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Hematology and Oncology: Current Research

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Systemic Cytokine Response during the Extracorporeal Circulation in Patients with Myocardial Revascularization

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Received: Nov 01, 2023

Accepted: Nov 27, 2023

Published Online: Nov 31, 2023

Journal: Hematology and Oncology: Current Research

Publisher: MedDocs Publishers LLC

Online edition: http://meddocsonline.org/

Keywords: Sirs; Cardiac surgery; Cytokines; Extracorporeal circulation.

Abstract

Revascularization of the myocardium under conditions of extracorporeal circulation cause clinical problems related to the effects of the intervention itself on the immune system and inflammatory response, especially systemic inflammatory response (Engl. Systemic Inflammatory Response Syndrome, SIRS).The aim of this study was to determine whether the use of extracorporeal blood flow system during surgical revascularization of the myocardium leads to an increased systemic inflammatory response, which contributes to the development of postoperative complications. This prospective study included 100 patients who underwent single, double and triple surgical revascularization of the myocardium, at the Clinic for Cardiac Surgery of CC Niš, in the period from January 15., 2020. until January 15, 2021. 22 female persons - 22.0% and 78 male persons - 78.0%) were included. Preoperatively, as well as 8 hours after the surgical intervention, the following parameters were determined for the subjects: blood count (erythrocyte count - Rbc, hemoglobin - Hgb, hematocrit - Hct, leukocyte count - Wbc, platelet count - Plt) and CRP, soluble CD14 molecule (sCD 14) - presepsin, levels of the following cytokines: TNFα, IL-1β, IL-2, IL-4, IL-6, IL-10, IL-12 p70 and IFN γ. Patients in whom the extracorporeal circulation method was applied had, postoperatively, a higher concentration of CRP ($p<0.001$), as well as a concentration of presepsin ($p< 0.05$) - a greater number of patients had a concentration of presepsin above the limit of 600 pg/ mL ($p< 0.01$). Four patients out of 51 (7.84%) in the ECB group had elevated presepsin values over 1000 pg/ml and high risk according to clinical criteria for the development of systemic bacterial infection and sepsis. No such patients were recorded in the off pump group. Also, in the group where the extracorporeal blood flow system was applied, 17.64% of patients (9/51) met the clinical criteria for SIRS with elevated presepsin values over

Cite this article: Lazarević M, Lazarević Z, Golubović M, Perić V, Marjanović P, et al. Systemic Cytokine Response During the Extracorporeal Circulation in Patients with Myocardial Revascularization. Hematol Oncol Curr Res. 2023; 6(2): 1023.

600 pg/mL, while in the other group of patients, 2 out of 49 (4.08%) met the clinical criteria and laboratory criteria for SIRS. Except for TNFa, the levels of IL-1β, IL-2, IL-6 and IL-10 showed an increase after the intervention in all groups compared to the preoperative period (p< 0.01). However, comparing the degree of cytokine increase in the postoperative period between the control (off pump) and the extracorporeal blood flow group did not show a statistical difference (IL-2; p=0.881, IL-1; p=0.709, IL-6; p=0.911, IL- 10, p=0.179). In the postoperative period, a higher incidence rate of SIRS was noted in patients in whom the ECB system was used, which also indicates its importance in the development of systemic inflammation and immune dysregulation.

Introduction

Surgical myocardial revascularization is a surgical procedure with a mortality of about 1% in elective cases. A condition for the controlled performance of this surgical procedure is the application of an Extracorporeal Blood Flow Device (ECB), which during the intervention ensures tissue oxygenation and thermoregulation, replacing cardiac action and pulmonary circulation. The greatest danger in its performance lies in ischemia-reperfusion injuries of the myocardium after revascularization with the development of hemorrhagic infarction with all its complications [1]. Revascularization of the myocardium under conditions of extracorporeal circulation has also opened up problems related to the effects of the intervention itself on the immune system and inflammatory response [2]. Due to its importance on the morbidity and mortality of patients, the appearance of a generalized, systemic inflammatory response (Engl. Systemic Inflammatory Response Syndrome, SIRS).

The strength of the inflammatory response depends on several factors: Biomaterials used oxygenator components, patient-related factors (age, sex, comorbidities) and surgical factors in terms of length of operation and surgical technique. In addition, the effects of anesthetic agents, techniques of perioperative myocardial protection and the use of pharmacological cardiosurgical supports can collectively modify the immune response and affect its characteristics [3].

Basically, the onset of the inflammatory response is considered to be a consequence of blood exposure to artificial materials as well as sheer stress during the passage of blood through an extracorporeal circulation machine. Then there is a disturbance of coagulation, activation of leukocytes and the complement system, as well as the release of inflammatory and vasoactive substances into the bloodstream [4].

