



## Independent role of interleukin-6 and interleukin-8 in the etiology of transfusion reactions to platelet concentrates in children

Nezavisna uloga interleukina-6 i interleukina-8 u etiologiji transfuzijskih reakcija nakon primene koncentrovanih trombocita kod dece

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### Abstract

**Background/Aim.** Transfusion reaction is an adverse event which manifests during or after administration of blood components to the patient. We aimed to show less known aspects of most common transfusion reactions (allergic and febrile non-hemolytic transfusion reactions – FNHTR) in the pediatric population at the platelet concentrates. The aim of this study was to determine the role of the accumulated cytokines interleukin-6 (IL-6), interleukin-8 (IL-8) and presence of anti-platelet antibodies in the etiology of transfusion reaction in children. **Methods.** The study included 239 pediatric patients, who received platelet concentrates. Data of reported transfusion reaction were collected and evaluated prospectively. The levels of IL-6 and IL-8 were determined using an immunoassay. Anti-human leukocyte antigen antibodies (anti-HLA) and anti-human platelet antigen antibodies (anti-HPA) were identified by Luminex flow cytometry. **Results.** Total of 70 transfusion reactions were recorded 52 patients. Allergic

reactions occurred in most of the cases (74.3%), followed by FNHTR (17.1%). Platelets derived from buffy coat caused the majority of reactions (73.5%). Patients with infection after platelet transfusion with FNHTR had the highest levels of IL-6,  $483.30 \pm 1,041.79$  pg/mL ( $p = 0.020$ ). Respectively, the febrile patients had IL-6,  $302.52 \pm 720.04$  pg/mL ( $p = 0.004$ ). The level of IL-8 in platelet units that caused transfusion reactions was  $95.66 \pm 319.10$  pg/mL, which was significantly higher ( $p = 0.001$ ) compared to the control platelet units. **Conclusion.** The predominant etiologic mechanism for FNHTR in our study was leukocyte derived cytokine accumulation during storage. Etiopathogenesis of FNHTR induced by IL-6 and IL-8 presented differently. We concluded that significant factors in the etiology of FNHTR by IL-6 were the factors related to the pediatric patient (infection, inflammation).

**Key words:** platelet transfusion; transfusion reaction; interleukin-6; interleukin-8; child.

### Apstrakt

**Uvod/Cilj.** Transfuzijske reakcije su neželjeni događaji koji se manifestuju tokom ili nakon primene krvnih komponenti. Prikazani su manje poznati aspekti najčešćih transfuzijskih reakcija (alergijske i febrilne nehemolizne transfuzijske reakcije – FNHTR) posle primene koncentrovanih trombocita u pedijatrijskoj populaciji. Cilj rada bio je da se ispita uloga akumuliranih citokina, interleukina-6 (IL-6) i interleukina-8 (IL-8) i prisustvo humanih anti-leukocitnih antitela (anti-HLA) i humanih anti-trombocitnih antitela (anti-HPA) kao etioloških faktora transfuzijskih reakcija kod dece. **Metode.** Studijom je bilo obuhvaćeno 239 pedijatrijskih bolesnika koji su dobili transfuziju

koncentrovanih trombocita. Podaci o prijavljenim transfuzijskim reakcijama prikupljeni su i obrađivani prospektivno. Nivoi IL-6 i IL-8 određivani su metodom indirektno hemiluminiscencije. Anti-HLA i anti-HPA identifikovana su Luminex protočnom citometrijom. **Rezultati.** Kod ukupno 52 bolesnika zabeleženo je 70 transfuzijskih reakcija. Najčešće su bile alergijske reakcije (74,3%), FNHTR (17,1%). Najveći broj reakcija izazvali su koncentrovani trombociti dobijeni iz "buffy coat" (73,5%). Bolesnici sa infekcijom su posle transfuzije trombocita na koje su ispoljili FNHTR imali najviši nivo IL-6 [ $483.30 \pm 1 041.79$  pg/mL ( $p = 0.020$ )], slede bolesnici sa febrilnošću [IL-6,  $302.52 \pm 720.04$  pg/mL ( $p = 0.004$ )]. Nivo IL-8 u jedinicama trombocita koje su izazvale transfuzijsku reakciju

