Aged versus fresh autologous platelet transfusion in a two-hit healthy volunteer model of transfusion-related acute lung injury

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**Abstract**

**Background:** Transfusion-related acute lung injury (TRALI) is a severe complication of blood transfusion that is thought of as a two-hit event: first the underlying patient condition (e.g., sepsis), and then the transfusion. Transfusion factors include human leukocyte antigen antibodies or biologic response modifiers (BRMs) accumulating during storage. Preclinical studies show an increased TRALI risk with longer stored platelets, clinical studies are conflicting. We aim to discover whether longer platelet concentrate (PC) storage time increases TRALI risk in a controlled human experiment.

**Abbreviations:** A-PLT, apheresis platelet concentrate; ARDS, acute respiratory distress syndrome; ANOVA, analysis of variance; BC-PLT, buffy coat-derived platelet concentrate; BRMs, biologic response modifiers; BAL, bronchoalveolar lavage; DLCO, diffusion capacity for carbon monoxide; ECG, electrocardiogram; HLA, human leukocyte antigen; HNA, human neutrophil antigen; IL, interleukin; LPS, lipopolysaccharide; PAS, platelet additive solution; P/F, PaO\textsubscript{2}/FiO\textsubscript{2}; PC, platelet concentrate; sCD40L, soluble CD40 ligand; TRALI, transfusion-related acute lung injury; TNF, tumor necrosis factor.

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Study Design and Methods: In a randomized controlled trial, 18 healthy male volunteers received a first hit of experimental endotoxemia (2 ng/kg lipopolysaccharide), and a second hit of fresh (2-day old) or aged (7-day old) autologous PC, or physiological saline. After 6 h, changes in TRALI pathways were determined using spirometry, chest X-ray, and bronchoalveolar lavage (BAL).

Results: All subjects reacted adequately to lipopolysaccharide infusion and satisfied SIRS criteria (increased pulse [>90/min] and temperature [>38°C]). There were no differences between the saline, fresh, and aged PC groups in BAL-fluid protein (95 ± 33 μg/ml; 83 ± 21 μg/ml and 104 ± 29 μg/ml, respectively) and relative neutrophil count (1.5 ± 0.5%; 1.9 ± 0.8% and 1.3 ± 0.8%, respectively), nor in inflammatory BAL-fluid BRMs (Interleukin-6, CXCL8, TNFα, and myeloperoxidase), clinical respiratory parameters, and spirometry results. All chest X-rays were normal.

Conclusions: In a human endotoxemia model of autologous platelet transfusion, with an adequate first hit and platelet storage lesion, transfusion of 7-day-old PC does not increase pulmonary inflammation compared with 2-day-old PC.

KEYWORDS
autologous blood transfusion, bronchoalveolar lavage, platelet activation, platelet transfusion, transfusion-related acute lung injury

1 INTRODUCTION

Transfusion-related acute lung injury (TRALI) is a severe complication of blood transfusion, with a high mortality rate and an especially high incidence among critically ill patients. TRALI causes dyspnea within 6 h of blood transfusion, with clinical symptoms alike to the acute respiratory distress syndrome (ARDS). TRALI is characterized by an increase in pulmonary inflammation, leading to endothelial damage, neutrophil influx and fluid leakage. It is thought to be a two-hit event, where an underlying clinical condition serves as a first hit and antibodies and inflammatory agents in the transfusion product serve as a second hit. TRALI can be mediated by human leukocyte antigen (HLA) or human neutrophil antigen (HNA) antibodies present in the transfusion product (antibody-mediated), but also by bioactive lipids and soluble CD40 ligand (sCD40L) that accumulate during storage (non-antibody-mediated). Non-antibody-mediated TRALI has been shown to occur in animal models, yet in human settings the lipids and cytokines involved have only been implicated in transfusion reactions in general. The incidence of antibody-mediated TRALI has been greatly reduced by the exclusion of female plasma donors and donors with HLA or HNA antibodies that were implicated in a previous TRALI case. However, this has no effect on non-antibody-mediated TRALI.

