The effect of open heart surgery on circulating lymphocytes and lymphocyte subsets in pediatric patients

Çocuk hastalarda açık kalp cerrahisinin dolaşımdaki lenfosit ve lenfosit alt gruplarına etkileri

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Background: Open heart surgery with cardiopulmonary bypass (CPB) is associated with humoral and cellular activation, leading to organ dysfunction and an increased risk for infections in the postoperative period. The goal of our study was to investigate the effect of CPB on cellular immune system in children.

Methods: We conducted a prospective study to investigate the response of circulating lymphocytes and their subpopulations in a sample of 28 consecutive cyanotic or acyanotic children undergoing cardiac surgery. Peripheral blood samples were obtained preoperatively, and at 48 hours and three months postoperatively to study total lymphocyte count, T lymphocytes, and T-lymphocyte subsets.

Results: There were no differences between cyanotic and acyanotic cases with regard to T-cell and subset counts before and after surgery ($p>0.05$). Overall, total lymphocyte count and absolute total T lymphocyte (CD3+) and absolute T helper cell (CD4+) counts decreased significantly within 48 hours after CPB ($p<0.05$). T suppressor (CD8+) and natural killer cell (NKC) levels also decreased in the early period ($p<0.05$). These values increased to preoperative values three months after the operation ($p<0.05$). The CD4/CD8 ratio increased from 1.31 to 1.55 in the early postoperative period ($p=0.002$); however, it then decreased to 0.93 in the late postoperative period ($p=0.001$).

Conclusion: Significant decreases in absolute NKC count, total lymphocyte count, total T cells and their subsets in the early period of CPB may be due to an extravasation and/or T-cell activation during and after the operation, predisposing pediatric patients to a higher risk for infections.

Key words: Cardiopulmonary bypass/adverse effects; child; immunity, cellular; lymphocyte activation; lymphocyte subsets; time factors.

Amaç: Kardiyopulmoner bypass (KPB) yardımıyla yapılan kalp cerrahisi humoral ve hücresel immünite sisteminde aktivasyona neden olarak organ disfonksiyonlarına ve ameliyat sonrası dönemde enfeksiyon riskinin artmasına neden olabilmektedir. Bu çalışmada, KPB'nin çocuk hastalarda hücresel immün sistem üzerindeki etkileri araştırıldı.

Çalışma planı: Bu prospektif çalışmada, açık kalp cerrahisi uygulanan siyanotik veya asiyanotik 28 çocuk hastada dolaşımdaki lenfosit ve lenfosit altgruplarının KPB'ye yanıtı araştırıldı. Ameliyat öncesinde ve ameliyat sonrası 48. saatte ve üçüncü ayda kan örnekleri alınarak periferik kanda total lenfosit, T lenfositleri ve T lenfosit altgrupları çalışıldı.

Bulgular: Siyanotik ve asiyanotik hasta grupları arasında ameliyat öncesi ve sonrası dönemlerde ölçülen T lenfosit ve altgruplarının sayımları açısından anlamlı fark saptanmadı ($p>0.05$). Tüm hasta grubu değerlendirildiğinde, total lenfosit sayımı, mutlak total T lenfosit (CD3+) and mutlak T yardımcı hücre (CD4+) sayımları KPB'den 48 saat sonra anlamlı düşüş gösterdi ($p<0.05$). Supressör T hücresi (CD8+) ve doğal öldürücü hücre sayılarında da anlamlı düşüş görüldü ($p<0.05$). Ameliyat sonrası üçüncü aydaki değerlendirmelerde, incelenen immün parametrelerin hepsinin ameliyat öncesi değerlere geldiği görüldü ($p<0.05$). CD4/CD8 oranı ameliyat sonrası erken dönemde 1.31'den 1.55'e artış gösterirken ($p=0.002$), üçüncü ayda bu değer 0.93'e düştü ($p=0.001$).