bio je  $95.66 \pm 319.10$  pg/mL, što je bilo značajno više ( $p = 0.001$ ) u poređenju sa kontrolom. **Zaključak.** Dominantan etiološki mehanizam za FNHTR u ovoj studiji su citokini produkovani leukocitima akumulirani tokom skladištenja. Etiopatogeneza FNHTR izazvane IL-6 i IL 8 je različita. Zato smo zaključili da značajan faktor u nastanku FNHTR

izazvane IL-6 u dečijoj populaciji predstavljaju faktori vezani za status bolesnika (prisustvo infekcije, inflamacije).

**Ključne reči:**  
**transfuzija trombocita; transfuzija, reakcija; interleukin-6; interleukin-8; deca.**

## Introduction

Platelet transfusions in the pediatric population are commonly used in hematology, oncology and hematopoietic stem transplantation. Acute reactions are defined as adverse events occurring during or within 4 to 6 hours of transfusion. The most common acute adverse events that occur to the transfusion of platelets in the pediatric population are allergic and febrile nonhemolytic transfusion reactions (FNHTR). If non-leukocyte-reduced platelets are used, FNHTR occurred in up to 37% of transfusions<sup>1</sup>, whilst allergic reaction comprises 13–33% of all transfusion reactions<sup>2</sup>. The residual leukocyte content and storage time as well as patient factors are the determining factors for the occurrence of this type of reaction<sup>2</sup>.

The pathogenesis of the FNHTR is multifactorial. The primary mechanism responsible for the FNHTR to platelets is leukocyte and platelet-derived biological response modifiers mechanism. Cytokine accumulation [regulated on activation, normal T cell expressed and secreted (RANTES), transforming growth factor beta (TGF- $\beta$ ), interferon gamma (IFN- $\gamma$ ), interleukin-1 (IL-1), interleukin-6 (IL-6) and interleukin-8 (IL-8)] in platelet concentrates (PC) during storage contribute not only to the FNHTR, but also to allergic reactions associated with transfusions<sup>3,4</sup>. Firstly, high levels of cytokines when infused to patient, cause fever by stimulation of the hypothalamus. Secondly, antigen-antibody hypothesis is believed that leukocyte antibody from patient's plasma reacts with leucocytes present in the PC. An antigen-antibody reaction occurs, resulting in the release of cytokines by the donor leucocytes. This mechanism accounts for less than 10% of the FNHTR to platelets<sup>1</sup>.

This study aimed to present the frequency and incidence of transfusion reaction to platelets, to determine what type of PC and which the number of units of PC resulted in transfusion reactions and in which particular patient age.

The specific aims were: first, to determine the level of cytokines IL-6 and IL-8 in patients with transfusion reaction and in a unit of the platelets which caused transfusion reaction; second, to determine the presence of anti-human leukocyte antigen antibodies (anti-HLA) and anti-human platelet antigen antibodies (anti-HPA) in patients with transfusion reactions and afterwards, to investigate whether the cause of transfusion reaction is accumulation of cytokines or anti-HLA/anti-HPA; thirdly, to investigate whether there is an association of inflammatory states and fever in patients with higher levels of IL-6 and IL-8.

## Methods

The study included patients (between one month and 18 years old) who received platelet transfusions between 2011 and 2014 at the Institute of Mother and Child Health Care of Serbia "Dr Vukan Čupić", a tertiary medical centre. Patients were treated at the Department of Hematooncology, the Department of Bone Marrow Transplantation as well as the Pediatric Intensive Care Unit.

