SCRIPTA MEDICA (BRNO) – 76 (6): 369–378, *December 2003*

PERIOPERATIVE IMMUNOLOGICAL PARAMETERS IN PATIENTS UNDERGOING CARDIAC SURGERY

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A b s t r a c t

In this study we evaluated the impact of cardiac surgery on the immune system, particularly on some commonly used laboratory parameters of cellular (CD3, CD3/4, CD3/8, CD3/HLA-DR, CD8/38, CD14/HLA-DR) and humoral immunity (acute-phase C-reactive protein; CRP). Our aim was, by immunological monitoring, to detect patients who were at increased risk of post-operative infectious complications, and had therefore poorer prognosis, as early as possible. We evaluated the predictive value of decreased expression of the HLA-DR surface marker on monocytes for the potential development of bacterial infections and, consequently, the patient's survival and the predictive value of increased expression of the HLA-DR activating marker on T- lymphocytes and increased expression of CD38 on cytotoxic lymphocytes for the potential development of viral infection. Fifty patients staying in the intensive care unit (ICU) for more than four days because of complicated post-operative outcomes due to bacterial endocarditis, other infections or circulatory problems, also usually accompanied by infections, were included in the risk group. This was further divided into survivor (SV, n=40) and non-survivor (NSV, n=10) subgroups. The control group (CG) comprised 30 consecutive patients without complications in whom pre- and post-operative immunological values were available. We used flow cytometry to assess cellular immunity parameters, and nephelometry to detect CRP levels. In the control group, most of the values of cellular immunity parameters that were markedly modified on post-operative day 1 approached the pre-operative values on day 4. However, in the risk subgroups, marked changes in these parameters remained detectable throughout the post-operative period. Persisting leukocytosis, lymphopenia and reduced HLA-DR expression on monocytes correlated well with post-operative complications and ICU stay for more than four days. Moreover, it was possible to take the reduced HLA-DR expression on monocytes as a prognostic marker for survival (SV *vs* CG, non-significant; NSV *vs* SV, $P < 0.005$). The increased expression of CD38 on cytotoxic lymphocytes (SV *vs* CG, $P<0.001$; NSV *vs* CG, $P<0.01$) and increased expression of HLA-DR on T lymphocytes (SV *vs* CG, $P<0.03$; NSV *vs* CG, $P<0.03$) were recorded in high-risk patients with prolonged ICU stay. It is speculated that these increased values may be related to cytomegalovirus reactivation, which may play a role in a slow recovery after cardiac surgery.

K e y w o r d s

Cardiac surgery, Post-operative infection, HLA-DR, Sepsis, CD38, Cytomegalovirus, Flow cytometry, Immunomonitoring, Intensive care unit patients

INTRODUCTION

Any surgery is a serious intervention in homeostatic systems of the organism, including the immune system. During cardiac surgery, the patient is subjected, in addition to the effects of anaesthesia and operative trauma, to extracorporeal circulation, contact activation of blood elements and reperfusion of ischaemic organs. As a result, systemic inflammatory response syndrome (SIRS) may develop and may further be complicated by infections, sepsis, septic shock or multi-organ failure.

It is known that SIRS is associated with counter-regulatory (compensatory) anti-inflammatory reactions aiming at prevention of undue tissue destruction from uncontrolled inflammation. These reactions may become dominant and may result in a state of immunological anergy with an increased risk of secondary infections (*1*). Monocytes are crucial components of resistance to infection. They engulf and digest pathogenic microorganisms, neutralise toxins produced by pathogens and, as antigen-presenting cells, they provide an important link between the innate resistance system and the highly specialised adaptive immune response. A decrease in monocyte HLA-DR expression has been reported to be an indicator of immunoparalysis as well as an indicator of an increased risk for septic complications and, therefore, a poor prognosis for survival in critically ill, septic patients (*1,2*). Moreover, functional paralysis of T lymphocytes after major surgery or trauma reduces cell-mediated immunity, which is fundamental in defence against viral infections. Most clinicians do not consider human cytomegalovirus (HCMV) infection to be a potential cause of various clinical manifestations, such as interstitial pneumonia, enteritis or encephalitis, in patients hospitalised in surgical intensive care units (ICU). Therefore, virological examination in these cases, which is an elaborate and expensive procedure, is the exception rather than the rule. However, recent reports have indicated that critically ill patients may be compromised by HCMV (*3*) because a pro-inflammatory reaction with T-cell activation itself may be essential to HCMV reactivation. It has been demonstrated that catecholamines directly stimulate HCMV promotors (*4*). During acute infections caused by viruses such as Epstein-Barr virus (EBV), HCMV, varicella zoster virus or influenza virus, there is an increase in CD8+ T-cell counts in the peripheral blood and lymph nodes. This expanded CD8+ population is a truly activated population because it expresses HLA-DR and CD25 as well as very high levels of CD38 antigens (*5*). This corresponds with the results of *Belles-Isles et al*. who demonstrated that, during HCMV and EBV infections in kidney transplant recipients, there was a dramatic increase in CD 3+8+38+ T-cell subset numbers in the active phase of disease (*6*).

