73 (-10.12 – 39.59)	0.27
Cell saver used intraoperatively	4 (17.4)	5 (27.8)	-10.39 (-36.23-15.46)	0.47

Data are expressed as number (%) unless otherwise indicated.

Abbreviations: OR, operating room; IQR, interquartile range.

Table 3. Efficacy outcomes: hemostasis

	Cryopreserved (n=23)	Liquid (n=18)	Difference (95% CI)	p
Estimated OR blood loss, mL, median (IQR)	925 (650-1175)	900 (675-1037)	25 (-323.3 – 373.3)	0.82
Blood in drains on ICU admission, mL, median (IQR)	140 (65-180)	110 (40-170)	30 (-46.5 – 106.5)	0.56
Blood in drains at 24hr, mL, median (IQR)	715 (540-915)	805 (591-1080)	-90 (-343.8 – 163.8)	0.41
Blood in drains at 48hr, mL, median (IQR)	980 (680-1215)	1075 (810-1540)	-95 (-476.0 – 286.0)	0.45
Requirement to return to OR for bleeding on day 1	2 (8.7)	3 (16.7)	-7.97 (-28.68 – 12.74)	0.64
Requirement to return to OR for bleeding on day 1-3	5 (21.7)	8 (44.4)	-22.71 (-51.18 – 5.77)	0.18
BARC4 bleeding [24]	7 (30.4)	10 (55.6)	-25.12 (-54.80 – 4.55)	0.10
Day postoperative aspirin commenced, median (IQR)	2 (2-3)	2 (2-3)	0 (-0.71 – 0.71)	0.76
Day postoperative prophylactic heparin commenced, median (IQR)	2 (2-2)	2 (2-3)	0 (-0.5 – 0.5)	0.41

Data are expressed as number (%) unless otherwise indicated. Abbreviations: IQR, interquartile range.

Abbreviations: OR, operating theatre; BARC4, Bleeding Academic Research Consortium criteria for significant postoperative hemorrhage in cardiac surgery, which requires one or more of: intracranial hemorrhage <48 hours postoperatively; reoperation after sternotomy closure for the purpose of controlling bleeding; transfusion of ≥ 5 units whole blood or red blood cells within 48 hours postoperatively; or chest tube output ≥ 2L within 24 hours postoperatively. [24]

Table 4. Efficacy outcomes: transfusion and hemostasis interventions required

	Cryopreserved (n=23)	Liquid (n=18)	Difference (95% CI)	p
RBC transfused in OR	11 (47.8)	13 (72.2)	-24.40 (-53.46 – 4.67)	0.20
RBC transfused at any stage	19 (82.6)	16 (88.9)	-6.28 (-27.51 – 14.95)	0.68
RBC transfused in OR, units, median (IQR)	2 (2-4)	3 (2-3)	-1 (-2.4 – 0.4)	0.83
RBC transfused outside OR, units, median (IQR)	2 (0-3)	0.5 (0-4)	1.5 (-0.8 – 3.8)	0.63
RBC transfused total, units, median (IQR)	3 (1-5)	4 (3-5)	-1 (-3.1 – 1.1)	0.23
Among those transfused RBC, RBC transfused in OR, units, median (IQR)	2 (2-4)	3 (2-3)	-1 (-2.4 – 0.4)	0.83
Among those transfused RBC, RBC transfused outside OR, units, median (IQR)	2 (0-3)	2 (0-4)	0 (-2.3 – 2.3)	0.70
Among those transfused RBC, RBC transfused total, units, median (IQR)	3 (2-5)	4 (3-5.5)	-1 (-3.0 – 1.0)	0.28
FFP transfused in OR	9 (39.1)	2 (11.1)	28.01 (3.35 – 52.69)	0.07
FFP transfused at any stage	18 (78.3)	5 (27.8)	50.48 (23.79 – 77.17)	0.002