Patients were stratified according to: age (infants - from 1 month to 1 year, children - 1 to 12 years and adolescents - 13 to 18 years), gender, diagnosis, and treatment of the underlying disease. The results were compared to levels of IL-6 and IL-8 in units of platelets which did not cause platelet transfusion reactions.

The patients received PC derived from the buffy coat (BC), single donor apheresis platelets and pooled platelets. The transplanted patients received the same platelet products but irradiated with 25 Gy per dose of platelets. The patients with aplastic anemia and the recurrent FNHTR received filtered platelets. The control group consisted of 156 patients who received a transfusion of platelets and did not have transfusion reactions.

Aliquots from transfused platelets were frozen and stored at  $-70$  °C until the analysis. These samples were then thawed at room temperature and centrifuged at 3,000 rpm for 5 min. The supernatant plasma was used for the analysis.

The following laboratory tests were done: blood count (Beckman Coulter, USA), immune-serology tests for blood groups, direct and indirect antiglobulin test with gel ID-Card method (Bio-Rad, DiaMed GmbH, Switzerland), C-reactive protein (CRP) (Roche, Switzerland). IL-6 was quantitatively measured using the automatic analyzer Access Immunoassay Systems (Beckman Coulter, USA) and the IL-6 Immunoassay. IL-8 was determined by immunoassay on an automated immunochemical analyzer IMMULITE (Siemens, Germany).

The testing of anti-HLA was performed by lymphocytotoxic test dependent on complement (LCT) and Luminex flow cytometry. Anti-HPA were analyzed by Luminex flow cytometry. All analyzes were performed according to commercial instructions. The used reference values represented the standard normal age-specific values for the pediatric setting.

Statistical analysis was performed using the IBM SPSS software. Numerical data were presented as mean  $\pm$  standard deviation (SD) or median, while categorical variables were presented as frequencies or percentages. Depending on the data types and the number of groups, differences between

independent samples were assessed using  $\chi^2$  test, Fisher test, Student's *t*-test, Kruskal-Wallis, Mann-Whitney *U* test and ANOVA, while differences between the related groups were examined by McNemar, Wilcoxon and Student's *t*-test for two related samples. Assessing the correlations, Pearson's and Spearman's tests were used. *p* values < 0.05 were considered to be significant.

## Results

A total of 239 patients treated with platelet transfusion were included in this study. Complications during or after platelet transfusion were recorded in 52 (21.7%) patients. The characteristics of the study population are shown in Table 1.

A total of 70 transfusion reactions were recorded. Allergic reactions occurred in majority of cases, with 52 of 70 (74.3%) manifestations, followed by 12 (17.2%) FNHTR manifestations.

The first manifestation of the transfusion reaction included 39 allergy-type reactions; one of them was anaphylactic reaction, 8 FNHTR, transfusion-associated dyspnea (TAD), hypotensive reaction, 4 reactions with combined reaction type: 2 allergic reaction with the FNHTR and 2 allergic reactions with gastrointestinal symptoms with abdominal colic, with nausea and emesis as seen in Figure 1.

Repeated reactions had 11 (21.1%) of patients. The second manifestation included 11 reactions: 7 allergic, 3 FNHTR and one TAD (Table 2). The third manifestation included 4 allergic and one FNHTR reaction. One patient had the fourth and the fifth reaction to platelet transfusion, both allergic types. Secondary pulmonary transfusion reactions which occur in the wake of another transfusion reaction – secondary TAD, were present in 10 patients (19.2%).

According to the probability of adverse events associated with platelet transfusion, the association was the most probable in 76.6%, certain in 19.1%, and only possible in 4.3% of the patients.

According to the severity of clinical presentation, transfusion reactions were the most commonly mild in 51.1%, moderate in 36.2% and severe in only 12.8% of the patients. The most severe clinical picture of transfusion reaction had the patients with repeated transfusion reaction (*p* = 0.001). Greater number of the patients had the reactions while receiving the platelet transfusion 31 (59.6%).