The aim of this study was to assess parameters of cellular and humoral immunity as a means of monitoring the patient's immunological status in the early post-operative period. The results can assist us in estimating disease prognosis

and, particularly, in evaluating the patient's potential risk of bacterial and/or viral complications.

MATERIALS AND METHODS

Patients

The immunological parameters of 80 patients undergoing cardiac surgery were evaluated. The control group (CG) consisted of 30 consecutive cardiac surgery patients with a non-infectious aetiology of the underlying disease, in whom data on immunological examination were available. The risk group included 50 patients with ICU stay for more than four days who were examined for immunological parameters because their clinical post-operative outcome was associated with complications or they were at an increased risk of infectious disease due to the infectious aetiology of their underlying disease (bacterial endocarditis); of them, 10 died during hospitalisation. For the purpose of evaluation, the group was divided into the survivor (SV, n=40) and non-survivor (NSV, n=10) subgroups.

Immunological assessment

The flow cytometry analysis, using EDTA, of whole blood samples was used to detect the following cellular immunity parameters: number of total lymphocytes (Ly); CD3 surface antigen on T cells (CD3+ lymphocytes); CD3 and CD4 on T-helper cell subsets (CD3+CD4+ lymphocytes); CD3 and CD8 on cytotoxic T-cell subsets (CD3+CD8+ lymphocytes); activating HLA-DR marker on CD3+ lymphocytes (CD3+HLA-DR+ lymphocytes); activating CD38 marker on cytotoxic lymphocytes (CD3+CD8+CD38+ lymphocytes); CD 14 and HLA-DR antigens expressed on the surface on monocytes (CD14+HLA-DR+ monocytes).

Peripheral blood immunophenotyping was performed by the whole blood non-wash method using a Coulter-Q-Prep System (Beckman Coulter, USA). The samples were analysed on an Epics-XL flow cytometer (Beckman Coulter, USA) as recommended by the manufacturer. Three-colour flow cytometry was performed by using a panel of monoclonal antibodies specific for CD3, CD4, CD8, CD14, HLA-DR, CD3/HLA-DR (Becton Dickinson, USA; Beckman Coulter,USA) conjugated with FITC, PE or PerCP with appropriate isotypic controls.

Serum levels of acute-phase C-reactive protein (CRP) were quantitatively determined by nephelometry on a BN II Behring Nephelometer (Dade Behring, Germany). This method is commonly used for the immnochemical determination of protein in serum.

Total leukocyte counts were assessed on a Coulter Counter M4 (Beckman Coulter, USA) in all patients.

Samples of blood and/or other biological materials (urine, sputum, etc.) were collected for microbiological examination from 22 CG patients and all risk-group patients.

In the control group, from the immunological data, the pre-operative results and those on post-operative days 1 and 4 were used for comparison. In the risk group, only the results of post-operative examination were available. Since these risk patients were referred for immunological examination because of post-operative complications, the day of the first blood sample collection was dependent on the patient's clinical outcome and not on a regular schedule.

Statistical analysis

The results were evaluated using Student's *t*-test and expressed as mean ± standard deviation (SD) values. They are presented as numerical values in the control group and as bar graphs in the patients

RESULTS

Cardiac surgery procedures in the control and risk groups are shown in *Table 1*.

In the control group, the values of immunological parameters in the early post-operative period, i.e., day 1 and day 4 after surgery, were compared with the pre-operative values (*Table 2*). They show that, on the first post-operative day, the patients had leukocytosis, lymphopenia, a moderate decrease in CD3+ lymphocytes and CD3+CD4+ lymphocytes; there was only a moderate decrease in HLA-DR expression on monocytes and a sharp increase in the CRP level. However, most of the cellular immunity parameters approached the pre-operative values by the fourth day.

