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# The effect of centrifugation speed and time on pre-analytical platelet activation

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## Abstract

**Background:** The results of laboratory analyses are affected by pre-analytical variables, and in particular can platelets be activated by shear handling stress and secrete granular substances. We therefore evaluated the effect of centrifugation speed and time on pre-analytical platelet activation.

**Methods:** Citrate- and EDTA-anticoagulated blood from healthy volunteers were centrifuged at 80–10,000 *g* for 5–15 min to prepare plasma and platelet-rich plasma. Pre-analytical platelet activation was assessed by flow cytometric measurement of platelet P-selectin (CD62p) expression. Blood cell counts, mean platelet volume (MPV), immature platelet fraction (IPF), and platelet distribution width (PDW) were measured. Platelet aggregation in platelet-rich plasma induced by arachidonic acid (AA), ADP or thrombin receptor activator peptide-6 (TRAP) was tested by 96-well aggregometry.

**Results:** The median percentage of platelets expressing P-selectin in citrate- and EDTA-plasma centrifuged at 2000 *g* for 10 min were 43% [interquartile range (IQR), 38%–53%] and 56% (IQR, 31%–78%), respectively ( $p=0.82$ ). Platelet-rich plasma prepared at 100–250 *g* for 10 min had significantly lower platelet P-selectin expression (11%–15%),  $p<0.001$ . Platelet count in plasma samples decreased with increasing speed but platelets were only completely removed if plasma was re-centrifuged. In platelet-rich plasma, increasing centrifugation speed significantly increased platelet yield but decreased contamination from other blood cells, platelet composition was altered as platelet parameters (MPV, IPF, and PDW) was lowered. Platelet

aggregation was not affected by the centrifugation speed platelet-rich plasma was prepared.

**Conclusions:** Proportional to centrifugation speed, platelets in plasma and platelet-rich plasma were activated with centrifugation speed, cell content and composition changed while platelet aggregation was unaltered.

**Keywords:** centrifugation; plasma; platelet aggregation; platelets; P-selectin.

## Introduction

Plasma circulates the bloodstream and extracellular spaces and contain substances originating from many tissues [1]. Plasma-based laboratory analyses are extensively used for diagnosing and monitoring various diseases. Plasma is isolated from whole blood using centrifugation, where the purpose of the centrifugation is to remove blood cells without altering the plasma composition. One exception is platelet function testing, which is primarily performed on whole blood or platelet-rich plasma [2]. Platelet-rich plasma is prepared by gentle centrifugation, hereby retaining platelets in the plasma, while removing red and white blood cells [3–5]. Generation of platelet-rich plasma may also be first step in a platelet isolation procedure [6]. Recent studies and guidelines conclude that centrifugation at 200–250 *g* for 10 min is optimal for preparing platelet-rich plasma for light transmission aggregometry [3–5]. However, studies of platelet activity or biology, such as platelet-released microvesicles, are using different protocols, which can potentially influence the results.

During preparation of plasma and platelet-rich plasma, it is warranted that platelets are not activated by the procedure. However, studies indicate that shear stress *in vitro* which may derive from the centrifugation procedure can activate platelets in a blood sample [6–10]. When platelets are activated, they secrete different substances from their granules, which could potentially influence subsequent laboratory test results. Even the slightest disturbance of the cells in the pre-analytical phase may lead to secretion of intracellular substances that can potentially affect the measurement of some components. The impact of pre-analytical factors becomes more important

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considering the increased interest in measuring ever-lower concentrations of plasma components. For instance are mi-RNA measurements sensitive to platelet contamination [11]. Further, it is warranted that plasma is cell-free before cryo-storage, as freeze/thaw mediated destruction of cells can lead to leakage that might affect plasma levels of analytes [12]. In order to implement analyses into everyday practice, it is crucial to minimize pre-analytical factors that can have impact on the result.

