Platelet transfusions and patient outcomes after cardiac surgery

Plucinski - van Hout, F.M.A.

CHAPTER 6

Storage duration of platelet concentrates and clinical outcomes in cardiac surgery patients

Fabienne M.A. van Hout, Camila Caram-Deelder, Aad Pors, Rutger A. Middelburg, Peter M.J. Rosseel, Leo M.G. van de Watering, Jean-Louis Kerkhoffs, Meindert Palmen, Johanna G. van der Bom

Submitted for publication

Background

Storage of platelet concentrates leads to "platelet storage lesion". In transfused patients this may influence haemostatic capacity and adverse events. We set out to investigate in cardiac surgery patients whether longer storage of platelet concentrates is associated with efficacy and safety.

Methods

Using an emulated trial design, we analysed data from patients of two hospitals from January 2005 to December 2017. We included cardiac surgery patients transfused with pooled platelet concentrates. Storage times were classified in 1-3 days (*fresh* platelets) and 4-7 days (*old* platelets). Endpoints were blood loss within 12 hours after surgery, reoperation for bleeding, stroke, myocardial infarction, infection, systemic inflammatory response syndrome, shock, multiorgan failure, and in-hospital mortality. Associations between storage duration and clinical endpoint incidences were quantified using logistic regression corrected for potential confounders.

Findings

In-hospital mortality among 2117 patients transfused with *old* platelets was 10•0% (212 patients); among 1439 patients who received *fresh* platelets it was 7.6% (109 patients); corrected odds ratio (cOR) 1•47, 95% confidence interval (CI) 1•13-1•91. Patients transfused with *old* platelets more often experienced blood loss ≥1000mL (102/285; 35•6%) than patients transfused with *fresh* platelets (87/326; 26•7%), cOR 1•74 (95%CI 1•19-2•52). Patients transfused with *old* platelets more often needed reoperation for bleeding (99/285; 34•7%) than patients transfused with *fresh* platelets (87/326; 26•7%) (cOR 1•62, 95%CI 1•12-2•35). There was no notable association with the other endpoints.

Interpretation

In conclusion, in our cardiac surgery population transfusion of *old* platelets was associated with higher in-hospital mortality, more blood loss, and more reoperations for bleeding compared with *fresh* platelets.

Funding

Sanquin (Amsterdam, the Netherlands; PPOC12-028)

Rationale

Patients undergoing cardiac surgery are at risk of excessive bleeding and surgical re-exploration. Both excessive blood loss and surgical re-exploration are associated with increased postoperative mortality and morbidity.[1-3] Thus, efficient prevention and treatment of bleeding is an important issue in cardiac surgery. Surgically induced injury is the most important cause of postoperative bleeding. In addition, a significant proportion of the observed bleeding can be explained by acquired haemostatic defects.[4,5] Impaired platelet function, mainly due to the effects of cardiopulmonary bypass and preoperative anti-platelet drug therapy, is one of the most important haemostatic factors leading to postoperative bleeding.[4-8] Platelet transfusions are administered to prevent or treat such bleeding.[9]

A possible adverse effect of *red blood cell* storage duration on clinical outcome in cardiac surgery patients has been studied extensively.[10-13] A possible detrimental effect of storage duration of platelet concentrates on safety and efficacy has been suggested but, thus far, scarcely supported by quantitative clinical evidence.[14]

In vitro studies show that, during storage, platelets undergo multiple changes in structure and function collectively known as "platelet storage lesion".[15-17] Such damage due to storage may reduce haemostatic capacity of the platelet concentrate.[18] Most clinical studies addressing hemostatic capacity this subject have been performed in non-bleeding haemato-oncological patients to treat thrombocytopenia. In a systematic review, transfusion of *old*er platelets has been associated with a shorter time to the next transfusion, a tendency towards a higher risk of bleeding, and, in haemato-oncological patients, an increased need for platelet transfusions.[14] However, whether these findings can be extrapolated to cardiac surgery patients is debatable. Besides leading to reduced haemostatic capacity, several features of the preparation and storage processes of platelet concentrates, lead to platelet activation.[19] Activated platelets might have improved haemostatic effects, but they also show increased CD62P exposure and GPIbα release which is associated with decreased platelet survival after transfusion.[20,21] It is unclear whether platelet activation affects outcomes of cardiac surgery patients and if so whether platelet activation has a favourable or an detrimental effect in these actively bleeding patients. Another concern is the release and accumulation of bioactive substances, like sCD40L and microparticles, during storage of platelets.[18,22-24] These bioactive substances are associated with pro-inflammatory and thrombotic events.[18,25-27]

Our aim was to examine in cardiac surgery patients whether transfusion of platelet concentrates, stored for more than three days, is associated with the occurrence of in-hospital mortality, (excessive) bleeding, reoperation for bleeding, infectious, inflam-

matory, and thromboembolic complications, when compared with administration of platelets with shorter storage duration.