Sonuç: Total lenfosit düzeyleri, mutlak doğal öldürücü hücre ve total T hücre sayılarında ve T hücrelerin diğer altgruplarında ameliyat sonrası erken dönemde görülen belirgin azalma T hücrelerin aktivasyonu veya başka nedenlerle ekstravazasyonu nedeniyle olmuş olabilir. Bu durum çocuk hastalarda enfeksiyon riskini artırmaktadır.

Anahtar sözcükler: Kardiyopulmoner bypass/yan etki; çocuk; immünite, hücresel; lenfosit aktivasyonu; lenfosit altgrupları; zaman faktörü.

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Since the beginning of open heart surgery, it is known that extracorporeal circulation (ECC) is highly traumatic to the components of peripheral blood, and that artificial circulation decomposes all blood elements. Because ECC is a nonphysiologic assisting device, it is considered to cause specific changes in immunity and lead to development of postoperative infections.[1-8] Although this pathologic condition has been discussed, the underlying mechanisms have not been clearly understood. In addition, to the best of our knowledge, there is only one report in the literature on the effects of ECC on lymphocyte counts, total T lymphocyte, and their subsets after cardiac operation in pediatric patients. In this study, we evaluated the effects of ECC on cellular immunity and whether the cell-mediated immune response was suppressed or activated in children older than six months in the early and late postoperative period.

PATIENTS AND METHODS

The study was conducted at Hacettepe University Cardiovascular Surgery Clinic. A total of 28 pediatric cases undergoing elective open heart surgery for congenital heart disease were included after obtaining informed parental consent. Institutional approval was obtained from the ethics committee of Hacettepe University. The study group consisted of 13 children with cyanotic heart disease and 15 with acyanotic heart disease. All patients underwent cardiopulmonary bypass (CPB) with a Dideco hollow fibre oxygenator (Dideco, Mirandola, Italy) at comparable flow rates. Samples of heparinized blood were drawn via a central venous catheter immediately before surgery (E1), 48 hours after the onset of CPB (E2), and three months after the operation. Blood samples were studied for *(i)* total lymphocyte counts, *(ii)* T-cell lymphocytes, *(iii)* T-cell subsets; CD3, CD4, CD8 and natural killer cells (NKC, CD16+, CD56+). Demographic data of the cyanotic and acyanotic patients are summarized in Table 1.

Exclusion criteria were any of the following: preoperative temperature greater than 37.5 °C, documented preoperative infection, active or prior history of autoimmune or collagen vascular disease, total CPB time over 200 min, and aortic cross-clamping time over 120 min. In addition, patients who had previous cardiac operations, and those with evidence for drug- or disease-induced immunosuppression, history of previous blood transfusion, or red cell alloantibodies were not included in the study.

Analyses of lymphocytes and lymphocyte subsets. Changes in peripheral blood lymphocytes and their subsets including CD3, CD4 (helper/inducer T lymphocyte), CD8 (cytotoxic/suppressor T lymphocyte) and CD16+, CD56+ (NKC) were measured on a FAC Scan flow cytometer (BDIS) (Becton Dickinson, Sunnyvale, California, USA) using monoclonal antibodies. From each patient, a blood sample of 2 ml was drawn and taken into tubes containing EDTA. The samples were homogenized for 15 minutes at 25 °C. An amount of 100/l blood and an amount of 10/l monoclonal antibodies marked with fluorescence were put together into tubes for each CD antigen (anti-CD3, anti-CD4, anti-CD8, and anti-CD16+, 56+). After flow cytometry, data were stored in list mode files and positive cells were identified.

Statistical analysis. The proportions of lymphocytes, T lymphocytes, and their populations were calculated. Data were analyzed using the SPSS 11.5 automated statistical program. Repeated-measures were analyzed using one-way ANOVA. Comparison of immune parameters in the preoperative and postoperative period was made using the Levene's test. In case of statistical significance, the difference between the pairs of medians was determined by the Scheffe or Tamhane tests. A p value of less than 0.05 was defined as statistically significant.

RESULTS

There were no significant differences between the two groups in terms of preoperative characteristics and operation time ($p>0.05$; Table 1).