Various authors have shown that cytokines have a crucial pathogenetic importance in the initiation and later maintenance of SIRS and sepsis, and their determination would also enable early diagnosis of these disorders. However, it is insufficiently known how the use of the extracorporeal blood flow system affects the level of cytokines of innate (IL-1β; IL-6 and TNF-α) and acquired immunity in terms of the polarization of the immune response in the direction of Th1 (IL-12p70 and IFN-γ), Th2 (IL-4), Th17 (IL-17) and regulatory T cells (IL-10) and how these changes are reflected in the postoperative recovery of patients. Today, special attention is paid to IL-6, which has been shown to increase the concentration of C-Reactive Protein (CRP). The soluble form of the CD14 molecule (presepsin) is also one of the more recently defined, early parameters of systemic inflammation. In the proposed study, by determining the level of the mentioned parameters in patients who undergo cardiac surgery with or without the use of extracorporeal blood flow systems as well as their postoperative monitoring, the systemic immune response would be looked at and parameters for the early identification of patients at risk of developing SIRS or sepsis would be determined. These findings would enable timely identification of high-risk patients in whom there is a possible need for additional therapeutic action.

The aim of this research was to determine whether the use of an extracorporeal blood flow system during myocardial surgical revascularization leads to an increased systemic inflammatory response of the body, which contributes to the development of postoperative complications. Analysis of the systemic cytokine response in these patients would indicate the influence of extracorporeal blood flow on the polarization of the immune response in the direction mediated by Th1 or Th2 cells.

Materials and Methods

This prospective study included 100 patients who underwent single, double and triple surgical revascularization of the myocardium, at the Clinic for Cardiac Surgery of CC Niš, in the period from January 15., 2020. Until January 15, 2021. 22 female persons - 22.0% and 78 male persons - 78.0%) were included. The average age of the studied population was 64.63±7.48 years (from 43 to 80 years).

After preoperative preparation, patients were operated according to standard cardiac surgical protocols. Patients were divided into 2 groups, group A (EKK group) in which a device for extracorporeal blood flow was used during the intervention (51 subjects) and group B (Off pump group), in which this system was not applied intra operatively (49 patients).

Preoperatively, as well as 8 hours after the surgical intervention, the following parameters were determined for the subjects:

1. blood count (erythrocyte count - Rbc, hemoglobin - Hgb, hematocrit - Hct, leukocyte count - Wbc, platelet count - Plt) and CRP (ng/ml) on the hematological analyzer Microsemi CRP LC-667G (Horiba Medical, France), from 4 mL of whole blood sampled in a tube with EDTA anticoagulant.

2. Soluble CD14 molecule (sCD 14) –presepsin (pg/ml), by the immunofluorescence method, using a cartridge on the Path fast device (Mitsubishi, Japan) from 4 mL of the patient's whole blood sampled in a test tube with EDTA anticoagulant.

   Presepsin values above 600 pg/mL were considered a state of presepsis, and this is also the cut-off value for distinguishing systemic inflammatory response syndrome (SIRS) from a septic state.

3. Levels of the following cytokines: TNFα, IL-1β, IL-2, IL-4, IL-6, IL-10, IL-12 p70 and IFN γ (pg/ml), by ELISA technique from serum obtained by centrifugation of a tube with 4 ml of sampled blood without anticoagulants.

The Magnetic Luminex® Performance assay was used to determine cytokines. Standard cytokine cocktail detectors, produced by Biotechne RnD systems, were used, and the reading was performed on a Flex Map3D-invitrogen flow analyzer, Thermo Fisher scientific, according to the manufacturer's instructions.

Early postoperative complications in terms of SIRS, sepsis and septic shock were monitored. According to the American Society of Anesthesiologists, the diagnosis of SIRS was made if two or more parameters were present: Temperature ≥ 38^{0}C or ≤ 36^{0}C, tachycardia ≥ 90/min, tachypnea ≥ 20/min or pCO_2 ≤ 32 mmHg, leukocytes ≥ 12,000 or ≤ 4000 /mm^3. Sepsis was defined as SIRS with proven infection; septic shock as sepsis with hypotension and marked organ hypoperfusion.

Statistical data processing

The data are presented in the form of arithmetic mean and standard deviation, minimum and maximum values, as well as in the form of absolute and relative numbers.

The normality of continuous variables was tested with the Kolomogor-Smirnov test. If the data distribution was normal, the preoperative and postoperative values were compared using the ANOVA test. If the data distribution is not normal, the Friedman test was used for this comparison. If the data distribution is normal, the comparison between the two groups was performed using the t test, if the data distribution is not normal, this comparison was performed using the Mann-Whitney test. The hypothesis was tested with a significance threshold of p<0.05. Data analysis was performed in the IBM SPSS 26.0.0 software package.