Methods

Study design and patient population

We used an emulated trial design, a method that is suited to simulate a randomised trial when a trial is not feasible, in a cohort of adult patients who underwent cardiac surgery and received one or more platelet transfusions in the Amphia Hospital in Breda, the Netherlands (hospital 1), between January 2005 and December 2017, or in the Leiden University Medical Center (LUMC; hospital 2) in Leiden, the Netherlands, between January 2006 and December 2017. We excluded patients who received apheresis platelets because in the Netherlands apheresis platelets are used for specific indications and tend to be transfused at different storage times and could thereby blur the results. Pseudonymized patient data were obtained from the two hospitals. Decisions regarding patient care were made by the responsible physician according to the applicable guidelines; transfusions were prescribed based on the hemodynamic and haemostatic status of the patient. Decisions regarding platelet transfusion were made independently from the duration of storage of the platelet concentrates, as the physicians are not aware of and cannot influence the storage time of the transfused concentrates. As soon as a platelet concentrate is ordered the concentrate is issued by the blood transfusion department according to the first-in-first-out principle. The medical ethical committees of the Amphia Hospital and of the LUMC approved the study protocol and granted a waiver for informed consent (reference P14.008).

Platelet product

The studied transfused platelet concentrates were all prestorage leukoreduced by filtration, prepared using five whole-blood-derived buffy coats collected and produced by Sanquin Blood Bank (Amsterdam, the Netherlands). The platelets were resuspended either in 100% plasma from one of the five whole blood donations (plasma-platelets) or in 65% platelet additive solution (PAS) and 35% plasma. Two types of PAS were used during the studied period: PAS-B (also known as PAS-2 or T-Sol, Baxter (Nivelles, France)) and PAS-C (also known as PAS-III or Intersol, Fenwal, a Fresenius company, La Châtre, France). The platelets transfused in hospital 1, from January 2005 to December 2012, were stored up to five days in PAS-B and from December 2012 they were stored up to seven days in PAS-C. Platelets transfused in hospital 2, from January 2006 to November 2014, were stored up to seven days in plasma and from November 2014 they were stored up to seven days in platelet additive solution C (PAS-C). Data regarding platelet products were extracted from the national database of Sanquin Blood Bank (eProgesa,

MAKsystems, Paris, France). The storage time of the platelets concentrates was defined as the number of calendar days between the first donation contributing to the pool of five donors and the date of transfusion, such that the donation date is day 0. The storage times were classified in two categories: 1 to 3 days, called *fresh* platelets throughout this manuscript, and more than three days, called *old* platelets. Patients transfused with multiple platelet concentrates were only included if all concentrates were in the same storage time category.

Postoperative clinical outcomes

We planned to evaluate the following endpoints for all patients in both hospitals: amount of blood loss within 12 hours after the end of surgery, reoperation for bleeding, stroke, myocardial infarction, infection, systemic inflammatory response syndrome, shock, multiorgan failure, and in-hospital mortality. In-hospital mortality was available in both hospitals; all other postoperative endpoints were only structurally available in hospital 1. The postoperative endpoints from hospital 1 were retrieved from its prospective peri-operative clinical data registry which is fully compliant with the national cardiac surgery data registry.[28] Herein stroke was defined as a new persistent cerebrovascular event leading to neurologic defects and was diagnosed by a neurologist. Acute kidney failure was defined as the need for postoperative renal replacement therapy when this was not indicated before and/or an increase in serum creatinine of more than 100%. The diagnosis postoperative myocardial infarction was made based on either the occurrence of new Q-waves on the electrocardiogram or ischemic ST-changes in combination with abnormal postoperative troponin-T levels (troponin-T level > 0•5 µg/L for coronary artery bypass grafting (CABG) surgery, troponin-T level > 0•8 µg/L for valve surgery and troponin-T level > 1•0 µg/L for combined CABG and valve procedures). Both serum creatinine and troponin-T were routinely measured in all patients postoperatively. Infection was categorized as pneumonia, mediastinitis, sepsis, and other infections. Diagnosis was stated if relevant organisms were isolated from culture(s). Systemic inflammatory response syndrome was diagnosed if two or more of the following criteria were present: temperature greater than 38 or less than 36°C; tachypnea (greater than 20 breaths per min) or hypocapnea (pCO_2 less than 32 mmHg); tachycardia (greater than 90 beats/min); or need for mechanical ventilation and leukocyte count greater than 12 or less than 4 $\times 10^9$/L. Multiorgan failure was defined as simultaneous or sequential dysfunction or failure of two or more organ systems. Shock was identified as a clinical diagnosis made by the attending physician and as registered in the clinical data registry of the hospital. Amount of blood loss was analysed as being high or low in two endpoints, one with 500 mL and one with 1000mL as the cut-off point.

Statistical analyses

Main analyses

A statistical analysis plan was prepared and agreed upon before starting the analyses. Continuous variables were described as mean (and standard deviation) or median (and interquartile range), as appropriate. Categorical variables were described as a percentage. The number of missing values per variable are presented in the supplemental material (sTable 1). Missing postoperative outcome variables were coded as "no", i.e. we assumed the outcome had not occurred. For the preoperative variable EuroSCORE I there were eight patient records with missing values. These values were imputed using the median of the non-missing. The missing values for the preoperative variables left ventricular (LV) function and recent myocardial infarction were not imputed; in the logistic regression models missingness was handled as a separate indicator category.

