Platelet storage time and cytokine (IL-2R, IL-8, TNF-α) levels

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العلاقة بين مدة تخزين الصفيحات وبين مستويات السيتوكين (الإنترلوكين –2R، و –8، وعامل نخر الورم – ألفا)

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الخلاصة: أُجريت هذه الدراسة لتقصِّي ما إذا كان تراكم السيتوكين في بلازما رُكازات الصفيحات المأخوذة من المتبرعين بطريقة الفصادة المُفْرَدَة، يتعدَّى الحدود المقبولة للتلوث بكُريات الدم البيضاء في أوقات مختلفة من التخزين. وقد تم الحصول على عيِّنات من 11 متبرعاً باستخدام الفصادة، وكانت كلها ملوَّثة بكثافة بالكريات البيضاء. وتم أخذ العينات من الصفيحات المخزَّنة بعد 0 و1 و3 و5 أيام، وأُجري عليها تعداد الصفيحات والكريَّات، وقياس السيتوكينات (الإنترلوكين –2R، والإنترلوكين –8، وعامل نخر الورم – ألفا). وقد أسفرت الدراسة عن وجود تراكم ملحوظ للسيتوكين في رُكازات الصفيحات الملوثة بالكريات البيضاء، خلال مدة خمسة أيام من التخزين، مما يوحي بأن مدة تخزين الصفيحات لها تأثير على مستويات الإنترلوكين –R.

ABSTRACT This study investigated whether cytokine accumulation in plasma of platelet concentrates obtained from single apheresis donors was beyond the acceptable limits of white blood cell contamination at different storage time-points. Samples were obtained from 11 voluntary apheresis donors. All products were heavily contaminated with leukocytes. Sampling times from stored platelets were 0, 1, 3, 5 days respectively. Platelet, white blood cell counts and cytokines (IL-2R, IL-8 and TNF-α) were measured. The study found significant cytokine accumulation during 5 days of storage time in leukocyte contaminated platelet concentrates, suggesting that the platelet storage time has an effect on IL-2R levels.

Temps de conservation des plaquettes sanguines et concentrations de cytokines (IL-2R, IL-8, TNF- α)

RÉSUMÉ Cette étude avait pour objectif de déterminer si l'accumulation de cytokines dans le plasma de concentrés plaquettaires d'aphérèse (CPA) provenant d'un donneur unique dépassait les limites acceptables de contamination par les leucocytes résiduels à différents points-temps de leur durée de conservation. Des échantillons ont été prélevés auprès de 11 donneurs par aphérèse volontaires. Tous les produits se sont avérés lourdement contaminés par les leucocytes. Les périodes d'échantillonnage sur les concentrés plaquettaires conservés ont été les suivantes : 0, 1, 3 et 5 jours. Les numérations plaquettaire et leucocytaire ainsi que la concentration en cytokines (IL-2R, IL-8 et TNF-α) ont été mesurées. L'étude a mis en évidence une accumulation significative de cytokines pendant la période de conservation de 5 jours, évoquant ainsi l'influence de la durée de conservation du concentré plaquettaire sur les taux d'IL-2R.

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Introduction

It has been suggested that cytokine accumulation in plasma of platelet concentrates (PC) during storage may contribute to development of non-haemolytic febrile reactions (NHFR) to platelet transfusion [*1*]. Interleukin-8 (IL-8) and tumour necrosis factor alfa (TNF-α) are some of the cytokines responsible for NHFR [*2*]. The degree of IL-8, TNF-α and interleukin-2 receptor (IL-2R) accumulation is related to the leukocyte content of the platelet component and can be reduced by prestorage leukocyte depletion [*3*]. NHFR are frequent side-effects that occur in 4% to 30% of platelet transfusions.

There are 2 different mechanisms that are considered to be possible causes of NHFR: white cell alloantibodies of the recipient that react with donor white blood cells (WBC) and proinflammatory cytokines in the supernatant of platelet concentrates [*4,5*]. According to earlier studies, during storage of platelet components, leukocytes and other cell types can secrete cytokines such as IL-1, IL-6, IL-8, TNF-α and regulated upon activation, normal T-cells, expressed and secreted (RANTES), which may be a reason for adverse reactions in some recipients [*6,7*]. It remains unclear whether WBC in PC are capable of producing these cytokines *de novo* or the cytokines are released from degranulation of WBC or even donor WBC may still continue to produce these cytokines *in vivo*. However, it is almost certain that there is a positive correlation between the pretransfusion storage time of PC and the onset of NHFR. Reaction rates are very high in stored PC from 3 days up to 5 days as compared with the 1-day to 2-day-old platelets [*6*].

