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Effect of cardiopulmonary bypass on neutrophil activity in pediatric open-heart surgery

Authors' Contribution:

- A** Study Design
- B** Data Collection
- C** Statistical Analysis
- D** Data Interpretation
- E** Manuscript Preparation
- F** Literature Search
- G** Funds Collection

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Summary

Introduction:

The nature of the participation of neutrophils in the post-cardiopulmonary bypass (CPB) inflammatory response is not very clear. The aim of our study was to investigate alterations in neutrophil phagocytic activity and adhesion molecule expression on these cells in children during and after CPB.

Materials and Methods:

Twenty-one children aged 6–33 months with congenital heart disease, scheduled for primary corrective surgery, were enrolled. The expressions of CD11b adhesion molecules and Fcγ receptor on neutrophils and their phagocytic activity were evaluated. The studied markers were sequentially measured before, at the initiation of, and after CPB.

Results:

During the course of the operation, CD11b molecule expression on neutrophils showed a slight elevation at the start of CPB (876.5±104.8 mean fluorescence intensity, MFI, vs. 768.1±178.2; p=0.0047), followed by a significant decrease to 689.01±166.7 MFI after completion of the procedure. The expression of CD11b molecule on neutrophils measured at the end of CPB inversely correlated with the duration of CPB (r= -0.68, p=0.00059). The expression of CD16 antigen dropped significantly at the start of CPB (1164.6±307.3 MFI vs. 1327.4±345.3 MFI; p=0.0007) and remained decreased until the end of CPB (814.0±198.1 MFI).

Conclusions:

These findings suggest that the characteristics of the neutrophil response to cardiac surgery appear to depend on many factors. We demonstrated a link between the duration of CPB and adhesion molecule expression on neutrophils.

Key words:

cardiopulmonary bypass • systemic inflammatory response syndrome • neutrophil • phagocytic activity

Abbreviations:

APTT – activated partial thromboplastin time, **CPB** – cardiopulmonary bypass, **FITC** – fluorescent isothiocyanate, **MFI** – mean fluorescence intensity, **SIRS** – systemic inflammatory response syndrome

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INTRODUCTION

Cardiac surgery is one of the most severe surgical stresses to which a child can be exposed and is clinically associated with systemic responses that can influence the post-operative course^{1, 13}. The inflammatory response to cardiac surgery is thought to be induced by exposing patients to pro-inflammatory factors. These include exposure of the blood to the foreign surface of the cardiopulmonary bypass (CPB) apparatus, myocardial reperfusion after de-clamping of the aorta, reduction in pulmonary blood flow during aortic cross-clamping, hypothermia, and the surgical stress response³. It is generally accepted that many mediators and immune cells take part in systemic inflammatory response syndrome (SIRS) after cardiac surgery. This process is associated with endothelial cell injury, adhesion molecule dysregulation, neutrophil activation, and initiation of the coagulation cascade^{15, 16}.

The nature of the participation of neutrophils and its time course in the post-CPB inflammatory response, however, is less clear. The extensive contact of the blood with the synthetic foreign substances on the surface of the CPB circuit is mainly responsible for neutrophil activation^{17, 23}. Activated neutrophils migrate through the vascular wall: they roll onto the endothelium, adhere firmly to it, spread over it, and finally migrate into the tissues^{17, 20}. After adhering to endothelial cells, the neutrophils release toxic agents. These products of neutrophil degranulation affect the function and structure of the endothelium^{20, 24}. This endothelial cell damage leads to an increase in the permeability of the vascular-endothelial barrier and a generalization of the inflammatory process^{5, 10}.

The aim of our study was to investigate alterations in neutrophil phagocytic activity and adhesion molecule expression on these cells in children during and after CPB.