The values obtained on post-operative day 4 in the control group were further compared with the first *(Fig.1)* and last *(Fig.2)* values available in the risk group, with a separate evaluation for the surviving and non-surviving patients.

The first values available for the SV patients were on average obtained on post-operative day 7 and those for the NSV patients on post-operative day 6. They were compared with the values for day 4 in the CG patients. In both risk subgroups we found persisting leukocytosis (CG, 8.4 x 10 E9/l; SV, 13.3 x10 E9/l, $P< 0.001$; NSV, 14.4 x 10 E9/l, $P< 0.001$), lymphopenia (CG, 18%; SV, 11%, $P< 0.001$; NSV, 6%, $P< 0.0001$), reduced CD3+CD4+ lymphocytes (CG, 49%; SV, 44%, $P<0.05$; NSV, 37%, $p<0.005$), decreased HLA-DR expression on monocytes (CG, 80%; SV, 67%, $P<0.01$; NSV, 46%, $P<0.001$) and reduced CD3+ lymphocytes in the non-survival subgroup only (CG, 72%; SV, 66%, non-significant; NSV, 51%, $p< 0.0001$). Greater differences are apparent between the non-survivor and control groups than between the survivor and control groups (*Fig. 1*).

The levels of activating markers, i.e., HLA-DR on CD3+ lymphocytes (CG, 2.7%; SV, 3.9%, ns; NSV, 2.8%, ns) and CD38 on cytotoxic lymphocytes (CG, 3.7%; SV, 8.6%, $P < 0.05$; NSV, 4.5%, ns) are comparable in all groups on the first examination. There was a sharp increase in the CRP level in all groups (CG, 104 mg/ml; SV, 126 mg/ml, ns; NSV, 121 mg/ml, ns).

The last values for both risk subgroups were available on post-operative day 16. The total leukocyte counts (CG, 8.4 x 10 E9/l; SV, 11.6 x 10 E9/l, $P<0.001$; NSV, 23.2 x 10 E9/l, $P<0.0001$), total lymphocyte numbers (CG, 18%; SV, 14%, $P< 0.05$; NVS,: 6%, $P<0.0001$), CD3+ subset numbers (CG, 72%; SV, 71%, ns; NSV, 59%, $P<0.01$) and values of HLA-DR expression on monocytes CG, 80%; SV, 82%, ns; NSV, 51%, $P<0.0001$) in the survivor subgroup on day 16 approached the control values on day 4, but considerable differences remained between the control group and non-survivor subgroup (*Fig. 2*).

Furthermore, the levels of activating markers, i.e., HLA-DR on CD3+ lymphocytes (CG, 2.7%; SV, 5.7%, $P<0.001$; NSV, 5.7%, $P<0.001$) and CD38 on cytotoxic lymphocytes (CG, 3.7%; SV, 17.9%, $P<0.0001$; NSV, 18.2%, $P<0.0001$)

Table 1

Surgical procedures in the risk and control groups

Surgical procedure	Risk group (n = 50)	Control group (n = 30)
Myocardial revascularisation	16	12
Aortic valve replacement	14	12
Mitral valve replacement	7	2
Revascularisation and replacement of aortic or mitral valve	7	4
Other procedures	6	0

Table 2

Values of pre- and post-operative immunological parameters in the control group (n=30)

	Before surgery	Post-operative day 1	Post-operative day 4	Before surgery	Post-operative day 1	Post-operative day 4	Before surgery	Post-operative day 1	Post-operative day 4
Range of values	Leu 4-10x10E9/l			Ly 20–55%			CD3 58–85%		
Mean	6.24	12.28*	8.35*	26.40	7.00*	18.10*	71.60	60.57*	71.67
Min	3.20	7.00	4.30	14.00	3.00	6.00	36.00	36.00	46.00
Max	9.90	18.10	14.10	38.00	15.00	30.00	87.00	78.00	82.00
S D	1.64	2.78	2.27	5.80	2.90	5.60	10.36	10.36	8.36
Range of values	CD3/4 30–60%			CD3/8 15–35%			CD3/DR 2.5–6.0%		
Mean	45.10	36.07*	49.40	23.57	21.93	20.63*	3.38	3.83	2.72
Min	19.00	18.00	36.00	7.00	10.00	9.00	0.80	0.70	0.30
Max	78.00	58.00	63.00	58.00	42.00	41.00	8.90	11.50	7.60
S D	12.36	9.06	8.00	11.09	9.18	7.77	2.27	2.34	1.74
Range of values	CD3/8/38 0–20%			CD14/DR 90–100%			CRP 0–10 mg/l		
Mean	3.73	4.45	3.73	97.20	81.70*	79.93*	7.07	71.57*	104*
Min	0.20	0.70	0.70	91.00	51.00	58.00	5.00	31.00	37.00
Max	16.00	17.00	19.40	99.90	99.00	99.00	22.00	135	185
S D	3.87	3.79	4.29	2.34	12.42	13.33	4.53	20.84	38.68