The two patient groups were also similar with respect to preoperative lymphocyte and lymphocyte subpopulation counts ($p>0.05$; Table 2). Similarly, lymphocyte, T cell, and NKC counts and the CD4/CD8 ratio remained similar in the two groups in both early and late postoperative periods ($p>0.05$; Table 2). The overall results of immune parameters are also tabulated in Table 2.

The absolute lymphocyte count decreased significantly 48 hours after the operation. The lowest total

Table 1. Characteristics of the cyanotic and acyanotic patients

	Cyanotic (n=13)		Acyanotic (n=15)	
	Mean	Range	Mean	Range
Age (months)	18.6	16-73	24.6	38-96
Weight (kg)	7.9	7.8-33.0	9.6	6.9-23.3
Height (cm)	84	63-110	77	71-115
Cardiopulmonary bypass time (min)	135	72-165	118	98-158

Table 2. The mean immune cell counts in the two patient groups preoperatively, and in the early and late postoperative periods

	Lymphocytes (cell/μl)	T cells (cell/μl)	CD4+ T cells (cell/μl)	CD8+ T cells (cell/μl)	NKC (cell/μl)	CD4/CD8 ratio
Cyanotic patients (n=13)						
Preoperative	3,482	1,450	582	360	119	1.24
Postoperative 48th hour	2,400	1,260	445	295	58	1.4
Postoperative 3rd month	3,450	2,529	776	430	251	0.9
Acyanotic patients (n=15)						
Preoperative	3,650	1,829	574	341	160	1.37
Postoperative 48th hour	2,600	1,566	423	259	61	1.52
Postoperative 3rd month	3,375	2,445	789	465	274	0.95
Overall results						
Preoperative	3,566	1,639	578	350	144	1.31
Postoperative 48th hour	2,500	1,563	434	277	60	1.55
Postoperative 3rd month	3,412	2,487	782	480	296	0.93

NKC: Natural killer cell.

lymphocyte levels were observed in the early postoperative period. In this period, absolute lymphocyte, T cell and their subset counts and the NKC level differed significantly from the preoperative values. The percentage of all immune cells decreased significantly on day 2 ($p<0.05$), but their levels increased above baseline levels three months after the operation (Table 2). The corresponding p values were p=0.000 and p=0.000 for total lymphocytes, p=0.002 and $p<0.001$ for CD3, p=0.025 and $p<0.001$ for CD4, and $p<0.001$, $p<0.001$ for CD8 counts.

CD3+CD4+ lymphocytes decreased significantly in all patients on day 2 ($p<0.05$), then increased above the baseline counts three months after surgery (Table 2). The absolute number of CD4+ cells also decreased (by 72% from baseline) on day 2, then increased in three months after the operation (Table 2). Similarly, the percentage of CD3+CD8+ cells decreased significantly in the post-bypass period (Table 2). Resulting from the decrease in total lymphocyte count, decrease in the absolute number of CD8+ cells on day 2 (65% decline from baseline) was also significant followed by an increase above the baseline level in the third month ($p<0.05$; Table 2).

The two patient groups were similar with respect to changes in the percentages of CD4+ and CD8+ T cells. As with total CD3+ cells, absolute numbers of CD4+ and CD8+ lymphocytes were significantly lower in the early postoperative period ($p<0.05$), again resulting from lower total lymphocyte counts.

These data demonstrated that ECC and CPB were associated with significant depression of all immune parameters and dramatic decreases in circulating lymphocytes, T cell and their subsets. The kinetics of the T-cell subpopulations are shown in Figure 1.

Scheffe test showed significant differences between consecutive samples as well as between the pre- and postoperative values.

The CD4/CD8 ratio increased from 1.31 to 1.55 on day 2 postoperatively (p=0.002); however, it then decreased to 0.93 in the late postoperative period (p=0.001; Table 2). This showed that both CD4+ and CD8+T lymphocyte counts increased during the postoperative period, but the increase in CD8+ T lymphocytes was greater than that of CD4+ T lymphocytes. In other words, the increment of CD4+ T lymphocytes during the postoperative period was not parallel to the increase in CD8+ T lymphocytes.

