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Persistent aggregates in apheresis platelet concentrates

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Background Aggregates often appear during apheresis. Sometimes, these persist throughout storage, causing product wastage. This study assessed product quality of apheresis concentrates containing persistent aggregates (PA) and aimed to identify the factors that contribute to their formation.

Methods Donation (n = 180) and platelet indices ($n \geq 10$) from apheresis concentrates with PA were compared with aggregate-free products.

Results The proportion of donors with at least one previous PA donation was two-fold higher in the PA group ($P < 0{\cdot}0001$) indicating a donor dependence. Significantly higher donor whole blood platelet counts (286 ± 50 vs. 266 ± 49 × 10^3/μl, $P < 0{\cdot}0001$) and higher apheresis yields (6·0 ± 1·6 vs. 5·4 ± 1·5 × 10^{11}, $P < 0{\cdot}0001$) were noted in the PA group. Haematocrit was also slightly higher, but age, gender and body mass were similar. The pH of PA products on day six postdonation was significantly lower ($P < 0{\cdot}001$), in line with higher lactic acid concentrations. Flow cytometry showed no differences in GPIbα levels or phosphatidylserine exposure. However, there was slightly more integrin activation as well as increased degranulation measured by P-selectin expression. Cytokine concentrations were also significantly higher in PA concentrates. Aggregation was normal in response to SFLLRN peptide and collagen stimulation, but agglutination at low-dose ristocetin was significantly higher (P = 0.01) in PA products. Finally, PA were disintegrated by plasmin-mediated thrombolysis but not by integrin $\alpha_{IIb}\beta_3$ inhibition.

Conclusion Products with PA have acceptable quality parameters, but additional functional studies are warranted. Furthermore, PA are more likely to recur in certain donors who have higher platelet counts.

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Introduction

Platelet concentrates may be prepared by pooling of buffy coats, by the platelet-rich plasma (PRP) method or by apheresis. Blood centres worldwide are familiar with the phenomenon of aggregates in platelet concentrates, and these have been reported in all three methods. In our blood centre, aggregates have mainly been found in apheresis products.

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Apheresis uses the inherent differences in buoyancy of blood cells to separate platelets from plasma and erythrocytes by centrifugation while donating, allowing to return the latter components to the donor in real time. During the procedure, the platelet fraction not only separates, but also packs (i.e. concentrates) and is then so transferred to a collection bag. In many cases, small but visible aggregates ('clumping') appear in the collection bag during and after apheresis. These are hyaline macroscopic particles that range in size, aspect and number. It is assumed that the particles are composed of (mainly) platelets that at a certain point during or after the procedure have aggregated, as has been shown for concentrates prepared from

whole blood by the PRP method [1]. Particles often dissipate during storage, but sometimes, the aggregates persist and products are discarded or prevented from being issued until particles have cleared. Neither there is, however, no consensus on what aggregate content – if any – is acceptable nor are there guidelines for the practical procedures to either remove aggregates or for the evaluation of concentrates with persisting aggregates.

There are multiple alleged factors more or less contributing to clumping like anticoagulant induced pH decrease [2], temperature [3] and collection modality [4]. All manufacturing methods have in common that blood platelet concentration is (temporarily) increased *ex vivo*, thereby inevitably raising chances of involuntary platelet–platelet contacts and intermolecular binding. This is especially the case for the PRP method as it involves a precipitation step that sediments the platelets to a tight 'button' and produces concentrates that appear more prone to containing particulate matter than buffy coat-derived products [5]. Yet, the problem is not restricted to one distinct method, all (apheresis) technologies are confronted with it. The different potential causes have been discussed recently in a review paper by Ringwald *et al.* and an International Forum by van der Meer *et al.* [6, 7].

The major questions on this topic are how these persistent aggregates arise, how they can be prevented from appearing and whether these concentrates are inferior to aggregate-free (AF) ones with consequences for clinical practice. There is currently no clinical evidence in literature that supports or refutes inferiority of these products but still many centres choose not to issue them. In the absence of clinical research, efforts may go to unravelling biological, chemical or mechanical pathways that underlie the origin of persisting aggregates so that these can be prevented by technological measures. In this paper, we provide descriptive data on donor, donation and product characteristics with persistent aggregates compared to AF concentrates prepared by dual component apheresis.

Materials and methods

Collection of platelet concentrates

Single donor platelet concentrates were collected at Belgian Red Cross-Flanders Blood Centres by Trima Accel (Terumo BCT, Lakewood, CO, USA) apheresis machines (software versions 5.2.1 and 6.0.6). In all cases, there was dual component donation of plasma and platelets. The whole blood to anticoagulant (acid–citric–dextrose) ratio was 10:1 in all cases. Following donation, the platelet bag was 'rested' label down, without flatbed agitation for at least 1 h at room temperature. Platelet concentrates were always supplemented with platelet additive solution (SSP+; Macopharma, Tourcoing, France) to finally contain on average 35% (v/v) plasma. Concentrates containing >400 ml volume or $>5 \times 10^{11}$ platelets were split in two separate products.