Results

Type of surgical revascularisation and basic parameters

The prospective study included 100 patients who underwent single, double and triple surgical revascularization of the myocardium, at the Cardiac Surgery Clinic of the CC Niš, in the period from January 15., 2020. Until January 15, 2021.

Figure 1 shows the representation of certain types of surgical revascularization - single, double or triple aorto-coronary bypass (CABG I, II or III). The largest number of patients (43.0%) underwent CABG I.

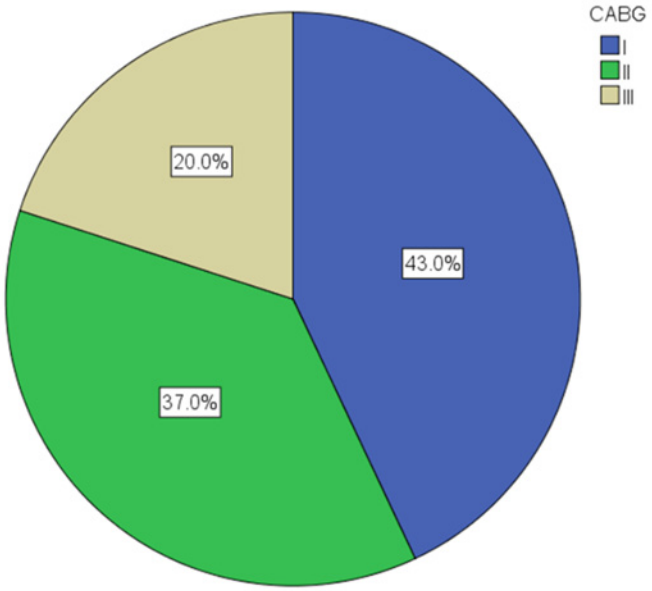

Figure 1: Representation of single, double and triple aorto-coronary bypass (CABG) in the studied group of patients.

Patients were divided into two groups in relation to the use of devices for extracorporeal blood flow. This method was used in 51 patients (51.0%).

Table 1 shows the intergroup differences in the preoperative parameters of the patients.

The only difference found between the two groups was in the type of surgical intervention - a device for extracorporeal blood flow was used more often in patients underwent multiple aorto-coronary bypass (p< 0.001). All subjects had normal preoperative presepsin values (≤ 600 pg/mL).

Table 1: Basic characteristics and preoperative parameters in patients with and without the use of an Extracorporeal Blood Flow Device (ECB).

		ECB (N=51)	off ECB (N=49)	t* ili χ^{2}** ili Z*** (p)
Age		63,90±7,49	65,39±7,48	0,992 (0,323)*
Gender (male)		39 (76,5%)	39 (79,6%)	0,018 (0,811)**
CABG	I	9 (17,6%)	34 (69,4%)	**35,834 (0,000)****
	II	22 (43,1%)	15 (30,6%)	**5,916 (0,000)*****
	III	20 (39,2%)	0 (0,0%)	
RBC (1x10^{12}/L)		4,45±0,46	4,49±0,49	0,376 (0,707)*
HGB (g/L)		136,80±11,68	138,80±10,25	0,905 (0,368)*
HCT (%)		38,78±4,48	39,58±3,80	0,965 (0,337)*
WBC (1x10^9/L)		7,34±1,51	6,95±1,13	1,431 (0,156)*
PLT (1x10^9/L)		241,72±70,05	232,51±56,70	0,721 (0,472)*
CRP (ng/mL)		9,0 (4,0-14,0)	10,0 (5,0-13,0)	0,252 (0,801)***
PSEP (pg/mL)		246,0 (167,0-366,0)	235,0 (166,0-337,0)	0,538 (0,591)***
PSEP (≥600pg/mL)		0 (0,0%)	0 (0,0%)	

* Student's t-test; ** χ2-test; *** Mann-Whitney U-test (CABG: aorto-coronary bypass; RBC: erythrocytes; HGB: hemoglobin; HCT: hematocrit; WBC: leukocytes; PLT: platelets; CRP: C reactive protein; PSEP - presepsin).

Table 2: Basic characteristics and preoperative parameters in patients with and without the use of an Extracorporeal Blood Flow Device (ECB).

