Yingting Huang [1,#], Xunbei Huang [1,#], Liqin Ling [1], Chaonan Liu [1], Si Chen [1], Jing Zhou [1], Lei Du [2], Jiyue Xiong [2]

[1] Sichuan University, West China Hospital, Department of Laboratory Medicine, Sichuan, China

[2] Sichuan University, West China Hospital, Department of Anesthesiology, Sichuan, China

[#] These authors contribute equally

# Prospective Reassessment of the Association Between Pro-Inflammatory Factors and Prognosis After on-Pump Cardiac Surgery

| | |
|---|---|
| *Aim* | Elevated levels of pro-inflammatory factors in plasma have been linked to worse prognosis after on-pump cardiac surgery, yet interventions that reduce the levels in patients have failed to improve prognosis. Therefore, we explored whether levels of pro-inflammatory factors are associated with prognosis of patients after valve surgery with cardiopulmonary bypass. |
| *Material and methods* | 244 patients were prospectively enrolled into observational study. Levels of tumor necrosis factor (TNF) – α, interleukin-8 and neutrophil elastase were measured once before and several times after cardiopulmonary bypass. The levels were compared between patients who experienced in-hospital adverse events or not, and between patients who experienced major adverse cardiac or cerebrovascular events (MACCEs) during three-year follow-up or not. |
| *Results* | Of the 244 patients enrolled, in-hospital adverse events occurred in 38 (15.6%); of the 237 patients who completed follow-up, MACCEs occurred in 30 (12.7%). Surgery led to significant increases in levels of all three pro-inflammatory factors, with levels returning to pre-bypass baseline on arrival in the intensive care unit (TNF-α), 4 h after arrival (interleukin-8) or 20 h after arrival (neutrophil elastase). However, pre- and post-bypass levels of all three factors did not differ significantly between patients who experienced adverse events in-hospital or not, or between patients who experienced MACCEs during follow-up or not. |
| *Conclusions* | Levels of TNF-α, interleukin-8 and neutrophil elastase may not be associated with poor prognosis after cardiopulmonary bypass. This may help explain why "cytokine clearance" strategies fail to improve clinical outcomes after on-pump cardiac surgery. |

| | |
|---|---|
| *Keywords* | TNF-α; interleukin-8; neutrophil elastase; Cardiopulmonary Bypass; adverse events |
| *For citations* | Yingting Huang, Xunbei Huang, Liqin Ling, Chaonan Liu, Si Chen, Jing Zhou et al. Prospective Reassessment of the Association Between Pro-Inflammatory Factors and Prognosis after on-Pump Cardiac Surgery. Kardiologiia. 2024;64(9):87–95. [Russian: Интинг Хуан, Сюньбэй Хуан, Лицинь Лин, Чаонань Лю, Си Чен, Цзин Чжоу и др. Проспективная переоценка связи между провоспалительными факторами и прогнозом после операции на сердце с искусственным кровообращением.Кардиология. 2024;64(9):87–95]. |

| | |
|---|---|
| *Corresponding author* | Jiyue Xiong. E-mail: xiongjiyue028@163.com |

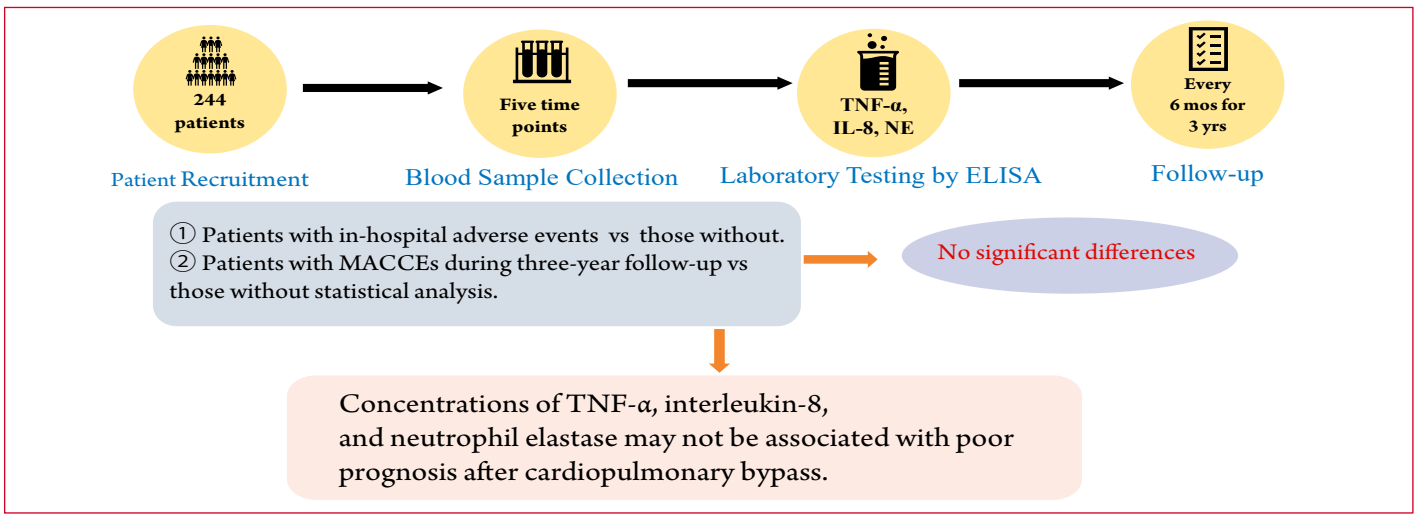
## Introduction

A substantial proportion of patients who undergo cardiac surgery with cardiopulmonary bypass (CPB) experience systemic inflammatory response syndrome [1, 2]. This response ranges widely from 28% to as high as 96% of cases, and it increases the risk of postoperative morbidity [1, 2]. In fact, this systemic inflammatory response syndrome has been linked to mortality rates as high as 10% [2]. How cardiopulmonary bypass induces systemic inflammation is not fully understood. The inflammatory response may begin when the blood contacts the foreign surfaces of the bypass apparatus, and then it may worsen as a result of the patient's ischemia-reperfusion injury, endotoxemia, coagulation activation, and the administration of heparin or protamine [3, 4]. Several studies have linked this syndrome to a massive release of pro-inflammatory cytokines, such as tumor necrosis factor (TNF) – α, interleukin-8, and neutrophil elastase [5–9]. The result being a drastic self-reinforcement of various feedback mechanisms, which can ultimately lead to systemic damage, multi-organ failure, and death [10].