Study design

Persistent aggregates are defined as free-floating precipitating hyaline particles, noticeable to the naked eye either during product processing or storage. Our blood centres utilize a practical guideline for detecting and handling products with PA as objectively as possible as published previously [7]. All products designated as PA positive were prevented from issuing and were isolated in a quarantined storage incubator. PA products may still have been released upon dissolution of the fragments as per our guideline.

Two types of data were collected: (1) donation and collection data, that is logbook parameters that relate to the donor, the apheresis product and the procedure and (2) laboratory data generated by (bio)chemical experiments to investigate the platelet quality in these products. For the donation and collection data, concentrates (n = 180) collected in all six donor centres in Flanders with persistent aggregates (PA) were compared to AF control products during the study period between August 2013 and January 2014. To generate the control group, the first AF donation preceding or following any PA donation in the same collection centre was used. To define whether donors had previously donated an apheresis concentrate with PA prior to the first detection in the study period, available historical registrations of PA incidence were analysed which range from the start of systematically recording this problem (18 June 2012) until the end of the study period (January 2014).

For the laboratory analyses, only unissued PA and AF products were used, and analysis was always 1 day beyond expiration (i.e. day 6) because of our guidelines that products may still be issued if the aggregates disappear spontaneously during quarantined storage. Furthermore, only products expiring on weekdays and from only one blood centre (nearest to the research facility, for logistic purposes) were used for laboratory analysis contrary to the products included for the donation and collection data set mentioned above. For these collective reasons, fewer products could be included in the biochemical study than in the donation and collection study. Furthermore, there was no systematic in the collection of control AF concentrates for the latter, so AF products that were examined for platelet quality were not necessarily the same as those used for donation data collection. Ethical approval and informed consent from the donors involved were obtained.

Metabolism and cell counts

Measurements of glucose and lactic acid were performed by a point-of-care blood gas analyzer (Siemens Rapidpoint® 500, Munich, Germany). pH was determined by glass electrode at room temperature of 20–22°C. Complete blood counts were performed on an automated XE 2100D haematology analyzer (Sysmex Corporation, Kobe, Japan) using a whole blood sample anticoagulated with ethylene diamine tetra acetic acid taken at the time of donation. Complete blood counts were all conducted in our central laboratory within 24 h following donation.

Aggregation and disaggregation

Platelet aggregation was determined by light transmission in a dual-channel lumi-aggregometer (Chrono-log; Helena Laboratories, Haverton, PA, USA) using three different platelet agonists, each at two concentrations; a high dose to boost aggregation and a low dose to investigate sensitivity. These concentrations were determined in separate serial dilution experiments. For collagen 25 and 15 μg/ml was used, ristocetin (both American Biochemical & Pharmaceuticals, Epsom, UK) was used at 1·5 and 0·6 mg/ml, and the PAR1 thrombin related activating peptide SFLLRN (PAR1AP; Sigma-Aldrich, St Louis, MO, USA) at 10 and 3·5 μM. Aggregation cuvettes contained platelets diluted to 250 000 platelets/μl with their corresponding autologous platelet-free plasma with additive solution. The latter was prepared by centrifugation at 4500 **g** for 10 min to pellet the platelet fraction.

Disaggregation was performed by isolation of PA from subject apheresis concentrates using gravity precipitation. The PA were subsequently gently washed twice in 20 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffered saline (HBS, pH 7·4) at room temperature to remove residual single platelets. The PA were resuspended in 500 μl heterologous pooled normal human plasma (n = 21) in an aggregation cuvette. From this suspension, a 2 μl sample was taken for counting CD41-positive events by flow cytometry. Next, 250 IU/ml of urokinase plasminogen activator or 220 μM of the integrin $\alpha_{IIb}\beta_3$ inhibitory tetrapeptide RGDS (Sigma-Aldrich) was added to the cuvette, and disaggregation was followed at 37°C in function of time. At the end of the experiment, another 2 μl sample of the urokinase-activated sample was used for recounting CD41-positive events by flow cytometry.