	ECB (N=51)	off ECB (N=49)	t* ili χ^{2}** ili Z*** (p)
RBC (1x10^{12}/L)	3,9 (3,6-4,3)	3,7 (3,5-4,0)	1,880 (0,060)***
HGB (g/L)	115,0 (110,0-119,0)	114,0 (108,5-116,0)	1,693 (0,090)***
WBC (1x10^9/L)	10,76±3,69	9,34±3,56	1,960 (0,053)*
PLT (1x10^9/L)	154,67±59,75	141,33±53,00	1,179 (0,241)*
CRP (ng/mL)	110,0 (90,0-132,0)	92,0 (76,5-104,5)	3,552 (0,000)***
PSEP (pg/mL)	419,0 (315,0-625,0)	343,0 (300,0-419,5)	2,552 (0,011)***
PSEP (≥ 600 pg/mL)	14 (27, 5%)	2 (4, 1%)	8,490 (0,002)**

*Student's t-test; ** χ2-test; *** Mann-Whitney U-test (RBC: erythrocytes; HGB: hemoglobin; HCT: hematocrit; WBC: leukocytes; PLT: platelets; CRP: C reactive protein, PSEP: presepsin).

Eight hours after the surgical intervention, hematological parameters and the concentration of CRP, presepsin and cytokines concentration were measured again. Postoperatively, there was a decrease in the number of erythrocytes (p< 0.001), hemoglobin (p< 0.001), the number of platelets (p< 0.001), and an increase in the number of leukocytes (p< 0.001), an increase in the concentration of CRP (p< 0.001) and presepsin (p< 0.001). An identical change was found in the group of patients with ECB, with a statistical significance of p< 0.001 for all measured variables.

Influence of extracorporeal circuit on basic postoperative parameters

Patients in whom the extracorporeal circulation method was applied had, postoperatively (**Table 2**.), a higher concentration of CRP (p< 0.001), as well as a concentration of presepsin (p< 0.05) - a greater number of patients had a concentration of presepsin above the limit of 600 pg/ mL (p< 0.01).

In patients in whom ECB was applied, postoperatively (**Table 3**) there was a smaller absolute decrease in hemoglobin value (p< 0.05). In addition, a greater increase in the absolute concentrations of CRP (p< 0.001) and presepsin (p<0.001) was found. However, analyzing the relative changes in CRP levels does not show a difference between patients on ECB compared to patients without (p= 0.137). On the other hand, a greater relative increase in the concentration of presepsin (p< 0.01) was found in patients on ECB in the postoperative period.

Four patients out of 51 (7.84%) in the ECB group had elevated presepsin values over 1000 pg/ml and high risk according to clinical criteria for the development of systemic bacterial infection and sepsis. No such patients were recorded in the off pump group. Also, in the group where the extracorporeal blood flow system was applied, 17.64% of patients (9/51) met the clinical criteria for SIRS with elevated presepsin values over 600 pg/mL, while in the other group of patients, 2 out of 49 (4.08%) met the clinical criteria and laboratory criteria for SIRS.

Table 3: Absolute and relative differences between postoperative and preoperative parameters in patients with and without the use of extracorporeal blood flow devices.

	ECB (N=51)	off ECB (N=49)	t* ili Z** (p)
Absolute differencies			
WBC (1x10^9/L)	3,8 (0,1-6,2)	2,2 (-0,2-5,2)	1,393 (0,164)**
CRP (ng/mL)	100,0 (89,0-119,0)	79,0 (68,0-98,0)	4,072 (0,000)**
PSEP (pg/mL)	200,0 (104,0-377,0)	109,0 (59,0-189,5)	3,427 (0,001)**
Relative changes			
WBC (1x10^9/L)	1,47 ±0.45	1,37 ±0.53	1,051 (0,296)*
CRP (ng/mL)	11.6(9.1-23.8)	9,9 (5.6-18.3)	1.486 (0,137)**
PSEP (pg/mL)	1.9 (1.5-2.5)	1,5 (1.2-2.0)	2,748 (0,006)**

*Student's t-test; ** Mann-Whitney U-test (RBC: erythrocytes; HGB: hemoglobin; HCT: hematocrit; WBC: leukocytes; PLT: platelets; CRP: C reactive protein; PSEP: presepsin).

Table 4: Cytokine levels before and after the intervention.