The associations between storage time of the platelet concentrate and the clinical endpoint incidences were evaluated using logistic regression derived odds ratios (ORs) with 95% confidence intervals (CIs). We calculated crude and corrected odds ratios, which were corrected for logistic EuroSCORE I (continuous), number of transfused platelet concentrates (continuous), age (continuous), female sex (yes/no), LV function (good (defined as >50%) / not good (<50%) / missing), recent myocardial infarction (yes/no/missing), isolated CABG (yes/no), and center (hospital 1/hospital 2). EuroSCORE I is a scoring system for the prediction of early mortality in patients undergoing cardiac surgery based on objective risk factors.[29]

Kaplan-Meier curves were made in which mortality was compared between patients receiving *fresh* platelets and patients receiving *old* platelets during the first 100 postoperative days. The adjusted Kaplan Meier curves were adjusted using inverse probability weighing. First, we calculated for all patients their predicted estimates of receiving *old* platelets using a logistic regression model with storage time (*old*/*fresh*) as dependent variable and all confounders (listed in the above) as independent variables; next, weights were assigned to all patients as the inverse of those predicted values. The presented adjusted Kaplan Meier curves are thereby corrected for the described confounders.[30]

For the analysis in which we studied mortality, we included all patients who had received platelets at any time during their hospital stay. For all other postoperative endpoints, we restricted the analysis to patients who had received platelet transfusions intraoperatively to assure that the transfusion took place before the occurrence of the endpoint. To evaluate whether the results were similar for different storage media and hospital, we repeated the analyses stratified for storage medium and hospital.

Sensitivity analyses

To explore the robustness of our findings we performed sensitivity analyses. We repeated the analyses in the selection of patients who had received not more than one platelet concentrate during their hospital stay. Also, we repeated the analysis in patients without any missing value for postoperative outcomes (supplemental material).

Role of the funding source

Three authors are employees of Sanquin, the funding source. All authors had full access to the data and the corresponding author had final responsibility for the decision to submit for publication.

Results

Patient characteristics

The database comprised a total of 33,758 (24,769 hospital 1 and 8,989 hospital 2) cardiac surgery patients, of whom 4,317 received one or more platelet transfusions. After exclusion of patients transfused with apheresis platelets and patients transfused with a mix of platelets of different storage time categories, 3,556 patients were included in the analyses (Figure 1). The baseline characteristics per storage time category are shown in Table 1. A small majority of the patients was male (56%), the mean age was 67 years (standard deviation 11 years) and most patients had a good left ventricular function (69%). In total, 2,117 patients were transfused with *old* platelets and 1,439 with *fresh* platelets. Most patients, 2,452/3,556 (69%) patients, received only one platelet concentrate during admission (Figure 1).

Platelet storage duration and in-hospital mortality

Among patients transfused with *old* platelets 212/2,117 (10•0%) died in the hospital whereas among those who received *fresh* platelets 109/1,439 (7•6%) died in the hospital; corrected odds ratio (OR) 1•47, 95%CI 1•13 to 1•91) (Table 2). Similar results were observed after stratification for storage medium and hospital (Table 2). Figure 2 presents Kaplan-Meier curves for in-hospital mortality after receiving *fresh* and *old* platelets. It shows that the increased mortality among patients transfused with *old* platelets compared to patients transfused with *fresh* platelets occurred largely during the first 20 postoperative days.

Platelet storage duration and post-operative bleeding & other clinical outcomes

Information on both postoperative outcomes and timing of transfusions (given in or outside the operating room) was available for 611 patients in hospital 1 (Figure 1). Patients