Platelets are sensitive cells that are prone to develop a platelet storage lesion during collection and storage of platelet concentrates (PCs). The storage lesion refers to a myriad of biochemical changes, such as the accumulation of leukocyte-derived cytokines (tumor necrosis factor [TNF]α, interleukin [IL] 1α, IL-6, CXCL8), platelet-derived sCD40L and extracellular vesicles in the PC, and increased expression of platelet apoptosis and activation markers (phosphatidylserine, CD62P). In a case-control study of TRALI-implicated and control PCs, sCD40L levels increased during storage in all products, but PCs implicated in TRALI showed significantly higher levels. In animal models, increased PC storage time is associated with TRALI, while clinical studies report conflicting data. In this study, we investigated the effect of PC storage time on pathways leading to the development of TRALI in a two-hit in vivo human autologous platelet transfusion model, and hypothesized that longer storage duration decreases respiratory function and increases pulmonary inflammation and neutrophil and fluid extravasation.

2 MATERIALS AND METHODS

The study protocol “Development of a ‘two-hit’ in vivo autologous platelet transfusion model in healthy volunteers” was approved by the Academic Medical Centre Medical Ethical Committee, part of Amsterdam UMC, on 18 November
2014 under reference number 2014_294#B2014961, and is in accordance with the Declaration of Helsinki. All volunteers provided written informed consent prior to study enrolment. The study is registered at the Netherlands Trial Register (NTR6493).

2.1 | Inclusion of volunteers

Between April 2018 and November 2021, we included 18 healthy male volunteers aged 18–35. Volunteers who dropped out of the study between the screening visit and subsequent study visits were replaced. Volunteers were screened on medical history, physical examination, blood and urine tests, electrocardiogram (ECG), chest X-ray, spirometry, and the lung diffusion capacity for carbon monoxide (DLCO). Any previous participation in a trial using lipopolysaccharide (LPS) was not allowed, and participation in another intervention trial was not allowed in the 3 months prior to inclusion and during the course of the study.

2.2 | Preparation of transfusion products

Participants were randomly allocated to one of three groups (n = 6 per group): autologous transfusion of fresh (2-day-old) PC, autologous transfusion of aged (7-day-old) PC, or infusion of an equal volume of normal saline (0.9% wt/vol NaCl). A randomization sequence was created by the investigators using R-Studio (version 1.3.1093; Boston, MA) and put into consecutively numbered opaque envelopes that were opened after inclusion of each participant. All volunteers donated one unit of apheresis PC, either 2 or 7 days before the experiment, including those who were allocated to the saline group (three of whom donated 2 days prior and three of whom donated 7 days prior to the experiment). PCs were stored in 100% platelet additive solution (PAS) E and kept at 22°C under gentle agitation, according to Dutch Blood Bank standards. On the day of the experiment, 50 ml PC was labeled with biotin, as previously described and then returned to the storage bag. Activation markers CD62P (BD, Franklin Lakes, NJ) and CD63 (BD, Franklin Lakes, NJ), and apoptosis marker phosphatidylserine (Biorbyt, Cambridge, UK) were measured on post-storage, pre-transfusion platelets using flow cytometry (BD, Franklin Lakes, NJ).

2.3 | Blinding

As volunteers had to donate either 2 or 7 days before the experiment, it was impossible to keep them blinded, and according to Good Clinical Practice, it was impossible to blind for saline or PC transfusion, so we performed an open-label randomized trial.

