

## Do manual and automated processes with distinct additive solutions affect whole blood-derived platelet components differently?

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Dear Sir,

Platelet component transfusions are still major contributors to adverse events, and avoiding storage lesions and the production of inflammatory products is of major importance during the production of these components. *Ex vivo* activation of platelets may lead to excess production of inflammatory factors that can cause acute transfusion reactions<sup>1</sup>. We compared the induction of inflammatory platelet storage lesion in platelet components produced by manual (*m*) and automated (*a*) procedures using the TACSI platform (Terumo France S.A., Guyancourt, France). Furthermore, for each procedure we compared platelet additive solutions (*i.e.*, PASIII [Fenwal, La Châtre, France] versus PASIIM [MacoPharma, Mouveaux, France]) with a mean range of 35% residual plasma. Differences in the composition of these platelet additive solutions have been described previously<sup>2</sup>. The comparison study involved five pooled whole blood buffy-coat-derived platelet components.

The TACSI platform has proven suitable for clinical grade platelet component production with regards to primary haemostasis<sup>3</sup> and platelet additive solutions have been developed to minimise the occurrence of transfusion-related acute lung injury (TRALI) and enable pathogen inactivation. However, little attention, if any, has been given to the impact of procedural changes on the pro-inflammatory lesions potentially inflicted by stored platelets.

The results of the quality control for each product (*m*PC/PASIII, *a*PC/PASIII, *m*PC/PASIIM, and *a*PC/PASIIM) are shown in Table I. All products fell within the range acceptable for use. Some significant individual differences were found, but they were corrected by reporting the quantity of product per issued platelet component. According to past experience, we focused on two markers, the activation platelet surface markers CD62p and CD40L, along with their soluble counterparts, which were measured under each condition. For reference values we used thrombin-receptor activating peptide (TRAP), an analogue of thrombin (50 µg/mL; Saint Quentin-Fallavier, France). Measurements were made on the contents of the quality control sampling bag 24 h after whole blood collection; the products in this study were not destroyed, but issued to patients as authorised, because all four types of platelet component are licensed by the notifying and regulatory body (Affsaps). Platelet membrane activation was tested by flow cytometry using fluorescein isothiocyanate-conjugated anti-CD41 monoclonal antibody (BD Biosciences, Le Pont de Claix, France) for gating the whole platelet population and allophycocyanin-conjugated anti-CD62p and phycoerythrin-conjugated CD40L monoclonal antibodies (BD Biosciences) (FACSvantage SE flow-cytometer and CellQuestS-Pro software, BD Biosciences). Soluble proteins were measured in supernatant fractions using specific enzyme-linked

**Table I -** Comparison of platelets obtained from different processing systems on day 1. Results are expressed as mean±SD (n=10 in each arm).

| Processing sytems | Volume (mL)    | Platelets (10 <sup>9</sup> /L) | Platelets (10 <sup>11</sup> /unit) | WBCs (x10 <sup>6</sup> /units) |
|-------------------|----------------|--------------------------------|------------------------------------|--------------------------------|
| PAS-III-m         | 348.8±3.23     | 1298.1±80.96                   | 4.525±0.27                         | <0.3                           |
| PAS-IIIIM-m       | 398.9±4.18 *   | 1195±61.67                     | 4.771±0.26                         | <0.3                           |
| PAS-III-a         | 329.8±7.59 ‡   | 1463.5±87.33 ‡                 | 4.822±0.3                          | <0.3                           |
| PAS-IIIIM-a       | 362.3±7.07 * ‡ | 1288.9±50.2 *                  | 4.664±0.15                         | <0.3                           |

### Legend

\*p <0.05 PASIII vs PASIIM, ‡ p <0.05 m (manual) vs a (automated -TACSI) using the Mann-Whitney test.

**Table II** - Mean fluorescence intensity (MFI) of CD40L/CD62p and sCD40L/sCD62p released from platelets with or without TRAP stimulation.

| PC                  | PAS-III     |               |                                           |                | PAS-IIIIM     |              |                                           |            |
|---------------------|-------------|---------------|-------------------------------------------|----------------|---------------|--------------|-------------------------------------------|------------|
|                     | MFI         |               | Concentration<br>(pg/10 <sup>9</sup> Plt) |                | MFI           |              | Concentration<br>(pg/10 <sup>9</sup> Plt) |            |
| <i>Without TRAP</i> |             |               |                                           |                |               |              |                                           |            |
|                     | CD40L       | CD62p         | sCD40L                                    | sCD62p         | CD40L         | CD62p        | sCD40L                                    | sCD62p     |
| Manual              | 10.67±0.139 | 31.05±4.5     | 349.3±97.5                                | 3516.7±294     | 9.27±0.1 *    | 16.6±0.8 *   | 1339±227 *                                | 2517±293 * |
| Automated           | 10.4±0.11 ‡ | 19.9±2.05 ‡   | 935.89±227.2 ‡                            | 1927.4±218.2 ‡ | 11.7±0.94 ‡ * | 19.5±5.7     | 1603±604 *                                | 2107±237   |
| <i>With TRAP</i>    |             |               |                                           |                |               |              |                                           |            |
|                     | CD40L       | CD62p         | sCD40L                                    | sCD62p         | CD40L         | CD62p        | sCD40L                                    | sCD62p     |
| Manual              | 11.3±0.41   | 117.8±18.24 # | 1747±248 #                                | 6819±842 #     | 9±0.56        | 40.8±8.7 #   | 1732±149 #                                | 3501±160 # |
| Automated           | 17.8±4.3 #  | 263.9±95.05 # | 1999±302 #                                | 2917±128 #     | 9.9±0.5       | 100.5±46.7 # | 2664±484 #                                | 3363±202 # |

**Legend** Results are expressed as mean±SD, n=10 experiments; \*p <0.05 PASIII vs PASIIIIM, ‡ p <0.05 m (manual) vs a (automated TACSI platform), # p <0.05 without TRAP vs with TRAP stimulation using the Mann-Whitney test.

immunosorbent assays. The monoclonal antibodies to CD62p and sCD40L were purchased from R&D Systems Europe Ltd, Lille, France and Bender MedSystems GmbH, Vienna, Austria, respectively. The reader was a Multiskan EX (Labsystem, Helsinki, Finland). Inter-experimental comparisons of data (10 platelet components in each arm) were performed using the Mann-Whitney U test. P-values <0.05 were considered statistically significant.

Next, we addressed the issue of whether all four types of platelet product were equivalent with regard to their propensity to be activated and secrete detectable inflammatory cytokines *ex vivo*. Slight differences in total CD40L and CD62p platelet surface expression were found between the products processed automatically or manually, irrespectively of the platelet additive solution (Table II). Secreted products were also significantly different between manually and automatically processed preparations. Platelets from all four types of preparations maintained the ability to express and secrete CD62p and CD40L following TRAP stimulation, demonstrating their viability and the absence of detrimental storage lesions (Table II). Significant differences were found but none was consistent with preferential conditions.

None of the 40 platelet components considered led to any reported adverse transfusion reaction, and the individual levels of secreted sCD40L, a molecule that we found correlates with adverse transfusion reactions (R=0.986) (Nguyen *et al.*, submitted), were far below the level necessary to mediate a bioactive effect on encountered cells<sup>4</sup> or that has been associated with TRALI<sup>5</sup>. These findings validate the use of automated platelet component processing with commercially available platelet additive solutions, an

advantage for homogenising the production of platelet components and increasing the production of pooled buffy coat-derived platelets compared to single donor aphaeresis platelet collection, which is currently considered advantageous for the donor and recipient.

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