Platelet activation can be assessed by a measurement of P-selectin expression on the platelet membrane. P-selectin is a component in the platelet  $\alpha$ -granules, which is expressed at the surface of the cell when the granules are exocytosed during activation [13]. This process is irreversible in vitro, and P-selectin is therefore a sensitive marker of platelet activation [14, 15]. To our knowledge, it is unknown to what extent different centrifugation settings activate the platelets during plasma preparation.

Our hypothesis is that pre-analytical platelet activation in plasma samples is positively associated with centrifugation speed and time, while contamination from other cells is negatively associated. Hence, we expect a relatively lower degree of platelet activation in platelet-rich plasma compared with plasma, due to the lower centrifugation speed during preparation. We hypothesize that very low centrifugation speed results in high yield of un-activated platelets and a higher platelet aggregation

capacity, compared with platelets in samples exposed to higher centrifugation speed.

The aim of the study was to evaluate how centrifugation speed and time affect pre-analytical platelet activation and platelet count in plasma and platelet-rich plasma. Additionally, we examined the effect of centrifugation on platelet parameters and platelet aggregation.

## Materials and methods

As shown in Table 1, experimental protocol 1 tested whether platelets were activated by the plasma preparation. In experimental protocol 2, cell concentrations in platelet-rich plasma and plasma were tested. In experimental protocol 3, platelet activation and aggregation in platelet-rich plasma samples prepared for platelet function testing were tested.

### Blood samples

For protocols 1 and 3 (Table 1), thirty-one healthy volunteers (16 women and 15 men) with a median age of 27 (IQR: 25–34) years were included in the study. None had taken medication known to affect platelet function within the previous 14 days. The participants had blood samples drawn from the antecubital vein using a minimal tourniquet pressure and a 21-gauge needle (Becton Dickinson, Franklin Lakes, NJ, USA). After discarding the first 3 mL, blood was collected

**Table 1:** Centrifugation settings and analyses performed.

Experiment protocol	1	2	3
Purpose	Test platelet activation in plasma samples	Test cell concentrations	Test activation and platelet aggregation for platelet function testing
Anticoagulant	Citrate plasma EDTA plasma	EDTA pool	Citrate PRP
Sample number, n	6	3 (pooled)	25
Centrifugation setting	2000 g, 10 min and recentrifugation at 2000 g 10 min	80 g, 5, 10, 15 min 100 g, 5, 10, 15 min 150 g, 5, 10, 15 min 200 g, 5, 10, 15 min 250 g, 5, 10, 15 min	100 g, 10 min 150 g, 10 min 200 g, 10 min 250 g, 10 min
Centrifugation of the remnant		1000 g, 5, 10, 15 min 1200 g, 5, 10, 15 min 1500 g, 5, 10, 15 min 2000 g, 5, 10, 15 min 3500 g, 5, 10, 15 min 5000 g, 5, 10, 15 min 10,000 g, 5, 10, 15 min	1500 g, 15 min 3500 g, 10 min 3500 g, 15 min 10000 g, 5 min
Results showed in Analyses	Figure 1	Figure 2	Figures 1 and 3, Tables 2 and 3
Platelet count	x	x	x
Platelet yield		x	x
WBC and RBC count	x	x	x
P-selectin expression	x		x
Platelet aggregometry			x

in 4.5 mL BD vacutainers containing 3.2% (109 mM) sodium citrate or ethylene-diaminetetraacetic acid (EDTA) (Becton Dickinson). After collection, the samples were gently mixed and allowed to rest for 15 min before further handling.

For protocol 2, EDTA-anticoagulated blood samples from anonymous individuals (n=93) with hemoglobin, leucocyte count, and platelet count within laboratory reference ranges were obtained from the departments previously collected samples. The samples were pooled in three pools and aliquoted into 4.5-mL sterile tubes without any additional additive (Becton Dickinson).

## Sample centrifugation

Centrifugation temperature was set at 21 °C. Plasma was separated with a 0.5-cm distance from the precipitate. Samples were centrifuged in an Eppendorf 5810R (Eppendorf, Hamburg, Germany) centrifuge, unless otherwise mentioned.