2.4 | Two-hit model

Experiments were conducted at the research unit of the intensive care department, and volunteers were monitored during the entire experiment (Figure S1). A peripheral venous catheter was inserted to administer 1 L of normal saline prehydration 1 h before the start of the experiment, after which maintenance fluids were set at 1.5 L of normal saline per 24 h until the end of the experiment. An arterial catheter was placed in the radial artery or, if anatomy obliged, the femoral artery. Recordings of continuous five-lead ECG, pulse oximetry, respiratory rate (patient monitor; Philips, Eindhoven, the Netherlands), and invasive arterial blood pressure ( Edwards Life Sciences, Irvine, CA) were automatically recorded in the electronic medical record (Epic, Verona, WI) from prehydration until 9 h after the start of the experiment (T9). Body temperature was measured hourly by an infrared tympanic thermometer (Genius II; Medtronic, Minneapolis, MN). The experiment started at timepoint T0 with the bolus administration of 2 ng/kg Escherichia coli LPS (National Institutes of Health Clinical Center, Bethesda, MD) as first hit. Two hours after the first hit (T2), volunteers received either fresh or aged PC or a saline infusion as second hit. The second hit was infused over the course of 50 min.

2.5 | Sample collection and analysis

Each hour during the experiment, self-reported symptoms (dyspnea, headache, myalgia, chills, nausea, and photophobia) were recorded on a five-point scale (mild–moderate–severe–life-threatening–fatal). Vital parameters were recorded simultaneously. Arterial blood was collected prior to LPS, prior to transfusion, 10, 30, and 60 min after the transfusion was fully administered and each hour afterwards until T9. Blood-gas variables were analyzed unadjusted for temperature and without oxygen supplementation, with a RapidLab 1265 (Siemens, Germany) in lithium heparin anticoagulated blood, and were used to calculate the PaO2/FiO2 (P/F) ratio. Five hours after the start of transfusion (T7), the spirometry, DLCO-measurement, and chest X-ray were repeated. Six hours after the start of transfusion (T8), a bronchoalveolar lavage (BAL) was performed by an experienced pulmonologist, in the middle lobe, using 8 fractions of 20 ml normal saline. The BAL was performed using a flexible
bronchoscope (Olympus, Tokyo, Japan) after local anesthesia with lidocaine 1% according to Dutch guidelines.27

Potassium EDTA anticoagulated blood was used for differential leukocyte count by the routine hospital laboratory before LPS, before transfusion, and every second hour afterwards. BAL-fluid (BALF) and potassium EDTA anticoagulated blood at T8 were used for differential leukocyte count by a specialized immunological laboratory. Total protein content was measured in BALF using a Lowry assay. IL-6, CXCL8, TNFα, and myeloperoxidase (MPO) were measured in potassium EDTA anticoagulated plasma (T0, T2, T4, and T8) and BALF. IL-6, CXCL8, and TNFα were measured using an enzyme-linked immunosorbent assay (ELISA; R&D Systems, Minneapolis, MN) and MPO was measured using a Luminex multiplex assay (R&D systems, Minneapolis, MN) read on a BioPlex 200 (BioRad, Hercules, CA).

2.6 | Outcomes

Co-primary outcomes were the BALF protein and neutrophil content. Secondary outcomes include respiratory parameters (P/F ratio, oxygen saturation, respiratory rate, spirometry, and DLCO) and inflammatory parameters (IL-6, CXCL8, TNFα, and MPO) in both BALF and plasma.

2.7 | Sample size

Based on the BALF protein content in a previous nested case–control study of TRALI cases (239 ± 85 μg/ml) and transfused controls (85 ± 26 μg/ml),28 a sample size of three subjects per group was calculated (α = 0.05, β = 0.2). To account for the mildness of the expected lung injury in this model using LPS-primed healthy volunteers instead of critically ill patients, the sample size was doubled to six subjects per group, which would give 80% power to detect a difference of 91 μg/ml in BALF protein content.