MATERIALS AND METHODS

Patients and clinical data

Twenty-one infants and children undergoing corrective surgery with CPB for congenital heart disease were studied at the Department of Cardiology of the Institute of the Polish Mother's Health Center in Łódź. The patients included in this study had no severe residual defects after repair and no known pre-existing causes of vascular injury, such as recent cardiac arrest, sepsis, or history of vasculitis. Neither had they been receiving immunosuppressive or anti-inflammatory drugs before surgical intervention. The

protocol of the study was accepted by the local Ethics Committee.

Midazolam was used for premedication. General anesthesia was performed using sulfenthanil (1 mg/kg), thiopental (3–5 mg/kg), and pavulon (0.1–0.15 mg/kg). In some children with body mass <15 kg, intramuscular ketamine in a dose of 7.5–10 mg/kg was administered. A naso-tracheal intubation was used. CPB was performed with a Jostra HL 20 (Jostra AG, Hirrlingen, Germany) apparatus and Safe Micro or Safe Mini (Polystan AS, Vaerlose, Denmark) oxygenators with laminar flow. Two central and two peripheral veins were cannulated. During CPB, central venous pressure, blood pressure, O₂ saturation, body temperature, hourly diuresis, ionogram, and hematocrit were measured. All patients received 3 mg/kg of non-fractionated heparin during surgery. Anticoagulation was controlled by the measuring activated partial thromboplastin time (APTT) with the aim to keep APTT >300 s. Heparin action was neutralized by the administration of prothamine sulfate in a dose of 2 mg per 1 mg of heparin.

After cardiac surgery was completed, body temperature was allowed to recover and aortic cross-clamping was stopped. CPB was discontinued when the hemodynamic state was stable, and a systemic perfusion pressure between 50 and 70 mm Hg was maintained. Surgical data (type of intervention, CPB and aortic cross-clamping time, minimum temperature on bypass) of each patient are shown in Table 1. Patients were considered to have received inotropic support if they received an infusion of dobutamine, epinephrine, or dopamine >5 µg/kg/min for more than 6 h.

Blood samples (2 ml) were collected in heparin-containing tubes from the arterial line of the patient or

Table 1. Clinical characteristic of the studied patients

Parameter	Studied patients (n=21)
Age at surgery (months)	11.3 (6–33)
Sex (female/male)	11/10
Type of defect	
transposition great arteries	3
tetralogy of fallot	7
aortic stenosis and aortic insufficiency	7
pulmonic atresia/stenosis	4
Cardiac operation duration (min)	148.6 (115–187)
CPB duration (min)	109.7 (85–145)
Aortic cross-clamping duration (min)	68.6 (51–95)
Lowest temperature (°C)	24.7 (19–25)
Post-operative inotropic agents (no. of patients)	6
Infection in the post-operative setting (no. of patients)	4

from the bypass circuit before anesthesia, at the start of CPB, 30 min after the start of bypass, at the cessation of CPB, and 24 h and 48 h after the cessation of CPB.

Preparation of leukocytes for flow cytometry

The blood samples were stained by the whole-blood technique. They were incubated with 20 μ l of the respective monoclonal antibodies for 15 min at room temperature in the dark. The erythrocytes were then lysed using Lysing Solution (BD-Biosciences, Heidelberg, Germany). Thereafter, the leukocytes were centrifuged for 5 min at $500 \times g$ and washed twice in 1 ml PBS and finally resuspended in 500 μ l PBS containing 0.5% paraformaldehyde (Sigma-Aldrich, St. Louis, Missouri, USA). The cells were maintained at 4°C for analysis within 24 h.