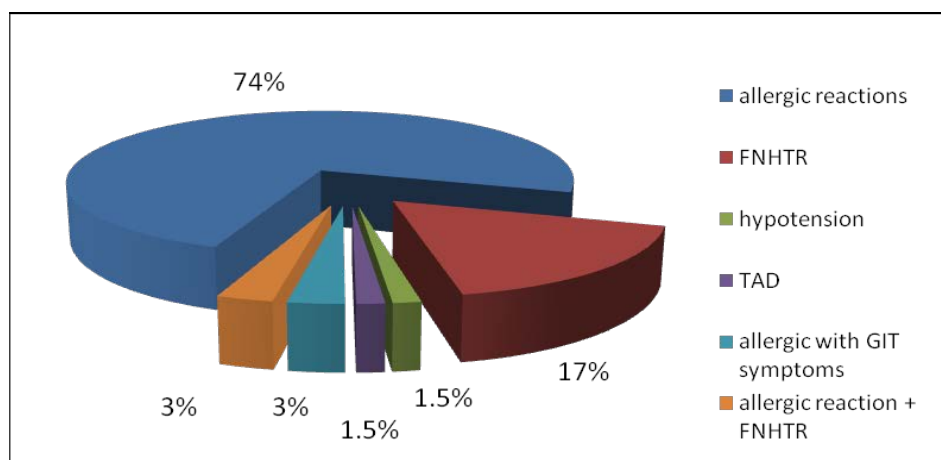
Occurrence of unwanted reactions increased in the older age group of the patients. In the group aged 1 month – 1 year, reactions occurred in 25% patients; in the group aged 1–12 years, 56% of the patients had transfusion reaction, and in the group aged 13 and more years the transfusion reaction had 69% patients.

**Table 1**

**Characteristics of the study population**

Variable	Patients (n = 52)
Age (years), <i>r</i> ± <i>SD</i>	10.2 ± 5.6
Gender, n (%)	
boys	35 (67.3)
female	17 (32.7)
The group of aged, n (%)	
1 month-1 year	1 (1.9)
1–12 years	30 (18.8)
13 and more years	21 (40.4)

*r* – mean value; *SD* – standard deviation.



**Fig. 1 – The frequency of certain types of transfusion reactions.**

FNHTR – febrile non-hemolytic transfusion reaction; TAD – transfusion associated dyspnea; GIT – gastrointestinal.

Table 2

Types of transfusion reaction regarding the diagnoses of the examined patients								
Diagnosis	Total number	Types of transfusion reaction (n)						
		allergic	FNHTR	other	TAD sec.	Recurrent reactions		
						allergic	FNHTR	TAD
ALL	26	13	3	1	3	5	3	1
ALL rec	2	1	1					
AML	11	4	1	1	2	5		
Neuroblastoma	7	4		1	1	2		
NHL	4	3		1				
MH	1	anaphylaxis			1			
AA	4	4			1			
MDS	1	1			1			
SA Ewing	3	2		1				
JMML	1		1					
CGD	1	1						
AM sec ALL	1	1						
APL	1	1						
FA	2		1				1	
Rabdomio SA	2	1				1		
TU testis	1	1			1			
TU Wilms	1	1						
CA hepatocelul.	1		1					

FNHTR – febrile non-hemolytic transfusion reaction; TAD – transfusion associated dyspnea; ALL – acute lymphoblastic leukemia; AML – acute myeloid leukemia; AA – aplastic anaemia; MDS – myelodyslasia; JMML – juvenile myelomonocytic leukemia; CGD – chronic granulomatous disease; MH – Morbus Hodgkin; NHL – non-Hodgkin lymphoma; APL – acute promyelocytic leukemia; FA – Fanconi anemia; SA – sarcoma; CA – carcinoma hepatocelulare; TU – tumor.

Among the patients who received the medications affecting the platelets (cephalosporin antibiotics, antifungal medications), 58.8% did not expose any reaction to platelet transfusion, 23.5% had allergic reaction, but without the FNHTR. The rest of patients (41.2%) who did not receive therapy affecting platelets number or function had both, allergic reactions (55.6%) and FNHTR (14.8%) ( $p = 0.015$ ).