2.8 | Statistical analysis

Normally distributed data are expressed as mean ± standard deviation and non-normally distributed data as median with interquartile range. Between-group differences were analyzed using analysis of variance (ANOVA), a Kruskal–Wallis test, Student’s t-test, or Wilcoxon signed rank-test as appropriate. Repeated measurements were analyzed using ANOVA repeated measurements with an interaction term for time and treatment allocation. Normality of data was assessed visually and using the Shapiro–Wilk test. Homogeneity of variance was assessed visually and using Levene’s test. Sphericity of data was assessed using Mauchly’s test and Greenhouse–Geisser corrections were applied as necessary. All statistical analyses were performed in R-Studio (version 1.3.1093; Boston, MA), using packages Rmisc, rstatix, grid, ggplot2, tableone, and tidyverse.29–34

3 | RESULTS

3.1 | Baseline characteristics

Thirty-one subjects were screened, of whom six were found ineligible due to abnormalities in spirometry, ECG, blood glucose level, or medical history. Seven eligible subjects withdrew from the study prior to the platelet apheresis appointment and were replaced by new subjects. There were no dropouts during the experiment. One subject randomized to the saline group was found to have abnormally high IL-6, CXCL8, and TNFα levels at T0 and was retrospectively excluded from the analysis (Figure S2). A sensitivity analysis was performed without exclusion of this subject, and showed no difference to the primary analysis (Table S1). Baseline characteristics are shown in Table 1. There were no serious adverse events.

3.2 | Transfusion products

All products were cleared for use by the blood bank and transfusion laboratory. However, the aged products expressed significantly more activation markers in flow cytometry analysis (Figure 1). The percentage of CD62P-positive platelets was 5.0 ± 4.8% in the fresh platelets group and 11.0 ± 3.2% in the aged platelets group, p = .03. The percentage of CD63-positive platelets was 1.1 ± 0.8% in the fresh platelets group and 3.2 ± 1.1% in the aged platelets group, p = .8. There was no difference in the percentage phosphatidylserine-positive platelets as a marker of apoptosis: 19.9 ± 5.6% in the fresh platelets group and 20.6 ± 3.6 in the aged platelets group, p = .004. There was no difference in the percentage phosphatidylserine-positive platelets as a marker of apoptosis: 19.9 ± 5.6% in the fresh platelets group and 20.6 ± 3.6 in the aged platelets group, p = .8. After stimulation with TRAP, all markers increased and no significant differences between fresh and aged platelets were found.

3.3 | First hit

All subjects reacted to the LPS infusion as expected. There were significant changes over time in LPS-related symptoms (Figure S3) and most vital parameters, with a
| Variable                      | Saline (n = 5) | 2d PLTs (n = 6) | 7d PLTs (n = 6) |
|-------------------------------|----------------|----------------|----------------|
| Age (year)                    | 25 (21–26)     | 23 (23–25)     | 27 (26–29)     |
| BMI (kg/m²)                   | 24 (2.6)       | 23 (0.8)       | 26 (2.8)       |
| Heart rate (beats/min)        | 60 (5.7)       | 75 (18)        | 64 (7.7)       |
| Mean arterial blood pressure (mmHg) | 92 (6.4)    | 94 (9.4)       | 99 (7.2)       |
| Platelet count (×10⁹/L)       | 270 (38)       | 295 (83)       | 261 (42)       |
| Leukocytes (×10⁹/L)           | 6.7 (1.0)      | 7.8 (2.4)      | 6.1 (0.7)      |
| Hemoglobin (g/dl)             | 15.0 (13.9–15.6) | 15.9 (14.9–16.4) | 14.3 (14.0–15.0) |
| FEV-1/VC ratio (%)            | 81 (4.2)       | 81 (6.7)       | 80 (3.4)       |
| Maximum vital capacity (L)    | 5.8 (5.4–6.3)  | 6.4 (5.8–6.8)  | 6.0 (5.6–6.2)  |
| Lung CO diffusion capacity (mmol/min/kPa) | 10.8 (1.4)  | 12.8 (1.9)     | 10.1 (1.4)     |

Note: Presented variables were measured during the screening visit, data are presented as mean (standard deviation) or as median (25th percentile–75th percentile).

Abbreviations: CO, carbon monoxide; FEV-1/VC, forced expiratory volume in 1 s divided by vital capacity.