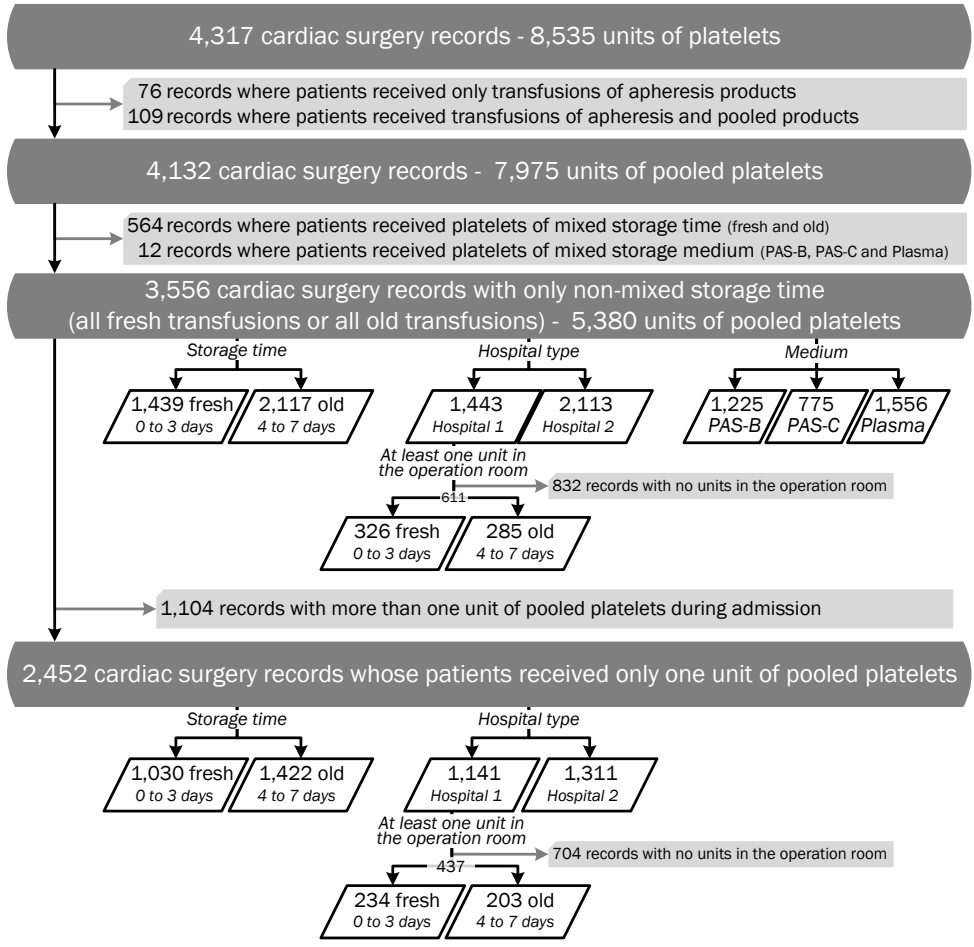

Figure 1: Flowchart of inclusion/exclusion steps

transfused with *old* platelets more often experienced blood loss ≥1000mL (102/285; 35•8%) than patients transfused with *fresh* platelets (87/326; 26•7%), corrected OR 1•74 (95%CI 1•19 to 2•52). Patients transfused with *old* platelets more often needed reoperation for bleeding 99/285 (34•7%) than patients transfused with *fresh* platelets (87/326; 26•7%) (corrected OR 1•62, 95% CI 1•12 to 2•35). There was no clear association between transfusion of *old* versus *fresh* platelets and the occurrence of stroke, myocardial infarction, infection, systemic inflammatory response syndrome, shock, or multiorgan failure (Table 3).

Table 1 - Characteristics and outcomes of cardiac surgery patients receiving platelets

	Storage time		
Characterisitics	**Fresh n=1,439**	**Old n=2,117**	**Overall n=3,556**
Distinct patients	1,434	2,103	3,520
Total – records	1,439	2,117	3,556
hospital 1	777	666	1,443
hospital 2	662	1,451	2,113
One concentrate – total	1,030	1,422	2,452
One concentrate –hospital 1	616	525	1,141
One concentrate – hospital 2	414	897	1,311
Age (years), mean (SD)	67•5 (10•7)	66•3 (11•6)	66•8 (11•2)
Female sex	582 (40•4)	968 (45•7)	1,550 (43•6)
BMI, mean (SD)	26•3 (4•0)	26•5 (4•2)	26•4 (4•1)
Previous cardiac surgery	199 (13•8)	327 (15•5)	526 (14•8)
Creatinine > 200 µmol/L	40 (3•0)	77 (4•0)	117 (3•5)
Active endocarditis	65 (4•7)	82 (4•1)	147 (4•4)
LV function good*	952 (67•0)	1,461 (70•1)	2,413 (68•8)
Recent myocardial infarction*	243 (19•2)	299 (15•8)	542 (17•1)
Emergency surgery	240 (17•4)	362 (18•2)	602 (17•9)
Isolated CABG	611 (42•7)	778 (36•8)	1,389 (39•1)
EuroSCORE I†, median (IQR)	7•9 (3•4 - 18•0)	7•5 (3•3 - 16•5)	7•7 (3•3 - 16•9)
Number of platelet transfusions, median (IQR)	1 (1 - 2)	1 (1 - 2)	1 (1 - 2)
in the operation room, median (IQR) ‡	0 (0 - 1)	0 (0 - 1)	0 (0 - 1)
outside the operation room, median (IQR) ‡	1 (0 - 1)	1 (0 - 1)	1 (0 - 1)
Number of RBC transfusions, median (IQR)	3 (1 - 6)	3 (1 - 6)	3 (1 - 6)
Outcomes	**n=1,439**	**n=2,117**	**n=3,556**
Death	109 (7•6)	212 (10•0)	321 (9•0)
	n=326	**n=285**	**n=611**
Blood loss ≥ 500 mL in first 12 hours‡	173 (53•1)	170 (59•7)	343 (56•1)
Blood loss ≥ 1000 mL in first 12 hours‡	87 (26•7)	102 (35•6)	189 (30•1)
Reoperation for bleeding‡	87 (26•7)	99 (34•7)	186 (30•4)
Stroke‡	10 (3•1))	8 (2•8)	18 (2•9)
Myocardial infarction‡	53 (16•3)	36 (12•6)	89 (14•6)
Infection‡	86 (26•4)	76 (26•7)	162 (26•5)
SIRS‡	67 (20•6)	75 (26•3)	142 (23•2)
Shock‡	85 (26•1)	84 (29•5)	169 (27•7)
Multi organ failure‡	50 (15•3)	36 (12•6)	86 (14•1)

Numbers represent number and percentages unless when stated otherwise

Percentages and statistics are calculated based on non-missing values (total valid observations)

CABG coronary artery bypass graft; IQR interquartile range; LV left ventricle; L liter; SD standard deviation; SIRS systemic inflammatory response syndrome

* Missings are included in the logistic regression models as an indicator category

† Single imputation (median)

‡ Only collected in one hospital and restricted to patients who received platelet transfusions in the operation room, denominators: *fresh*=326, *old*=285 and overall=611

Note: for all but "Distinct patients" all numbers refer to records. Patients are counted twice when there was more than one surgery during the study time.