PC-BC caused the majority of transfusion reactions (73.5%). Of those, 59.2% were non-irradiated, and 14.3% irradiated. Non-irradiated apheresis platelets counted for 12.2%, and irradiated apheresis platelets counted for 10.2% reactions. Non-irradiated platelet units (71.8%) more frequently provoked allergic transfusion reactions than irradiated ones ( $p = 0.027$ ). The number of received units of PC in patients who had manifested the FNHTR was  $25.63 \pm 25.43$  (3–84),  $p = 0.853$ , and in patients with manifested allergic reactions it was  $14.53 \pm 17.58$  (1–73),  $p = 0.384$ . The patients without a transfusion reactions received  $26.69 \pm 28.38$  (1–118) PC units. The most frequent adverse reactions occurred with platelets units after 3 days of storage (33.3%). The oldest (5 days) platelet units caused only 7.8% of adverse reactions.

#### Incidence of transfusion-associated adverse reactions

Cumulative incidence of all unwanted reactions, allergic reactions and the FNHTR related to number of units and quantity of PC in the 5-year period is shown in Table 3.

#### IL-6 and IL-8

Values of IL-6 and IL-8 were measured in the patients' samples before and after the transfusion reaction, as shown in Table 4. IL-8 did not differ significantly in patients with and without infection, before and after transfusion. The control group of healthy children had IL-6  $1.91 \pm 0.93$  pg/mL (1.4–4.68 pg/mL), and IL-8 of all samples were  $< 5$  pg/mL (below the measurement threshold analyzer).

There was a significant correlation between CRP and IL-6 values after platelet transfusion. Spearman's rho correlation coefficient was 0.453. Test of significance of the Spearman's rho correlation coefficient with selected two-tailed probabilities was  $p = 0.023$ , as seen in the Figure 2.

The number of neutrophils correlated with the IL-6 values after transfusion ( $p = 0.019$ ), and the correlation itself was negative (Spearman's rho correlation coefficient – 0.484) as shown in Figure 3.

The mean IL-6 values in platelets units that caused the reactions were  $6.06 \pm 13.94$  pg/mL (1.54–66.36 pg/mL), which was more than in control units  $2.65 \pm 1.62$  pg/mL, (1.59–6.11 pg/mL), but it was not statistically significant ( $p = 0.197$ ).

The IL-8 in platelets units that caused the reactions was  $95.66 \pm 319.10$  pg/mL (4 to 2121.0 pg/mL), which was statistically significant ( $p = 0.001$ ) comparing with the control units,  $4.9 \pm 1.34$  pg/mL (4.0–5.9 pg/mL).

Table 3

Cumulative incidence of adverse transfusion reactions in a five-year period			
Parameter	Transfusion reaction total	Allergic reaction	FNHTR
Per 1,000 units transfused platelets	2.1	1.7	0.4
Per liter transfused platelet product	0.03	0.02	0.005

FNHTR – febrile non-hemolytic transfusion reaction.

Table 4

Value of interleukin-6 (IL-6) and interleukin-8 (IL-8) in patients before and after febrile non-hemolytic transfusion reaction (FNHTR)

Patients	IL-6 (pg/mL) r ± SD	p	IL-8 (pg/mL) r ± SD	p
Before transfusion reaction	32.53 ± 43.47		55.67 ± 68.44	
After transfusion reaction (FNHTR)	94.17 ± 164.73	0.252	123.0–183.17	0.244
Without infection before platelet transfusion	26.45 ± 39.56		28.82 ± 24.20	
With infection before platelet transfusion	29.05 ± 43.24	0.732	83.97 ± 93.83	0.623
Without infection after platelet transfusion with FNHTR	63.33 ± 144.57		243.06 ± 380.47	
With infection after platelet transfusion with FNHTR	483.30 ± 1041.79	0.020	1223.61 ± 2775.03	0.491
Non-febrile, before platelet transfusion	3.27 ± 3.54		14.95 ± 5.73	
Febrile, before platelet transfusion	51.20 ± 42.87	0.032	66.71 ± 66.86	0.073
Non-febrile, after platelet transfusion with FNHTR	10.28 ± 13.79		137.67 ± 308.27	
Febrile, after platelet transfusion	302.52 ± 720.04	0.003	762.78 ± 1958.32	0.070

r – mean value; SD – standard deviation.

