

Effects of ischemic heart disease operations with use of Cardiac Pulmonary Bypass (CPB) and without it, using the Off-Pump Coronary Artery Bypass (OPCAB) technique on the chosen subpopulations of lymphocytes: potential usefulness in the routine clinical diagnostics



Zmiany w obrębie wybranych subpopulacji limfocytów u pacjentów operowanych z powodu choroby niedokrwiennej serca z zastosowaniem krążenia pozaustrojowego oraz bez krążenia z zastosowaniem techniki OPCAB

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Abstract

Background: The use of less invasive operative techniques, such as OPCAB (off-pump coronary artery bypass), can significantly reduce the inflammatory response, thus decrease the mortality as well as the postoperative complications in patients after the cardiovascular operation.

Aim: The objective of this study was to compare the number of selected immunocompetent cells in two groups of patients with ischemic heart disease operated with the usage of cardiac pulmonary bypass and those operated using the OPCAB technique. Moreover, a trial was made in order to define the usefulness of examined receptors in routine clinical diagnostics for estimation of postoperative complication risk in the investigated groups.

Material and methods: Patients were divided into two groups: A – those operated using CPB, B – those operated using OPCAB. Blood for the investigation was taken from patients at the following time intervals: before heparin administration, and next samples were taken 5, 15, 30, 60, 120 and 240 minutes after the start of the surgical procedure. The CD4, CD8, CD3, CD16, CD19 and CD25 receptors were investigated.

Results: The most significant differences between two groups of patients were observed for the cells expressing CD16, CD19 and CD25 receptors. In the OPCAB group, the CD16 cells increase significantly 60 min after the start of the surgical procedure and decrease below the control value in the 240th minute. Our study shows also a decrease in CD3, CD4 and CD8 recep-

Streszczenie

Wstęp: Zastosowanie krążenia pozaustrojowego jest powszechnie akceptowaną metodą w kardiologicznym leczeniu choroby niedokrwiennej serca. Istnieje jednak szereg doniesień wskazujących na możliwość stymulowania reakcji zapalnej tzw. SIRS (*Systemic Inflammatory Response Syndrome*) podczas stosowania techniki krążenia pozaustrojowego. Reakcja ta jest wynikiem kontaktu krwi ze sztuczną powierzchnią oksygenatorów i drenów. W wyniku tej reakcji może dochodzić do pobudzenia elementów układu odpornościowego. Reakcja zapalna może być przyczyną szeregu powikłań pooperacyjnych, takich jak zmiany zatorowe, niewydolność wielonarządowa czy zaburzenia neuropsychologiczne. Z tego względu strategia wykonywania operacji zespolenia naczyń wieńcowych koncentruje się m.in. na możliwości redukcji stymulowania reakcji zapalnej. Wydaje się, że techniki mało inwazyjne typu OPCAB mogą w efektywny sposób ograniczyć stymulowanie reakcji zapalnej, a tym samym przyczynić się do zmniejszenia śmiertelności i liczby powikłań wśród pacjentów operowanych z powodu choroby niedokrwiennej serca.

Cel: Celem pracy było zatem porównanie zmian ilościowych wybranych komórek immunokompetentnych u pacjentów operowanych z powodu choroby niedokrwiennej serca z zastosowaniem krążenia pozaustrojowego oraz bez krążenia pozaustrojowego techniką OPCAB. Ponadto celem pracy było określenie czynników przydatnych w ocenie stopnia inwazyjności prowadzonych zabiegów kardiologicznych.

Słowa kluczowe: krążenie pozaustrojowe, OPCAB, limfocyty.

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tors in the CPB group. The CD19 positive cells decrease significantly in the CPB group during the surgical procedure, back to the control value 240 min after the start of the procedure. The CD25 positive cells increase significantly in the CPB group. No significant differences for the investigated receptors were observed in the OPCAB group.