Flow cytometry analysis

We used monoclonal antibodies against integrin molecules: CD11b (BD-Biosciences, Heidelberg, Germany), against Fc γ receptor: CD16 (Dako, Glostrup, Denmark), and the appropriate isotype control antibodies (BD-Biosciences, Heidelberg, Germany). Antibodies were labeled with the fluorescent dyes fluorescein isothiocyanate (FITC) or phycoerythrin. A minimum of 20,000 leukocytes per sample were analyzed using a FACScan flow cytometer, (BD-Biosciences, Heidelberg, Germany) calibrated with Calibrate Beads (BD-Biosciences, Heidelberg, Germany). The neutrophil data were extracted and analyzed separately by gating these populations using morphological characteristics displayed on a dot-plot of FSC (a measure of cell size) vs. SSC (a measure of cell granularity). Flow cytometry data were analyzed with the CellQuest computer software package (BD-Biosciences, Heidelberg, Germany).

Phagocytic activity analysis

The phagocytic activity of neutrophils in whole blood was evaluated with the PhagoTest reagent kit (Orphegen Pharma, Heidelberg, Germany) using flow cytometry. The method of quantitative assay for investigating phagocytic activity was described in detail previously⁷. Two assays of each sample ($2 \times 100 \mu$ l) were incubated with FITC-marked opsonized *E. coli*, either at 0°C (control) or at 37°C for 10 min. During the incubation period, FITC-marked *E. coli* were ingested in the 37°C assay. To exclude extracellular bacteria from measurement they were quenched with a staining solution. The phagocytic activity of the leukocytes was determined by the content of FITC-marked *E. coli* in the phagocytic cells, expressed as mean channel fluorescence per cell.

Statistical study

The statistical evaluations were carried out with Statistica for Windows version 5 (StatSoft Inc., USA). Data are expressed as mean $\pm$ SD. Time-dependent variations of biologic variables were analyzed by the Wilcoxon test. Alpha-adjustment for repeated comparisons was performed according to Bonferroni-Holm. The Spearman correlation coefficient was calculated for correlation analysis. A probability level of $p < 0.05$ was considered significant. A multiple regression model was used to explore whether maximum CD11b, CD16, or phagocytic activity were independently associated with duration of CPB, aortic cross-clamping, circulatory arrest, or with inotropic agent administration.

RESULTS

Twenty-one consecutive children undergoing cardiac surgery with CPB were included in the study. They were 11 girls and 10 boys, with a median age of 11.3 (range: 6–33) months. Seventeen children made an uncomplicated recovery. Four patients developed minor wound infection. Table 1 summarizes patient epidemiological and operative data.

The expression of CD11b molecule before anesthesia was 768.1 ± 178.2 ; it increased at the start of CPB (876.5 ± 104.8 ; $p = 0.0047$), and then returned to the baseline (Fig. 1). The expression of CD16 dropped significantly at the start of CPB and remained decreased until the end of CPB. On the first post-operative day the expression of this molecule increased significantly in comparison with that before anesthesia. The lowest phagocytic activity was observed in the patients after the completion of surgery. It was significantly decreased in comparison with other times of measurement. On the first post-

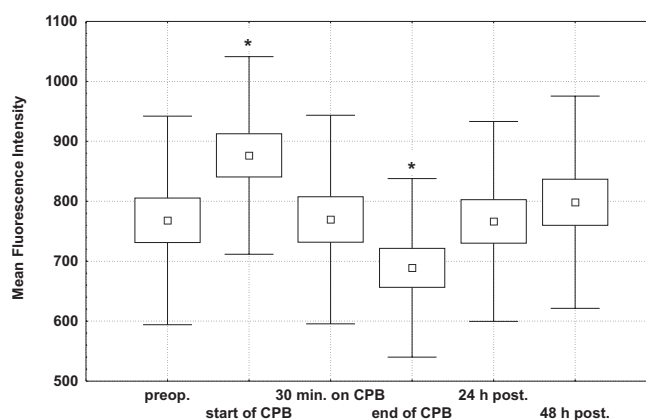


Figure 1. CD11b expression on neutrophils. * $p < 0.001$ level before anesthesia versus level at time-point.