Flow cytometry

Expression of glycoprotein Ibα (GPIbα) (anti-CD42b~fluorescein; Life Technologies, Carlsbad, CA, USA), activated integrin $\alpha_{IIb}\beta_3$ (PAC-1~fluorescein; BD Biosciences, Erembodegem, Belgium), P-selectin (anti-CD62P~phycoerythrin; BD Biosciences), integrin α_{IIb} (anti-CD41~R-PE; Life Technologies) and Annexin V (Annexin V~Peridinin chlorophyll-Cy5·5; BD Biosciences) was determined using the acoustic focusing Attune® flow cytometer (Life Technologies). Platelets were incubated with labelled antibodies or ligand for 10 min at room temperature in HBS, supplemented with 1 mM $MgSO_4$ and diluted 1000-fold immediately before read-out. For Annexin V measurements, buffers were supplemented with 2 mM $CaCl_2$. For measurements of integrin $\alpha_{IIb}\beta_3$ activation on stimulated platelets, PAR1AP was added at a final 30 μM [from a 575 μM stock solution in 0·1% (v/v) trifluoro acetic acid]. Median fluorescence intensities and percentage positive events were determined of 10 000 cells staining positive for the platelet marker CD61 (anti-CD61~allophycocyanin; Life Technologies).

Cytokine measurements

The platelet cytokines transforming growth factor beta (TGFβ1), chemokine (C-C motif) ligand 5 (CCL5 or RANTES) and platelet factor 4 (PF4 or CXCL4) were determined by commercial immunosorbent assays following the instructions of the provider (R&D Systems, Minneapolis, MN, USA). These assays were based on the sandwich principle and included validated calibration standards provided by the manufacturer.

Statistical analysis

Comparison of means of laboratory data was with t-test or Mann–Whitney U-testing for parametric or nonparametric data sets, respectively. A significance level of 0·05 was used. Analyses were performed with PRISM® software version 6.01 (GraphPad Software Inc., La Jolla, CA, USA) or SAS version 9.3 (SASInstitute, Cary, NC, USA).

Results

Donor-related factors

The data set was collected between August 2013 and January 2014 and revealed a PA incidence of 3·6% on a total of 5178 successful donations in six centres in Flanders. Table 1 shows the results of the collection and donation data retrieved from the central data logging system. From this, we found that the proportion of donors with at least one previous PA donation was twofold higher in the PA group meaning a donor-related factor is involved. The number of preceding donations was not different between control and subject groups. The donor whole blood platelet count was on average higher in PA products than AF controls (Fig. 1a). The apheresis driver software requires the

Table 1 Collection and donation data

Parameter	Aggregate-free (n = 180)	Persistent aggregates (PA) (n = 181)	P (<0·05)[c]
Number of donations[a] [median (range)]	6 (0–36)	7 (0–37)	0·59
Gender: % male and % female donors	65% male/35% female	72% male/28% female	0·14
Preceding PA donation[b] Yes or No (%)	15% Yes/85% No	33% Yes/67% No	**<0·0001**
Age (mean ± SD)	49·2 ± 13·4	49·2 ± 13·6	0·98
Body mass index (mean ± SD)	27·0 ± 3·9	26·3 ± 3·7	0·097
Haematocrit (%, mean ± SD)	44·4 ± 2·4	45·1 ± 2·7	**0·023**
Platelet count donor ($\times 10^3$/μl, mean ± SD)	266 ± 48	286 ± 50	**<0·0001**
Predicted yield ($\times 10^{11}$, mean ± SD)	5·53 ± 1·47	6·39 ± 1·46	**<0·0001**
Procedure time (min, mean ± SD)	63 ± 12	67 ± 10	**0·0028**
Processed blood volume (ml, mean ± SD)	3498 ± 758	3746 ± 657	**0·0012**
Collection volume (ml, mean ± SD)	199 ± 55	228 ± 53	**<0·0001**
Final product volume (ml, mean ± SD)	452 ± 119	514 ± 128	**<0·0001**
Final platelet conc ($\times 10^6$/μl, mean ± SD)	1·20 ± 0·13	1·17 ± 0·11	**0·0053**
Effective yield ($\times 10^{11}$, mean ± SD)	5·43 ± 1·48	6·01 ± 1·57	**0·0003**
Time to PAS addition (min, mean ± SD)	231 ± 171	217 ± 180	0·21

min, minutes; conc, concentration; PAS, platelet additive solution.

[a]The median number of donations by each donor in a 1·5-year period prior to the study period.

[b]Preceding PA donation is defined as whether or not the donor has previously given PA concentrates.

[c]P values in bold indicate statistical significance <0.05.