Conclusion: It seems that the examination of the selected immunocompetent cells can be useful in the routine clinical diagnostics. CPB, as compared to the OPCAB technique, seems to cause major suppression of immunological response which can make the patients more susceptible to infections. However, it should be noticed that in the OPCAB group, the primary number of cells positive to a given receptor is lower than in patients operated with the usage of CPB. Therefore, it could turn out that the risk of postoperative complications is similar in both groups of patients.

Key words: coronary artery/pulmonary CPB bypass, OPCAB, lymphocyte.

Background

Usage of cardiac pulmonary bypass is a commonly accepted method in surgical treatment of ischemic heart disease. However, there are opinions indicating that the usage of that method can be responsible for the stimulation of inflammatory response (SIRS – Systemic Inflammatory Response Syndrome), as a result of blood contact with the artificial surface of oxygenators and drains. The inflammatory cascade includes: bradykinin release, complement activation, cytokine release. The inflammatory factors can contribute to development of multiorgan failure, renal and respiratory dysfunction, bleeding disorders, neuropsychological failure and other postoperative complications.

There are not many papers describing leukocyte activation and alteration of cell-mediated immune response after CPB and OPCAB operations. It is known from these papers that the lymphocyte subset numbers in response to the alterations provided by the invasive surgical techniques can be decreased, resulting in the inflammatory response [1-4]. The lymphocyte can be reduced not only in the number of the cells but also in the lymphoproliferative responsiveness to the antigen [5]. The mechanism by which the cell-mediated immune response is reduced includes the decrease in the CD4+ and IL-2R+ T cells. The proper T cell proliferation and differentiation depends on the number of IL-2 producing cells and on the number of IL-2 responsible T helper cells. Additionally, the T cell and monocyte interaction is important for the adequate cell-mediated immune response. The reduced number of the monocytes observed in the CPB is the key factor that influences the PGE synthesis [6, 7], an essential factor involved in the downregulation of the immune response [7-9]. As a result of the alterations in the cell-mediated immune response described below, the increase in the infection risk in the patients can be observed. To avoid the possible alterations in the cell-mediated immune response, those the increase risk of inflammatory response in the patients after the improvement of circuits are proposed. Among many methods, the improvement of biocompatibility

of circuits may be one of the factors which can reduce the inflammatory response. There have been several reports about heparin-coated surfaces that can affect the complement activation, thus prevent postoperative complications. Complement inhibition with a soluble complement receptor can also effectively decrease the complement activation of both classic and alternative pathways and reduce the induced organ injury. It seems that the usage of less invasive operative techniques such as OPCAB can significantly reduce the inflammatory response, thus decrease the mortality as well as the postoperative complications in patients after cardiovascular operations.

However, it is important to indicate that most data concern the inactivation of complement or cytokine cascade, which are very important immunological factors, but the inhibition of these factors cannot entirely exclude the possibility of activation of the immunological response; there is a complex reaction in which a lymphocyte response must be also included. However, usage of less invasive methods cannot completely exclude the possibility of inflammation response. Patients undergoing cardiac operations subjected to other factors such as surgical trauma, which may cause induction of the inflammatory cascade.

Therefore, the main goal of this study was to define whether the level of immunological response, evaluated according to the quantitative changes of T and B lymphocyte receptors, is different for two groups of patients: operated with and without the usage of the OPCAB technique, in the case of ischemic heart disease treatment. Another goal of the study was to evaluate whether analysed receptors can be useful in routine clinical diagnostics.

Aim

The objective of this study was to compare the number of selected immunocompetent cells in two groups of patients with ischemic heart disease operated with the usage of cardiac pulmonary bypass and those operated using the OPCAB technique. Moreover, a trial was made in order to

define the usefulness of examined receptors in routine clinical diagnostics for estimation of postoperative complication risk in the investigated groups.

Material and methods

Patients

The analysed group consisted of 24 patients who underwent the surgical treatment of ischemic heart disease at the Silesian Centre of Heart Diseases, Zabrze, Poland. Patients were divided into two groups:

A – patients who underwent the operation using CPB,

B – patients who underwent the operation using the OPCAB technique.