-operative day the phagocytic activity increased in comparison with the pre-surgery level (Table 2). We analyzed the relationship between the expression of CD11b molecules, CD16 antigen, phagocytic activity, and patient demographic and intraoperative data. We found that the duration of CPB inversely correlated with the expression of CD11b on neutrophils measured at the end of CPB, but not with other times of measurements (Fig. 2).

Table 2. Phagocytic activity of and Fcγ receptor expression on neutrophils

	MFI	%	CD16
Before anesthesia	237.6±81.5	87.3±2.8	1327.4±345.3
Start of CPB	247.4±78.5	88.7±3.6	1164.6±307.3*
30 min into CPB	234.6±77.9	87.3±5.9	992.1±224.6*
End of CPB	194.7±59.8**	80.8±9.4	814.0±198.1*
24 h after CPB	350.5±109.8**	86.0±9.8	1508.7±123.9**
48 h after CPB	297.5±99.8	87.9±9.6	1577.4±304.7

Explanations: MFI – mean fluorescence intensity; % – percentage of cells phagocytizing *E. coli*.

* $p < 0.001$ level before anesthesia vs. level at time-point; ** $p < 0.05$ level before anesthesia vs. level at time-point.

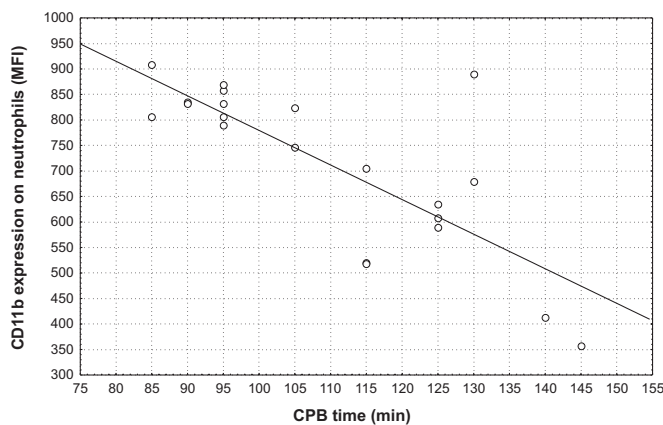


Figure 2. Relationship between cardiopulmonary bypass duration and CD11b expression on neutrophils at the end of CPB. Spearman correlation coefficient $r = -0.68$; $p = 0.00059$.

DISCUSSION

Neutrophils seem to play an important role in the prevention of infections. Activation of these cells, with the release of lysosomal enzymes and phagocytic activity, is a part of the bactericidal defense mechanism^{15, 20}.

In our study we demonstrated a significant decrease in phagocytic activity following CPB, with its lowest value immediately after surgical intervention. The lowest neutrophil phagocytic activity was also associ-

ated with a decrease in Fcγ (CD16) receptor expression on these cells. The pattern of response of neutrophil phagocytic activity to the CPB circuit in infants had not been previously analyzed. However, our findings in infants do agree with the results of the few published studies that address neutrophil phagocytosis in response to CPB in adults. Hamano et al.⁸ studied phagocytosis of granulocytes during CPB in adults using chemoluminescence. They found that the reduction in phagocytic activity remained until 24 h after CPB. Demircioglu⁶ also showed a transient decrease in phagocytosis during CPB in adults. Furthermore, they demonstrated a correlation between the duration of the bypass procedure and the inhibition of leukocyte phagocytosis. Burrows et al.⁴, in contrast, demonstrated that phagocytic activity of neutrophils in neonates did not show significant changes during and after CPB.

Recent studies have provided evidence that in the active phase of other severe systemic inflammatory diseases, the phagocytic activity of and Fcγ receptor expression on neutrophils are also decreased. In septic shock, for instance, low levels of both phagocytosis and CD16 expression are related with greater clinical severity and mortality^{14, 27}. In this regard it is possible to suggest that the lack of phagocytotic response could predispose the patient to serious infectious diseases and can complicate the post-operative period in infant patients.