Several investigators (Becker S, Liu MH, Magoon R, et al) have explored whether reducing concentrations of pro-inflammatory cytokines in the blood can improve the prognosis of patients after on-pump cardiac surgery. Unfortunately, such "cytokine clearance" strategies have failed to substantially improve clinical outcomes in several clinical studies [11–15]. These findings raise the possibility that elevated concentrations of pro-inflammatory factors in plasma may not contribute much to the poor prognosis after on-pump cardiac surgery, and are therefore they are unreliable prognostic predictors.

To explore this possibility, we compared concentrations of pro-inflammatory factors in plasma between patients who experienced adverse events and those who did not after on-pump cardiac surgery. Our results suggest that focusing on

Central illustration. Prospective Reassessment of the Association Between Pro-Inflammatory Factors and Prognosis After on-Pump Cardiac Surgery




reducing pro-inflammatory factors is not sufficient to improve prognosis of patients after on-pump cardiac surgery.

## Material and methods

### *Patients*

This is a re-analysis of a prospective cohort study. The patients in this study have been described previously [16, 17]. Based on the following inclusion and exclusion criteria, 244 of 457 patients were included in the study. Inclusion criteria: Patients aged 18 to 65 yrs with rheumatic valve disease and who underwent valve surgery with cardiopulmonary bypass between November 2011 and September 2012 at West China Hospital, Sichuan University.

*Exclusion criteria:* 1) Known organ dysfunction, including adult respiratory distress syndrome, kidney failure, or New York Heart Association (NYHA) class IV heart failure. 2) Preoperative pulmonary diseases, such as chronic obstructive pulmonary disease, pneumonia, and pulmonary hypertension. 3) Confirmed systemic inflammatory response syndrome. 4) History of any previous heart surgery. 5) Required a second operation due to hemorrhage or other adverse events. 6) Patients who have been or are currently participating in other clinical studies.

The study protocol was approved by the Ethics Committee of Sichuan University (approval number: 2011–133) and registered in the Chinese Clinical Trial Registry (ChiCTR-OCH-12001922). All patients gave written informed consent before enrollment.

Patients underwent surgery and were managed according to routine practice at our hospital. After the initiation of CPB, the ascending aorta was occluded. Cardioplegia solution was then infused through the root of the aorta, with a temperature of approximately 6–10°C (average 8°C). The infusion pressure was maintained at 120–160 mmHg, and the infusion duration of the cardioplegia solution was 5 min.

After discharge, patients were followed up every 6 mo for 3 yrs by telephone or during outpatient visits. The last follow-up was conducted in August 2015. During the follow-up period, the patients or their family members were asked to report whether and when the patients had been hospitalized in any hospitals. Patients without any hospitalization were considered to not have experienced major adverse cardiac or cerebrovascular events (MACCEs). For patients who were hospitalized in our hospital, MACCEs were assessed during the hospitalization by our researchers. For patients were hospitalized in other hospitals, MACCEs were assessed based on medical records provided by the hospitals. Researchers conducting follow-up were blinded to the patients' laboratory testing results.

### *Blood sampling and analyses*

Blood samples were collected at five time points:
1) After anesthesia induction but before the start of cardiopulmonary bypass;
2) Immediately before weaning off bypass;
3) Arrival in the intensive care unit (ICU);
4) 4 h after arrival in the ICU;
5) 20 h after arrival in the ICU. Within 4 h after collection, the blood samples were centrifuged at 4 °C for 15 min at 1000 g, and the plasma was removed and stored at –80 °C until assayed for tumor necrosis factor (TNF)-α, interleukin-8, and neutrophil elastase.

These assays were performed using commercial enzyme-linked immunosorbent kits (R&D Systems, Minneapolis, MN, USA).

### *Data collection and outcomes*

Data were collected on preoperative variables including patient clinic demographics, smoking history, preoperative comorbidities, cardiac disease, NYHA class, and medica-


ISSN 0022-9040. Кардиология. 2024;64(9). DOI: 10.18087/cardio.2024.9.n2682

tion history. In addition, data were collected on intraoperative variables including type of valve replacement, duration of cardiopulmonary bypass, and duration of cross-clamping.

Data were collected on the following adverse events during hospitalization: 1) acute respiratory distress syndrome; 2) acute kidney injury; 3) acute liver dysfunction; 4) neurological adverse events, including seizures, coma, cerebral hemorrhage, transient ischemic attack or stroke; 5) cardiovascular adverse events, including heart failure, myocardial infarction, and life-threatening arrhythmia. Data were also collected on the MACCEs that occurred during the three-year follow-up after discharge: 1) stroke; 2) heart failure; 3) myocardial infarction; 4) life-threatening arrhythmia; 5) transient ischemic attack; 6) MACCE-related death. Multiple rehospitalizations of the same patient were counted only once. All of these adverse events have been described in detail elsewhere [16, 17].

### *Statistical analysis*

Data were analyzed using Prism 9.0 (GraphPad, San Diego, CA, USA). Categorical data are reported as number (%), while continuous data are reported as the median (interquartile range). Normal distribution was tested using the Anderson–Darling test at the 5% significance level and the data were found to follow a normal distribution. Intergroup differences were assessed for significance by using the chi-squared test or Fisher's exact test in the case of categorical data, or by using the independent-samples t test or one-way ANOVA in the case of continuous data. Intergroup differences of pro-inflammatory factors at different time points were assessed using two-way, repeated-measures ANOVA. Differences associated with two-sided $p< 0.05$ were considered significant. In some analyses, subsets of patients were matched to each other using propensity scoring based on demographics, cardiac function, comorbidities, medications before surgery, and surgical procedures. Matching was performed within SPSS 26.0 (IBM, Chicago, IL, USA).

## Results

Of the 244 patients enrolled in the study, 38 (15.6%) experienced adverse in hospital events (Table 1). These events comprised the following: 1) acute respiratory distress syndrome, 17 patients; 2) acute kidney injury, 9 patients; 3) neurological adverse events, 3 patients; 4) cardiovascular adverse events, 3 patients; 5) combinations of these events, 6 patients. Of the 237 patients who completed three-year follow-up, 30 (12.7%) suffered MACCEs, comprising of the following: 1) heart failure progression, 6 patients; 2) life-threatening arrhythmia, 6 patients; 3) stroke, 8 patients; 4) transient ischemic attack, 1 patient; 5) myocardial infarction, 1 patient; 6) MACCE-related death, 8 patients. Among the patients who completed the follow-up period, the risk of MACCEs during follow-up was significantly higher among those who experienced adverse events during hospitalization than among those who did not (relative risk 1.304, 95% confidence interval 1.066–1.789).