The examined group consisted of 6 females and 17 males, whose age ranged from 34 to 74 years. All patients underwent the operation as planned. The selection of patients was based on the following exclusion criteria: coagulopathy, implemented anti-inflammatory or anticoagulation treatment, age over 75 years, insulin-dependent diabetes, serious non-cardiologic disease, abnormal liver function, earlier usage of steroids, numerous transfusions before the operation and re-operations. The average period of circulation was 85 minutes and ranged from 48 to 114 minutes. The required dose of heparin ranged from 100 to 410 mg. However, significant differences in heparin dosage could be observed between group A (CPB) and B (OPCAB), for which the average dosage was 254 mg and 182 mg, respectively. The period of ACT ranged from 325 to 1082 units. The average ACT in the group operated using the OPCAB technique was 444 units, while in the CPB group it increased significantly to 684 units.

Methods

The analysis of immunological parameters was performed in whole blood drawn from the artery at intervals set according to the literature data [Saito, 2000] and own experiences:

1. control (before the heparin administration), 2. 5 minutes

after the circulation start, 3. 15 minutes after the circulation start, 4. 30 minutes after the circulation start, 5. 60 minutes after the circulation start, 6. 120 minutes after the circulation start, 7. 240 minutes after the circulation start.

The basic criterion of blood draw moment selection was the dynamics of examined receptors' quantitative changes. Drawn blood samples were prepared and analysed according to the literature on the subject [Ormerod, 1990]. With the usage of a flow cytometry technique (FC), T and B lymphocyte cell receptors were determined: CD4 (helper lymphocytes), CD8 (cytotoxic lymphocytes), CD3 (mature lymphocyte receptors), CD16 (NK cells), CD19 (B lymphocytes) and CD25 (interleukin 2 receptors).

Results

Statistical analysis

Lymphocytes CD4

The dynamics of changes regarding the number of cells with the CD4 receptor was similar in both groups of patients, independently of the applied surgical technique. Both in group A (CPB) and in group B (OPCAB technique), the average percentage of CD4 cells in control samples amounted to 40%. A decrease in those values of about 7% on average in group A and 4% on average in group B, took place in 5th minute after beginning of the operation. In the 120th minute of operation, the number of cells with CD4 receptor increased up to 41% in the CPB group and up to 46% in the OPCAB group. The return to control values took place in the 240th minute in group B and in the CPB group (group A) at the same time; the decrease in CD4 cells percentage occurred – 5% decrease in comparison with control values. At particular moments, for group A statistically significant differences in the number of cells with the said receptor were not observed, while in group B the number of mentioned receptors in the 120th minute of operation was significantly different from those parameters observed in the 5th and 15th minute of operation (Fig. 1).

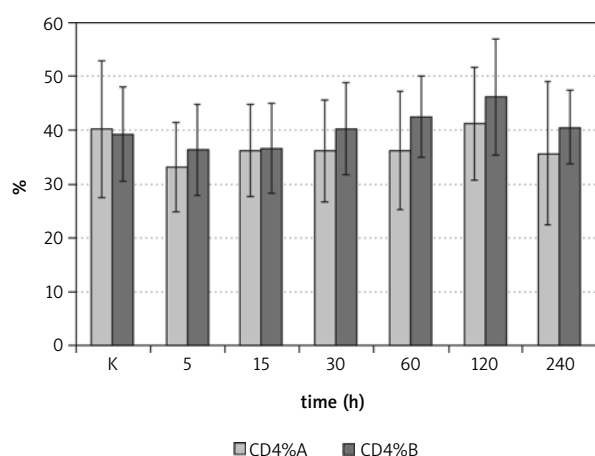


Fig. 1. Changes of the mean percentage value of CD4 positive lymphocyte in: A – patients who underwent the operation using the CPB, B – patients who underwent the operation with the usage of the OPCAB technique

Lymphocytes CD8

Response from the immunological system was different for both groups in the 30th and 60th minute of operation, which was expressed by an increase in these receptors in the CPB group. In group A, the number of CD8 cells was in control of 31%, in the 15th minute of operation increased to the level of 38%, and after that, in the 240th minute after circulation start, a significant decrease in the number of the said cells to 23% occurred. In group B, more stability in the number of cytotoxic lymphocytes was observed. At all period intervals, the percentage of CD8 cells was about 28%. In the 240th minute of operation only a slight decrease to 25% was observed (Fig. 2).