FFP transfused in OR, units, median (IQR)	0 (0-2)	0 (0-0)	0 (-0.5 – 0.5)	1.0
FFP transfused outside OR, units, median (IQR)	0 (0-0)	0 (0-0)	0 (0-0)	0.68
FFP transfused total, units, median (IQR)	2 (0-2)	0 (0-1)	2 (1.35-2.65)	0.009
Among those transfused FFP, FFP transfused in OR, units, median (IQR)	0.5 (0-2)	0 (0-2)	0.5 (-1.3 – 2.3)	0.93
Among those transfused FFP, FFP transfused outside OR, units, median (IQR)	0 (0-0)	0 (0-0)	0 (0-0)	0.66
Among those transfused FFP, FFP transfused total, units, median (IQR)	2 (1-3)	2 (2-3)	0 (-1.8 – 1.8)	0.72
Cryoprecipitate transfused in OR	10 (43.5)	6 (33.3)	10.14 (-19.60 – 39.89)	0.54
Cryoprecipitate transfused at any stage	15 (65.2)	9 (50.0)	15.23 (-14.99 – 45.42)	0.36
Cryoprecipitate transfused in OR, units, median (IQR)	0 (0-6)	0 (0-6)	0 (-3.6 – 3.6)	0.73
Cryoprecipitate transfused outside OR, units, median (IQR)	0 (0-0)	0 (0-0)	0 (0-0)	0.88
Cryoprecipitate transfused total, units, median (IQR)	5 (0-10)	1 (0-8)	4 (-1.9 – 9.9)	0.16
Among those transfused cryoprecipitate,	5 (0-8)	6 (0-10)	-1 (-7.0 – 5.0)	0.56

cryoprecipitate transfused in OR, units, median (IQR)				
Among those transfused cryoprecipitate, cryoprecipitate transfused outside OR, units, median (IQR)	0 (0-0)	0 (0-0)	0 (-2.4 – 2.4)	0.71
Among those transfused cryoprecipitate, cryoprecipitate transfused total, units, median (IQR)	10 (5-10)	8 (5-10)	2 (-1.3 – 5.3)	0.22
PCC given in OR	8 (34.8)	9 (50.0)	-15.22 (-45.42 – 14.99)	0.36
PCC given at any stage	10 (43.5)	9 (50.0)	-6.52 (-37.24 – 24.20)	0.76
Factor VIIa given in OR or ICU	4 (17.4)	0 (0)	17.39 (1.90 – 32.88)	0.12
Tranexamic acid given in OR or ICU	15 (65.2)	14 (77.8)	12.56 (39.91 – 14.78)	0.50
Anti-D given in OR or ICU	2 (8.7)	0 (0)	8.70 (-2.82 – 20.21)	0.50
Study platelet units transfused				
1 unit	7 (30.4)	12 (66.7)		
2 units	9 (39.1)	5 (27.8)		
3 units	7 (30.4)	1 (5.6)		0.04
Median number of study platelet units (IQR)	2 (1-3)	1 (1-2)	1 (-0.03 – 2.0)	0.012
Open-label platelets transfused prior to 3 units study platelets	3 (13.0)	0 (0)	13.04 (-0.7 – 26.80)	0.24
Open-label platelets transfused after 3	2 (8.7)	0 (0)	8.70 (-2.82 – 20.21)	0.50

units study platelets exceeded

Data are expressed as number (%) unless otherwise indicated. Abbreviations: IQR, interquartile range.

Abbreviations: RBC, red blood cells; FFP, fresh frozen plasma; OR, operating room; ICU, intensive care unit; PCC, prothrombin complex concentrate.

Table 5. Efficacy outcomes: laboratory indices

	Cryopreserved (n=23)	Liquid (n=18)	Difference (95% CI)	p
Platelet concentration day 1, /μl, median (IQR)	112 (80 - 153)	150 (124 - 192)	-38 (-79.6 – 3.6)	0.02
Hemoglobin concentration day 1, g/dL, median (IQR)	84 (77 - 98)	89.5 (83 - 94)	-5.5 (-14.8 – 3.8)	0.42
INR day 1, median (IQR)	1.4 (1.2 - 1.6)	1.35 (1.2 - 1.8)	0.05 (-0.3 - 0.3)	0.64
APTT day 1, median (IQR)	42 (35 - 54)	38.5 (30 - 52)	3.5 (-8.9 - 15.9)	0.53
Fibrinogen day 1, g/L, median (IQR)	2.5 (2.3 - 2.9)	2.5 (2.1 - 3.0)	0 (-4.7 - 4.7)	0.80