*, statistically significant at $P<0.01$

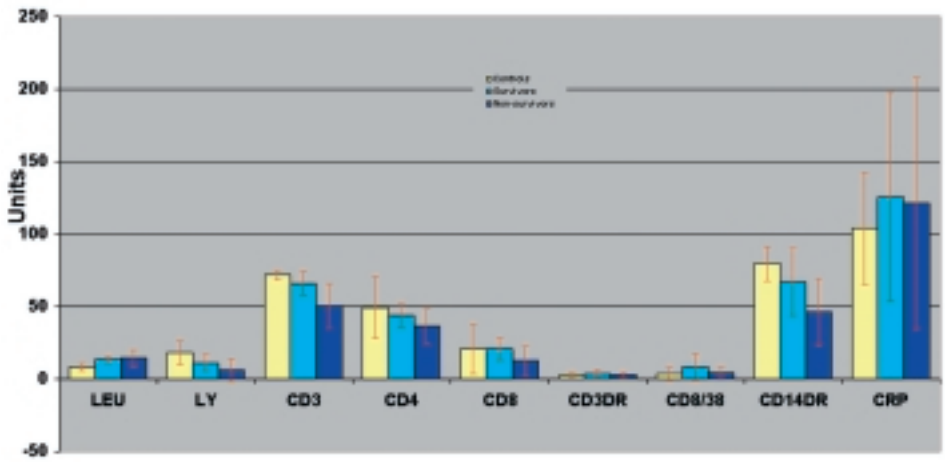

Fig. 1

Immunological parameters in the control and risk groups. In the control group, they were assessed on post-operative day 4. In the survivor and non-survivor subgroup, the values were obtained on average on post-operative days 6 and 7, respectively. **LEU**, total leukocyte counts (x 10^9 cells/l). **LY**, total lymphocyte numbers; **CD3**, CD3+ lymphocytes; **CD4**, CD3+CD4+ lymphocytes; **CD8**, CD3+CD8+ cytotoxic lymphocytes; **CD3DR**, CD3+ HLA-DR+ lymphocytes; **CD8/38**, CD3+ CD8+CD38+ lymphocytes; **CD14DR**, CD14+HLA-DR+ monocytes; the values are expressed in percentages. **CRP**, C-reactive protein (mg/l).

were increased in both risk subgroups. There was a sharp increase in the CRP level in all groups (CG, 104 mg/ml; SV, 98 mg/ml, ns; NSV, 103 mg/ml, ns).

Microbiological findings were positive in 13 CG patients (43%), in 36 SV patients (90%) and in 10 NSV patients (100%).

DISCUSSION

Patients after cardiac surgery generally suffer from mild leukocytosis and moderate to severe lymphopenia, as indicated by immunological markers in our control group; this may be caused by haemodilution and partially also by re-distribution of lymphocytes among the bone marrow, lymphatic tissue and peripheral blood. T lymphocyte counts were reduced particularly in CD3+CD4+ lymphocytes; a decrease in T-helper cell subset numbers has been associated with a high risk of infections, especially those caused by potentially pathogenic microorganisms (*7*).