Lymphocytes CD3

In group A, the percentage of cells with CD3 receptor was in the 5th, 15th, 30th, 60th and 120th minute of operation was similar to the control values (70%). In this group, in the

240th minute, a significant (in comparison to the values obtained at other moments) decrease of about 12% in the number of cells with the said receptor took place. Usage of the OPCAB technique was characterized by a tendency of increase in the number of cells with CD3 receptor during the operation. The highest values for that receptor, significantly different from the control values, were observed in the 120th minute of operation (Fig. 3).

Lymphocytes CD16

In a group of patients who underwent the operation with the CPB usage, the average percentage of cells with CD16 receptor was about 12% in the control group and showed the increasing tendency, achieving the maximum value (19%) in the 60th minute of operation. In the 240th minute, the level of the said cells in this group of patients decreased more than twice in comparison with maximum values. In the OPCAB group, the number of cells with CD16 receptor was stable (15% on average), independently of the moment of blood draw (Fig. 4).

Lymphocytes CD19

The operation performed with the usage of CPB was characterised by a decrease of, on average, a half of CD19 cell number, in comparison with the control values, starting from the 15th minute of operation. The value of CD19 receptor returned to the control level in the 240th minute of operation. The significant differences in the quantity of cells with the said receptor took place between the control and the 15th, 30th, 60th and 120th minute. Additionally, the lymphocyte values observed in the 15th, 30th, and 120th minute were different from those observed in group A in the 240th minute of operation. In the OPCAB group, the level of CD10 cells was similar, though in the 240th minute a tendency of decrease was observed. However, the differences between values of this receptor in this group of patients at particular moments were not statistically significant. The differences in immuno-

logical response of CD19 receptor in both groups referred to the 30th and 240th minute, but only the values at the latter time were significantly lower in the OPCAB group (Fig. 5).

Lymphocytes CD25

In group B, the number of CD25 cells was on a stable level at all times of measurement and was 3% on average. In group A, the values of the said receptor were almost twice as high, except those observed in the 240th minute of operation, when the number of cells increased to nearly 70% in comparison with the control. This value was significantly different from the values observed at other moments for group A. Values of CD25 receptor (three times as high) in the 240th minute of operation in the CPB group patients, compared with the OPCAB group, can indicate that the performed operation technique is an important factor, mobilizing the group of T lymphocytes (Fig. 6).

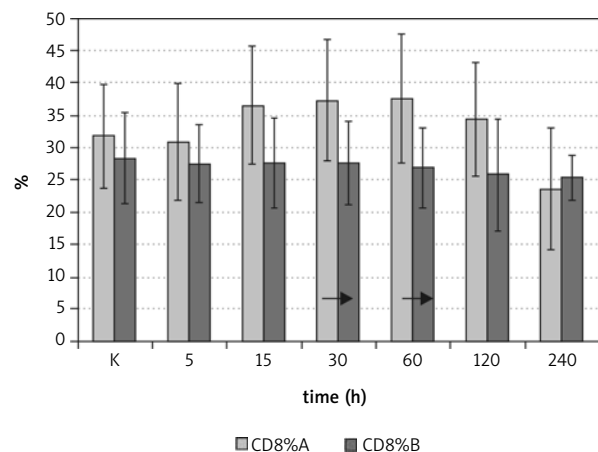


Fig. 2. Changes of the mean percentage value of CD8 positive lymphocyte in: A – patients who underwent the operation using the CPB, B – patients who underwent the operation with the usage of the OPCAB technique (arrows indicate the differences between the groups)