For protocol 1, plasma was centrifuged at 2000 g for 10 min with brake and the supernatant was re-centrifuged at the same settings. For protocol 2, pooled samples were centrifuged at 15 different settings at speeds between 80 and 250 g for 5–15 min with brake. For protocol 3, samples for testing platelet activation and aggregation in platelet-rich plasma were centrifuged at 100–250 g, for 10 min without brake. The remaining blood from each platelet-rich plasma preparation was pooled, transferred into 1.5 mL tubes, and plasma was isolated following re-centrifugation at 1000–10,000 g for 5–15 min with brake in a MiniSpin centrifuge (Eppendorf, Hamburg, Germany).

## Hematology parameters

Numbers of platelets, white (WBC) and red (RBC) blood cells were measured using a Sysmex XN-9000 analyzer (Sysmex, Kobe, Japan). By the same procedure, the following additional platelet parameters were recorded: mean platelet volume (MPV), which describes the platelet size, immature platelet fraction (IPF), which reflect the fraction of immature platelets, and platelet distribution width (PDW), which describes the variation in platelet size.

For some purposes the platelet yield, that is the total number of platelets in the sample, is of relevance, for instance in the process of isolating platelets, where a high platelet yield after the centrifugation is preferable. The platelet yield was calculated, using plasma density=1025 mg/cm<sup>3</sup>, by the following equation:

$$\frac{\text{Weight (mg)}}{\text{Plasma density}} \times \text{Platelet count} = \text{Platelet yield}$$

## Flow cytometry

Platelet P-selectin (CD62p) expression was used as marker for platelet activation. P-selectin expression was evaluated by mixing anti-CD62p antibody or an appropriate isotype control (eBioscience, San Diego, CA, USA) with 5 µL whole blood, plasma or platelet-rich plasma diluted in tyrodes-HEPES buffer [NaCl 134 mM, KCl 2.9 mM, MgCl<sub>2</sub> 1 mM, glucose 5.6 mM, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), pH 7.4]. The P-selectin expression on platelets

in whole blood was measured and used as a reference. Samples were incubated for 10 min at room temperature in the dark and were fixed with 0.5% formaldehyde diluted in phosphate-buffered saline (PBS) [16]. Samples were stored at 4 °C and analyzed within 16–20 h from preparation on a FACS Canto II flow cytometer (Becton Dickinson, NJ, USA). The flow cytometer was set to count 10,000 events determined by platelet light scattering properties on a forward-side scatter plot. The results were reported as the percentage of platelets positive for P-selectin compared with the results using a matching isotype control, which was used to define a negative sample. It reflects the percentage of activated platelets.

## 96-well aggregometry

Platelet aggregation was measured using 96-well aggregometry according to a previous published protocol [17]. Briefly, 5 µL of agonist or PBS was applied to individual wells of half-area 96-well microtiter plates (Greiner Bio-One; Stonehouse, Gloucestershire, UK). The agonists were ADP (12.8 µM), arachidonic acid (AA) (0.5 mM), and thrombin receptor activator peptide-6 (TRAP) (10 µM), respectively. ADP was obtained from Sigma Aldrich (St. Louis, Missouri, USA) and all other agonists were from Roche Diagnostics (Mannheim, Germany). All plates were prepared in one batch and stored at –80 °C and used within 24 days from preparation. Prior to testing, 45 µL of platelet-rich plasma from citrated blood was added to wells with agonists. As reference, 45 µL of platelet-rich plasma or plasma centrifuged for 15 min at 1500 g was added to wells with PBS instead of agonists. Plates were shaken at 900 rpm for 10 min at 37 °C, where after the optical density in each well was determined using Victor X5 (Perkin Elmer, Turku, Finland). Aggregometry was conducted in replicates and within 2 h from blood sampling. Aggregation was calculated from mean optical density (OD) in wells with agonists in reference to platelet-rich plasma (PRP) (set to 0% aggregation) and plasma (set to 100% aggregation), using the following equation:

$$\frac{(\text{OD PRP} - \text{OD sample})}{(\text{OD PRP} - \text{OD Plasma})} * 100 = \text{Aggregation \%}$$

## Statistical analysis

The distribution of continuous data was evaluated using D'Agostino and Pearson omnibus normality test and by inspection of histograms. Data were non-normally distributed and thus presented as median and interquartile ranges (IQR). Differences between three or more groups of samples centrifuged at different speeds and times were tested with Kruskal-Wallis test. Dunn's multiple comparison test was used for internal contrasts. When comparing two groups, Mann-Whitney's test was used. p-Values <0.05 were considered significant. Statistical analyses were performed using GraphPad Prism 5 (GraphPad Software, Inc., La Jolla, CA, USA).