In contrast to the other neutrophil functions, cardiac surgery with CPB enhanced the CD11b expression on neutrophils at the start of CPB, which then fell significantly during cardiac surgery. Patrick et al.²⁰ also showed that a rise in the expression of adhesion molecules on neutrophils commenced toward the start of bypass. The authors concluded that the increased CD11b expression on neutrophils at the start of CPB was associated with the priming of these cells. Our recent study supported this hypothesis that the increase in CD11b expression on neutrophils is the earliest result of their priming¹⁹. Some authors suggest that CPB directly primes neutrophils and also potentiates the priming of neutrophils by tumor necrosis factor α ²⁰. This state of priming, which can be considered as a result of increased plasma levels of inflammatory mediators, may suggest a mechanism of predisposition to organ dysfunction following CPB.

The current finding suggests that CPB may affect neutrophil functions. We found that there was a significant negative correlation between CD11b expression on neutrophils at the end of CPB and CPB duration. Our results are consistent with other reports^{9, 25}.

It is generally accepted that the fall in adhesion molecule expression on neutrophils during cardiac surgery is associated with a sequestration of these cells^{2, 24}. Activated neutrophils accumulate within the pulmonary microvasculature after exposure to CPB. The mechanism by which these cells are retained intravascularly includes upregulation of endothelial and neutrophil adhesion molecules with subsequent neutrophil rolling and sticking. Although activated neutrophils possess many enzymes important in the killing of microorganisms, these cells may also produce tissue injury. Therefore, the neutrophils accumulated within the capillary bed of the lung may damage the endothelium and increase vascular permeability, thus increasing the risk of SIRS^{2, 22}.

Limitations of the study

One of this study's limitations is that it does not specify adequately the genotypic differences among the patients tested. Recent publications have paid particular attention to the genetically determined individual diversity of the immune system's response to operational intrusion²⁸. The highest concentrations of pro- and anti-inflammatory cytokines in the patients' serum after a cardiac operation may depend on polymorphisms of genes for those cytokines^{21, 28}. It is important to specify the risk of the occurrence of post-operational inflammatory complications by defining the antigen expression of the HLA-DR system^{18, 21}.

The authors of this study pointed out the considerable increase in CD11b expression on neutrophils at the moment the operation was started. One of the possible mechanisms to explain this fact could be neutrophil priming²⁰. However, other results described in this paper do not support this notion.

There was no correlation found between CD11b expression on neutrophils measured at the start of the operation and CPB duration nor was there an observed increase in neutrophil phagocytic activity in the later stages of the operation or after the operation had ended. The phenomenon of neutrophil sequestration in the lung circulation could be a possible explanation for higher CD11b expression only at the beginning of the operation. As mentioned by the other authors, it is mostly those neutrophils which show high CD11b expression on their surface that gather within the lung blood vessels^{20, 21}. The functional immaturity of the cells in newborns and early infancy is conducive to the sequestration of the granulocytes²¹.

The development of post-operational inflammatory complications after heart surgery is connected with the procedures used during the operation. The correlation between CD11b molecule expression on neutrophils and CPB duration shown in this study could be a confirmation of the above hypothesis. The statistical analysis, however, did not show any other dependencies between the tested parameters of the immune system and clinical factors. In investigations performed on adults it was observed that interdependencies occurred between the tested parameters of the inflammatory reaction and the operation's duration, the duration of aorta cross-clamping, the extent of hypothermia, and the use of inotropic drugs^{7, 11, 12, 26}.

This study included children with various heart disorders. It seems that further research concerning the correlation between the procedures used during the operation and parameters of the immune system should be limited to more homogenous groups (e.g. separate groups of children with cyanotic disorder and a separate group of those with a leak disorder).