	Cytokine	ECB	Off ECB	t* or Z** (p)
Before operation	TNFα (pg/ml)	261.39±53.12	239.03±44.23	2.283 (0.025)*
	IL-2 (pg/ml)	47.5 (43.0-49.6)	45.3 (40.6-47.5)	1.828 (0.068)**
	IL-1β (pg/ml)	17.2 (15.6-20.1)	18.7 (13.7-22.6)	0.962 (0.336)**
	IL-6 (pg/ml)	79.9 (70.0-92.3)	84.8 (54.7-109.0)	0.017 (0.986)**
	IL-10 (pg/ml)	32.26±18.43	36.69±19.19	1.151 (0.252)*
After operation	TNFα (pg/ml)	261.2 (232.8-315.5)	246.7 (221.3-271.5)	1.914 (0.056)**
	IL-2 (pg/ml)	65.8 (41.7-81.7)	62.6 (47.5-81.5)	0.445 (0.656)**
	IL-1β (pg/ml)	34.2 (25.8-40.1)	30.9 (22.6-39.1)	0.528 (0.598)**
	IL-6 (pg/ml)	905.20±361.19	857.30±399.69	0.629 (0.531)*
	IL-10 (pg/ml)	114,9 (93.9-151.2)	85.9 (58.6-146.3)	2.500 (0.012)**

Tnfa: Tumor Necrosis Factor Alpha; ECB: Extra Corporeal Blood Flow.

Cytokines response

Patients that were not submitted to ECB had lower pre-operative levels of TNFα (p< 0.05), as well as lower post-operative levels of IL-10 (p< 0.05).

After comparing the preoperative and postoperative values (**Table 5**), we have observed that, with an exception of TNFα, there was a statistically significant increase in all interleukin levels measured in all study patients (p< 0.001), as well as in both subgroups – ECB (p< 0.001) and off ECB (p< 0.001). When assessing the absolute increase in cytokine levels, the only statistically significant difference found was in IL-10 changes – greater increase (by 125.3 vs. 77.3) was calculated in ECB patients (p< 0.05).

Table 5: Preoperative vs. postoperative levels of cytokines in ECB, off ECB patients and total study group (Wilcoxon signed rank test Z (p)).

Cytokine	ECB	Off ECB	Total
TNFα (pg/ml)	0.825 (0.409)	1.547 (0.122)	1.565 (0.118)
IL-2 (pg/ml)	5.415 (0.000)	5.410 (0.000)	7.532 (0.000)
IL-1β (pg/ml)	6.095 (0.000)	5.000 (0.000)	7.888 (0.000)
IL-6 (pg/ml)	6.216 (0.000)	6.025 (0.000)	8.658 (0.000)
IL-10 (pg/ml)	6.216 (0.000)	6.094 (0.000)	8.682 (0.000)

Tnfa: Tumor Necrosis Factor Alpha; ECB: Extra Corporeal Blood Flow.

After comparing the preoperative and postoperative values, we have observed that, with an exception of TNFα, there was a statistically significant change in all interleukin levels measured in all CABG I patients, as well as in both subgroups (**Table 6**). When assessing the absolute increase in cytokine levels, there were no statistically significant differences between ECB and off ECB patients (**Table 7**).

Table 6: Preoperative and postoperative levels of cytokines in CABG I patients.

	Cytokine	ECB	Off ECB	t* or Z** (p)
Preoperative	TNFα (pg/ml)	259.13±54.78	253.31±30.79	0.387 (0.978)*
	IL-2 (pg/ml)	46.43±4.97	44.89±3.54	1.103 (0.275)*
	IL-1β (pg/ml)	18.7 (15.6-20.1)	21.4 (15.6-28.3)	1.643 (0.100)**
	IL-6 (pg/ml)	83.75±25.30	67.72±26.40	2,062 (0.044)*
	IL-10 (pg/ml)	20.9 (15.0-48.3)	42.2 (15.0-57.6)	2.071 (0.038)**
After operation	TNFα (pg/ml)	267.06±65.04	256.97±35.81	0.725 (0.472)*
	IL-2 (pg/ml)	62.6 (49.6-81.7)	57.4 (47.5-70.5)	0.648 (0.517)**
	IL-1β (pg/ml)	34.2 (25.8-42.3)	33.0 (29.6-46.2)	0.600 (0.549)**
	IL-6 (pg/ml)	913.70±298.43	869.42±394.23	0.446 (0.658)*
	IL-10 (pg/ml)	114 (93.9-151.2)	135.7 (91.0-175.7)	0.358 (0.721)**

Tnfa: Tumor Necrosis Factor Alpha; ECB: Extra Corporeal Blood Flow.

Table 7: Preoperative vs. postoperative levels of cytokines in ECB, off ECB patients and total study group (Wilcoxon signed rank test Z (p)).