**FIGURE 1**  Aged platelets were significantly more activated than fresh platelets. Platelet activation (A/B: CD62P and C/D: CD63) and apoptosis (E/F: phosphatidylserine) markers were measured after storage, after biotinylation of 50 ml platelet concentrate out of a total volume of 300–350 ml, but prior to transfusion. Markers were measured using flow cytometry. Significance levels: *p < .05; **p < .01.
maximum change approximately 4 h after LPS administration (Figure 2). Notably, temperature increased from 36.6 ± 0.5°C at baseline to 38.7 ± 0.8°C, pulse increased from 68/min ± 14 to 105/min ± 9, mean arterial pressure decreased from 91 ± 10 mmHg to 75 ± 10 mmHg, and oxygen saturation decreased from 100 ± 0.6% to 98 ± 1.3%. Blood neutrophil count increased until the end of the experiment, from 2.8 × 10⁹/L ± 1.0 at baseline to 13.9 × 10⁹/L ± 3.4, while IL-6, CXCL8, and TNFα increased within 2 h (to 278 ± 129 pg/ml, 240 ± 146 pg/ml, and 296 ± 241 pg/ml, respectively) and then returned to normal levels (Figure 3). There were no significant interaction effects for time and treatment allocation. All subjects met the SIRS criteria, with their pulse above 90 beats/min and temperature above 38°C.35

3.4 | Second hit

There were no significant between-group differences in BALF protein content (95 ± 33 μg/ml, 83 ± 21 μg/ml, and 104 ± 29 μg/ml, respectively for the saline, fresh, and aged PC group) and relative neutrophil count (1.5 ± 0.5%, 1.9 ± 0.8%, and 1.3 ± 0.8%, respectively, for the saline, fresh, and aged PC group; Figure 4 and Table S2). There were also no significant between-group differences with respect to BALF IL-6, CXCL8, TNFα, and MPO levels (Table 2). BALF recovery was similar across groups. When comparing pre-transfusion and post-transfusion values for respiratory parameters, spirometry, DLCO, and N-terminal prohormone of brain natriuretic peptide (NT-proBNP), there were no between-
group differences (Table 3). None of the subjects met TRALI criteria: the P/F ratio remained above 300 mmHg for all subjects and all chest X-rays were normal.

4 DISCUSSION

In this study, we hypothesized that transfusion of PC with longer storage duration decreases respiratory function and increases pulmonary inflammation and neutrophil and fluid extravasation during human experimental endotoxemia. Our data showed no difference between 2-day stored and 7-day stored autologous PCs. These results add to existing evidence from animal studies that showed a relation between PC storage length and TRALI development, and evidence from clinical studies, showing conflicting results. Our setup differs in some important aspects from previous studies, which might explain these results.

First, the conflicting evidence in the clinical setting is mainly derived from observational data, and thus likely suffers from bias and confounding. Two case–control studies found a difference in PC storage duration between TRALI cases and transfused controls, while two other case–control studies did not. Two small randomized studies on PC storage duration found no TRALI cases, but were likely underpowered to find any, as their focus was on platelet count increment and transfusion intervals. An individual patient data meta-analysis of PC storage duration found no relation with in-hospital mortality, and neither did two retrospective and one prospective cohort study.
Second, when comparing our results with the positive findings in previous animal models, it is important to note that the studies by Tung et al. and Silliman et al. used cross-species transfusions of only the platelet supernatant,\textsuperscript{14,15,17} the studies by McVey et al. used different strains of mice as donor and as model animals,\textsuperscript{18,19} and the study by Vlaar et al., which used the same strain of rats both as donor and as model animal, only found an effect in lung coagulopathy, but not in lung inflammation and lung injury.\textsuperscript{16} Moreover, in the studies by Vlaar et al. and McVey et al., the murine platelets were stored in smaller volumes than regular human PCs,\textsuperscript{16,18,19} which might have increased their ex vivo activation, as was previously shown in human PCs.\textsuperscript{26} Another important difference concerns the dose of lipopolysaccharide, which was 7.5–1000 times higher in the animal models, representing a very severe phenotype that cannot be used ethically in a human volunteer study. Instead, in this study, we were interested in the pulmonary inflammatory reaction that precedes and accompanies TRALI, with BALF protein, neutrophil, IL-6, CXCL8, TNF$\alpha$, and MPO content serving as surrogate outcomes for the development of full-blown TRALI.