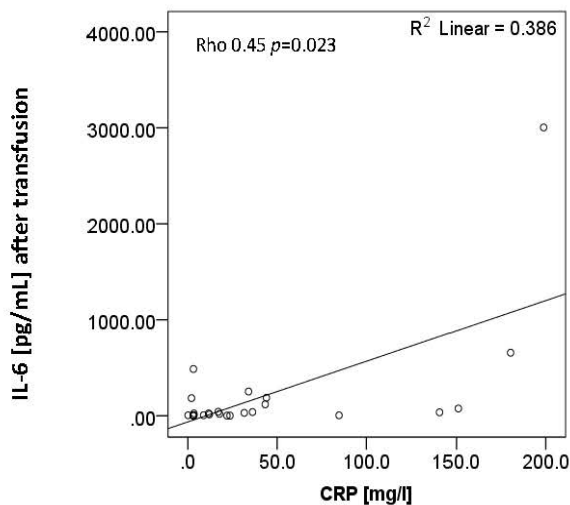


Fig. 2 – The correlation between values of interleukin-6 (IL-6) and C-reactive protein (CRP).

#### Anti-HLA, anti-HPA and transfusion reactions

Anti-HLA antibodies were present in 8 patients; 2 of them had anti-HLA and anti-HPA. Most frequent manifestation of presence of anti-HLA antibodies were allergic reactions, 5 of 8 (62.5%). The frequency of the FNHTR in the patient with anti-HLA antibodies was 12.5%. Connection of antibodies, anti-HLA and anti-HPA with occurrence of transfusion reaction was as follows: the patients with anti-HPA antibodies did not expose any reaction. Anti-HLA antibodies had 9.8% (5/51) of the

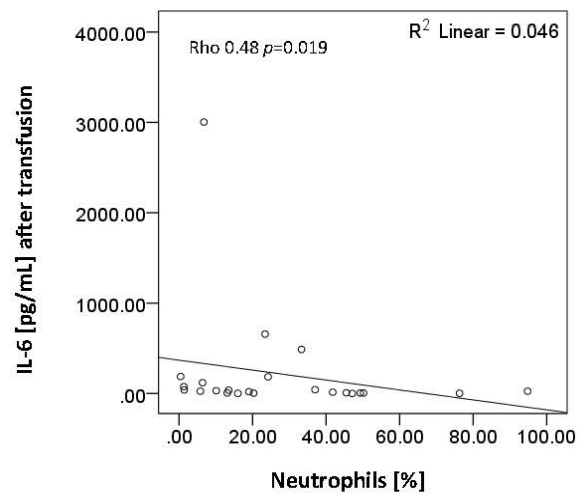


Fig. 3 – Correlation of interleukin-6 (IL-6) level and the number of neutrophils.

patients with allergic reactions, and only one, 8.3% (1/12) ( $p = 0.007$ ) of the patients with the FNHTR, ( $p = 0.004$ ). The patients with antibodies, anti-HLA and anti-HPA, did not have the FNHTR, and 14.3% of the patients had allergic reaction ( $p = 0.004$ ).

