

## Brief Report

# GENERATION OF IL-8 AND TNF-ALPHA IN PLATELET CONCENTRATES DURING STORAGE

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**Background:** Platelet transfusion is accompanied by febrile nonhemolytic transfusion reactions. The generation of cytokines (like IL-1 beta, IL-6, IL-8, and TNF-alpha) in platelet concentrates by white cells is suggested to be responsible for febrile nonhemolytic transfusion reactions. The number of white cells in the platelet concentrates is crucial to cytokine generation.

**Methods:** This study was performed to determine whether WBC reduction in platelet concentrates by prestorage leukodepletion filters or inactivation by gamma radiation reduced the levels of these cytokines during storage for 3 days. Each of the platelet concentrates (n = 54) was prepared from a single random donor by platelet-rich plasma. This was then divided into four groups: 1) unfiltered, nonirradiated random-donor platelet concentrates (n = 13); 2) unfiltered, gamma-irradiated random-donor platelet concentrates (n = 16); 3) filtered, nonirradiated random-donor platelet concentrates (n = 14); and 4) filtered, gamma-irradiated random-donor platelet concentrates (n = 11).

Cytokine levels in platelet concentrates supernatants were measured by ELISA kits according to the manufacturer's recommendations.

**Results:** Our results showed that IL-8 was detected in unfiltered, nonirradiated, and gamma-irradiated random-donor platelet concentrates but not in the filtered random-donor platelet concentrates. TNF-alpha was only detected in unfiltered, nonirradiated units. Compared with unfiltered platelet concentrates, prestorage filtration prevented a rise in the IL-8 and TNF-alpha on day 3 of storage. The concentration of IL-1 beta was lower than the minimum concentration value of the kit used for this purpose. IL-6 was detected only in 7 units of all filtered platelet concentrates on day 3.

**Conclusion:** These data indicate that gamma irradiation can not prevent generation of IL-8 in platelet concentrates during storage, but prestorage leukoreduction of platelet concentrates can prevent accumulation of IL-6, IL-8, and TNF-alpha during storage.

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## Introduction

Febrile nonhemolytic transfusion reactions (FNHTRs) are a frequent complication with blood and platelet concentrates (PCs) transfusion.<sup>1</sup> Transfusion of nonleuko-

reduced red blood cells (RBCs) are associated with 0.5 – 6% risk, whereas transfusion of PCs carry a risk as high as 2 – 37% in adults. The incidence of such reactions in children is not known.<sup>2</sup>

Many studies suggested that cytokines such interleukin 1 beta (IL-1 beta), interleukin 6 (IL-6), interleukin 8 (IL-8), and tumor necrosis factor alpha (TNF-alpha) may play a role in mediating transfusion reactions.<sup>1</sup> The contaminating white blood cells (WBCs) are the main source of cytokines in the PCs.<sup>1, 2</sup> Cytokines can also be released by activated donor WBCs present in PCs, or possibly by recipient WBCs.<sup>2</sup> It's not entirely

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understood what stimulates WBCs to produce cytokines during platelet storage. The platelets themselves could contribute to these reactions through the release of platelet-specific cytokine after activation or damage to platelets with component preparation and storage.<sup>3</sup>

## Materials and Methods

In this study, we measured levels of IL-1 beta, IL-6, IL-8, and TNF-alpha in the supernatant of stored unfiltered nonirradiated, irradiated, filtered, and filtered irradiated PC units by enzyme immuno assay (EIA) to determine if WBC reduction or inactivation in PCs by prestorage leukodepletion filters and gamma irradiation could reduce the levels of these cytokines during storage for 3 days.

Single random-donor platelet concentrates (RD-PCs, n = 54) were prepared from whole blood into CPDA using the platelet-rich plasma (PRP) method. In this method, collected whole blood (triple bag-system, Boin Medical Co. Ltd.) was first spun at low speed for 3 – 4 minutes within 8 hours of collection. The resulting PRP was then spun at a higher speed for 5 minutes to pellet platelets. All but about 60 mL of PRP was removed and the pellet was left undistributed for 1 hour.<sup>4</sup> RD-PCs were divided into four groups:

Group 1: Unfiltered, nonirradiated RD-PCs (n = 13) were stored in a polyvinyl chloride bag flatbed agitator at 22°C up to 3 days in a temperature-controlled incubator.