Allogeneic transfusions are the clinical standard and were also used in the previously mentioned animal studies, which makes it hard to discern whether any effect arises from an increase in storage length or from patient–donor mismatch and/or HLA antibodies. The use of autologous transfusions in our study has allowed us to selectively study the effect of platelet storage duration, while disregarding any antibody-mediated effects. The difference between 2 days of storage and 7 days of storage is as large a difference as feasible, and even larger than possible in regions where PCs are stored for a maximum of 5 days. Indeed, 7-day-stored platelets showed significantly increased activation compared with two-day stored platelets, yet did not induce changes in any of the clinical and laboratory outcomes in this experimental human model. Moreover, we found no difference between either autologous PC group and the saline control group, suggesting that an allogeneic factor might be needed for TRALI to occur.

Our study has some limitations. First, we only studied male subjects, so we cannot preclude that a difference does exist for females, especially since females tend to show a stronger immune response to LPS treatment.\textsuperscript{40}
TABLE 3 Respiratory parameters

| Variable                        | Pre-transfusion       | Post-transfusion      |
|---------------------------------|-----------------------|-----------------------|
|                                 | Saline (n = 5)        | 2d PLTs (n = 6)       | 7d PLTs (n = 6) | Saline (n = 5) | 2d PLTs (n = 6) | 7d PLTs (n = 6) | p (change) |
| P/F ratio (mmHg)\(^a\)          | 454 (57)              | 460 (51)              | 496 (25)       | 396 (34)       | 398 (35)       | 410 (39)       | 0.75       |
| Oxygen saturation (%)\(^b\)     | 99 (0.6)              | 100 (0.4)             | 100 (0.0)      | 97 (1.3)       | 98 (1.0)       | 98 (1.4)       | 0.35       |
| Respiratory rate (/min)\(^c\)   | 17 (4.2)              | 19 (5.4)              | 19 (3.1)       | 21 (2.3)       | 24 (2.1)       | 23 (2.5)       | 0.97       |
| Subjective dyspnea\(^d\)        | 0 (0–0)               | 0 (0–0)               | 0 (0–0)        | 0 (0–0)        | 0 (0–0)        | 0.5 (0–1)      | 0.69       |
| FEV-1/VC ratio (%)\(^e\)        | 98 (5.3)              | 98 (7.5)              | 96 (3.5)       | 92 (8.6)       | 95 (12.1)      | 94 (2.9)       | 0.29       |
| Maximum vital capacity (%)\(^f\) | 108 (8.5)             | 105 (9.7)             | 111 (13.7)     | 106 (9.7)      | 102 (8.1)      | 109 (12.0)     | 0.99       |
| Lung CO diffusion capacity (%)\(^g\) | 95 (6.8)          | 105 (10.5)            | 92 (13.6)      | 97 (6.3)       | 101 (6.9)      | 90 (15.4)      | 0.58       |
| NT-proBNP (ng/L)\(^h\)          | 49 (49–49)            | 49 (49–49)            | 49 (49–49)     | 152 (106–219)  | 49 (49–111)    | 135 (68–154)   | 0.34       |

Note: The last column represents the p-value of the difference in relative change in pre- and posttransfusion between the different experimental groups and is calculated with analysis of variance if normally distributed with homogeneous variance and Kruskal–Wallis test if not. Data are presented as mean (standard deviation) or as median (25th percentile–75th percentile).

Abbreviations: CO, carbon monoxide; FEV-1, forced expiratory volume in 1 s; NT-proBNP, N-terminal prohormone of brain natriuretic peptide; P/F, arterial partial oxygen pressure/inhaled oxygen fraction; VC, vital capacity.