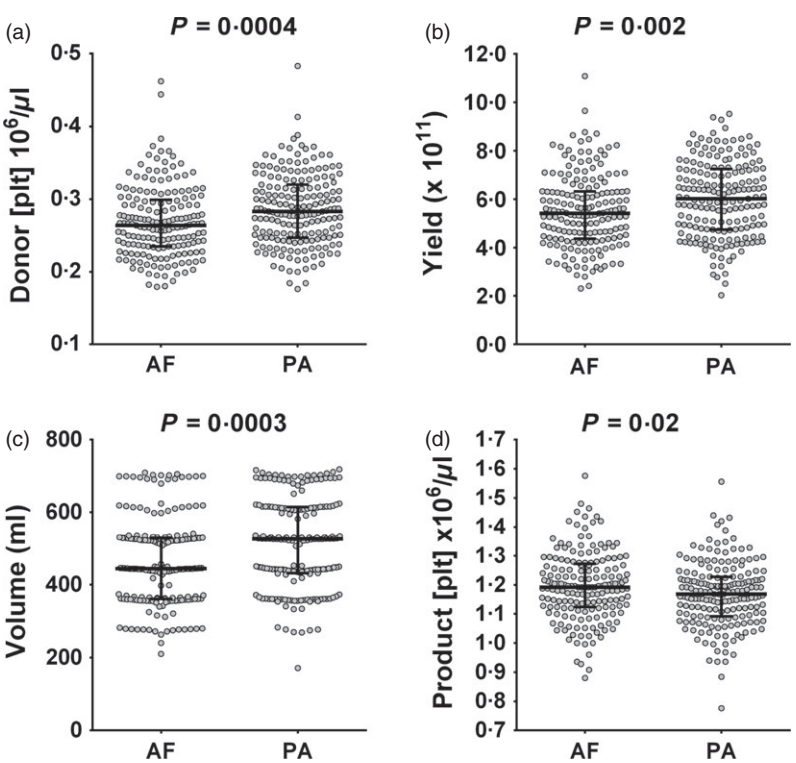

Fig. 1 Products with persistent aggregates (PA) have increased content from donors in the higher whole blood platelet count quantile. (a) Whole blood platelet concentration of the donor. (b) Product platelet yield. (c) Total volume (ml) of the final product, following dilution with additive solution. (d) Platelet concentration in the final product, following dilution with additive solution. All individual data are shown as dots for products without (aggregate-free, n = 180) or with (PA, n = 181) persistent particles. Median and interquartile range are shown as a horizontal line with whiskers, respectively.

anticipated product yield to be manually set, allowing to adjust the targeted yield to the donor parameters including whole blood platelet count. Possibly but not necessarily linked with the latter, we found that PA concentrates had higher yields (Fig. 1b). This was associated with higher volumes (Fig. 1c) (both collection and final) because the standard operating protocol aims towards fixed platelet concentrations (Fig. 1d). Note that there is an even lower

average platelet concentration in PA concentrates than AF controls. There were no statistical differences in donor-related variables for gender, body mass index and age. However, a small but significantly higher haematocrit (Hct) (Table 1) was found in donors giving PA concentrates.

Metabolism in PA products is increased

Metabolic parameters glucose and lactic acid were measured on day six (the day following product expiration) and revealed that products containing PA produced more lactic acid than AF ones (Fig. 2). This points to increased anaerobic respiration in these concentrates causing lower pH values (Fig. 2c).

Platelets in PA products express more P-selectin

Consistent with increased storage lesion on day six as measured by lower pH values, median P-selectin levels were increased in PA concentrates (Fig. 3a). This difference was small, but significant. Figure 3b, moreover, indicates that there was a small but significant difference in platelet activation status as measured by integrin $\alpha_{IIb}\beta_3$ conformational change in resting platelets. Activation by 30 μM PAR1AP was not different between groups (Fig. 3c). Binding of annexin V was not different between PA and AF concentrates (Fig. 3d) indicating no increased phosphatidylserine/-ethanolamine expression.

Increased ristocetin sensitivity in concentrates with PA

Light transmission aggregation experiments were performed on day six, and no differences were seen when stimulated with collagen or PAR1AP at both low and high agonist concentrations (Fig. 4a–d). From the aggregation experiments with ristocetin though, an increased response was noted for PA concentrates in response to low- but not high-dose agonist concentrations (Fig. 4e, f).

Cytokines are elevated in concentrates with persistent aggregates

Platelet cyto-/chemokines TGFβ1, CCL5 and PF4 were measured in the concentrate supernatants because an increase in P-selectin expression pointed to increased platelet degranulation. All three molecules were significantly increased indeed pointing to platelet vesicle release at some point during collection or storage (Fig. 5).