REFERENCES

- Alcaraz A. J., Sancho L., Manzano L., Esquivel F., Carrillo A., Prieto A., Bernstein E. D. and Alvarez-Mon M. (2002): Newborn patients exhibit an unusual pattern of interleukin 10 and interferon γ serum levels in response to cardiac surgery. *J. Thorac. Cardiovasc. Surg.*, 123, 451–458.
- Asimakopoulou G., Smith P. L., Ratnatunga C. P. and Taylor K. M. (1999): Lung injury and acute respiratory distress syndrome after cardiopulmonary bypass. *Ann. Thorac. Surg.*, 68, 1107–1115.
- Baue A. E., Durham R. and Faist E. (1998): Systemic inflammatory response syndrome (SIRS), multiple organ dysfunction syndrome (MODS), multiple organ failure (MOF): are winning the battle? *Shock*, 10, 79–89.
- Burrows F. A., Steele R. W., Marmer D. J., van Deventer S. H. and Westerman G. R. (1987): Influence of operations with cardiopulmonary bypass on polymorphonuclear leukocyte function in infants. *J. Thorac. Cardiovasc. Surg.*, 93, 253–260.
- Cecchia P. A., Backer C. L., Bronicki R. A., Baden H. P., Crawford S. E., Green T. P. and Mavroudis C. (2003): Dexamethasone reduces postoperative troponin levels in children undergoing cardiopulmonary bypass. *Crit. Care Med.*, 31, 1742–1745.
- Demircioglu F. F. (1989): Leukocyte phagocytosis during cardiopulmonary bypass in adolescents. *Turk. J. Pediatr.*, 31, 127–135.
- Haddad R., El-Hassan D., Araj A., Hallal A. and Abdelnoor A. M. (2001): Some inflammation-related parameters in patients following normo- and hypothermic cardio-pulmonary bypass. *Immunopharmacol. Immunotoxicol.*, 23, 291–302.
- Hamano K., Ito H., Katoh T., Fujimura Y., Tsuboi H. and Esato K. (1996): Granulocyte phagocytic function is impaired during cardiopulmonary bypass. *Ann. Thorac. Surg.*, 62, 1820–1824.
- Hamsch J., Osmancik P., Schneider P. and Tarnok A. (2002): Neutrophil adhesion molecule expression and serum concentration of soluble adhesion molecules during and after pediatric cardiovascular surgery with or without cardiopulmonary bypass. *Anesthesiology*, 96, 1078–1085.