**Table 1.** Clinicodemographic characteristics stratified by whether or not the patients experienced adverse events during hospitalization or follow-up

| Characteristic | All patients | Adverse events in hospital | | | MACCEs during follow-up | | |
|---|---|---|---|---|---|---|---|
| | (n = 244) | Yes (n = 38) | No (n = 206) | p | Yes (n = 30) | No ( n=207 ) | p |
| **Demographics** | | | | | | | |
| Age, yrs | 47 (42, 55) | 48 (43, 57) | 47(42, 55) | 0.908 | 48 (44, 57) | 46 (41, 55) | 0.323 |
| Male | 78 (34.8) | 16 (42.1) | 62 (30.1) | 0.185 | 7 (23.3) | 68 (32.9) | 0.295 |
| Body mass index, kg/m$^2$ | 22.04 (20.2, 23.6) | 23.5 (21.2, 24.6) | 21.9 (20.1, 23.4) | 0.569 | 22.48 (20.89, 23.92) | 21.96 (20.22, 23.58) | 0.848 |
| Ever smoker | 52 (21.3) | 13 (34.2) | 39 (18.9) | 0.051 | 3 (10.0) | 47 (22.7) | 0.111 |
| **Cardiac function** | | | | | | | |
| LVEF, % | 63 (57,68) | 61 (58, 66) | 63 (57, 68) | 0.146 | 63 (60, 70) | 63 (57, 68) | 0.505 |
| NYHA class | | | | 1.000 | | | 0.603 |
| II | 37 (15.2) | 6 (15.8) | 31 (15.0) | | 3 (10.0) | 31 (15.0) | |
| III | 207 (84.8) | 32 (84.2) | 175 (85.0) | | 27 (90.0) | 175 (85.0) | |
| **Comorbidities** | | | | | | | |
| Diabetes | 5 (2.0) | 2 (5.3) | 3 (1.5) | 0.174 | 1 (3.3) | 4 (1.9) | 0.495 |
| Atrial fibrillation | 118 (52.7) | 19 (50.0) | 99 (48.1) | 0.861 | 15 (50.0) | 99 (47.8) | 0.824 |
| Hypertension | 18 (7.4) | 6 (15.8) | 12 (5.8) | 0.043 | 3 (10.0) | 15 (7.2) | 0.870 |
| Left atrial thrombus | 33 (13.5) | 5 (13.2) | 28 (13.6) | 1.000 | 4 (13.3) | 28 (13.5) | 1.000 |
| Cerebral infarction | 8 (3.3) | 2 (5.3) | 6 (2.9) | 0.362 | 2 (6.7) | 5 (2.4) | 0.218 |
| **Medications before surgery** | | | | | | | |
| Warfarin | 3 (1.2) | 1 (2.6) | 2 (1.0) | 0.400 | 0 (0.0) | 3 (1.4) | 1.000 |
| Aspirin | 11 (4.5) | 2 (5.3) | 9 (4.4) | 0.683 | 1 (3.3) | 10 (4.8) | 1.000 |
| Calcium antagonists | 2 (0.8) | 0 | 2 (1.0) | 1.000 | 0 (0.0) | 2 (1.0) | 1.000 |
| β-blocker | 19 (7.8) | 4 (10.5) | 15 (7.3) | 0.509 | 2 (6.7) | 17 (8.2) | 1.000 |

**Table 1. Continued.** Clinicodemographic characteristics stratified by whether or not the patients experienced adverse events during hospitalization or follow-up

| Characteristic | (n = 244) | Yes (n = 38) | No (n = 206) | p | (n = 244) | Yes (n = 38) | No (n = 206) |
|---|---|---|---|---|---|---|---|
| Digoxin | 26 (10.7) | 2 (5.3) | 24 (11.7) | 0.389 | 1 (3.3) | 24 (11.6) | 0.290 |
| Insulin | 1 (0.4) | 0 | 1 (0.5) | 1.000 | 0 (0.0) | 1 (0.5) | 1.000 |
| ACEI | 9 (3.7) | 3 (7.9) | 6 (2.9) | 0.150 | 0 (0.0) | 8 (3.9) | 0.579 |
| Diuretics | 26 (10.7) | 4 (10.5) | 22 (10.7) | 1.000 | 3 (10.0) | 22 (10.6) | 1.000 |
| Diseased valve(s) and surgical procedure | – | – | – | 0.122 | – | – | 0.780 |
| Aortic valve | 39 (16.0) | 10 (26.3) | 29 (14.1) | – | 6 (20.0) | 33 (16.0) | – |
| AVR | 29 (11.9) | 7 (18.4) | 22 (10.7) | – | 6 (20.0) | 23 (11.1) | – |
| AVR+Maze | 3 (1.2) | 0 | 3 (1.5) | – | 0 | 3 (1.4) | – |
| AVR+AAP | 1 (0.4) | 0 | 1 (0.5) | – | 0 | 1 (0.5) | – |
| Bentall | 6 (2.5) | 3 (7.9) | 3 (1.5) | – | 0 | 6 (2.9) | – |
| Atrioventricular valves | 103 (42.2) | 12 (31.6) | 91 (44.2) | – | 13 (43.3) | 86 (41.5) | – |
| MVR | 36 (14.8) | 2 (5.3) | 34 (16.5) | – | 3 (10.0) | 31 (15.0) | – |
| MVR+Maze | 11 (4.5) | 1 (2.6) | 10 (4.9) | – | 2 (6.7) | 9 (4.3) | – |
| MVR+Maze+TVP | 23 (9.4) | 4 (10.5) | 19 (9.2) | – | 2 (6.7) | 20 (9.7) | – |
| MVR+TVP | 28 (11.5) | 4 (10.5) | 24 (11.7) | – | 4 (13.3) | 23 (11.1) | – |
| MVR+TVR | 1 (0.4) | 1 (2.6) | 0 | – | 1 (3.3) | 0 | – |
| MVR+TVP+TVR | 1 (0.4) | 0 | 1 (0.5) | □ | 0 | 1 (0.5) | □ |
| MVR+Maze+TVR | 1 (0.4) | 0 | 1 (0.5) | – | 1 (3.3) | 0 | – |
| TVR | 2 (0.8) | 0 | 2 (1.0) | – | 0 | 2 (1.0) | – |
| Aortic and atrioventricular valves | 102 (41.8) | 16 (42.1) | 86 (41.7) | – | 11 (36.7) | 88(42.5) | – |
| AVR+TVR | 1 (0.4) | 0 | 1 (0.5) | – | 0 | 1 (0.5) | – |