Cytokine	ECB	Off ECB	Total
TNFα (pg/ml)	0.852 (0.394)	0.285 (0.776)	0.745 (0.456)
IL-2 (pg/ml)	4.473 (0.000)	3.076 (0.002)	5.348 (0.000)
IL-1β (pg/ml)	5.347 (0.000)	2.222 (0.026)	5.740 (0.000)
IL-6 (pg/ml)	5.445 (0.000)	3.418 (0.001)	6.395 (0.000)
IL-10 (pg/ml)	5.445 (0.000)	3.418 (0.001)	6.395 (0.000)

After comparing the preoperative and postoperative values, we have observed that in patients who received CABG II intervention, with an exception of TNFα, there was a statistically significant increase in all interleukin levels measured in all study patients (p< 0.001), as well as in both subgroups – ECB (p< 0.001) and off ECB (p< 0.001) (**Table 8**). When assessing the absolute change in cytokine levels, there were no statistically significant differences between ECB and off ECB patients (**Table 9**).

Table 8: Preoperative and postoperative levels of cytokines in CABG II patients.

	Cytokine	ECB	Off ECB	t* or Z** (p)
Preoperative	TNFα (pg/ml)	268.73±48.83	232.73±48.06	2.222 (0.031)*
	IL-2 (pg/ml)	45.8 (41.2-52.4)	45.3 (41.5-47.5)	0.642 (0.521)**
	IL-1β (pg/ml)	16.13±5.03	18.45±6.65	1.103 (0.276)*
	IL-6 (pg/ml)	75.0 (55.6-106.4)	92.3 (57.4-175.4)	0.602 (0.547)**
	IL-10 (pg/ml)	43.36±17.52	35.75±19.82	1.175 (0.246)*
After operation	TNFα (pg/ml)	260.0 (238.7-309.2)	246.7 (201.8-271.5)	1.578 (0.114)**
	IL-2 (pg/ml)	72.34±13.66	68.37±21.38	0.600 (0.551)*
	IL-1β (pg/ml)	30.0 (17.3-39.4)	30.9 (22.0-35.3)	0.075 *0.940)**
	IL-6 (pg/ml)	877.57±533.15	851.95±407.84	0.172 (0.864)*
	IL-10 (pg/ml)	126.0 (84.9-398.3)	71.4 (56.2-139.9)	2.706 (0.007)**

Tnfa: Tumor Necrosis Factor Alpha; ECB: Extra Corporeal Blood Flow.

Table 9: Preoperative vs. postoperative levels of cytokines in ECB, off ECB patients and total study group (Wilcoxon signed rank test Z (p)).

Cytokine	ECB (N=12)	Off ECB	Total
TNFα (pg/ml)	0.474 (0.636)	1.411 (0.158)	1.459 (0.145)
IL-2 (pg/ml)	3.078 (0.002)	4.361 (0.000)	5.348 (0.000)
IL-1β (pg/ml)	3.078 (0.002)	4.833 (0.000)	5.579 (0.000)
IL-6 (pg/ml)	3.078 (0.002)	4.969 (0.000)	5.831 (0.000)
IL-10 (pg/ml)	3.078 (0.002)	5.089 (0.000)	5.907 (0.000)

Tnfa: Tumor Necrosis Factor Alpha; ECB: Extra Corporeal Blood Flow.

Moderate positive correlation was observed between postoperative TNFα levels and preoperative TNFα (p< 0.001), postoperative IL-2 (p< 0.001) and IL-1b (p< 0.001), as well as between postoperative IL1b, IL-2 and IL-6 (p< 0.001). Week negative correlation was observed between preoperative IL-10 and preoperative TNF α (p< 0.01) and IL-6 (p< 0.05) and postoperative IL-2 (p< 0.01), as well as between postoperative IL-10 and preoperative IL-6 (p< 0.01) and postoperative IL-2 (p< 0.05) (**Table 10**).

Table 10: Correlation between cytokines.

	Pre opIL-2	**Pre op**IL-1beta	**Pre op**IL-6	**Pre op**IL-10	***After op*** TNF alpha	***After op*** IL-2	***After op*** IL-1beta	***After op*** IL-6	***After op*** IL-10
Pre op TNF alpha	**.167***	.003	.006	**-.180***	**.400****	.026	**.161***	-.052	.127
	.019	.962	.929	**.010**	**.000**	.709	**.020**	.447	.065
Pre op IL-2		.088	.029	.001	.018	.093	.079	.015	-.122
		.225	.683	.986	.799	.196	.268	.836	.087
Pre op IL-1beta			**.154***	-.006	.050	.020	.022	.121	-.007
			.029	.928	.475	.781	.758	.087	.917
Pre op IL-6				**-.150***	-.026	-.053	.091	**.193****	**-.184****
				.033	.705	.450	.188	**.005**	**.008**
Pre op IL-10					-.105	**-.154***	-.096	-.125	.062
					.134	**.030**	.174	.074	.372
After op TNF alpha						**.316****	**.377****	**.245****	-.037
						.000	**.000**	**.000**	.596
After op IL-2							**.307****	**.329****	**-.141***
							.000	**.000**	**.043**
After op IL-1beta								**.437****	-.086
								.000	.215
After op IL-6									.009
									.896

Average presepsin level was 231.70±111.06, without any significant difference between ECC and off ECC patients (t=0.816, p=0.417), but higher in CABG II patients compared to CABG I (265.24±120.79 vs. 203.13±94.04, t=0.832, p< 0.01).