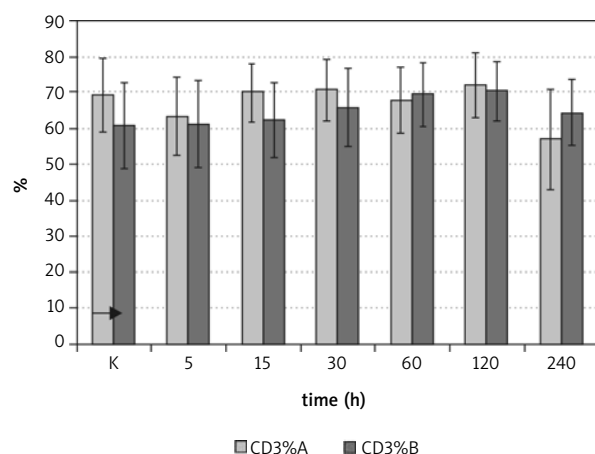


Fig. 3. Changes of the mean percentage value of CD3 positive lymphocyte in: A – patients who underwent the operation using the CPB, B – patients who underwent the operation with the usage of the OPCAB technique (arrows indicate the differences between the groups)

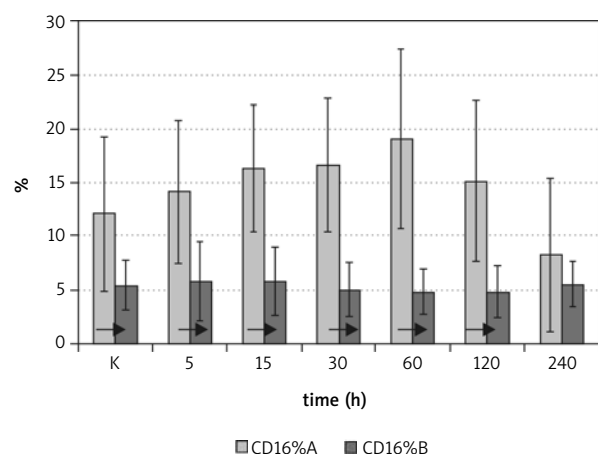


Fig. 4. Changes of the mean percentage value of CD16 positive lymphocyte in: A – patients who underwent the operation using the CPB, B – patients who underwent the operation with the usage of the OPCAB technique (arrows indicate the differences between the groups)

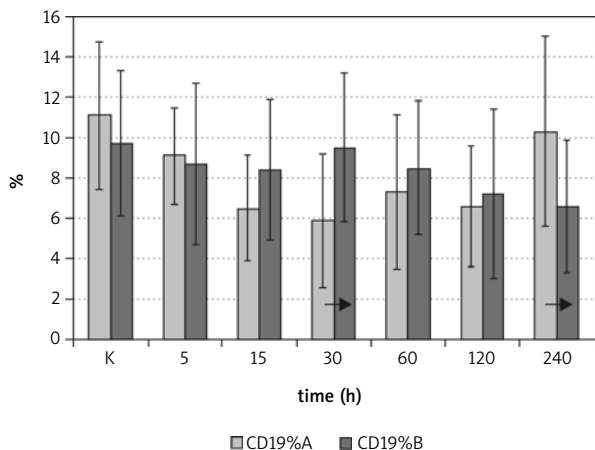


Fig. 5. Changes of the mean percentage value of CD19 positive lymphocyte in: A – patients who underwent the operation using the CPB, B – patients who underwent the operation with the usage of the OPCAB technique (arrows indicate the differences between the groups)