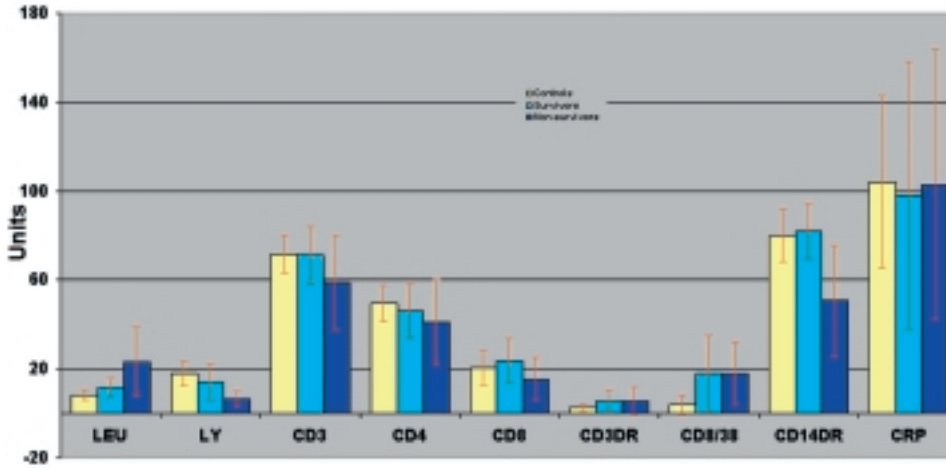

Fig. 2
Immunological parameters in the control and risk groups. In the control group, they were assessed on post-operative day 4. In both the survivor and non-survivor subgroups, the values were obtained on average on post-operative day 16. **LEU**, total leukocyte counts (x 10^9 cells/l). **LY**, total lymphocyte numbers; **CD3**, CD3+ lymphocytes; **CD4**, CD3+CD4+ lymphocytes; **CD8**, CD3+CD8+ cytotoxic lymphocytes; **CD3DR**, CD3+ HLA-DR+ lymphocytes; **CD8/38**, CD3+ CD8+CD38+ lymphocytes; **CD14DR**, CD14+HLA-DR+ monocytes; the values are expressed in percentages. **CRP**, C-reactive protein (mg/l).

Our results showed that, out of the immunological parameters tested, those correlating best with the clinical status of patients were the total leukocyte count, total lymphocyte number, percent of monocytes carrying HLA-DR antigens, and percent of CD3+lymphocytes and CD3+CD4+lymphocytes. It is in agreement with our findings that a decrease in HLA-DR expression on monocytes is often described as a sign of post-operative and post-traumatic immunosuppression (*8*). The occurrence of HLA-DR antigens is closely associated with the role of monocytes as antigen-presenting cells (*9*). Monocytes can be de-activated by anti-inflammatory cytokines, particularly IL-10, TGF-beta and PGE_2 prostaglandin (*10*). The post-traumatic reaction also has an effect on hormone production or catecholamine levels, which may increase and affect HLA-DR expression on monocytes (*7*).

Bacterial lipopolysaccharides can also act as inhibitors of the monocyte-macrophage system. When bound to CD14 lipopolysaccharide receptors, either membrane-associated or soluble, they may trigger SIRS onset. If a compensatory

anti-inflammatory response is not sufficient and there is an overproduction of pro-inflammatory cytokines, sepsis and multi-organ failure may develop. On the other hand, if the compensatory anti-inflammatory response becomes predominant, this gives rise to compensatory anti-inflammatory response syndrome (CARS), that may eventually lead to the development of immunological anergy; the patient suffering from persistent primary infection may further be endangered by secondary infections. CARS is characterised by reduced HLA-DR expression on monocytes, an increase in the synthesis of anti-inflammatory cytokines (IL-10, TGF-beta and PGE_2) and a decrease in pro-inflammatory cytokines, which results in inhibition of the cell-mediated immune response. The condition in which the proportion of monocytes expressing HLA-DR remains lower than 40% for a minimum of 2 days is known as immunoparalysis. If this state lasts longer than 7 days, it implies a poor prognosis for the patient (*1, 2, 11–14*).

Our results did not unanimously confirm a causal relationship between immunoparalysis and the subsequent development of an uncontrolled infectious disease although there were positive bacterial or fungal findings in most of the risk patients (SV, 90%; NSV, 100%). However, the relationship between persisting reduced expression of the HLA-DR on monocyte and a poor disease prognosis and that between an increase in HLA-DR expression on monocytes and clinical outcome improvement were confirmed. The mechanism responsible for the development of immunoparalysis should not be associated with infectious causes only. Low HLA-DR expression on monocytes does not necessarily indicate sepsis and, on the other hand, a patient with sepsis need not be in the state of immunoparalysis. Larger groups of patients will be needed to analyse and draw conclusions from relationships between HLA-DR expression on monocytes and various clinical manifestations unrelated to infectious aetiology, such as cardiac tamponade, bilirubinemia, etc., or relationships of CD14DR expression to other markers, such as procalcitonine, that indicate the presence of sepsis (*15, 16*).