TNF has direct pyrogenic activity and can mediate inflammatory reactions, whereas IL-8 works as a neutrophil-chemotactic and neutrophil-activating factor. The concentrations of T-cell derived IL-2R are increased in mixed lymphocyte cultures. Although leukoreduction prevents transmission of cytomegalovirus and decreases alloimmunization to human leukocyte antigen (HLA), it does not prevent allergic reactions. Whether all PCs should be leukoreduced, including PCs prepared from apheresis of single donors or from pooled samples, is not still universally accepted.

We undertook this study to investigate whether the cytokine accumulation in PCs obtained from single apheresis donors was beyond the acceptable limits of WBC contamination at different storage time-points, to quantify the effects of storage on platelets.

Methods

All PCs were obtained from healthy volunteer donors by apheresis (CS-3000 Plus, Baxter Fenwall, Deerfield, Illinois, USA) and kept unfiltered on a flatbed platelet agitator (PS-900 Helmer) at 22 °C and samples were taken aseptically on days 0, 1, 3 and 5. Measurements of pH and lactate were obtained from supernatant of PC, using a pH analyser (9110, AVL® Medical Instruments, Graz, Austria) and Vitros 750 XRC (Johnson & Johnson, Rochester, New York, USA) respectively. Platelet and WBC counts were measured using an automated cell counter (XT-2000i, Sysmex, Kobe, Japan), which was calibrated using the reference standards provided by the manufacturer. The sensitivity of this method is 1 WBC per μL. Cytokine assays (IL-8, IL-2R and TNF-α) were estimated by chemiluminescence in Immulite® One (DPC, USA). The absolute range of these assays were 62 pg/L, 391 U/L and 8.1 pg/L respectively and the limits of detection were 2.0 pg/L, 5 U/L and 1.7 pg/L.

Statistical analysis

For data that were essentially normally distributed, comparison between groups was made using a paired 2-tailed *t*-test, and association between variables was assessed using Pearson's correlation. For data that were non-Gaussian with a positive skew, comparison was made using the Wilcoxon rank test. A *P*-value of < 0.05 was considered significant.

Results

In the current study, for the first time, the cytokine of IL-2R levels as a marker of WBC activation were investigated in PCs together with beyond the acceptable limits of WBC contamination and at different storage time-points. Regarding the leukocyte subsets, our study design resembles well the situation in standard random-donor PC. The concentrations of cytokines and other analytes in PCs are summarized in Table 1.

At the beginning (day 0), low concentrations of IL-2R, IL-8 and TNF-α were detected in the supernatant of PCs. These PCs also showed a significant increase in the concentration of cytokines at some points of the storage period. In contrast, only IL-8 showed persistent, statistically significant higher levels in the supernatant of PCs during storage time ($P < 0.01$). The peak median value (740 pg/L) of IL-8 was reached after 5 days of storage. TNF-α remained within low levels during the storage time, although there was a significant increase of TNF-α between day 0 and day 3 ($P < 0.01$). The peak median value of TNF-α was 14.8 pg/L after 5 days of storage. The concentrations of IL-2R remained within low levels in all units. There was a significant increase in IL-2R during storage ($P < 0.05$). However, these increases showed peak mean values for IL-2R on day 1 and for TNF-α on day 3 of storage, with IL-2R concentrations up to 525 U/L and TNF-α concentrations up to 16.9 pg/L. For IL-8 a wide range of cytokine levels were observed, with concentrations from 5 to 7500 pg/L.

We detected a gradual increase of IL-8 concentrations during storage, giving a 835% increase on day 5 (Table 1). As expected, lactate increased and pH decreased persistently during storage ($P < 0.01$). All leukocyte levels in these units were beyond the acceptable limits of 5×10^6 per PC unit (Table 1).

Table 1 Relationship between concentrations of cytokines, pH, lactate and storage time

Parameter	Day 0	Day 1	Day 3	Day 5
pH [Mean (SD)]	7.12 (0.16)	7.22 (0.09)	7.13 (0.20)	7.06 (0.31)
Lactate [Mean (SD) mmol/L]	1.6 (0.41)	3.6 (0.97)	7.8 (4.2)	11.6 (6.0)
TNF [Mean (min–max) pg/L]	5.9 (4–13)	12.2 (4–54)	16.9 (4–23)	14.8 (4–100)
IL-8 [Mean (min–max) pg/L]	8.9 (5–23)	86.3 (5–810)	441 (5–4370)	740 (5–7500)
IL-2R [Mean (min–max) U/L]	394 (162–1113)	525 (171–1614)	406 (168–1452)	422 (182–1319)
WBC [Mean (SD)/unit $\times 10^6$]	142 (101)			

TNF = tumour necrosis factor; IL-8 = interleukin-8; IL-2R = interleukin-2 receptor.
WBC = white blood cells.