Abbreviations: INR, international normalized ratio; APTT, activated partial thromboplastin time

Table 6. Safety outcomes

	Cryopreserved (n=23)	Liquid (n=18)	Difference (95% CI)	p
Maximum temperature day 1, mean (SD)	37.1 (36.5-37.4)	37.1 (36.3-37.3)	0 (-0.5 - 0.5)	1.0
Temperature 3hrs after 1st platelet transfusion, mean (SD)	35.7 (35.4-36.3)	36.0 (35.3-36.4)	-0.3 (-0.9 – 0.3)	0.69
PaO_2/FiO_2 ratio 3hrs post transfusion, median (IQR)	280 (220-435)	277 (208-314)	3 (-76.0 – 82.0)	0.88
Lowest PaO_2/FiO_2 ratio day 1	238 (194-381)	256.5 (175-306)	-18.5 (-117.1 – 80.1)	0.72
Hospital length of stay, days, median (IQR)	18 (10-28)	23 (16-34)	5 (-16.4 – 6.3)	0.28
28-day mortality	0 (0)	0 (0)	0 (0-0)	1.0
Wound infection	3 (13.0)	0 (0)	13.04 (-0.7 – 26.81)	0.24
Systemic infection	4 (17.4)	5 (27.8)	-10.39 (-36.23 – 15.46)	0.43
Deep venous thrombosis	1 (4.3)	1 (5.6)	-1.2 (-14.68 – 12.26)	1.0
Acute myocardial infarction postoperatively	0 (0)	0 (0)	0 (0-0)	1.0
Requirement for postoperative renal replacement therapy	1 (4.3)	3 (16.7)	-12.3 (-31.44-6.81)	0.3
Peak postoperative creatinine, μmol/L, median (IQR)	128 (85-202)	105.5 (90-181)	22.5 (-31.2 – 76.2)	0.60

Data are expressed as number (%) unless otherwise indicated. Abbreviations: IQR, interquartile range.