Sensitivity analyses

Among 2,452 patients who received one concentrate of platelets the association for in-hospital mortality was similar, with a broader, in part non-statistically significant, confidence interval, OR 1•24 (95%CI 0•88 to 1•74), (Table 2). Similar results were observed for blood loss ≥1000mL, OR 1•50 (95%CI 0•94 to 2•37) and for reoperation for bleeding, OR 1•95 (95%CI 1•23 to 3•10), (Table 3). For the other postoperative outcomes (stroke, myocardial infarction, infection, systemic inflammatory response syndrome, shock, or multi organ failure) the analyses among patients transfused with only one platelet concentrate also yielded similar results as the main analysis.

In the main analysis missing postoperative outcome variables were coded as "no", i.e. we assumed the outcome had not occurred. To explore the effect of this assumption we selected the patients who did not have missing values on bleeding complications and repeated the main analysis. The results of this sensitivity analysis among the patients who did have information on blood loss in the first 12 hours ≥500mL, blood loss in the first 12 hours ≥1000mL and/or for reoperation for bleeding were similar to the main analysis, confirming the robustness of our findings (supplemental Table 2).

Table 2 - Odds ratio for in-hospital mortality; *old* vs *fresh* concentrates in the total population and stratified per storage medium and hospital

	Number of deaths / number of patients (% deaths)		OR (95% CI) of *old* vs *fresh* platelets	
	Fresh	**Old**	**Crude**	**Corrected***
Overall	109/1,439 (7•6)	212/2,117 (10•0)	1•36 (1•01 to 1•73)	1•47 (1•13 to 1•91)
Storage fluid				
PAS-B	64/711 (9•0)	65/514 (12•7)	1•46 (1•02 to 2•11)	1•49 (1•00 to 2•21)
PAS-C	19/230 (8•3)	59/545 (10•8)	1•35 (0•78 to 2•32)	1•36 (0•73 to 2•52)
Plasma	26/498 (5•2)	88/1,058 (8•3)	1•65 (1•05 to 2•59)	1•88 (1•16 to 3•03)
Hospital				
Hospital 1	73/777 (9•4)	85/666 (12•8)	1•41 (1•01 to 1•97)	1•41 (0•99 to 2•02)
Hospital 2	36/662 (5•4)	127/1,451 (8•8)	1•67 (1•14 to 2•44)	1•72 (1•15 to 2•59)
Only one concentrate				
Overall	67/1,030 (6•5)	104/1,422 (7•3)	1•13 (0•83 to 1•56)	1•24 (0•88 to 1•74)
Hospital 1	48/616 (7•8)	53/525 (10•1)	1•33 (0•88 to 2•00)	1•26 (0•82 to 1•95)
Hospital 2	19/414 (4•6)	51/897 (5•7)	1•25 (0•73 to 2•15)	1•31 (0•74 to 2•31)

CI confidence interval; OR odds ratio; PAS platelet additive solution

*corrected for number of transfusions, logistic EuroSCORE I, age, gender, left ventricular function, recent myocardial infarction isolated CABG, and hospital (unless the OR was already stratified for hospital)