#### Discussion

Children represent a vulnerable population with more frequent transfusion-related reactions comparing to adults. Specifically, pediatric patients have a significantly higher

incidence of transfusion reactions associated with platelet transfusion (6.2 per 1,000 transfusions compared to 2.4 in adults,  $p < 0.001$ )<sup>5</sup>. According to Oakley et al.<sup>5</sup>, the incidence of allergic reactions in pediatric transfusion is 2.7/1,000 in relation to the adults of 1.1/1,000 ( $p < 0.001$ ), for the FNHTR is 1.9/1,000 in children, and 0.47/1,000 ( $p < 0.001$ ) in adults. Oakley et al.<sup>5</sup> also determined that male gender was predominant in the pediatric population with transfusion reactions (7.9/1,000 in boys and 4.3/1,000 in girls). The incidence of transfusion reactions in adults was not different based on gender.

Our 5-year cumulative incidence of allergic (1.7/1,000) and febrile reactions (0.4/1,000) is closer to the data for the adult population, which explains the high number of adverse reactions in adolescent patients aged 13 and over (40.1%). Transfusion reactions also occurred more frequently in boys (67.8%) in our study.

Clinical manifestations of acute transfusion reactions in pediatric patients differ significantly from study to study, depending on the department where the data were collected. The frequency of acute allergic reaction ranged from 6.7% to 57.8% and for the FNHTR from 12.5% to 60%<sup>6-8</sup>. Study from Pakistan<sup>8</sup> and our study present data from the Department of Haematology that has a higher incidence of the FNHTR and allergic reactions, while in intensive care wards were higher incidence of hypotension and transfusion reactions with dyspnea. Increased incidence of allergic reactions and the FNHTR in our study compared to previously published studies<sup>6, 7</sup> was most likely due to application of the non-leucoreduced platelet products.

PLADO study came to the conclusion that the source of platelets (e.g. apheresis single donor or PC-BC), length of storage and ABO status were not significantly associated with the occurrence of any reaction. The number of transfused platelet units represented the most important characteristic that was associated with transfusion reactions<sup>9</sup>.

Seghatchian et al.<sup>10</sup> in comparative analysis between PC-BC and apheresis platelets found the highest levels of IL-6 and IL-8 in the early stage of storage of platelets. An additional increase of interleukins was not found over the next 5 days. The level of interleukins present in units of platelets was affected by different process of collection, the degree of contamination of leukocytes and changes during storage. The use of filtration in line during the preparation of PC reduces the generation of cytokines at units of platelets during storage, and thus contributes to the reduction of transfusion reactions<sup>10</sup>.

In our study there was no association between the transfusion reactions and age of the units of platelets, as well as no association with the number of units of platelets. The values of the cytokine IL-6 and IL-8 derived from platelets in units of different ages in our study did not differ significantly, which was in accordance with all the above-mentioned literature.

Muyllle et al.<sup>11, 12</sup> showed that reaction could be caused by the administration of plasma containing large amounts of cytokines. The correlation found between the increased IL-6 plasma levels in PC and the transfusion reactions suggested

that the cytokine was responsible for the reactions<sup>11, 12</sup>. The residual reactions that occurred with the plasma-removed product still correlated with IL-6, providing further support for the cytokine theory and suggesting that even low cytokine levels may cause reactions in some patients<sup>13</sup>.

IL-6 serum levels are often elevated before onset of clinical symptoms and before routine laboratory test, such as measurement of high-sensitivity CRP, become positive. Owing to its short life, human serum IL-6 levels change rapidly. According to Schefold et al.<sup>14</sup>, IL-6 may be used as a highly sensitive diagnostic tool for the early identification of sepsis in both newborns and adult. Also, IL-6 may be useful in the longitudinal monitoring of patients with sepsis. The bedside densitometric point-of-care IL-6 test (with a turn-around time of 20 min) may help to initiate early goal-directed therapy. The correlation between level of IL-6 after transfusion and the CRP test results of our patients was statistically significant indicating that both test measured the immunological host response consisted of pro-inflammatory cytokine release. The negative correlation between IL-6 and the neutrophil count showed that patients with a decrease in neutrophils had an increased level of IL-6 after transfusion reactions. A possible explanation is that the destruction of certain quantities of neutrophils during the transfusion reaction leads to their degranulation and the consequent release of cytokines.