Presepsin was moderately positively correlated with CRP (ρ=0.313, p< 0.001). Correlations with cytokines measured are shown in

Table 11 Presepsin was negatively correlated with postoperative IL-1b (p< 0.01), while CRP was positively correlated with preoperative IL-10 (p< 0.01).

In off ECB patients, presepsin positively correlated with preoperative CRP (p< 0.001), TNFα (p< 0.01) and IL-6 (p< 0.001), but negatively with postoperative Il-1b (p< 0.05). In ECB patients, presepsin did not significantly correlated with none of the measured cytokines, but was in week positive correlation with CRP (p< 0.01).

Table 11: Correlation of presepsin with cytokines.

		Pre op TNFa	Pre op IL-2	Pre op IL-1beta	Pre op IL-6	Pre op IL-10	*After op TNF alpha*	*After op IL-2*	*After op IL-1beta*	*After op IL-6*	*After op IL-10*
Presepsin	Correlation coefficient	.034	.079	.013	.091	.125	.024	.032	**-.192****	.011	-.065
	Sig. (2-tailed)	.619	.266	.851	.189	.073	.725	.646	**.005**	.877	.343
CRP	Correlation Coefficient	-.073	-.118	-.020	.067	**.220****	.070	.025	.002	.112	.055
	Sig. (2-tailed)	.300	.104	.783	.342	**.002**	.317	.726	.974	.110	.435

Tnfa: Tumor Necrosis Factor Alpha; ECB: Extra Corporeal Blood Flow.

Discussion

Cardiosurgical intervention is mutilating in itself, regardless of whether it is performed under ECB conditions or without a pump. Access to the heart through a medial sternotomy, using vibration testers and electrocautery, causes the release of tissue fragments into the circulation and the expression of Damage-Associated Molecular Patterns (DAMPs). These molecules act on multiple receptors from the PRR family and initiate an intracellular signaling pathway that ultimately leads to the activation of the transcription factor NF-κ B and p38 with the subsequent initiation of an inflammatory response.

An early increase in reactants of the acute phase can also occur due to ischemia - that is, due to reperfusion injury of the brain, heart, lungs, kidneys and liver, due to cross-clamping of the aorta. Gut perfusion may be reduced even when whole-body perfusion indices are normal due to the consequent release of vasopressin and catecholamines that reduce flow through the mesenteric arteries [6]. The use of ECB potentiates the hypoperfusion effect on the mucosa of the gastrointestinal tract, with a consequent increase in intestinal permeability and facilitated penetration of bacterial endotoxins. In this way, additional conditions are created for the occurrence of SIRS and damage to other organs. After the use of ECB, 2-4% of patients undergo gastrointestinal surgery, due to bleeding, peritonitis or intestinal obstruction, with a mortality rate of up to 30% [7].

It can be assumed that the early inflammatory response, present after cardiac surgery, is a consequence of the cumulative action of the above-mentioned factors on the immune system. Therefore, the findings of elevated values of presepsin, CRP and leukocytes after cardiac surgery are not surprising, with a proposal for their use as independent predictors of SIRS in cardiac surgery [8]. In this sense, for example, presepsin values of 600-700 pg/mL have been defined as clinical significant for the development of infectious complications after surgery [9]. In our study, the postoperative increase in the level of CRP, prespsin and leukocytes after revascularization, recorded in all investigated groups, fully correlates with the results of other researchers.

Proinflammatory cytokines play a key role in stimulating the inflammatory process, with the concentration of specific cytokines in plasma, such as IL-1β and IL-6, predicting the outcome in certain critical groups of patients. Tumor necrosis factor α (TNF-α) and IL-1β are elevated early after cardiac surgery, and IL-6 and IL-8 peak later. Although a direct cause-and-effect relationship has not been proven, the elevation of pro-inflammatory cytokines is strongly associated with an unfavorable outcome after cardiac surgery. Patients who develop SIRS show a significant elevation of cytokine concentrations compared to patients with an uncomplicated course after cardiac surgery [10].

In our study, convincing findings of an increase in IL-1, IL-2 and IL-6 were obtained postoperatively in all groups, but the differences between the control -off pump group and patients with extracorporeal blood flow are not convincing. TNFα levels did not differ between groups.