Natural killer cells. As with T cells and their subsets, the percentage of CD16+, CD56+ (NKC) decreased significantly in all patients on day 2 and then rose three months after surgery ($p<0.05$; Table 2). The actual number of NK cells also decreased on day 2 along with their relative percentage. The absolute count was also lower on day 2. However, NK cells were the only lymphoid subset that rose to the normal range three months after surgery ($p<0.05$).

DISCUSSION

Open heart surgery leads to activation of compartments of the immune system because ECC is highly traumatizing to the components of blood cells, and the artificial heart-lung system decomposes the whole blood elements. Since a nonphysiologic assisting device is used in open heart operations, specific changes in the immune system are inevitable, leading to the development of postoperative infections.[1-8] Therefore, the CPB seems to play an important role for the inflammatory or immune cellular response after cardiac surgery.

Previous studies include adult cardiac surgery cases.[5,9,10] However, the postoperative cytokine and

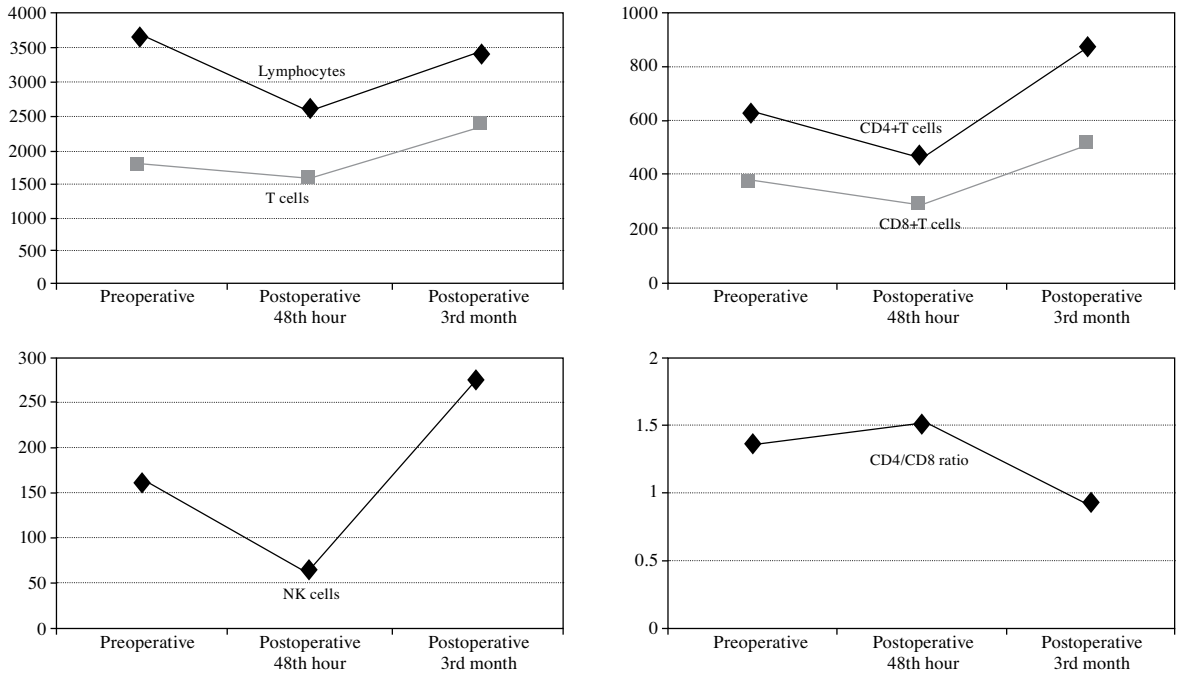

Fig. 1. Lymphocyte, T cell, CD4, CD8, NKC counts and the ratio of CD4/CD8 in preoperative, early and late postoperative periods.

immune response in pediatric patients is different from that in adults.[11,12] In addition, there is a limited number of studies on immune system changes in children undergoing open heart surgery.[13] Therefore, we investigated the immune cell modulation after open heart surgery in pediatric cases. On the other hand, for a better understanding of the cellular immune response after open heart surgery, it is necessary to investigate the cellular components of the immune system such as T lymphocytes and their subsets. Therefore, we evaluated the effects of CPB on kinetics of lymphocytes, T cells, and their subsets and, in addition, the influence of cardiac operations with ECC on total lymphocyte, differential T cell, and the NKC counts.