\(^a\)Values were assessed hourly, values pre-transfusion are at timepoint 2 or, if missing, the most recent available timepoint, and values post-transfusion are the worst value at timepoints 3–8.

\(^b\)Values pre- and posttransfusion are at screening and at timepoint 7, respectively. Values represent the percentage of the expected value, based on age, sex, height, and weight.

\(^c\)Values pre- and posttransfusion are at timepoint 0 and at timepoint 8, respectively. Values below the detection limit (<50) are represented as 49.

However, sex and gender are no clinical risk factors for TRALI.\(^3\) Also, since we found no effect whatsoever in all primary and secondary outcomes, it is unlikely that inclusion of female subjects would alter these results. Second, although LPS infusion results in a pronounced immune response, it does not give the same degree of severity as seen in critically ill patients. However, we think that the LPS model is sufficient to investigate our hypothesis for several reasons: the current LPS model was directly translated from animal experiments where TRALI was observed; bolus injection of LPS results in sustained transcriptomic changes in circulating neutrophils, and adequately primes circulating neutrophils, which ought to be a sufficient first hit.\(^41,42\) Moreover, our sample size was doubled to account for the expected less severe clinical condition as compared with critically ill patients. Another limiting factor is that PC storage performance consistently differs between donors, with older donors and diabetic donors showing worse storage performance than young and healthy donors.\(^43,44\) Therefore, the use of younger instead of older volunteers, and healthy instead of diabetic volunteers, might have influenced our results. This was, however, a choice made out of concern for the safety of our human volunteers, who are both donor and recipient in this autologous model. A follow-up study could potentially include these older and diabetic subjects, but would need to balance volunteer risk with scientific potential.

The use of apheresis PC (A-PLT) instead of the buffy-coat-derived PCs (BC-PLT), necessitated by the autologous nature of this study, could be considered both a strength and a limitation, as different regions have different preferences for either A-PLT or BC-PLT, and there are important differences between the two. In one study, A-PLTs were shown to be more activated than BC-PLTs after production, but showed a smaller increase in activation during storage.\(^45\) In line with these results, PAS storage medium was shown to decrease TRALI incidence for BC-PLTs, but not for A-PLTs.\(^10\) However, in another study, there was increased activation in BC-PLTs after production and no difference in additional storage-related activation between BC-PLTs and A-PLTs.\(^46\) Therefore, we cannot say with certainty whether the use of A-PLTs instead of BC-PLTs influenced the results of our study.

While we were able to study the isolated effect of storage duration, in the absence of HLA or HNA antibodies, it remains an open question whether an interaction between these two TRALI mechanisms exists. Knockout of CD62P in a murine model decreased pulmonary edema and both pulmonary and systemic inflammation during antibody-mediated TRALI.\(^47\) Since platelets show increased CD62P expression during storage, it could be hypothesized that aged platelets play an intermediary role in antibody-mediated TRALI. It would be interesting...
to further study such an interaction in a clinical trial, but given the incidence rate of TRALI that study would require a very large sample size.

Meanwhile, several steps have already been taken to prevent TRALI and other transfusion reactions. Universal leukoreduction prevents leukocyte-derived cytokines from accumulating during storage and decreases the incidence of transfusion reactions.\textsuperscript{38,49} Similarly, the use of PAS for storage of platelets, as opposed to plasma, has decreased the incidence of transfusion reactions.\textsuperscript{50} The incidence of antibody-mediated TRALI has decreased since the exclusion of antibody-positive donors.\textsuperscript{2,9,10}

In this study, we have shown that autologous transfusion of longer stored platelets in the presence of endotoxemia does not increase pulmonary fluid and neutrophil leakage nor decrease respiratory function in a human experimental model of mild TRALI, suggesting that increased storage duration alone does not increase TRALI risk. We propose instead that future research focus more on further reduction of antibody-mediated TRALI.

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CONFLICT OF INTEREST
The authors have disclosed no conflicts of interest.

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

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