## Ethical considerations

All samples were obtained in accordance with the declaration of Helsinki guidelines and the study was approved by the Ethical

Committee of the Region of Southern Denmark. All participants signed an informed consent form.

## Results

### Plasma preparation

#### P-selectin expression

The percentage of activated platelets in citrate and EDTA whole blood prior to centrifugation was 4% [3–5] and 9% [7–19], respectively. For plasma centrifuged at 2000 g for 10 min, the percentage of activated platelets were equally high: citrate 43% (38–53) and EDTA 56% (31–78),  $p=0.82$ . An additional centrifugation of the plasma at the same speed and time followed by re-suspension of the sample lead to no further activation (Figure 1). When re-suspending centrifuged samples, the platelet P-selectin expression was equal to the value measured prior to centrifugation.

In plasma prepared from the samples where platelet-rich plasma was initially removed (protocol 3), platelet P-selectin expression was higher than for platelet-rich plasma, but lower than for plasma (Figure 1). No difference was found between different centrifugation settings (all medians were between 23.2% and 24.2%, Kruskal-Wallis test,  $p=0.96$ ). Therefore, results were pooled into one group named “plasma from PRP remnant”.

#### Platelet count

None of the tested centrifugation settings were able to fully remove platelets from plasma by a single centrifugation

(Figure 2A). When centrifuging for 15 min at 10,000 g (the most vigorous setting tested), a median of  $2 \times 10^9$  platelets/L still remained in the plasma.

The platelet count did not differ between citrated and EDTA plasma samples prepared at the same speed and time. When plasma initially centrifuged for 10 min at 2000 g was re-centrifuged at the same speed and time and the upper layer was preserved, platelet count in the supernatant was below the hematology equipment’s detection limit ( $<1 \times 10^9/L$ ).

In plasma samples from both citrated and EDTA-anticoagulated blood, there was a low degree of contamination with WBC and no significant difference in WBC numbers between centrifugation settings (WBC,  $0-0.04 \times 10^9/L$ ,  $p=0.25$ ). Numbers of RBCs were un-measurably low in all the citrated samples, and only a very low number were present in EDTA samples (RBC,  $0-0.01 \times 10^{12}/L$ ).

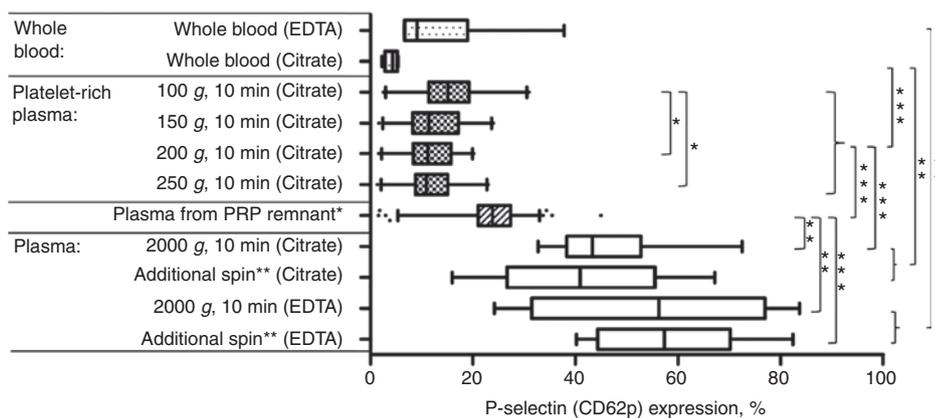
### Platelet-rich plasma preparation for platelet function testing

#### P-selectin expression

In citrated platelet-rich plasma samples, platelet P-selectin expression was significantly higher in the samples centrifuged at 100 g compared with 200 g and 250 g of platelet-rich plasma (Figure 1).