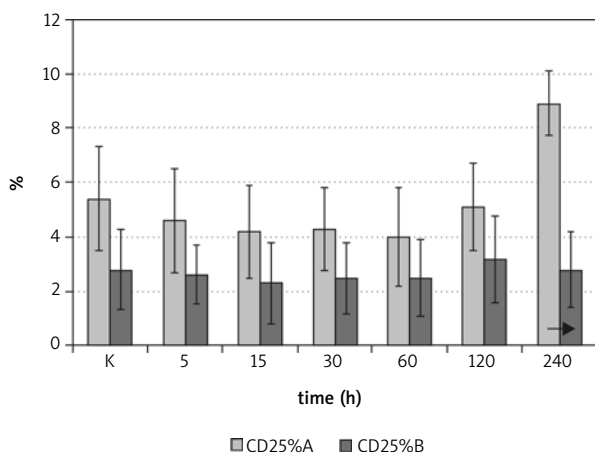


Fig. 6. Changes of the mean percentage value of CD25 positive lymphocyte in: A – patients who underwent the operation using the CPB, B – patients who underwent the operation with the usage of the OPCAB technique (arrows indicate the differences between the groups)

Analysis of Variance (ANOVA/MANOVA)

Analysis of variance revealed significant influence of the type of operation (the usage of the CABG or OPCAB technique) on the mean percentage level of evaluated sub-populations of lymphocytes. Also the time of operations significantly influence the percentage of estimated lymphocytes.

Discussion

In the investigations concerning changes in the immunological system, caused by the usage of CPB, an evaluation of lymphocyte receptors typical of particular T and B lymphocyte sub-populations seems to be also very important. The hitherto investigations show that the usage of CPB technique usually causes the suppression of the cell response. Suppressive impact of CPB on cell response has been acknowledged in the literature [1, 5, 10, 11]. It is emphasized at the same time that this kind of immunological

response attenuation increases the risk of infection in the early postoperative period and leads to the sepsis development [5]. Investigations of Tajima et al. [12] show that during the operations performed with the usage of CPB, mostly the number of helper lymphocytes CD4 as well as B lymphocytes decreases. The suppression of cell response, which is a result of particular lymphocyte subpopulation quantity decrease, reached its maximum usually within twenty-four hours after the operation. In this study, the longest measurement period reached only up to 240 min of the operation, but already in this period the tendency of suppression in the CPB group was observed. The decreased number of lymphocytes can usually hold on for up to one week since the operation's day [5, 11, 12]. In the case of NK cells as well as CD8 cytotoxic lymphocytes, an increase in their quantity during the operation is mostly observed. Therefore, the statement that during the CPB a response from the immunological system is moved into the direction of cytotoxic reaction seems to be valid [12]. Except the quantitative changes, NK cells increase also their activity, however those changes are temporary and hold on usually up to two days since the operation; after that period their number decreases [13-15]. Similarly in the presented study, the increase in the number of CD16 lymphocytes in the CPB group was a temporary phenomenon. The maximum number of analysed leucocytes was observed 120 min after the circulation beginning on average, whereupon the rapid decrease in their number was observed. Temporary activity intensification of this lymphocyte subpopulation probably results from the increase in the adrenaline level in blood. Also the investigations carried out by Akbas et al. [16] acknowledge a decrease in the number of helper lymphocytes, B lymphocytes and NK cells in patients operated with the usage of the CPB technique, just after the operation as well as in the first 24 hours after the operation. At the same time, the authors did not observe such changes in patients operated using the OPCAB technique. The extracorporeal circulation causes more changes to the immunological system, and the possible suppression of cell response observed in OPCAB patients is not stronger than in the case of other types of surgical intervention [16]. Comparison investigations, including three groups of cardiac surgery patients operated with the usage of CPB, less invasive LAD technique and finally the OPCAB technique, present a slightly different opinion. In each group, the changes of leucocytes, CD4 lymphocytes, B lymphocytes and NK cells showed a similar course during the operation. Such results can suggest that the leukocyte reaction in the course of coronary artery bypass operation is connected more with the traumatic effect, which is characteristic of each kind of surgical intervention, than with the activity itself [17]. However, taking into consideration the results obtained in the presented study, it seems that the suppression of cell response is connected mainly with the CPB activity, while the traumatic effect is one of the sources of influence on lymphocytes and can contribute to intensification of changes caused by the extracorporeal circulation.