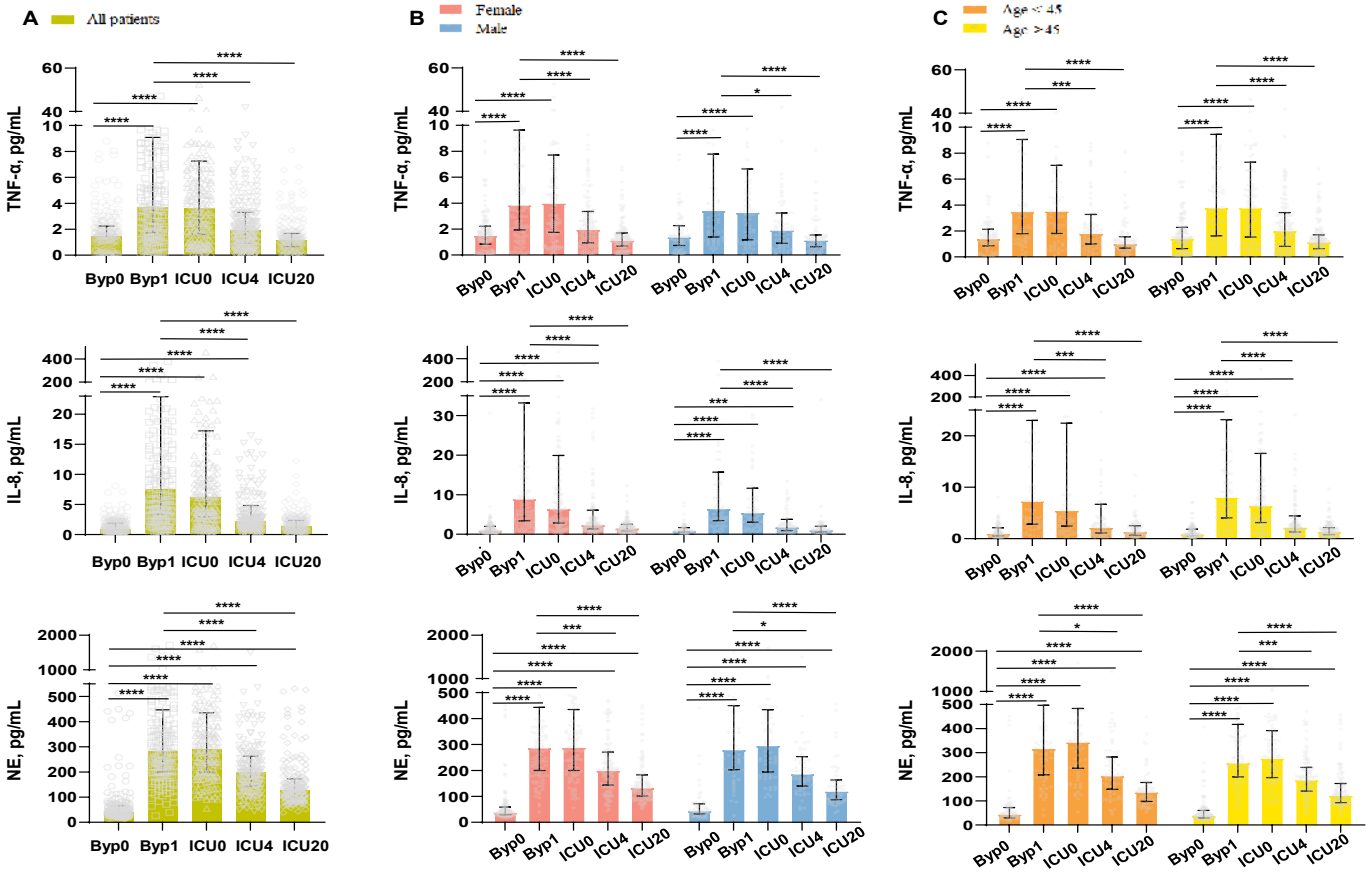
| Characteristic | (n = 244) | Yes (n = 38) | No (n = 206) | p | (n = 244) | Yes (n = 38) | No (n = 206) |
|---|---|---|---|---|---|---|---|
| DVR | 34 (13.9) | 3 (7.9) | 31 (15.0) | – | 2 (6.7) | 31 (15.0) | – |
| DVR+Maze | 7 (2.9) | 1 (2.6) | 6 (2.9) | – | 0 | 7 (3.3) | – |
| DVR+Maze+TVP | 21 (8.6) | 4 (10.5) | 17 (8.3) | – | 3 (10.0) | 17 (8.2) | – |
| DVR+Maze+TVP+AAP | 1 (0.4) | 0 | 1 (0.5) | – | 0 | 1 (0.5) | – |
| DVR+TVP | 37 (15.2) | 8 (21.1) | 29 (14.1) | – | 5 (16.7) | 31 (15.0) | – |
| DVR+AAR | 1 (0.4) | 0 | 1 (0.5) | – | 1 (3.3) | 0 | – |
| **Transfusion** | | | | | | | |
| **During cardiopulmonary bypass** | | | | | | | |
| Red blood cells | 55 (22.5) | 7 (18.2) | 48 (23.3) | 0.673 | 9 (30.0) | 45 (21.7) | 0.352 |
| Fresh frozen plasma | 11 (4.5) | 1 (2.6) | 10 (4.9) | 1.000 | 0 | 11 (5.3) | 0.368 |
| Within 20 h after bypass | | | | | | | |
| Red blood cells | 26 (10.7) | 5 (13.2) | 21 (10.2) | 0.571 | 3 (10.0) | 23 (11.1) | 1.000 |
| Platelets | 1 (0.4) | 0 | 1 (0.5) | 1.000 | 0 | 1 (0.5) | 1.000 |
| Fresh frozen plasma | 9 (3.7) | 1 (2.6) | 8 (3.9) | 1.000 | 0 | 9 (4.3) | 0.608 |
| Cryoprecipitate | 2 (0.8) | 0 | 2 (1.0) | 1.000 | 0 | 2 (1.0) | 1.000 |
| **Cardiopulmonary bypass** | | | | | | | |
| Duration of bypass, min | 112 (91, 137) | 122(102, 137) | 111(90, 137) | 0.188 | 112 (96,143) | 113 (91, 137) | 0.989 |
| Duration of cross-clamping, min | 74 (55, 97) | 88 (69, 98) | 72 (54, 93) | 0.110 | 72 (57, 109) | 75 (55, 95) | 0.382 |