Persistent aggregates are sensitive to thrombolysis

Disaggregation experiments in which urokinase plasminogen activator was added to isolated PA in the presence of

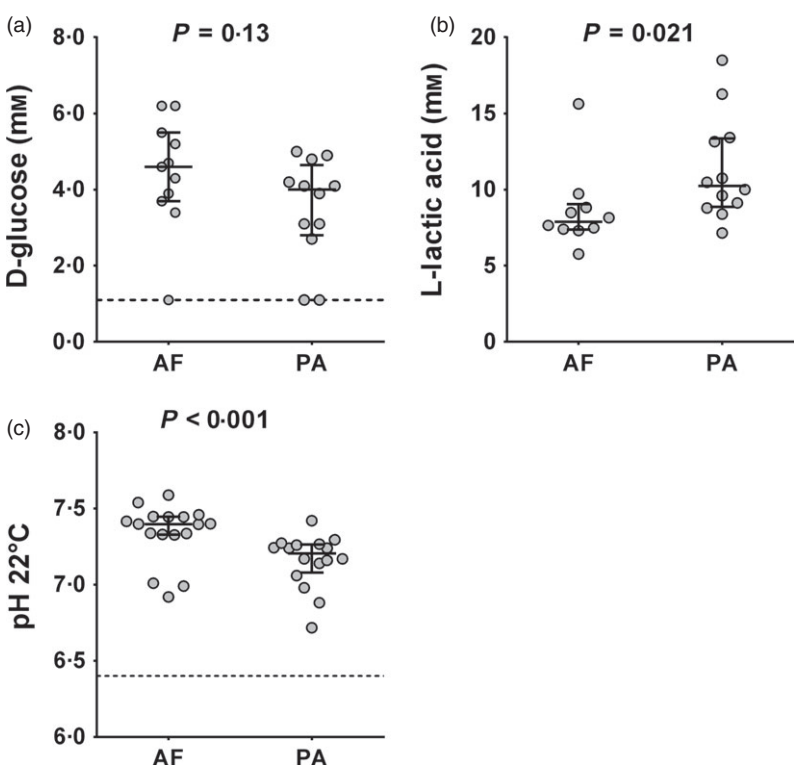

Fig. 2 Increased anaerobic metabolism in products with persistent aggregates (PA). (a) Glucose and (b) lactic acid (mM) concentrations are shown for concentrates with (PA, $n = 12$) or without (aggregate-free, AF, $n = 10$) PA. The dashed horizontal line indicates the lower limit of detection. (c) The pH at room temperature for products with (PA, $n = 16$) and without (AF, $n = 17$) PA. The dashed horizontal line indicates the lower acceptance criterion (6·4) for pH of platelet concentrates. All individual data are shown as dots, and the median and range are shown as horizontal line and whiskers, respectively.

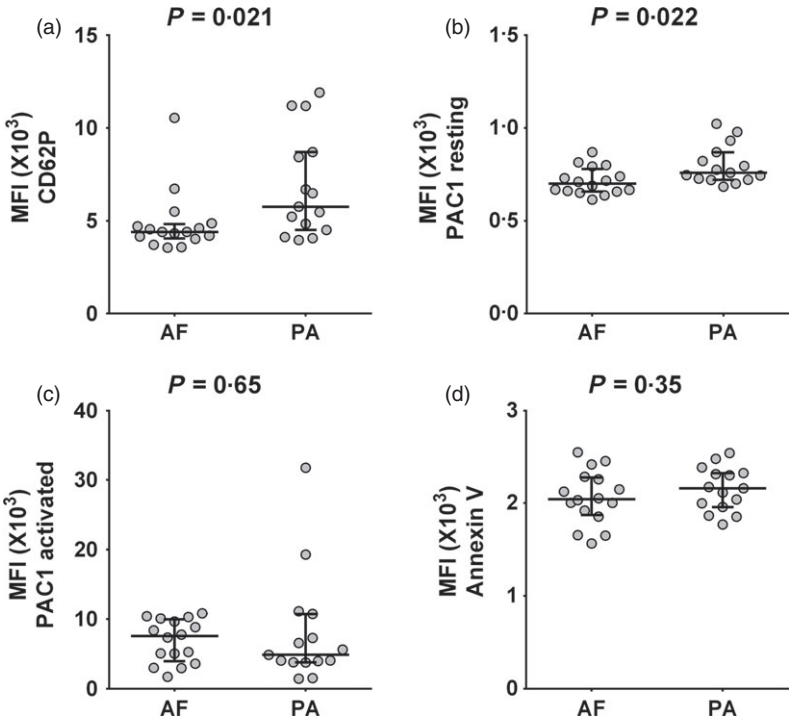

Fig. 3 Platelet receptor expression. Median fluorescence intensities (MFI) indicate the median level of platelet receptor or phospholipid expression. (a) Degranulation using an anti-P-selectin antibody. (b, c) Expression of activated integrin $\alpha_{IIb}\beta_3$ in (b) resting platelets and in (c) platelets activated with 30 μM PAR1AP. (d) Expression of phosphatidylserine/-ethanolamine phospholipids as measured by Annexin V binding. All data were collected on day six postdonation. Median and interquartile range are shown as a horizontal line and whiskers, respectively, for concentrates with (persistent aggregates, PA, $n = 15$) and without (aggregate-free, $n = 16$) PA. Individual data are shown as dots.