Group 2: Sixteen unfiltered RD-PCs were

gamma irradiated at the dose 2500 – 3000 Gy.

Group 3: Fourteen units were filtered immediately after preparation to reduce leukocytes using purecell PL (Pall Corp, Italy) leukodepletion filters.

Group 4: Eleven filtered PCs were also gamma irradiated.

Five mL samples were withdrawn aseptically through the bag on days 0 and 3 and the platelets were separated from the supernatants by centrifugation for 15 – 20 minutes ( $\times 1000$  g). The supernatants were aliquoted and stored at  $-70^{\circ}\text{C}$ .

Platelet and WBC numbers in PCs on days 0 and 3 in unfiltered units were analyzed by means of an automatic analyzer and microscopically by improved Neobar for filtered units. WBC counts were performed by microscopic counting using a Nageotte chamber (Pall), and 100  $\mu\text{L}$  of cells was added to 900  $\mu\text{L}$  of a cell suspension fluid.<sup>4</sup>

We measured the following cytokines by ELISA: IL-1 beta, IL-6, and TNF-alpha (Biosource International, CA) and IL-8 (Roche) according to the manufacturers' recommendations. The minimum detectable concentration of the kits were 1 pg/mL, 2 pg/mL, 1.7 pg/mL, and 6.2 pg/mL, respectively.

All data were expressed as mean  $\pm$  SE. To compare data on different days of storage in each group, the paired *t*-test was performed. To analyze between the groups, the variance of analysis was calculated. To analyze the correlation of the cytokines with each other, the Pearson correlation coefficient was used.

**Table 1.** Mean of cytokine concentration (pg/mL) and standard error (SE) on day 0 and day 3, in different groups.

Groups	IL-1 Day 0	IL-1 Day 3	IL-8 Day 0	IL-8 Day 3	IL-6 Day 0	IL-6 Day 3	TNF-alpha Day 0	TNF-alpha Day 3
<b>Group1</b>								
Mean $\pm$ SE	*	*	48.5 $\pm$ 29.47	239.4 $\pm$ 129.56	6.4 $\pm$ 2.08	7.2 $\pm$ 2.35	15.44 $\pm$ 5.32	24.06 $\pm$ 11.04
Min-Max n = 13			19.4 – 99.5	95.6 – 514.4	4.3 – 10.7	4.6 – 10.8	12 – 32	9 – 44
<b>Group2</b>								
Mean $\pm$ SE	0.763	0.531	37.3 $\pm$ 20.25	62.3 $\pm$ 23.98	9.33 $\pm$ 11.22	5.63 $\pm$ 2.93	33.1 $\pm$ 11.99	24.39 $\pm$ 10.11
Min-Max n = 16	*	*	14 – 80.6	27.9 – 114.6	0 – 45.5	0 – 8.6	8.4 – 46	10.5 – 38
<b>Group3</b>								
Mean $\pm$ SE	*	*	47.32 $\pm$ 19.56	45.04 $\pm$ 19.53	8.78 $\pm$ 9.79	2.73 $\pm$ 3.04	35.27 $\pm$ 6.82	32.18 $\pm$ 7.58
Min-Max n = 14			21.5 – 68.4	19 – 65.5	0.4 – 5.2	0.1 – 7.7	15 – 42.3	15.3 – 40.2
<b>Group4</b>								
Mean $\pm$ SE	*	0.6	50.1 $\pm$ 18.5	48.47 $\pm$ 18.6	2.4 $\pm$ 0.85	5.6 $\pm$ 2.97	33.92 $\pm$ 6.48	28.7 $\pm$ 9.32
Min-Max n = 11		*	21.5 – 89	23.2 – 85.7	0.3 – 2.8	3.5 – 7.7	18.5 – 39.5	14.7 – 40.8

\* = lower than minimum detectable concentration of the kit ( $< 1$  pg/mL).