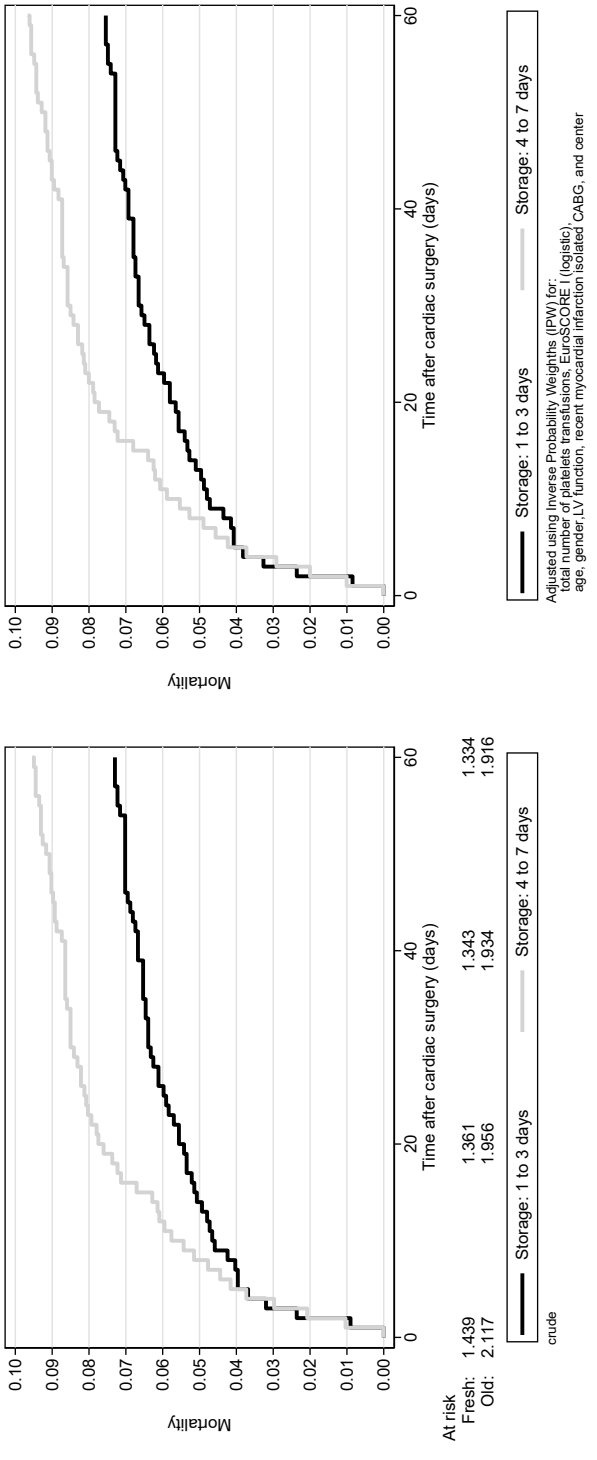

Figure 2: Crude and adjusted Kaplan-Meier curves for the occurrence of in-hospital mortality according to time (days) after cardiac surgery.

Table 3 - Odds ratio for postoperative outcomes according to administrating *old* vs *fresh* concentrates

	Number of outcomes / number of patients (% outcome)		OR (95% CI) of *old* vs *fresh* platelets	
	Fresh	Old	Crude	Corrected*
Other outcomes				
Blood loss ≥ 500 mL first 12 hours†	173/326 (53•0)	170/285 (60•0)	1•31 (0•95 to 1•80)	1•38 (0•98 to 1•92)
Blood loss ≥1000 mL first 12 hours†	87/326 (26•7)	102/285 (35•8)	1•53 (1•08 to 2•16)	1•74 (1•19 to 2•52)
Reoperation for bleeding†	87/326 (26•7)	99/285 (34•7)	1•46 (1•03 to 2•07)	1•62 (1•12 to 2•35)
Stroke†	10/326 (3•1)	8/285 (2•8)	0•91 (0•35 to 2•34)	0•90 (0•34 to 2•33)
Myocardial infarction†	53/326 (16•3)	36/285 (12•6)	0•74 (0•47 to 1•18)	0•87 (0•62 to 1•21)
Infection†	86/326 (26•4)	76/285 (26•7)	1•01 (0•71 to 1•46)	0•99 (0•68 to 1•44)
SIRS†	67/326 (20•6)	75/285 (26•3)	1•38 (0•95 to 2•01)	1•35 (0•92 to 1•99)
Shock	85/326 (26•1)	84/285 (29•5)	1•18 (0•83 to 1•69)	1•16 (0•79 to 1•69)
Multi organ failure	50/326 (15•3)	36/285 (12•6)	0•80 (0•50 to 1•27)	0•73 (0•45 to 1•19)
Other outcomes - Only one concentrate				
Blood loss ≥ 500 mL first 12 hours‡	108/234 (46•2)	107/203 (52•7)	1•30 (0•89 to 1•90)	1•35 (0•92 to 1•98)
Blood loss ≥1000 mL first 12 hours‡	47/234 (20•1)	54/203 (26•6)	1•44 (0•92 to 2•25)	1•50 (0•94 to 2•37)
Reoperation for bleeding‡	44/234 (18•8)	58/203 (28•6)	1•73 (1•10 to 2•70)	1•95 (1•23 to 3•10)
Stroke‡	5/234 (2•1)	7/203 (3•5)	1•64 (0•51 to 5•24)	1•78 (0•55 to 5•81)
Myocardial infarction‡	37/234 (15•8)	24/203 (11•8)	0•71 (0•41 to 1•24)	0•63 (0•35 to 1•15)
Infection‡	57/234 (24•7)	48/203 (23•7)	0•96 (0•62 to1•49)	0•88 (0•56 to 1•38)
SIRS‡	49/234 (20•9)	56/203 (27•6)	1•44 (0•93 to 2•23)	1•50 (0•95 to 2•36)
Shock‡	57/234 (24•4)	54/203 (26•6)	1•13 (0•73 to 1•73)	1•09 (0•69 to 1•74)
Multi organ failure‡	32/234 (13•7)	22/203 (10•8)	0•77 (0•43 to 1•37)	0•71 (0•39 to 1•31)

CI confidence interval; OR odds ratio; SIRS systemic inflammatory response syndrome

*corrected for number of transfusions, logistic EuroSCORE I, age, gender, left ventricular function, recent myocardial infarction, and isolated CABG

† restricted to patients who received one or more platelet transfusions in the operation room

‡ restricted to patients who received one platelet transfusion in the operation room

Discussion

The main finding of this study is that, in a cardiac surgery population, transfusion of *old* platelets was associated with a higher in-hospital mortality when compared to transfusion of *fresh* platelets. Moreover, patients transfused with *old* platelets more often developed blood loss ≥1000 mL in the first 12 hours after surgery and more often required reoperation for bleeding than patients transfused with *fresh* platelets. In our study hospital mortality was higher than in the general cardiac surgery population,

which is explained by the specific population that was selected. Patients who have an indication for platelet transfusion have a worse clinical condition than patents who don't require platelet transfusions.