heterologous plasma showed that the particles were sensitive to this type of thrombolytic treatment (Fig. 6a). The particles did not disaggregate in response to high concentrations of RGDS peptide meaning that the aggregates were not formed solely by $\alpha_{IIb}\beta_3$-fibrinogen interactions. Flow cytometry with the platelet-specific anti-α_{IIb} (CD41) marker before and after this *in vitro* thrombolysis confirmed that the aggregates contained significant amounts of platelet-specific antigen.

Discussion

This explorative study was designed to gather information on apheresis products (Trima® Accel) with PA in a preset period of routine collections in one regional blood service. The main goal was to detect the differences between PA and AF products, hereto two comprehensive analyses were performed, (i) statistical comparison of apheresis collection and donation data and (ii) laboratory analysis of platelet functional and behavioural parameters to investigate the quality of PA concentrates.

Our data show that there is a twofold increased likelihood of yielding a PA concentrate if the particular donor had previously donated a product with PA. This indicates the existence of a link between the donor and PA occurrence. Since apheresis platelet concentrates are by definition derived from a single donor, genetic [8, 9] and acquired [10] differences in platelet function including the susceptibility of these anucleate cells to changing environmental cues are specific to each donor [11]. The link we found is, however, not absolute because a previous PA donation does not always predict a next one, showing that auxiliary factors must contribute also. The significantly higher whole blood platelet concentration in donors giving PA products corroborates with such a donor-related factor, but by itself neither reliably predicts a PA incident because there are also aggregates in the moderate to low platelet count population (Fig. 1). Moreover, historical adjustments to the anticoagulant to whole blood ratio [12], which influences product acidity (pH), but also changes in temperature [3] and optimizations to the apheresis technicalities [13] have significantly decreased the PA prevalence, indicating that a technical contributor is likely to also be involved [6]. Consequently, despite a demonstrable role of a donor factor(s), a PA incident requires multiple concerting variables.

We found no differences for gender, age or body mass index of the donor. This is different from a previous retrospective study [14] in which 803 single donor products (100% plasma) indicated a significantly higher incidence of PA among women. Correspondingly, these donors had a lower Hct. The authors hypothesized that the consequently higher plasma volume may increase the incidence of aggregates based on less anticoagulation relative to donors with higher Hct. Their findings are nevertheless in contrast to our data in which a significantly higher Hct was found in the PA group. This difference was, however, small and therefore should be interpreted with caution