Figure 1. Patient flow diagram

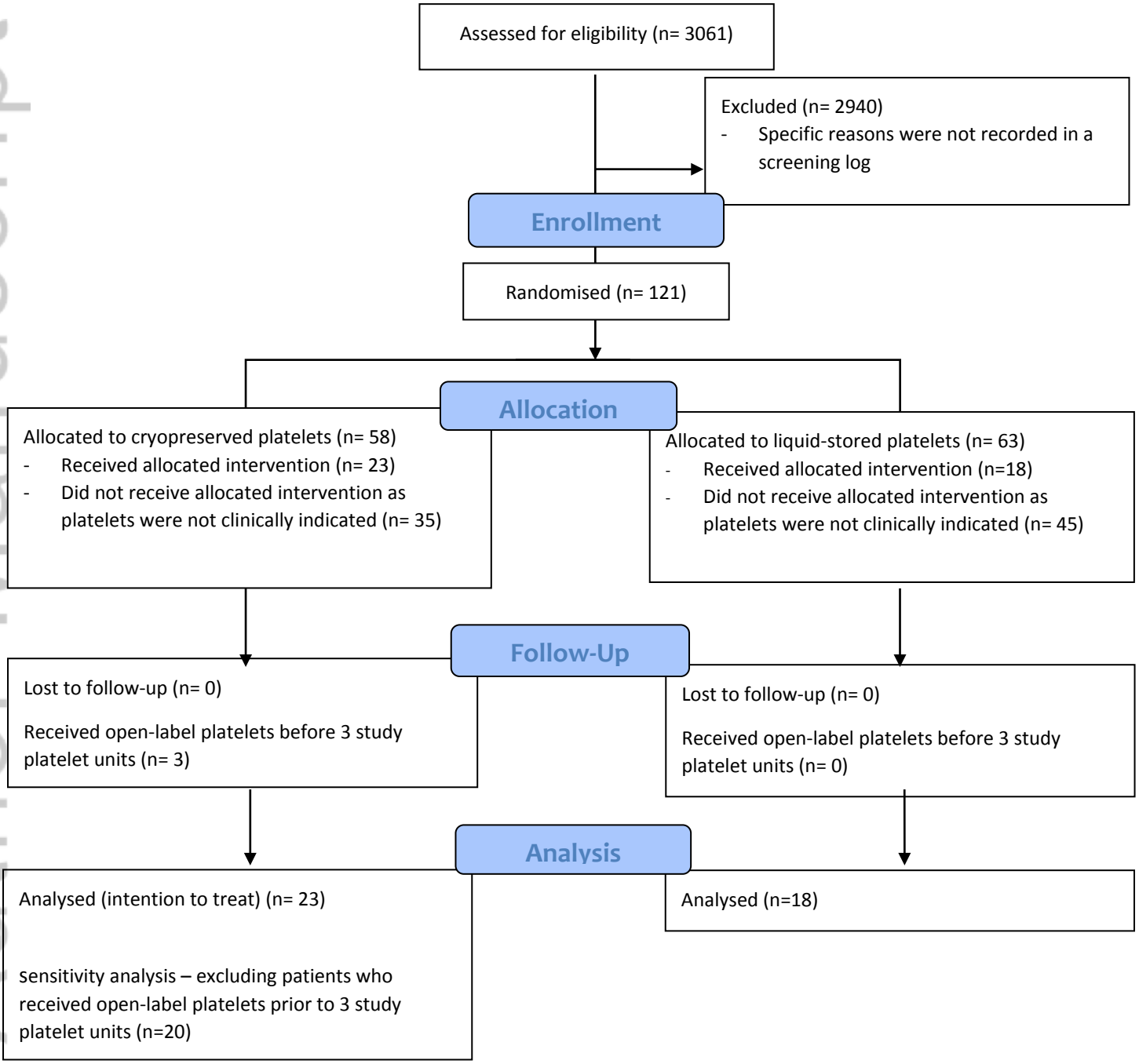
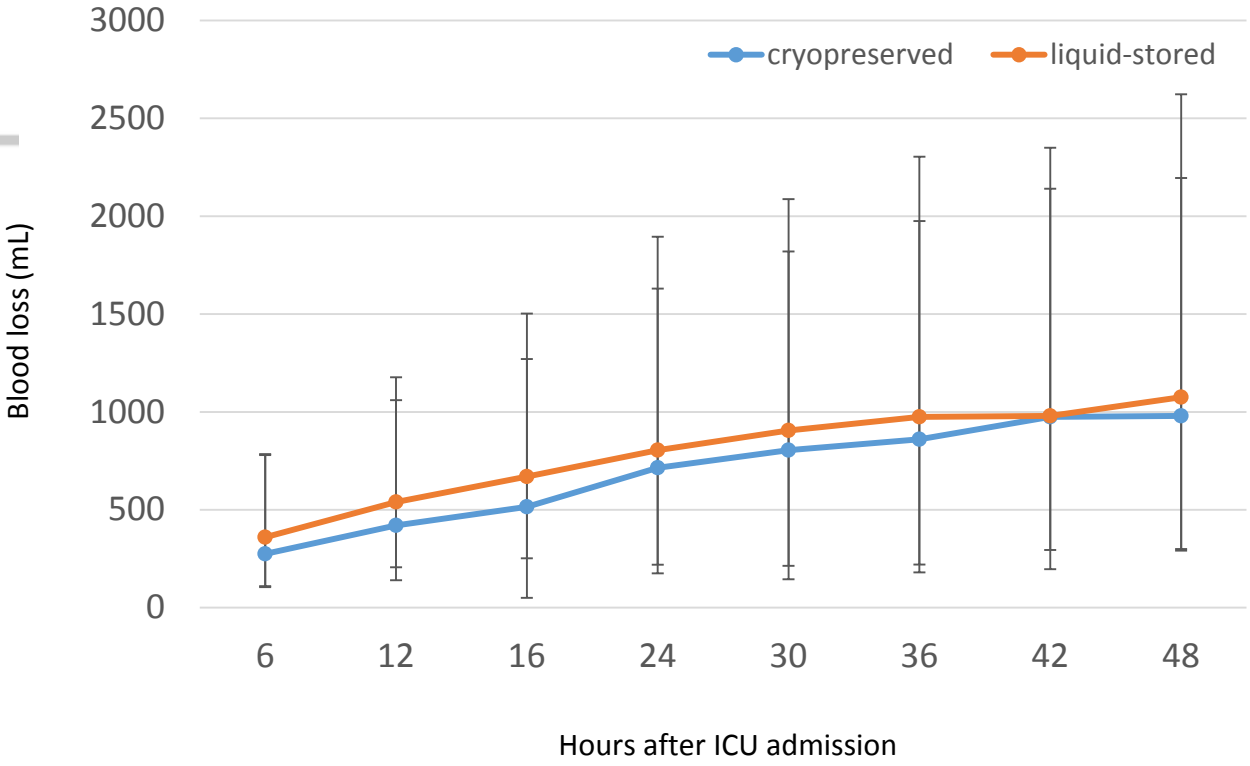
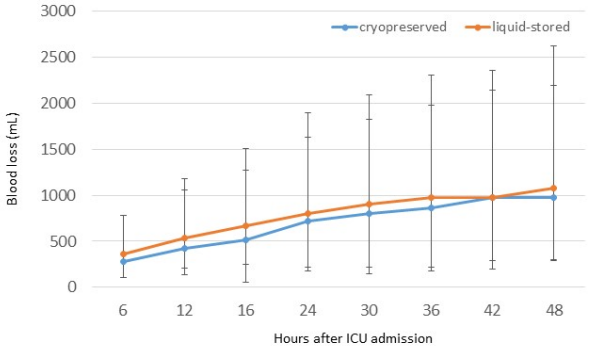
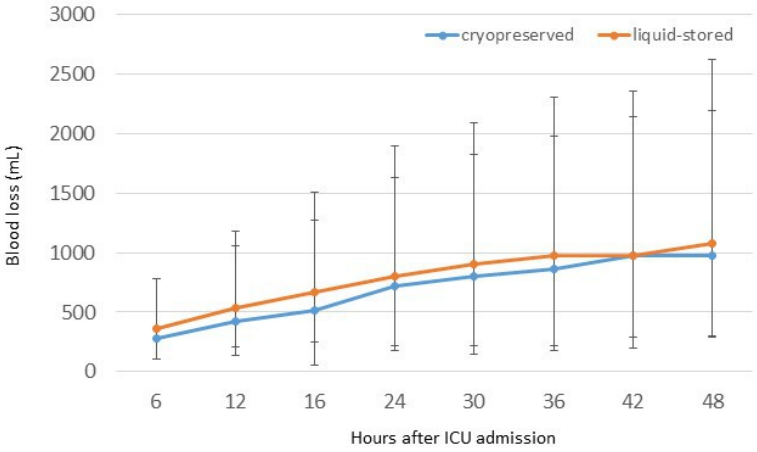


Figure 2.







TRF_15423_Reade et al CLIP Figure 2.jpg

Author/s:
Reade, MC;Marks, DC;Bellomo, R;Deans, R;Faulke, DJ;Fraser, JF;Gattas, DJ;Holley, AD;Irving, DO;Johnson, L;Pearse, BL;Royse, AG;Wong, J;Weinberg, L;Eastwood, G;Peck, L;Young, H;Sidiropoulos, S;Baulch, S;Dalyell, A;Kolar, D;Martinelli, T;Reidy, Y;Caldwell, N;Royse, A;Tivendale, L;Bisignano, M;Hausler, M;Williams, Z;Dong, N;Buhr, H;Bannon, P;Cartwright, B;Turner, L;Gibson, J;Blayney, B;Beattie, L;Hutch, D;Coles, JWJ;Pearse, B;Faulke, D;Zeigenfuss, M;Tesar, P;Fraser, J;Perel, J;Kahn, C;Vincent, B;O'Brien, D;Holley, A;Irving, D

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