We selected cardiac surgery patients who received solely *old* platelets or solely *fresh* platelets. That enabled a clear comparison without any influence of exposure to both types of platelets. We explored possible differences between platelet storage fluids and per hospital. Similar results in those subgroups confirmed that storage duration was indeed associated with mortality and bleeding outcomes in various settings. Differences in outcomes between patients exposed to platelets with different storage time may have been due to other risk factors for the outcome. Yet, clinical decisions to transfuse *fresh* or *old* platelet concentrates were made independently from the storage time of the platelet concentrates, as the treating physicians was not aware of and could not influence the storage time of the transfused concentrates. Consequently, one can assume that assignment to *fresh* or *old* platelets was independent of the clinical condition of the patient. This is confirmed by the results in Table 1, showing that risk factors for mortality and clinical outcomes did not differ between patients treated with *fresh* and *old* platelet concentrates. Platelets are issued according to the first-in-first-out principle. Therefore, the storage time can be influenced by the number of platelets a patient receives, because the more concentrates a patient received, the bigger the chance that that patient will receive a *fresh* concentrate (as these concentrates lie in the back of the shelf). Therefore, patients with unfavourable risk profiles and worse prognosis tend to receive more platelet concentrate units with a higher chance of receiving *fresh*er platelets. To minimize this potentially confounding factor of the amount of platelet transfusions, our analyses were corrected for number of platelet transfusions administered. Even if residual confounding remained despite the applied correction, then we expect it to result in an underestimation of the actual effect, because the patients with a less favourable prognosis were more likely to receive younger platelets than the patients with a better prognosis. The analyses were also corrected for "logistic EuroSCORE I", age, gender, left ventricular function, recent myocardial infarction, isolated CABG, and hospital. EuroSCORE I is an internationally accepted scoring system for the prediction of early mortality in cardiac surgical patients (in Europe) based on objective preoperative risk factors.[29] So by correcting for EuroSCORE I factors that are considered relevant for early mortality in cardiac surgery are taken into account. We therefore conclude that it is unlikely that bias or confounding explain our findings.

The literature on the association between platelet storage time and clinical outcomes after thoracic surgery is scarce. A systematic review and meta-analysis that evaluated the effect of platelet storage time on platelet measurements (count increment, corrected count increments, platelet recovery, survival and half-life) after platelet transfusion[31]

concluded that *fresh* platelets were superior to *old* platelets for all these laboratory platelet measurements. Another systematic review and meta-analysis examined the effect of storage time of transfused platelet concentrates on the clinical outcomes.[14] This review concluded that prolonged storage decreases the efficacy of platelet transfusions, resulting in a shorter interval to the next transfusion. Also, a trend towards a higher risk of bleeding and an increased need of platelet transfusions was observed. However, most of the papers included in this review describe the outcomes of haematological patients. Results in this patient population may not be transportable to cardiac surgery patients. Another systematic review also studied the association between storage time of platelets and clinical endpoints.[32] In this review most studies, 13 of 18, concerned haematological patients, and five of the 18 studies described critically ill patients. Critically ill patients consisted of trauma patients, cardiac surgery patients and a heterogeneous population of critically ill patients. The conclusions were that no association was found between storage time of platelets and clinical outcomes, including bleeding, sepsis, or mortality. This paper also stated that there is an absence of evidence to draw definitive conclusions, especially in critically ill patients. So, both reviews don't yield clear clinical results that apply to (cardiac) surgery patients.

To the best of our knowledge there is one other study that investigated the association between storage time of platelets and clinical outcomes of cardiac surgery patients.[33] In contrast to our study, this study did not find a statistically significant association between storage time of platelets and adverse outcomes. A possible explanation for the difference between the findings is that the study of Welsby et al only contained nonemergent CABG patients transfused with apheresis platelets with a maximum storage of five days while our study included both elective and non-elective cardiac surgery patients transfused with pooled buffy-coat platelets with a maximum storage of seven days. So, our database contains more high-risk patients who might also be more vulnerable for the potential influence of transfusion with *old* platelets. This difference in risk-profile of the patient populations is also reflected in the hospital mortality (of 5•2%) observed by Welsby et al, that is lower than the hospital mortality we observed (9•0%). Besides, possibly the "platelet storage lesion" is different in apheresis than in pooled platelets and the two days longer maximal storage duration in our pooled platelets might lead to more "storage lesion". Our study had a higher statistical power because our main analysis is performed in a larger population and our population consisted of patients transfused solely with *old* or solely with *fresh* platelets to avoid bias.

Our findings suggest that in-hospital mortality, postoperative (first 12 hours) blood loss >1000 mL and reoperation for bleeding occur more frequently following transfusion of *old* platelets. Based on these results the most obvious potential explanation for the higher hospital mortality are higher blood loss and the resulting higher need

for reoperation. If this is the actual explanation for the higher hospital mortality after transfusion with *old* platelets, this would suggest that *old* platelets are less effective in preventing and/or stopping bleeding in cardiac surgery. This possible explanation for our findings is plausible in the light of the discoveries earlier studies uncovered over storage time: increasing platelet activation, decreasing platelet responsiveness to agonist and declining platelet viability and function, together called the "platelet storage lesion".[14,15,17,31,34-36] Besides the possibly reduced efficacy of *old* platelets compared to *fresh* platelets, other possible (partial) explanations of the higher in-hospital mortality could be a higher risk of TRALI, other transfusion reactions, or other complications that were observed with longer storage of platelets.[37-40]

If prolonged storage time indeed negatively affects mortality one might consider to preferably transfuse *fresh*er platelet concentrates to cardiac surgery patients. Yet, a single observation from a non-experimental study does not provide sufficient proof for a change in clinical practice. Therefore, our findings need confirmatory evidence.