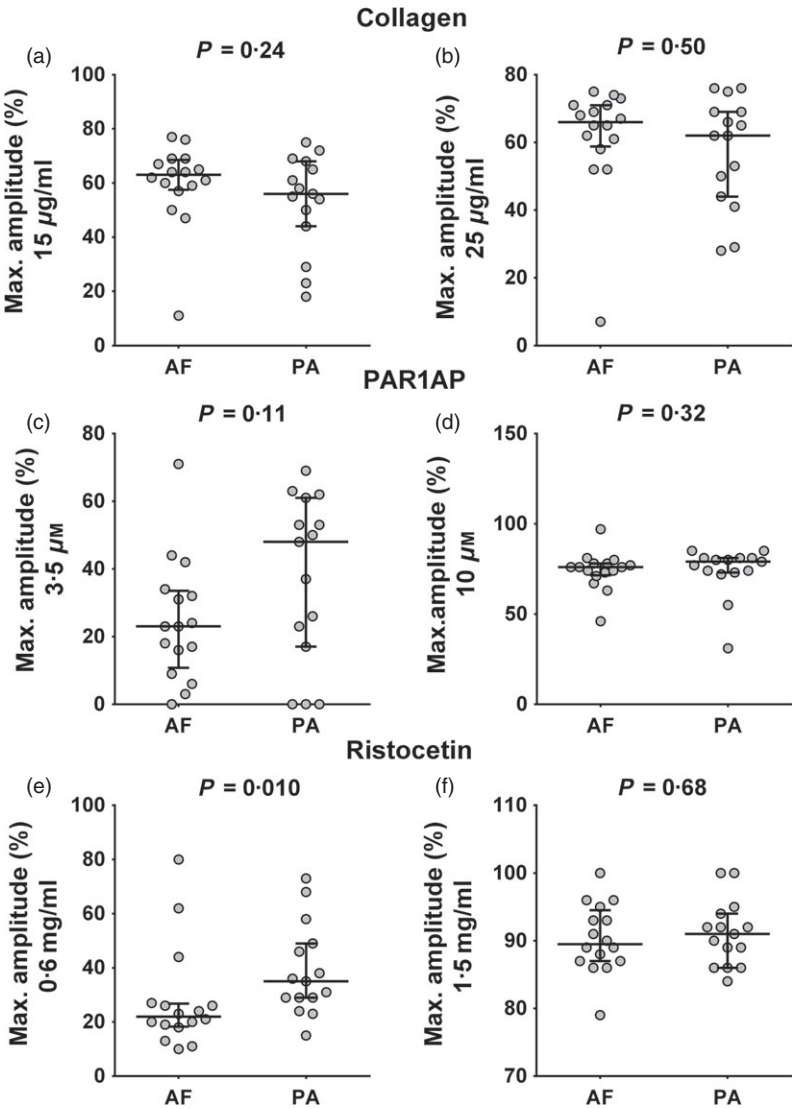

Fig. 4 Ristocetin agglutination is increased in concentrates with persistent aggregates (PA). Aggregation by light transmission was performed with three agonists as indicated on top of the panels; collagen (a, b), PAR1AP (c, d) and ristocetin (e, f) each in a low (a, c and e) and high (b, d and f) concentration. The percentage of maximum aggregation (amplitude) recovered from the aggregation tracings is shown in dots for the individual data points of concentrates with (PA, $n = 15$) and without (aggregate-free, $n = 16$) PA. The median value and interquartile range are shown by horizontal line and whiskers, respectively.

although a higher Hct could theoretically increase viscosity and shear force-regulated interplatelet interactions [15, 16].

As mentioned above, the differences in cell concentration are unlikely to be the sole underlying cause of PA, because PA are also found in low yield concentrates. Furthermore, during circulation in the human body, platelets are locally also highly concentrated because the biorheological process of margination [17] causes tight packing of platelets near the inner blood vessel lining. More importantly, the vicinity of the healthy vessel wall *in vivo* assures platelet pacification by hormones such as prostacyclin [18], nitric oxide [19] and ectonucleotidases [20] that scavenge adenosine phosphates. These biochemical regulators are all absent during apheresis. So, it is evident that biochemical signals (or lack thereof) are also important in the generation of platelet aggregates. The laboratory data gathered at day six postdonation provide such insights. The increased P-selectin expression and the higher cyto-/chemokine content in the supernatant indicate increased vesicle release [21, 22]. Although not dramatically different to AF products, it is logical to find markers of vesicle release in PA concentrates because even minimal perturbations cause vesicle release without platelet activation [23]. Alternatively, the irreversible nature of the observed platelet aggregation likely requires amplificatory signals at some stage which must originate from the cell's granules [24]. Amplification and subsequent stable aggregation is indirectly confirmed in the disaggregation experiment which showed that although the persistent aggregates were sensitive to thrombolysis by plasmin (Fig. 6), they were not dislodged by incubation with the strong integrin $\alpha_{IIb}\beta_3$–fibrinogen inhibitor RGDS. This indicates that the persistent aggregates