| Characteristic | (n = 244) | Yes (n = 38) | No (n = 206) | p | (n = 244) | Yes (n = 38) | No (n = 206) |
|---|---|---|---|---|---|---|---|
| Other information | | | | | | | |
| Duration of mechanical ventilation, h | 15 (10, 20) | 18 (11, 26) | 15 (10, 19) | 0.051 | 16 (12, 22) | 15 (10, 20) | 0.471 |
| ICU stay, h | 46 (41, 69) | 69 (45, 142) | 46 (41, 67) | <0.001 | 66 (45, 80) | 46 (41, 68) | <0.001 |
| Hospital stay, d | 9 (8, 10) | 10 (8, 15) | 9 (8, 10) | <0.001 | 10 (8, 16) | 9 (8, 10) | <0.001 |

Values are median (interquartile range) or n (%). AAP, ascending aortoplasty; AAR, ascending aorta replacement; ACEI, angiotensin-converting enzyme inhibitors; AVR, aortic valve replacement; DVR, double-valve replacement; ICU, intensive care unit; LVEF, left ventricular ejection fraction; MACCE, major adverse cardiac and cerebrovascular events; MVR, mitral valve replacement; NYHA, New York Heart Association; TVP, tricuspid valvuloplasty; TVR, tricuspid valve replacement.


ISSN 0022-9040. Кардиология. 2024;64(9). DOI: 10.18087/cardio.2024.9.n2682

**Figure 1.** Concentrations of tumor necrosis factor (TNF)-α, interleukin (IL)-8 and neutrophil elastase (NE) at various times before and after cardiopulmonary bypass




**(A)** Based on analysis of all patients. **(B)** Based on analysis of patients with sex difference. **(C)** Based on analysis of all patients with age difference. Histograms show the median and interquartile range, while individual measurements are shown as translucent gray dots. Concentrations were measured before the start of cardiopulmonary bypass (Byp0), immediately before the end of bypass (Byp1), at arrival in the intensive care unit (ICU0), 4 h after arrival (ICU4) and 20 h after arrival (ICU20). ****, p< 0.0001, ***, p < 0.001, *, p < 0.05.

None of the patient or surgical characteristics that were examined differed significantly between patients who experienced adverse events in hospital or not, except that patients who experienced such events were more likely to have had a history of hypertension (Table 1). Similarly, none of the characteristics differed significantly between patients who experienced MACCEs during follow-up and those that did not (Table 1). These results were confirmed in comparisons of the corresponding subsets of propensity score-matched (PSM) patients (Table 2).

Surgery significantly increased the concentrations of all three of the pro-inflammatory cytokines that we assayed, and their concentrations peaked at the end of cardiopulmonary bypass or at arrival in the ICU (Figure 1). These concentrations had returned to the pre-bypass baseline, or nearly so, by arrival in the ICU in the case of TNF-α, by 4 h after arrival in the case of interleukin-8 or by 20 h after arrival in the case of neutrophil elastase. These results did not vary with patient sex or age.

In the entire patient sample, we observed no significant differences, at any of the five blood samplings, in concentrations of any of the three pro-inflammatory factors between patients who suffered adverse events in hospital and those who did not (Figure 2), or between patients who experienced MACCEs during follow-up and those who did not (Figure 3). Similar results were observed for the corresponding subsets of propensity score-matched patients.

## Discussion

The causes and processes responsible for the systemic inflammatory response syndrome in patients who undergo on-pump cardiac surgery remain obscure, though it is clear that they involve upregulation of pro-inflammatory factors in the circulation [3–10]. This has led to numerous efforts to reduce the inflammatory syndrome by adsorbing pro-inflammatory factors from the blood, yet these efforts have not reproducibly demonstrated clinical benefits [11–15].

Our analysis of patients undergoing valve surgery with cardiopulmonary bypass suggests no association between elevations in three pro-inflammatory factors and risk of adverse events either in hospital or during three-year follow-up. These findings persisted even after we controlled for several potential confounders through propensity score matching. This may be explained, in part, because the expression of pro-inflammatory factors is merely an intermediate step in a highly complex biochemical

**Table 2.** Clinical and demographic characteristics stratified by whether or not the patients experienced adverse events during hospitalization or follow-up after PSM

| Characteristic | PSM adverse events in hospital | | | PSM MACCEs during follow-up | | |
|---|---|---|---|---|---|---|
| | Yes (n = 33) | No (n = 33) | p | Yes (n=25) | No (n=25) | p |
| **Demographics** | | | | | | |
| Age, yrs | 48 (43, 57) | 49 (42, 58) | 0.738 | 48 (44, 58) | 50 (44, 57) | 0.986 |
| Male | 13 (39.4) | 10 (30.3) | 0.606 | 6 (24.0) | 11 (44.0) | 0.260 |
| Body mass index, kg/m² | 23.45 (21.17, 24.62) | 22.44 (21.08, 23.51) | 0.333 | 22.44 (19.78, 23.98) | 22.09 (20.61, 23.25) | 0.777 |
| Ever smoked | 10 (30.3) | 8 (24.2) | 0.783 | 3 (12.0 ) | 5 (20.0) | 0.709 |
| **Cardiac function** | | | | | | |
| LVEF, % | 62 (58, 67) | 63 (56, 70) | 0.936 | 64 (58, 71) | 62 (52, 70) | 0.531 |
| NYHA class | – | – | 1.000 | – | – | 1.000 |
| II | 5 (15.2) | 5 (15.2) | – | 3 (12.0) | 4 (16.0) | – |
| III | 28 (84.8) | 28 (84.8) | – | 22 ( 88.0) | 21 (84.0) | – |
| **Comorbidities** | | | | | | |
| Diabetes | 1 (3.0) | 2 (6.1) | 1.000 | 1 (4.0) | 1 (4.0) | 1.000 |
| Atrial fibrillation | 15 (45.5) | 18 (54.5) | 0.623 | 12 (48.0) | 10 (40.0) | 0.794 |
| Hypertension | 4 (12.1) | 2 (6.1) | 0.672 | 2 (8.0) | 3 (12.0) | 1.000 |
| Left atrial thrombus | 4 (12.1) | 7 (21.2) | 0.511 | 4 (16.0) | 2 (8.0) | 0.672 |
| Cerebral infarction | 1 (3.0) | 0 | 1.000 | 1 (4.0) | 0 | 1.000 |
| **Medications before surgery** | | | | | | |
| Warfarin | 1 (3.0) | 1 (3.0) | 1.000 | 0 | 0 | – |
| Aspirin | 2 (6.1) | 1(3.0) | 1.000 | 1 (4.0) | 0 | 1.000 |
| Calcium antagonists | 0 | 0 | – | 0 | 0 | – |
| β-blocker | 4 (12.1) | 2 (6.1) | 0.672 | 1 (4.0) | 1(4.0) | 1.000 |