In conclusion, in our cardiac surgery population transfusion of *old* platelets was associated with a higher hospital mortality, more blood loss, and more reoperations for bleeding compared with *fresh* platelets. In our opinion these results instigate further research into this topic because if *old* platelets do cause higher hospital mortality in cardiac surgery patients this asks for a change in clinical practice.

Acknowledgements

We would like to thank the employees of Amphia hospital, Leiden University Medical Center and Sanquin Blood Supply for their support to the collection and management of the data and Sanquin Blood Supply for funding this study.

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Supplemental material

Table 1 Missings

	Storage time				Overall	
	Fresh		Old			
Preoperative	**n=1,439**	**%**	**n=2,117**	**%**	**n=3,556**	**%**
Age	0	0%	0	0%	0	0%
Gender	0	0%	0	0%	0	0%
BMI	475	33%	1,004	47%	1,479	42%
Previous cardiac surgery	0	0%	0	0%	0	0%
Creatinine > 200 µmol/L	83	6%	171	8%	254	7%
Active endocarditis	60	4%	128	6%	188	5%
LV function good*	17	1%	33	2%	50	1%
Recent myocardial infarction*	171	12%	218	10%	389	11%
Emergency surgery	58	4%	125	6%	183	5%
Isolated CABG	0	0%	0	0%	0	0%
EuroSCORE I†	1	0%	7	0%	8	0%
Number of platelet transfusions	0	0%	0	0%	0	0%
in the operation room‡	0	0%	0	0%	0	0%
outside the operation room‡	0	0%	0	0%	0	0%
Number of RBC transfusions	0	0%	0	0%	0	0%
Postoperative§						
Death	0	0%	0	0%	0	0%
Blood loss ≥ 500 mL in first 12h‡§	14	4%	19	7%	33	5%
Blood loss ≥1000 mL in first 12h‡§	14	4%	19	7%	33	5%
Reoperation for bleeding ‡§	112	34%	86	30%	198	32%
Stroke‡	417	54%	313	47%	730	51%
Myocardial infarction‡	25	3%	82	12%	107	7%
Infection‡	7	1%	9	1%	16	1%
SIRS‡	5	1%	3	0%	8	1%
Shock‡	5	1%	12	2%	17	1%
Multi organ failure‡	4	1%	3	0%	7	0%

Reported percentages refer to the population included in the analyses (see items ‡ and § below)

* Missings are included in the logistic regression models as an indicator category

† Single imputation (median)

‡ Only collected in the hospital 1, denominators: fresh=777, old=666 and overall=1,443

§ Restricted to patients who received one or more platelets transfusions in the operation room, denominators: fresh=326, old=285 and overall=611

Table 2 Sensitivity analyses – Excluding patients who had missings for bleeding-related outcomes

Missing	Storage time				Overall	
	Fresh		Old			
	n=1,439	%	n=2,117	%	n=3,556	%
Outcomes[§]						
Blood loss ≥ 500 mL in first 12h[‡§]	14	4%	19	7%	33	5%
Blood loss ≥1000 mL in first 12h[‡§]	14	4%	19	7%	33	5%
Reoperation for bleeding [‡§]	112	34%	86	30%	198	32%

	Number of outcomes / number of patients (% outcome)		OR (95% CI) of old vs fresh platelets	
	Fresh	Old	Crude	Corrected*
Other outcomes				
Blood loss ≥ 500 mL in first 12h[†]	173/326 (53.0)	170/285 (60.0)	1.31 (0.95 to 1.80)	1.38 (0.98 to 1.92)
Sensitivity analyses[1]	173/312 (55.5)	170/266 (63.9)	1.42 (1.01 to 1.98)	1.51 (1.07 to 2.16)
Blood loss ≥1000 mL in first 12h[†]	87/326 (26.7)	102/285 (35.8)	1.53 (1.08 to 2.16)	1.74 (1.19 to 2.52)
Sensitivity analyses[1]	87/312 (27.9)	102/266 (38.4)	1.61 (1.14 to 2.28)	1.83 (1.25 to 2.69)
Reoperation for bleeding[†]	87/326 (26.7)	99/285 (34.7)	1.46 (1.03 to 2.07)	1.62 (1.12 to 2.35)
Sensitivity analyses[2]	87/214 (40.7)	99/199 (49.8)	1.44 (0.98 to 2.13)	1.70 (1.11 to 2.61)

*corrected by number of transfusions, logistic EuroSCORE, age, gender, LV function, recent myocardial infarction, and isolated CABG

† restricted to patients who received one or more platelet transfusions in the operation room

1 sensitivity analyses: excludes patients with missing data on blood loss. In the main paper they where recoded as "non experiencing the outcome"

2 sensitivity analyses: excludes patients with missing data on reoperation for bleeding. In the main paper they where recoded as "non experiencing the outcome"