IL-8 had a different impact on the occurrence of reaction; its increased concentration in a unit of platelets was sufficient to cause transfusion reactions in our patients. Increased amounts of IL-8 in the plasma of PC may be related to white blood cells (WBC) activation or its lysis<sup>15</sup>. Transfusion-related adverse reactions were attributed to the presence of substantially high levels of IL-8. Gamma irradiation could inhibit IL-6 accumulation, but did not prevent IL-8 production during storage in unfiltered irradiated units of PC<sup>16</sup>.

The predominant etiologic mechanism for the FNHTR in our study was leukocyte derived cytokine accumulation during storage (96.2%). The FNHTR caused by the anti-HLA antibodies was only 3.8%. Also, the FNHTR occurred only if the applied units of PC contained increased concentrations of cytokines and the recipient had the disease or condition with the inflammatory process. According to the literature the additive effects of the cytokine IL-6 produced by the recipient along with donor's IL-6, represented the amount of the cytokine IL-6 sufficient to cause symptoms and signs of the FNHTR. Rate of metabolism of the cytokines and the presence of soluble IL-6 receptor in the recipient also influence on FNHTR incidence<sup>14</sup>.

The presence of anti-HPA antibodies was associated only with allergic reactions in our patients. In literature anti-HPA-1a was shown to induce the release of the chemokine CCL5 RANTES from platelets, a proinflammatory chemokine that was implicated in allergic transfusion reactions<sup>17</sup>. Conceptual model of allergic transfusion reactions (ATRs) similar to the FNHTR was described by Savage et al.<sup>18, 19</sup>. ATRs may result from a combination of recipient atopic predisposition (chronic-genetic or subacute-

acquired) and a necessary plasma mediator in the blood component. The degree of recipient susceptibility at the time of transfusion and magnitude of the plasma mediators may determine the severity of an ATR<sup>18,19</sup>.

Application of premedication has not been proven effective in allergic reactions and in the FNHTR<sup>2</sup>. Significant proportions in the etiology of the FNHTR had patient-related factors. Possible prevention of the recurrent FNHTR is the treatment of local infection and inflammation in the patient's body. Reducing the concentration of cytokines in PC units, the risk for recurrent transfusion reaction also reduces. The cytokines produced during storage in platelet units cannot be removed by leukodepletion filtration. Additive solutions were developed to attenuate platelet activation. Adding magnesium and potassium ions to the commercial platelet additive solutions (PAS) II and PAS III completely prevented platelet activation<sup>20</sup>. A study that evaluated the cost-effectiveness of PAS to prevent allergic transfusion reactions concluded that using PAS may be financially and clinically beneficial when compared to current practice – washing platelets<sup>21</sup>.

Our choice of cytokine reduction in PC units was to use plasma reduced platelets, resuspended in additive solution. We applied the platelets in additive solution to 2 children

with allergic reactions. Evaluation of the effectiveness of this therapy is in progress.

### Conclusion

In the study group of pediatric patients allergic transfusion reactions had the highest incidence and frequency. The greatest number of reactions was provoked by PC-BC units in the group of patients aged 13 to 18 years (69%). There was no difference between the number of received PC in the patients with transfusion reactions and without reactions. The predominant etiologic mechanism for the FNHTR in our study was leukocyte derived cytokine accumulation during storage (96.2%). The FNHTR incidence caused by the anti-HLA antibodies was only 3.8%. The mean IL-6 values in PC units that caused the transfusion reactions was not significantly different than those in the control PC units, but, IL-8 level was significantly higher in the PC units that caused the reactions. In addition, we determined that etiopathogenesis of FNHTR was different depending on whether it is induced by IL-6 or IL-8. The significant roles in the development of FNHTR induced by IL-6 have factors related to the status of a patient (presence of infection, inflammation, sensitivity).

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