| Characteristic | Yes (n = 33) | No (n = 33) | p | Yes (n = 33) | No (n = 33) | p |
|---|---|---|---|---|---|---|
| Digoxin | 2 (6.1) | 2 (6.1) | 1.000 | 1 (4.0) | 0 | 1.000 |
| Insulin | 0 | 0 | □ | 0 | 0 | □ |
| ACEI | 3 (9.1) | 1 (3.0) | 0.613 | 0 | 0 | |
| Diuretics | 1 (3.0) | 3 (9.1) | 0.613 | 2 (8.0) | 1 (4.0) | 1.000 |
| Diseased valve (s) | | | 0.848 | | | 0.524 |
| Aortic | 9 (27.3) | 7 (21.2) | – | 5 (20.0) | 8 (32.0) | – |
| AVR | 6 (18.2) | 5 (15.2) | – | 5 (20.0) | 6 (24.0) | – |
| AVR+Maze | 0 | 1 (3.0) | – | 0 | 1 (4.0) | – |
| AVR+AAP | 0 | 1 (3.0) | – | 0 | 0 | – |
| Bentall | 3 (9.1) | 0 | – | 0 | 1 (4.0) | – |
| Atrioventricular | 11 (33.3) | 12 (36.4) | – | 9 (36.0 ) | 6 (24.0 ) | – |
| MVR | 2 (6.1) | 3 (9.1) | – | 2 (8.0) | 2 (8.0) | – |
| MVR+Maze | 1 (3.0) | 2 (6.1) | – | 1 (4.0) | 0 | – |
| MVR+Maze+TVP | 3 (9.1) | 2 (6.1) | – | 2 (8.0) | 4 (16.0) | – |
| MVR+TVP | 4 (12.1) | 4 (12.1) | – | 4 (16.0) | 0 | – |
| MVR+TVR | 1 (3.0) | 0 | – | 0 | 0 | – |
| MVR+TVP+TVR | 0 | 0 | – | 0 | 0 | – |
| MVR+Maze+TVR | 0 | 1 (3.0) | – | 0 | 0 | – |
| TVR | 0 | 0 | – | 0 | 0 | – |
| Aortic and atrioventricular | 13 (39.4) | 14 (42.4) | – | 11 (44.0 ) | 11 (44.0) | – |
| AVR+TVR | 0 | 0 | – | 0 | 0 | – |
| DVR | 3 (9.1) | 5 (15.2) | – | 2 (8.0) | 2 (8.0) | – |
| DVR+Maze | 1 (3.0) | 0 | – | 0 | 0 | – |
| DVR+Maze+TVP | 4 (12.1) | 2 (6.1) | – | 3 (12.0) | 3 (12.0) | – |
| DVR+Maze+TVP+AAP | 0 | 0 | – | 0 | 0 | – |
| DVR+TVP | 5 (15.2) | 7 (21.2) | – | 5 (20.0) | 6 (24.0) | – |