Discussion

The aim of this study was mainly to investigate cytokine accumulation in PCs during storage. Numerous studies have reported a positive correlation between storage time and leves of certain cytokines [*7–10*]. IL-1, IL-6, IL-8 and TNF-α were among the cytokines investigated in this context. In the present study increased levels of IL-2R were detected in stored PCs and additionally IL-8 and TNF-α were also significantly increased as expected [*11–13*]. Release of IL-2R in PCs during storage was not increased consistently. Increased levels of IL-2R have been detected in stored bags and we believe that the methods used for preparation of PC may affect the release and accumulation of this cytokine from WBC. Most studies have compared different apheresis methods or different filters using *in vitro* tests, e.g. measurement of pH, IL-6, IL-8, TGF-1, TNF-α and platelet-activation markers as indicators of platelet quality in PCs [*14–18*]. However, no reports have examined the levels of this cytokine (IL-2R) in PCs. We are unaware of any report in the literature linking IL-R levels in platelet apheresis to storage time.

Interleukin-2 was an appropriated cytokine for amplifying T-cell culture and the first T-cell growth factor that was cloned molecularly [*10*]. *In vitro*, IL-2 can greatly increase production of T-cells. So it was assumed that IL-2 induces T-cell production *in vitro* and therapeutic strategies have been developed for modulating IL-2 signal strength in clinical use [*4,10*]. However, to increase the number and function of T-cells, solutions of IL-2 were given to patients with cancer or AIDS [*10*]. In contrast, inhibited IL-2 signalling suppresses rejection in transplantation patients. This evidence guided many studies in the past decade and showed that lack of IL-2 or IL-2R genes developed T-cell-mediated autoimmune disease [*10*].

Synthesis of IL-2 is regulated at the mRNA level by the T-cell receptor and co-stimulating modules such as CD28 [*10*]. The receptor complex of IL-2 consists of 3 subunits designated IL-2R (CD25), IL-2R (CD122), and common-chain (γ-CD132). IL-2 needs subunits for high-affinity binding. IL-2R and CD122 can form an intermediate receptor that can signal in the absence of IL-2R expression. Nevertheless, the high-affinity receptor seems to be a physiologically pair of the IL-2R, as CD25-deficient mice are phenotypically the same as IL-2-deficient mice [*10*].

Although several studies in PCs showed a distinct connection between WBC contamination and cytokine contents, the degree of platelet activation may also play an important role [*10,11*]. As we did not study platelet activation markers, we could not comment on this issue; however, collection technique by CS-3000 plus apheresis machine is based on dry platelet production that we used in this study, may activate platelets more than we assume. However, the clinical significance of platelet activation is unclear.

Increased expression of CD62P on the surface of activated platelets may be linked to rapid clearance of platelets from the circulation. According to studies there is a weak negative correlation between CD62P expression and the percentage recovery and lifespan of platelets post-transfusion [*12*]. Thus, comparison of platelets which were mixed with sufficient thrombin to release granule contents and unstimulated platelets shows they have no significantly different survival time in the circulation [*13*].

Reports show that platelets and plasma proteins may interact with the artificial materials in filters, apheresis machines and storage bags. This can activate coagulation,

complement systems, proteolytic fragmentation and cellular injury. The amount of platelet activation enhances with length of PCs storage time [*6,12–19*].

In our study, WBC contamination was found well beyond the acceptable limit of 5 million per unit. IL-8 levels were mainly related to the quantity of WBC in PCs [*12*]. We therefore measured levels of IL-8 in PCs on day 0, 1, 3, 5 of storage to investigate the relationship with WBC contamination and found strong evidence that the source of IL-8 was due to contaminated leukocytes (Pearson correlation, data not shown). Conversely, accumulation of IL-8 during storage can serve as an indicator of the performance of the WBC-reduction process and therefore the quality of the platelet product [*17*]. However, it is still unknown what is the level of WBC contamination critical for the accumulation of inflammatory cytokines in PCs [*12,20*]. A reduction in WBC number may reduce cytokine production and so transfusion reactions. Numerous studies have shown decreased levels of WBC in PCs prevents cytokines being released from WBC during storage. Nevertheless, transfusion reactions may continue despite minimal WBC in PCs as related to platelet products [*6*].

Adverse transfusion reactions include immunologic reactions and passively transferred biologic response modifiers. In fact, febrile non-haemolytic reactions after platelet transfusion are not immune-mediated but probably caused by cytokines. In order to prevent adverse immunologic reactions, transmission of infectious agents and immunosuppression, blood banks are introducing universal leukoreduction by prestorage filtration of blood components [*20–22*]. A correlation has been demonstrated between transfusion reactions and the leukocyte content of blood components, generation of pro-inflammatory cytokines and storage time.

Our results showed that in leukocyte contaminated single donor apheresis platelet concentrates, cytokine accumulation, especially IL-8 and TNF-α, significantly occurs during 5 days of storage time.

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