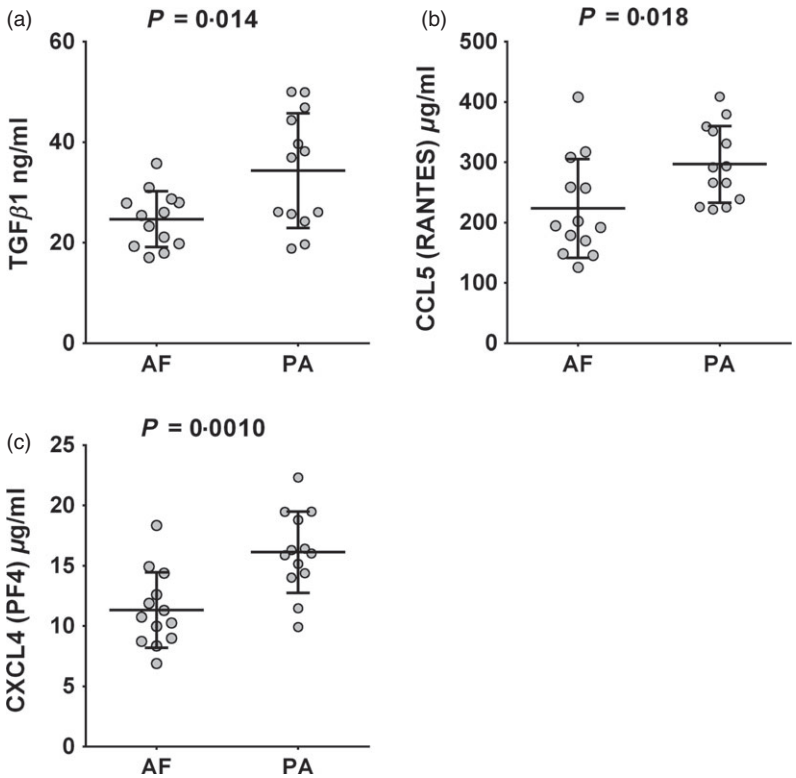

Fig. 5 Cytokines were increased in platelet concentrates with persistent aggregates (PA). The concentration of platelet-related cyto- and chemokines (a) TGFβ1 in ng/ml, (b) CCL5 in µg/ml and (c) CXCL4 in µg/ml is shown. Individual data are shown as dots for platelet concentrates with (PA) and without (aggregate-free) particles (n = 13). Mean and standard deviation are shown as a horizontal line and whiskers, respectively.

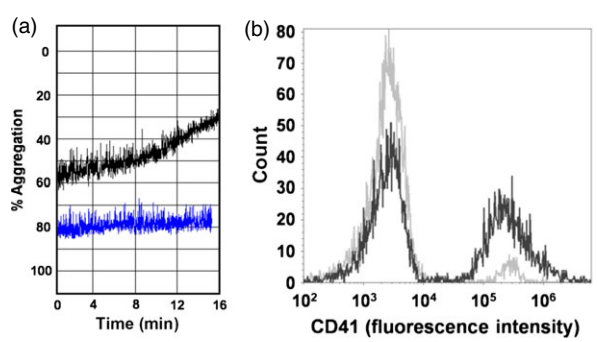

Fig. 6 Persistent aggregates are sensitive to thrombolysis and contain platelets. (a) Incubation of isolated persistent aggregates with urokinase (black tracing) or RGDS (blue tracing) in heterologous pooled normal human plasma was followed in function of time with stirring in an optical aggregometer. Significant increase in turbidity was seen in the presence of urokinase, but not RGDS. (b) CD41-positive events were determined by measuring the R-PE signal in samples taken before (grey) and after (black) urokinase incubation. Data are representative of three repeats.

involve multiple interacting molecular species and are possibly stabilized by fibrin. Why this 'activation balance' of platelets is tilted towards pro-aggregatory signalling only in certain apheresis donations, however, remains an open question, but the inherent diversity in platelet reactivity in the human population is a likely contributor [25].

Platelets from concentrates with PA are more sensitive to low doses of ristocetin in agglutination experiments (Fig. 4). Both VWF and GPIbα are polymorphic complex biomolecules [26, 27]; and therefore, it is likely that certain donors bear variants of these proteins or in the proteins that regulate VWF [28] and GPIbα [29]. This may influence the affinity and/or the degree of activation following primary sensitization of platelets *ex vivo*. Together with increases in viscosity leading to altered blood rheology and higher platelet counts, phenotypes with such higher GPIbα-VWF affinities or heightened primary activation may form agglutinates more easily. A follow-up study must answer questions on how biomolecular differences in the GPIbα-VWF axis influence the incidence of PA in our products. Comparable research in the context of ventricular assist devices indicates the importance of the influence of extracorporeal shear stress on this ligand receptor couple [30].

Despite this functional difference, the current laboratory data indicate that the presence or generation of PA is not of great influence to the typical *in vitro* quality parameters which is in line with earlier studies on aggregates following leucocyte filtration [1] or in the context of resting periods [31]. The final platelet concentration of products with PA was a little lower than in those without. This difference of roughly 2·5% might be explained by the incorporation of platelets in

the aggregates [7]. Although a slight increase in anaerobic metabolism reduced the pH, the mean acidity was well above international standards. Moreover, the remaining single platelets responded normally to thrombin-related activatory stimuli as shown by PAC1 measurements. Therefore, the presence of PA may be largely a problem of perception, more so because its mere ascertainment is already (considered) subjective or 'operator-dependent'.

In conclusion, persistent visible aggregates are more likely to be generated in donors with previous PA donations and in the higher platelet count quantile. The products with PA have a higher yield and a slightly increased anaerobic metabolism, but in general, the quality parameters on day six are within acceptance criteria. Therefore, one may opt to filter products with PA which is feasible without influencing product yields [7]. Additional studies on platelet function throughout storage, with a focus on rheological influences on GPIbα-VWF are required to elucidate a possible mechanistic explanation for the onset of PA. Innovation in apheresis technologies as well as increased knowledge of the donor population may furthermore aid in reducing this practical issue in blood banking.

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Authorship

HBF, JC, HP, RD, BVA and VC contributed in study design and data analysis; PVDK, VC and JC contributed essential research reagents and facilitated research; HP and HBF performed statistical analyses; RD and BVA performed experiments; HBF wrote the manuscript; all authors critically read and reviewed the manuscript.

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