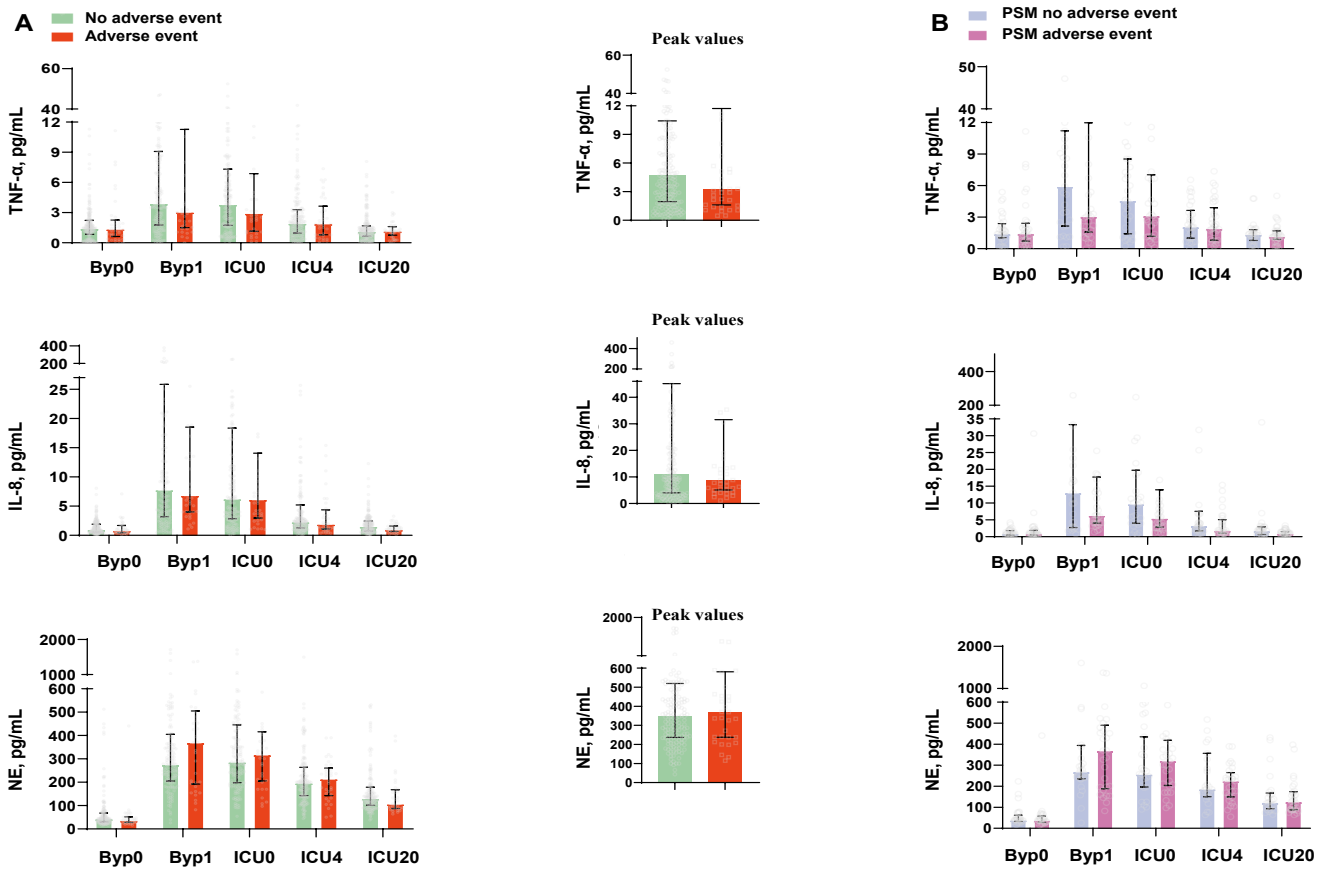

ISSN 0022-9040. Кардиология. 2024;64(9). DOI: 10.18087/cardio.2024.9.n2682

**Table 2. Continued.** Clinical and demographic characteristics stratified by whether or not the patients experienced adverse events during hospitalization or follow-up after PSM

| Characteristic | Yes (n = 33) | No (n = 33) | p | Yes (n=25) | No (n=25) | p |
|---|---|---|---|---|---|---|
| DVR+AAR | 0 | 0 | □ | 1 (4.0) | 0 | □ |
| **Transfusion** | | | | | | |
| **During bypass** | | | | | | |
| Red blood cells | 7 (21.2) | 8 (24.2) | 1.000 | 9 (36.0) | 4 (16.0) | 0.196 |
| Fresh frozen plasma | 1 (3.0) | 1 (3.0) | 1.000 | 0 | 1 (4.0) | 1.000 |
| Within 20 h after bypass | | | | | | |
| Red blood cells | 4 (12.1) | 5 (15.2) | 1.000 | 2 (8.0) | 2 (8.0) | 1.000 |
| Platelets | 0 | 0 | | 0 | 1 (4.0) | 1.000 |
| Fresh frozen plasma | 1 (3.0) | 2 (6.1) | 1.000 | 0 | 1 (4.0) | 1.000 |
| Cryoprecipitate | 0 | 0 | – | 0 | 0 | – |
| **Cardiopulmonary bypass** | | | | | | |
| Duration of bypass, min | 122 (106, 148) | 112 (97, 119) | 0.097 | 112 (96, 146) | 117 (98, 149) | 0.097 |
| Duration of cross-clamping, min | 89 (72, 98) | 75 (66, 83) | 0.072 | 72 (55,111) | 78 (62, 106) | 0.938 |
| Other information | | | | | | |
| Duration of mechanical ventilation, h | 17 (11, 27) | 13 (10,16) | 0.026 | 16 (12, 20) | 18(11,25) | 0.605 |
| ICU stay, h | 69 (45, 154) | 45 (40,59) | 0.002 | 66 (45,76) | 45 (41,68) | 0.135 |
| Hospital stay, d | 10 (8, 16) | 8 (7, 10) | 0.007 | 10 (8, 13) | 8 (9, 10) | 0.252 |

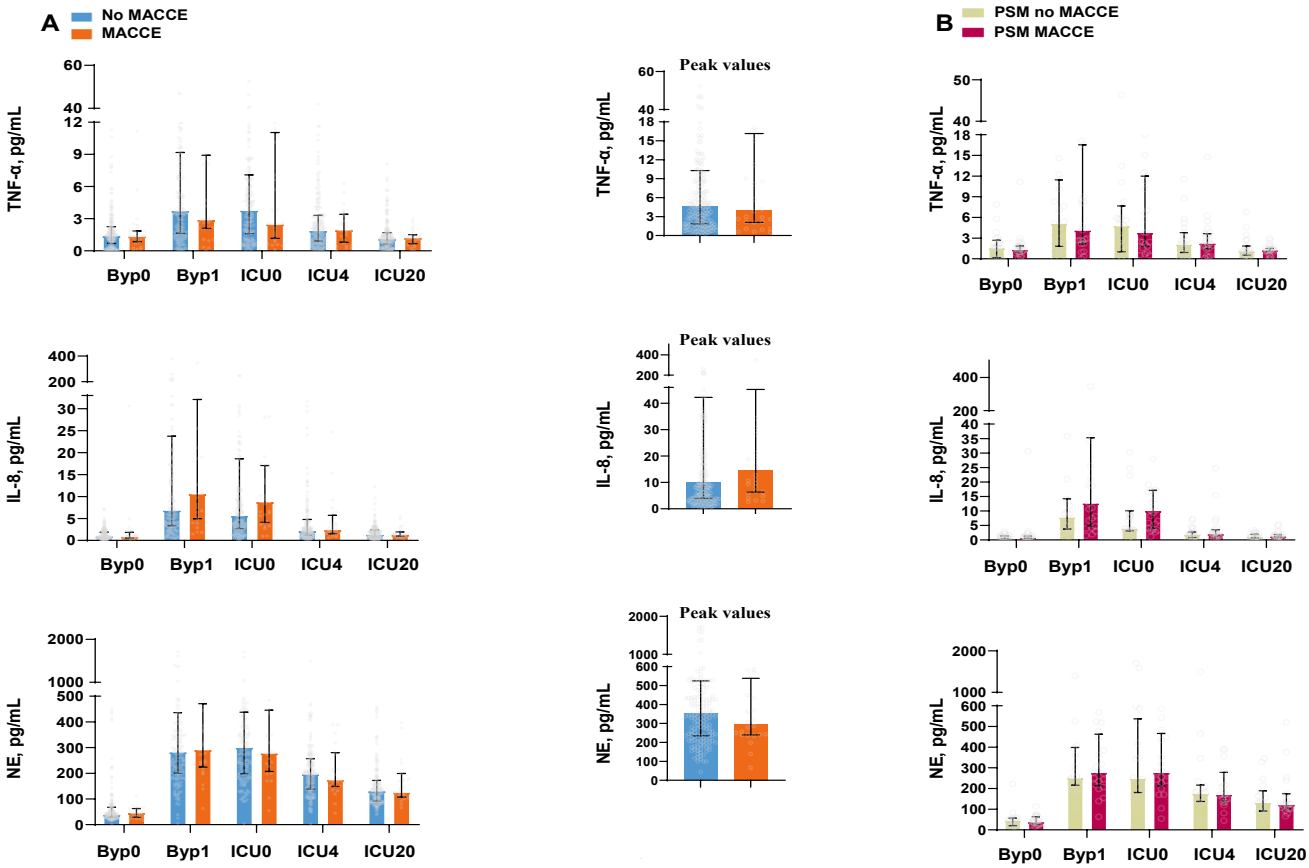
Values are median (interquartile range) or number (%). AAP, ascending aortoplasty; AAR, ascending aorta replacement; ACEI, angiotensin-converting enzyme inhibitors; AVR, aortic valve replacement; DVR, double-valve replacement; ICU, intensive care unit; LVEF, left ventricular ejection fraction; MACCE, major adverse cardiac and cerebrovascular events; MVR, mitral valve replacement; NYHA, New York Heart Association; TVP, tricuspid valvuloplasty; TVR, tricuspid valve replacement;Bentall, aortic valve and root replacement surgery; Maze, surgical treatment for atrial fibrillation.