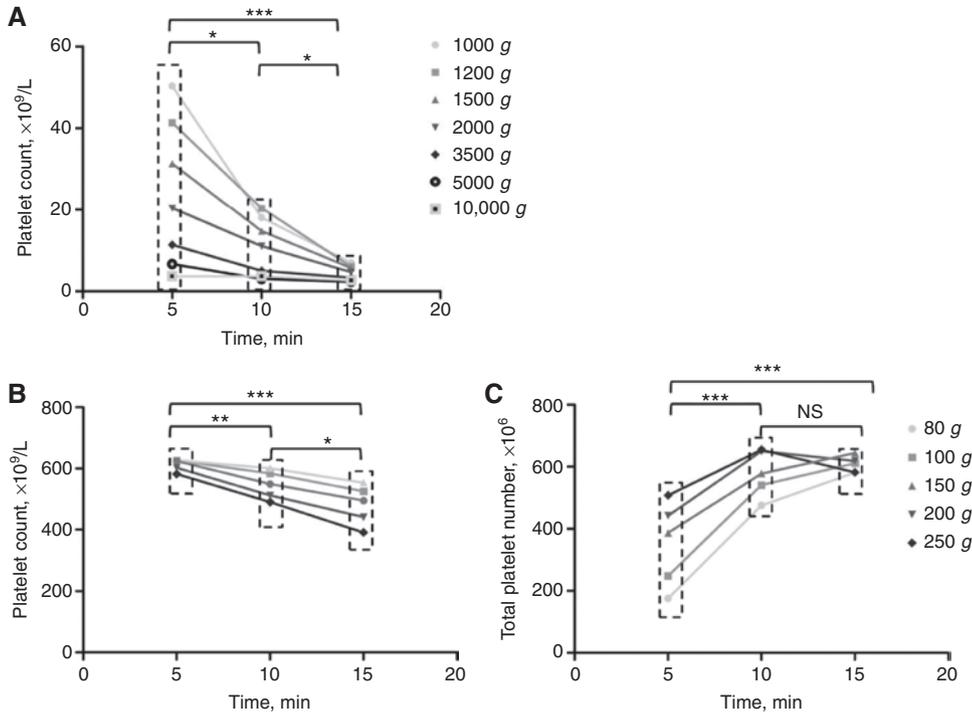
#### Platelet count and contamination with other blood cells

Platelet count in platelet-rich plasma decreased with increasing centrifugation time at different speeds of centrifugation (Figure 2B). Significantly higher platelet yield



**Figure 1:** Percentage of platelets in a sample that express P-selectin.

Measurements divided into groups by used centrifugation setting and anticoagulant. Comparisons between the groups are calculated with Mann-Whitney’s test. \* $p<0.05$ , \*\* $p<0.01$ , \*\*\* $p<0.001$ . \*Plasma prepared from samples where platelet-rich plasma is removed, centrifuged between 1500 g for 15 min and 10,000 g for 5 min. \*\*Plasma centrifuged at 2000 g for 10 min spun an additional time at the same setting and re-suspended.



**Figure 2:** Platelet count and platelet yield: results from protocol 2.

(A) Platelet count in plasma, (B) platelet count in platelet-rich plasma, (C) platelet yield in platelet-rich plasma. Comparisons among the groups within the dotted lines are calculated with Mann-Whitney's test. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ .

**Table 2:** Cell counts in citrated whole blood and platelet-rich plasma centrifuged at 100–250  $g$  for 10 min.

	Whole blood	100 $g$	150 $g$	p-Value	200 $g$	p-Value	250 $g$	p-Value
Platelet count, $\times 10^9/L$	266 (229–283)	335 (269–414)	366 (318–420)	NS	342 (295–440)	NS	336 (267–398)	NS
Platelet yield, $\times 10^6$	1197 (1028–1277)	277 (183–446)	391 (340–507) <sup>a</sup>		467 (404–547) <sup>b</sup>		507 (374–585) <sup>c</sup>	
White blood cells, $\times 10^9/L$	6.89 (5.74–7.56)	0.02 (0.01–0.11)	0.01 (0–0.01) <sup>b</sup>		0 (0–0.01) <sup>c</sup>		0 (0–0.01) <sup>c</sup>	
Red blood cells, $\times 10^{12}/L$	4.71 (4.42–5.08)	0.03 (0.02–0.03)	0.02 (0.01–0.03) <sup>a</sup>		0.01 (0.01–0.02) <sup>c</sup>		0.01 (0.01–0.01) <sup>c</sup>	

p-Values describe the differences between 100  $g$  and each of the other groups calculated with Mann-Whitney's test. <sup>a</sup> $p < 0.05$ , <sup>b</sup> $p < 0.01$ , <sup>c</sup> $p < 0.001$ .

was obtained in plasma samples centrifuged for 10 min compared with 5 min, while no significant difference was found between 10 min and 15 min of centrifugation (Figure 2C). In platelet-rich plasma (protocol 3, Table 1), platelet yield increased significantly with increasing centrifugation speed (Figure 2C, Table 2) The numbers of WBCs and RBCs were low in all platelet-rich plasma samples, but decreased significantly with increasing speed (Table 2).

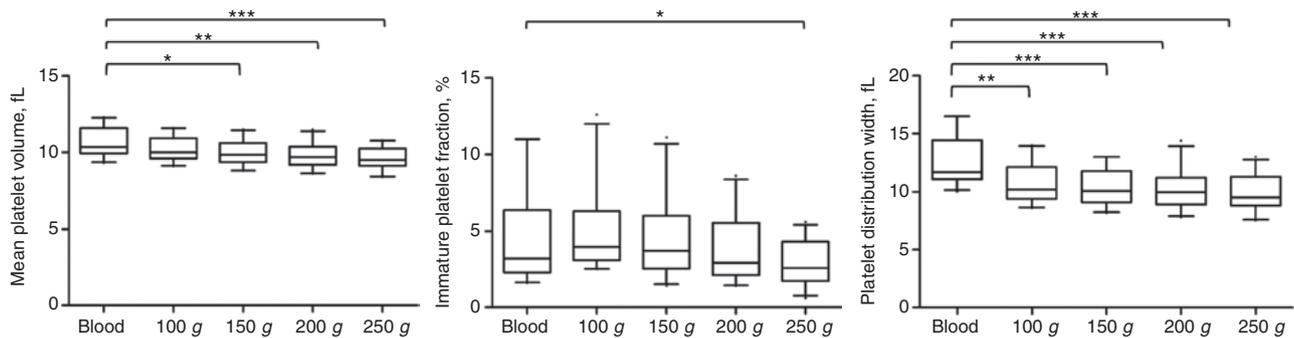
### Platelet markers and platelet aggregation

The platelet parameters MPV, PDW and IPF decreased with increasing speed in all platelet-rich plasma samples

(Figure 3). In platelet-rich plasma (protocol 3), platelet aggregation induced by AA, ADP and TRAP did not differ significantly across the groups of platelet-rich plasma prepared at 100–250  $g$  (Table 3).

## Discussion

In this study, we evaluated the effect of centrifugation speed and time on pre-analytical platelet activation defined by P-selection expression. Our results showed that platelet activation increases with increased centrifugation speed. While approximately half of the platelets present



**Figure 3:** Platelet parameters: results from protocol 3.

Platelet parameters in citrated whole blood and platelet-rich plasma centrifuged at 100–250 g for 10 min. Comparisons between whole blood and the other groups are calculated with Mann-Whitney's test. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ .

**Table 3:** Platelet aggregation.

	100 g	150 g	200 g	250 g	p-Value
AA	65 (60–78)	71 (65–78)	69 (58–72)	69 (60–77)	0.48
ADP	69 (63–78)	64 (59–74)	67 (63–77)	72 (60–79)	0.63
TRAP	74 (62–79)	71 (58–78)	75 (54–81)	74 (55–82)	0.10

in plasma from both EDTA- and citrate-anticoagulated samples were activated by centrifugation, a smaller percentage of platelets expressed P-selectin in platelet-rich plasma (11%–15%). Platelet count in plasma samples decreased with increasing speed but platelets were only completely removed if plasma was re-centrifuged. In the preparation of platelet-rich plasma for platelet function testing, increasing centrifugation speed from 100 to 250 g significantly increased platelet yield from  $277$  to  $507 \times 10^6$  platelets and decreased the platelet parameters (MPV, IPF and PDW). Contamination with WBCs and RBCs also decreased with increasing centrifugation speed. Of note, platelet aggregation capacity did not change with centrifugation speed.

We found that approximately 50% of platelets from both EDTA and citrate plasma were activated after a standard plasma centrifugation (2000 g, 10 min). To our knowledge, only few studies describe that centrifugation leads to platelet activation, but none of these evaluate the effect of increased centrifugation speed and time [6, 9, 10]. There is controversy about the effect of EDTA on platelets in a sample. EDTA inhibits platelet aggregation and dense granule secretion induced by platelet agonists [18, 19]. Hence, less activation of platelets in EDTA plasma compared to citrate plasma is expected. On the other hand, studies have shown higher spontaneous P-selectin expression in EDTA than citrate samples [20, 21]. We found that platelets in citrate and EDTA plasma were activated to the same extent.

The impact of centrifugation on platelet activation was related to centrifugation speed. When comparing with plasma, the more gentle centrifugation during platelet-rich plasma preparation resulted in less platelet activation (11%–15%). The existing literature is scarce and the only comparable study found is a test of three individuals, where the percentage of platelet P-selectin expression was 4% in platelet-rich plasma samples centrifuged at 100 g for 20 min [9]. A finding in our study that contradicts the relationship between centrifugation speed and platelet activation is that platelet-rich plasma samples centrifuged for 10 min at 100 g showed a higher P-selectin expression than samples centrifuged at 200–250 g (Figure 1). Other factors influencing the proportion of activated platelets in plasma must be considered. One explanation could be a shift in the ratio between activated and resting platelets at different centrifugation speeds. Platelets swell upon activation [22] and our results showed that the MPV decreased with increasing centrifugation speed, indicating that the larger platelets are removed more easily at higher centrifugation speed than smaller platelets. Hence, the higher P-selectin expression on platelets in the samples centrifuged at 100 g might be due to a higher number of the larger platelets in the sample, which are known to produce a larger spontaneous as well as an agonist-induced aggregation [23]. Other factors such as buoyancy might affect the composition of platelets that are present in plasma after centrifugation; if a relatively higher proportion of resting platelets compared with activated platelets were centrifuged out of the plasma, the proportion of activated platelets might erroneously appear higher.

Notably, we found a large inter-individual variation in the platelet activation among plasma samples. This indicates that the effect of centrifugation cause inter-individual differences in the amount of secreted analytes during processing. Thus, it is likely to be unpredictable how centrifugation alter the result and not only result in bias on the measurements.

We found no further platelet activation following re-centrifugation of plasma prepared at 2000 g for 10 min. This suggests that all platelets capable of being activated by centrifugation are activated during the first centrifugation. Such a double centrifugation procedure is currently used to remove platelets, e.g. prior to testing mi-RNA, since contamination of plasma with platelets and microvesicles can interfere with the measurements [11, 24]. Our study support the use of a double centrifugation procedure to eliminate platelets as the platelets could not be fully eliminated by a single centrifugation at the tested centrifugation settings. By an additional centrifugation step, the platelet count was reduced below the hematology equipment's detection limit ( $1 \times 10^9$ ). It may, however, be speculated that unwarranted substances released from activated platelets may still be present in plasma after centrifugation.