## Results

The results are summarized in Table 1. There wasn't any significant differences in IL-6, TNF-alpha, and IL-8 levels on day 0 between the different groups. We couldn't detect IL-1 beta in all samples, as the IL-1 beta level was lower than the minimum detectable concentration of the used kit ( $< 1$  pg/mL).

We found a significant difference in IL-8 and TNF-alpha levels in the first group ( $P = 0.011$  and  $= 0.021$ , respectively). It means that there was a significant increase in IL-8 and TNF-alpha levels from day 0 to day 3 in unfiltered, nonirradiated RD-PCs.

IL-6 level didn't show any significant difference from day 0 to day 3 ( $P = 0.45$ ), which means that IL-6 didn't increase during storage in this group.

We sought to evaluate the effectiveness of gamma radiation (2500 – 3000 Gy) before storage to prevent cytokine generation. Our data showed that IL-8 level on day 3 was significantly increased ( $P = 0.006$ ) in the second group. Data also showed that TNF-alpha level was significantly decreased from day 0 to day 3 in this group ( $P = 0.011$ ) and that IL-6 level didn't show any significant difference from day 0 to day 3 ( $P = 0.177$ ). Our data showed that gamma irradiation could inhibit TNF-alpha generation and IL-6 accumulation, but didn't prevent IL-8 production during storage in unfiltered irradiated units.

After prestorage filtration, WBC numbers decreased in all cases ( $< 250000$  cells/unit) and IL-8 didn't show any significant change from day 0 to day 3 in groups 3 and 4 ( $P = 0.7$  and  $P = 0.67$ , respectively), but TNF-alpha significantly decreased in both groups ( $P = 0.037$  and  $P = 0.04$ , respectively).

IL-6 was only detected in 7 filtered units on day 3 (from 25 filtered units in both groups 3 and 4) and in the remaining filtered PCs, IL-6 level after filtration was lower than the minimum detectable concentration of the kit ( $< 2$  pg/mL).

## Discussion

This study showed that the levels of TNF-alpha and IL-8 were significantly elevated during storage of unfiltered, nonirradiated RD-PCs and prestorage filtration could prevent cytokine accumulation in PCs. Gamma irradiation (2500 – 3000 Gy)

prevented TNF- alpha and IL-6 accumulation in irradiated units but didn't affect IL-8 generation during storage.

These results were similar to the results reported by Aye et al.<sup>5</sup> Chalandon et al<sup>6</sup> measured TNF-alpha and IL-6 in single donor-PCs and they found increased levels of these cytokines during storage. Fujihara et al showed that prestorage inactivation of white cells by ultraviolet B, but not by gamma irradiation, was effective in preventing the generation of cytokine during storage.<sup>1</sup> Lin et al studied the influence of gamma irradiation and storage on apheresis platelets and reported that gamma irradiation of apheresis platelets inhibited the secretion of IL-1 beta and IL-8 after 5 days storage.<sup>7</sup> These different results may be due to different preparation of PCs.

The cytokine generation is suggested due to new synthesis and release of cytokines from residual WBCs.<sup>1</sup> The principal source of IL-1 beta, IL-6, IL-8, and TNF-alpha that is found in PCs is monocytes.<sup>8</sup>

Prestorage leukoreduction has been employed to reduce numbers of WBCs and cytokine generation during storage<sup>1</sup> if WBCs are reduced to a concentration lower than  $5 \times 10^6$  per unit.<sup>9</sup> Our results also confirmed that WBC reduction with prestorage filtration could prevent cytokine accumulation in RD-PCs during storage. Many complications after platelet transfusion are reported such as FNHTRs.<sup>1, 2</sup> It is suggested that prestorage leukodepletion of PCs and red cells diminished the accumulation of leukocyte-derived cytokines during storage; however, transfusion reactions were not eliminated.<sup>10</sup> We didn't investigate FNHTRs during PC transfusion as it was not our aim, but we suggest that further studies should investigate the relationship between these cytokines and FNHTRs.

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