The frequent activation of herpetic infections in ICU patients, including other than cardiac surgery patients, is well confirmed and opens the question of the role of viral infections in the patient's post-operative outcome and recovery (*5,6,17,18,19*). Diseases suspected of being related to HCMV infection include restenosis following cardiac surgery, interstitial pneumonia rapidly developing into respiratory insufficiency, hepatitis, meningitis and other diseases (*19*). In our patients, the potential development of viral (particularly HCMV) infection was monitored by means of an increase in CD38 expression on cytotoxic T cells. This was usually accompanied by an increase in HLA-DR expression on CD3+lymphocytes. In both risk subgroups, a gradual increase in CD3+CD8+CD38+lymphocytes was observed in the patients with a prolonged ICU stay. At this stage our immunological findings can only be taken as suggestive of HCMV reactivation and must further be confirmed by other

laboratory methods for CMV detection (anti-CMV antibodies or PCR detection of CMV-DNA). This will be the topic of our continuing study.

In this context, the question of whether prolonged ICU hospitalisation, slower post-operative progress, need for long-term mechanical ventilation, the presence of intermittent fever, lassitude and apathy resulting in poor communication, and other potential complications are due to a viral infection or whether a viral infection is one of the manifestations of the patient's overall clinical status still remains to be answered. Besides, if we consider the post-operative development of infectious complications to be a simultaneous action of viral and bacterial agents, it is very difficult to distinguish which of the agents is responsible.

Moreover, there are further questions to be answered. Can a high-risk cardiac-surgery patient, in whom a decrease in cellular immunity has been documented, still be regarded as "immunocompetent" in the post-operative period or is he/she jeopardised by infection to a similar degree as a patient regarded as "immunocompromised" ? In other words, are viral infections as dangerous to high-risk cardiac-surgery patients as they are to organ transplant recipients?

A c k n o w l e d g e m e n t

The authors thank to Professor RNDr. Anna Gerylovová, Faculty of Medicine in Brno, and Dr. Jiří Damborský, National Centre for Research on Biomolecules in Brno, for their assistance with statistical data processing.

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PERIOPERAČNÍ IMUNOLOGICKÉ PARAMETRY U KARDIOCHIRURGICKÝCH PACIENTŮ

S o u h r n

Studovali jsme ovlivnění imunologických parametrů kardiochirurgickou operací. Cílem byla selekce pacientů s vyšším rizikem rozvoje infekčních komplikací již v časném pooperačním období. Zhodnocení role exprese HLA-DR povrchových znaků na monocytech v predikci možného rozvoje septických komplikací a prognózy dalšího klinického vývoje u již septických nemocných a úlohy zvýšené exprese CD38 znaku na cytotoxických lymfocytech svědčící pro možnou reaktivaci latentního CMV. Metodou průtokové cytometrie byly vyšetřeny následující parametry buněčné imunity:zastoupení lymfocytů, exprese znaků CD3, CD3/4, CD3/8, CD3/HLA-DR, CD8/38 na lymfocytech a CD14/HLA-DR na monocytech. Nefelometricky byl v séru kvantifikován parametr humorální imunity C-reaktivní protein akutní fáze (CRP). Kontrolní skupinu tvořilo 30 pacientů s nekomplikovaným pooperačním průběhem. Imunologické parametry byly vyšetřeny před operací, první a čtvrtý pooperační den. Do rizikové skupiny bylo zařazeno 50 pacientů s komplikovaným pooperačním průběhem. Většina ze sledovaných buněčných parametrů se v kontrolní skupině čtvrtý pooperační den blížila hodnotám předoperačního vyšetření. Přetrvávající leukocytóza, lymfopenie a snížená exprese HLA-DR znaku na monocytech u rizikových pacientů dobře korelovala s horší prognózou . Zvýšená exprese CD38 znaku na cytotoxických lymfocytech a zvýšená exprese HLA-DR na T lymfocytech zejména u dlouhodobě hospitalizovaných svědčí pro možnou reaktivaci CMV a možný podíl virových infekcí na pomalé rekonvalescenci a neuspokojivém klinickém stavu.

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