Our findings demonstrated that CPB produced an absolute decrease in the number of circulating cells in most lymphocyte subsets, as did previous studies. The most profound decreases were seen in the absolute numbers of all lymphocyte subsets in the early postoperative period. In our patients, T cells and subpopulation counts decreased after ECC in the early postoperative period.

Consisted with the findings of Habermehl et al.,[13] we also found that the median number of T cells decreased after ECC. Although a limited number of cases exhibited increments in the number of total lymphocytes and T cells after CBP, the majority of cases showed decreased immune cells after ECC. T cells and their subsets were profoundly reduced 48 hours after open heart surgery. Similar findings have been reported in some studies including adult cases.[5,14]

As in previous reports,[3,15,16] total T cells and the CD4+ T cell subset demonstrated the greatest relative decreases, with the percentage of CD4+ cells falling to 84% of baseline on the first postoperative day. This decrease in the percentage of CD4+ cells was further amplified by the decrease in the absolute number of circulating lymphocytes, making the percentage of CD4+ cells 26% of baseline on day 2 and resulting in a mean absolute count of only 273 CD3+CD4+ cells/μl. The absolute number of CD4+ cells in the third month approached the values seen in severely immunocompromised patients. The CD4/CD8 ratio was increased to 1.55 on day 2, but it fell from 1.3 at baseline to 0.93 in the third month.

In our study, we observed that CPB induced drastic decreases in the number of circulating T cells, predominantly in NKC and total lymphocyte cells. Total lymphocytes and NK cells fell from 3,566 to 2,500 and from 144 to 60, respectively (Table 2). In both patient groups, the lowest levels of immune cells were observed in the counts of total lymphocyte cells and NKCs.

Similar to previous studies,[5,10,14,15,17] we noted that the CD4+ T cells, which play a major role in response to infection, decreased as well as the CD8+ T-cell subset and NKC after ECC.

The CD4/CD8 ratio increased from 1.31 to 1.55 48 hours after the operation. Compared to its preoperative

level, we found a lower ratio three months after surgery. Although the counts of both T-cell subsets increased significantly during the same period, the ratio was the lowest three months after the operation. These findings may suggest that T helper cells are depressed more profoundly than the cytotoxic T cells and that the increments of the two subsets are not parallel to each other.

Although we did not see any evidence for infection postoperatively, in our opinion, the risk of infection in our cases continued in the late postoperative period. It is possible that some compensatory mechanisms such as humoral immune system might be activated as a preventive factor from severe infection after the operation. In addition, in parallel to previous reports,[3,16,18] we noted a profound decrease in NKCs in the early postoperative period. Total lymphocyte and CD3+ cell counts were decreased after the operation, but returned to preoperative values three months after surgery.

The cellular immune response to ECC involves all compartments of this immune system. We found a sustained decrease in total T lymphocytes and their subpopulations such as T helper cells and T suppressor cells in peripheral blood. Almost 75% of the study population had T helper cell count below 500 μ/l 48 hours after surgery. However, CD4/CD8 ratio continued to be within the normal range. We suggest that the activation of T cells leads to an extravasation of a significant number of T helper cells to sites of inflammation after ECC. The low counts of these cells in the peripheral blood, which may contribute to the postoperative immune response, appear not to be correlated with clinical findings. Further studies are needed to support the findings of previous studies and our hypothesis that ECC may play a role in T-cell activation and subsequent extravasation of T lymphocytes.

In conclusion, considering all data together, we suggest that the downregulation of cellular immune response may be the reaction of a cellular stress response, induced by anesthesia together with ECC. The morbidity and mortality due to postoperative infections associated with pediatric cardiac surgery may be partially attributable to the use of CPB. We believe that the cellular immunity is not only affected by ECC but also by general anesthesia. The findings of this prospective study show that the risk of infection continues during the three months postoperatively.

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