The reasons of lymphocyte suppression observed during the CPB indicate several mechanisms. The destruction and self-digestion of immunocompetent cells are possible. Moreover, with cell integrity preserved, the damages of extracellular receptors can occur, which leads to the loss of ability of response to monoclonal antibodies, and finally to the reduction in the number of lymphocytes [12]. Furthermore, it is suggested that the quantitative changes of lymphocytes, manifested by the decrease in the number of CD4 helper lymphocytes, can be connected with insufficient cell redistribution between peripheral blood and bone marrow [12, 18]. It is also conceivable that these disturbances can be responsible for quality changes in particular lymphocyte subpopulations, mainly as a result of activated complement fixation of C3 fragment to lymphocyte receptors [10, 19]. It is probable that the weakness of cell response results from the disturbed interaction between monocytes and T lymphocytes. During the extracorporeal circulation, an increase in inhibitor monocyte quantity takes place; these monocytes are characterised by increased secretion of PGE₂, which reduces presentation ability of T lymphocyte and inhibits the secretion of IL-1 [20]. Hormonal stress is another significant factor that can be responsible for the decrease in lymphocyte quantity, and as a result, for the weakening of cell response [15]. Particularly, cortisone is a strong immunoregulator, which quietens immunological response. Investigations proved that in patients operated with the usage of CPB, apart from the increase in the said glucocorticoid level, a higher level of catecholamines was also observed. Those changes were reflected in reduction of NK cells activity, with simultaneous lymphopenia of CD4, CD3 as well as CD 8 lymphocytes [15]. Therefore, it seems that in patients operated with the usage of invasive methods, a stronger exposure to stress can be an explanation of an increased immunosuppression level in comparison with patients operated using the OPCAB technique.

In extracorporeal circulation, apart from the decrease in the number of lymphocytes circulating in peripheral blood, an impairment of these cell functions should be also noted [21]. The ability of lymphocytes reaction to mitogen is weakened, as well as their response to alloantigen stimulation [1]. In the case of B lymphocytes, the reduction in ability of immunoglobulin synthesis is also observed [21]. It can be assumed that during the CPB, factors inhibiting the ability of lymphocytes proliferation are secreted. The analysis of results of investigations performed for this study indicates that in patients operated with the usage of CPB, apart from the decrease in lymphocyte quantity, also their function was impaired. In this group of patients, a significant increase in the number of lymphocytes with CD25 receptor for IL-2 was observed. In physiological conditions, such kind of changes should correlate with increased lymphocyte proliferation, thus an increase in the number of particular lymphocyte subpopulations. The reduction in the quantity of CD3, CD4 and CD8 lymphocytes can therefore indicate their function impairment as a result of extracorporeal circulation activity.

Conclusions

Cardiac Pulmonary Bypass, in comparison with the OPCAB technique, seems to cause stronger suppression of immunological response, expressed in decrease in lymphocyte quantity in a given subpopulation. The inhibiting of immunological response in patients operated with the usage of CPB brings about a potentially higher risk of complications in this group of patients. However, it should be noticed that though the modulation of immunological response in the OPCAB group is lower, at the same time the primary number of cells positive to a given receptor is lower than in patients operated with the usage of CPB. Thus, it could turn out that in both groups of patients, the risk of postoperative complications is similar. In patients operated using a less invasive technique this risk can be mostly dependent on the primary immunological status, while in the second group of patients it is more dependent on the influence of the immunological system. The immunological status of a patient seems to significantly determinate the possibility of inflammation response as well as occurrence of infectious complications. Therefore, it seems reasonable to include the immunological factor into the criteria of qualification to a particular type of operation. The receptors examined in this study can be useful in that kind of routine clinical diagnostics of patients operated due to ischemic heart disease in extracorporeal circulation as well as with the usage of a less invasive technique and the most important differences were observed in the mean percentage values for CD 16, CD 3, CD 8, CD 19 positive cells. The estimations of CD 25 (IL-2R) 240 min after the circulation start can be also useful in the routine diagnostics. It should be noted that the estimations of the selected receptors are relatively simple and fast, and in the case of routine clinical diagnostics, the methodological factors should be also included.

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