10. Harlan J. M. and Winn R. K. (2002): Leukocyte-endothelial interactions: clinical trials of anti-adhesion therapy. *Crit. Care Med.*, 30 (suppl.), 214–218.
11. Hayashi Y., Sawa Y., Fukuyama N., Miyamoto Y., Takahashi T., Nakazawa H. and Matsuda H. (2003): Leukocyte-depleted terminal blood cardioplegia provides superior myocardial protective effects in association with myocardium-derived nitric oxide and peroxynitrite production for patients undergoing prolonged aortic crossclamping for more than 120 minutes. *J. Thorac. Cardiovasc. Surg.*, 126, 1813–1821.
12. Honore P. M., Jacquet L. M., Beale R. J., Renauld J. C., Valadi D., Noirhomme P. and Goenen M. (2001): Effects of normothermia versus hypothermia on extravascular lung water and serum cytokines during cardiopulmonary bypass: a randomized, controlled trial. *Crit. Care Med.*, 29, 1903–1909.
13. Hill G. E. (1998): Cardiopulmonary bypass-induced inflammation: is it important? *J. Cardiothorac. Vasc. Anesth.*, 12 (suppl. 1), 21–25.
14. Jensen R. H., Storgaard M., Vedelsdal R. and Obel N. (1995): Impaired neutrophil chemotaxis after cardiac surgery. *Scand. J. Thorac. Cardiovasc. Surg.*, 29, 115–118.
15. Joashi U., Tibby S. M., Turner C., Mayer A., Austin C., Anderson D., Durward A. and Murdoch I. A. (2002): Soluble Fas may be a proinflammatory marker after cardiopulmonary bypass in children. *J. Thorac. Cardiovasc. Surg.*, 123, 137–144.
16. Kawahito K., Kobayashi E., Ohmori M., Harada K., Kitoch Y., Fujimura A. and Fuse K. (2000): Enhanced responsiveness of circulatory neutrophils after cardiopulmonary bypass: increased aggregability and superoxide producing capacity. *Artif. Organs*, 24, 37–42.
17. Kress H. G., Gehrsitz P. and Elert O. (1987): Predictive value of skin testing, neutrophil migration and C-reactive protein for post-operative infections in cardiopulmonary bypass patients. *Acta Anaesthesiol. Scand.*, 31, 397–404.
18. Oczenski W., Krenn H., Jilch R., Watzka H., Waldenberger F., Koller U., Schwarz S. and Fitzgerald R. D. (2003): HLA-DR as a marker for increased risk for systemic inflammation and septic complications after cardiac surgery. *Intensive Care Med.*, 29, 1253–1257.
19. Paśnik J., Tchórzewski H., Baj Z., Luciak M. and Tchórzewski M. (1999): Priming effect of met-enkephalin and β -endorphin on chemiluminescence, chemotaxis and CD11b molecule expression on human neutrophils *in vitro*. *Immunol Lett.*, 67, 77–83.
20. Patrick D. A., Moore E. E., Fullerton D. A., Barnett C. C., Meldrum D. R. and Silliman C. C. (1999): Cardiopulmonary bypass renders patients at risk for multiple organ failure via early neutrophil priming and late disability. *J. Surg. Res.*, 86, 42–49.
21. Strohmeyer J. C., Blume C., Meisel C., Doecke W. D., Hummel M., Hoefflich C., Thiele K., Unbehauen A., Hetzer R. and Volk H. D. (2003): Standardized immune monitoring for the prediction of infections after cardiopulmonary bypass surgery in risk patients. *Cytometry B Clin. Cytom.*, 53, 54–62.
22. Suzuki T., Fukuda T., Ito T., Inoue Y., Cho Y. and Kashima I. (2000): Continuous pulmonary perfusion during cardiopulmonary bypass prevents lung injury in infants. *Ann. Thorac. Surg.*, 69, 602–606.
23. Tárnok A., Bocsi J., Pipek M., Osmancik P., Valet G., Schneider P. and Hamsch J. (2001): Preoperative prediction of postoperative edema and effusion in pediatric cardiac surgery by altered antigen expression patterns on granulocytes and monocytes. *Cytometry*, 46, 247–253.
24. Tárnok A., Bocsi J., Rössler H., Schlykow V., Schneider P. and Hamsch J. (2001): Low degree of activation of circulating neutrophils determined by flow cytometry during cardiac surgery with cardiopulmonary bypass. *Cytometry*, 46, 41–49.
25. Zahler S., Massoudy P., Hartl H., Hahnel C., Meisner H. and Becker B. F. (1999): Acute cardiac inflammatory responses to postischemic reperfusion during cardiopulmonary bypass. *Cardiovasc. Res.*, 41, 722–730.
26. Zilberstein G., Levy R., Rachinsky M., Fisher A., Greemberg L., Shapira Y., Appelbaum A. and Roytblat L. (2002): Ketamine attenuates neutrophil activation after cardiopulmonary bypass. *Anesth. Analg.*, 95, 531–536.
27. Zimmerman J. J. and Ringer T. V. (1992): Inflammatory host response in sepsis. *Crit. Care Clin.*, 8, 163–189.
28. Zimmermann A. K., Simon P., Seeburger J., Hoffmann J., Ziemer G., Aebert H. and Wendel H. P. (2003): Cytokine gene expression in monocytes of patients undergoing cardiopulmonary bypass surgery evaluated by real-time PCR. *J. Cell Mol. Med.*, 7, 146–156.