Variable detection of certain cytokines after Coronary Artery Bypass Graft Surgery (CABG) is not a rare finding in the literature. Conflicting results also refer to the time course of detection of maximal cytokine levels. There are numerous reasons for these differences: sampling time, assay sensitivity, circadian variation in cytokine release, short half-life of TNF-α and IL-1β, all of which may affect the measurement of desired parameters during CABG surgery [11,12].

In their research, Diegeler et al. indicated that Cardiopulmonary Bypass (CPB) causes systemic release of inflammatory cytokines. This study compares the humoral immune response in patients undergoing CABG with standard, minimally invasive and "off-pump" techniques [13]. In support of the increased systemic inflammation, the significantly increased release of the breakdown products of complement activation C5a and C3d as well as IL-8 speak. This trend was most pronounced in patients who underwent EKK during the initial period and in the short period of time after perfusion. Also, TNF-α receptors p55 and p75 were increased and at the same time prolonged expression (up to 48 hours) in the CPB group compared to the off-pump group. Interestingly, in this study, IL-6 did not show a different release among the three surgical groups over the entire period. There was no significant difference in any parameter measured in relation to the type of operative approach.

An interesting finding in our study is the postoperative increase in IL-10 levels in all analyzed groups, but greater increase in ECB group. The finding of increased production of IL-10 after cardiac surgery has been described in the literature and may represent a compensatory mechanism for suppressing excessive activity of the pro-inflammatory cytokines IL-6 and IL-8 [14-16]. IL-10 is a strong inhibitor of the production of other cytokines such as TNF-α, IL-1β, which is why it is assumed that the clinical outcome after cardiopulmonary bypass depends on the balance between pro- and anti-inflammatory cytokines. It is believed that prior administration of steroids and use of aprotinin can significantly improve IL-10 production in hepatocytes in patients undergoing cardiopulmonary bypass [17].

Finally, the authors of this research believe that the absence of changes in IL-4, IL-12p70 and IFNγ levels between groups and different time intervals may be due to the study design. It is likely that the blood sampling interval of 8 hours after reperfusion surgery is too short for the polarization, development of Th1 and Th2 lymphocytes and the secretion of detectable concentrations of their cytokines, specifically IL-12p70, IFN-γ and IL-4.

Table 11: Correlation of presepsin with cytokines.

		Pre op TNFa	Pre op IL-2	Pre op IL-1beta	Pre op IL-6	Pre op IL-10	*After op TNF alpha*	*After op IL-2*	*After op IL-1beta*	*After op IL-6*	*After op IL-10*
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Tnfa: Tumor Necrosis Factor Alpha; ECB: Extra Corporeal Blood Flow.

Contradictions in the findings of cytokine levels in our work and in the literature indicate that there are still no definitive answers and point to the need for new studies with a larger number of patients. Unfortunately, there are no triple coronary bypass surgeries performed without the aid of ECB systems, which reduces the weight of the conclusions. Also our study population was relatively small, so research should be expanded with more patients.

Conclusions

Based on the obtained results, the following conclusions were drawn:

1. After surgical revascularization of the myocardium, a greater relative increase in the concentration of presepsin, the number of leukocytes and CRP was found in the ECB group of patients compared to the control group, which proves the significant influence of the ECB system on the immune status of patients.

2. Postoperative increase in the levels of IL-1, IL-2, IL-6 and IL-10 was noted in all patients in all groups, but the level of increase between the ECB group and the control had no statistical significance.

3. After 8 hours of the intervention, there were no detectable levels of cytokines involved in the response mediated by Th2 cells (IL-4) nor cytokines secreted by Th1 cells (IFNγ and IL-12 p70), which is why the influence of extracorporeal blood flow on the polarization of the adaptive immune response must be evaluated by determining the cytokine level in later time intervals.

4. In the postoperative period, a higher incidence rate of SIRS was noted in patients in whom the ECB system was used, which also indicates its importance in the development of systemic inflammation and immune dysregulation.

Author contributions: Conceptualization, Dragan Milic and Milan Lazarevic; methodology, Isidora Milić.; software, Pavle Marjanovic; validation, Vesna Marjanovic; formal analysis, Sasa Zivic; investigation, Zorica Lazarevic; resources, Mlađan Golubovic.; data curation, Velimir Peric; writing—original draft preparation, Aleksandar Kamenov and Vladimir Stojiljkovic.

Funding: Please add: This research received no external fund in.

Institutional review board statement: The study was conducted in accordance with the Declaration of Helsinki, and approved by Ethics Committee of Clinical Center Nis Serbia 5257/20 06.01.2020.

Informed consent statement: Informed consent was obtained from all subjects involved in the study.

Conflicts of interest: The authors declare no conflict of interest.

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