When testing platelet function *in vitro*, a sample with high platelet count and low contamination with RBCs and WBCs is preferable. In this regard, we found that centrifugation for 10 min is adequate with no further gain at increasing centrifugation time. Further, a sample that resembles whole blood the most in terms of platelet parameters (MPV, IPF, and PDW) and degree of pre-analytical activation would be desirable. Platelet parameters similar to whole blood were best obtained for samples centrifuged at the lowest speed (100 g). However, the risk of RBC and WBC contamination was slightly higher at 100 g. A lower platelet yield and platelet count was obtained at a lower centrifuge speed, and it is therefore necessary to collect a larger starting volume of blood from the patient to gain the same platelet yield that is obtainable at a higher centrifugation speed. The samples centrifuged at 100 g had a higher pre-analytical activation than samples centrifuged at 200–250 g. Moreover, the isolation of platelet-rich plasma is difficult due to an unclear separation of platelet-rich plasma and the remaining blood cells. Overall, we can not recommend any changes in the existing guidelines of centrifugation in light transmission aggregometry [5]. There was no difference in platelet aggregation between the groups of samples prepared at different speed. This indicates that the difference in platelet-rich plasma composition and platelet pre-activation did not affect the analysis considerably. Other studies concluded that decreased platelet aggregation at high centrifugation speed (300–500 g) could be due to the decreased MPV [3, 4]. However, we did not find this relationship in our study, suggesting that the MPV decrease at the highest speed tested (250 g) is not sufficient to lower aggregation when measured with the used aggregation method.

This study is strengthened by the wide and thorough investigation of centrifugation settings. As centrifugation

of samples is a daily laboratory routine, the present result should be of interest for researchers in many fields. To our knowledge, this is the first study to investigate how centrifugation speed and time affect platelet P-selectin expression. A limitation of the study is a small sample size in some of the analyzed groups. However, we chose to focus on a wider range of test settings rather than a large sample size. It was beyond the scope of the present study to investigate the relevant relationship between pre-analytical platelet activation and particular plasma measurements.

P-selectin measurement is one of many known markers of platelet activation. Specific products of platelet activation in plasma such as  $\beta$ -thromboglobulin or ATP-release also can be used to evaluate the platelet granule secretion upon activation [25, 26]. In future research, a combination of different platelet activation markers is likely to give a clearer image of the effect the pre-analytical settings have on plasma composition.

The present findings should encourage researchers to find means to minimize the effect of the plasma centrifugation, for example by inhibiting the platelets before processing. Prostaglandin E1 and acid citrate dextrose solution A are used in the platelet isolation process to prevent platelet activation [27, 28]. Similar precautions regarding the plasma centrifugation process could be taken.

Platelets are often viewed as contamination in a sample, but it is likely that various components measurable in plasma in fact originate from platelets. Studies have suggested that platelets can receive and transport information from cells throughout the body, including tumor cells. A recent study showed that specific RNAs extracted from platelets could differentiate cancer patients from healthy donors, and the researchers could even locate the origin of the primary tumor [29]. In the future, platelets might in some cases be preferable over plasma for measuring certain analytes in a patient. Again, a well-defined procedure for the pre-analytical handling will be crucial for the obtained results.

In conclusion, the present study show that platelets are activated during centrifugation determined by increasing percentage of P-selectin-positive platelets. It applied for platelet-rich plasma, plasma and was independent of anticoagulant. For plasma preparation, platelets were not completely eliminated by a single centrifugation up to 10,000 g for 5 min, while by double centrifugation at  $2 \times 2000$  g for 10 min platelet count was  $< 1 \times 10^9$ . For platelet-rich plasma preparation, increasing centrifugation speed from 100 to 250 g significantly increased platelet yield and decreased contamination with WBCs and RBCs.

However, the composition of platelets were altered with increasing centrifugation speed as platelet parameters (MPV, IPF and PDW) were lowered. Our findings support the current centrifugation guidelines for platelet-rich plasma for platelet aggregometry.

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**Honorarium:** None declared.

**Competing interests:** The funding organization(s) played no role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the report for publication.

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