**Figure 2.** Comparison of concentrations of tumor necrosis factor (TNF)-α, interleukin (IL)-8, and neutrophil elastase (NE) between patients who did or did not experience an adverse in hospital event




The peak values across all five time points are also shown. **(A)** Analysis of all patients who experienced an adverse event (n = 206) or did not (n = 38). **(B)** Analysis of propensity-score matched (PSM) patients who experienced an adverse event (n = 33) or did not (n = 33). Histograms show the median and interquartile range, while individual measurements are shown as translucent gray dots. Time points are defined in the legend to Figure 1.

**Figure 3.** Comparison of concentrations of tumor necrosis factor (TNF)-α, interleukin (IL)-8, and neutrophil elastase (NE) between patients who did or did not experience a major adverse cardiac or cerebrovascular event (MACCE) during follow-up




The peak values across all five time points are also shown. **(A)** Analysis of all patients who experienced an MACCE (n = 207) or did not (n = 30). **(B)** Analysis of propensity-score matched (PSM) patients who experienced an MACCE (n = 25) or did not (n = 25). Histograms show the median and interquartile range, while individual measurements are shown as translucent gray dots. Time points are defined in the legend to Figure 1.

pathway of the systemic inflammatory response syndrome induced by cardiopulmonary bypass. Therefore, the concentrations of pro-inflammatory factors in blood cannot fully represent this syndrome. Our study highlights the need to clarify which molecules and processes upregulate pro-inflammatory factors in the first place. These may be more effective targets for clinical interventions than adsorbing the cytokines from the blood.

In our patient sample, the concentrations of the three pro-inflammatory factors in plasma peaked and fell at different times consistent with their roles in the inflammatory responses. TNF-α and interleukin-8 peaked at the end of cardiopulmonary bypass, while neutrophil elastase peaked later at admission to the ICU. TNF-α and interleukin-8, which are produced primarily by mononuclear phagocytes [18, 19], activate neutrophils to secrete neutrophil elastase. Concentrations of TNF-α had fallen to pre-bypass concentrations by 4 h after admission to the ICU, suggesting that mononuclear phagocytes did not remain activated for long after bypass. Concentrations of interleukin-8, in contrast, did not fall to the pre-bypass baseline until 20 h after admission to the ICU, probably because IL-8 has more complicated sources, including mononuclear phagocytes, vascular endothelial, and smooth muscle cells [20].

Concentrations of neutrophil elastase, for their part, remained significantly above the pre-bypass baseline even by 20 h after admission to the ICU. These results imply that efforts to control inflammation during bypass should focus on inhibiting both mononuclear phagocytes and neutrophils, while efforts after bypass should focus more on neutrophils.

## Conclusions

This study found no association between the elevated pro-inflammatory factors (TNF-α, interleukin-8, and neutrophil elastase) and the risk of adverse events during hospitalization or over a three-year follow-up period. Our results call into question to what extent concentrations of pro-inflammatory factors in plasma can be used as biomarkers of inflammatory response induced by cardiopulmonary bypass, thereby justifying the continuing use of more generalized indices such as white blood cell count, heart rate, temperature and breathing rate [1, 2]. Our results argue against using cytokine concentrations to predict prognosis. Future research should explore other factors that can reliably predict prognosis after on-pump cardiac surgery. Such factors likely should take into account a broad range of biological processes, given that the inflammatory syndrome


ISSN 0022-9040. Кардиология. 2024;64(9). DOI: 10.18087/cardio.2024.9.n2682

induced by cardiopulmonary bypass involves immune responses, coagulation, fibrinolysis, complement, and other processes [3, 4]. The involvement of these diverse mechanisms may explain why neither sex nor age influenced concentrations of proinflammatory factors in our study, though both variables strongly influence immune responses in other contexts [21, 22].

## Availability of Data and Materials

The original data of this article can be requested from the corresponding author on reasonable grounds.

## Author Contributions

Conceptualization: YH, XH, LL. Data curation: LL, YH. Formal analysis: YH, XH, CL. Investigation: XH, SC, YH. Methodology: YH, JZ, LD. Project administration: JX, JZ, LD. Resources: LL, SC. Supervision: YH, XH, JX. Validation: YH, LL, JZ. Visualization: YH, CL. Writing – original draft: YH, JZ. Writing – review and editing: YH, XH, LL. All authors read and approved the final manuscript. All authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

## Ethics Approval and Consent to Participate

The study protocol was approved by the Ethics Committee of Sichuan University (approval number: 2011–133) and registered in the Chinese Clinical Trial Registry (ChiCTR-OCH-12001922).

***Funding***

*This work was supported by a grant to LL from the National Natural Science Foundation of China (82302601).*

*No conflict of interest is reported.*

**The article was received on 15/04/2024**

## REFERENCES

1. Squiccimarro E, Labriola C, Malvindi PG, Margari V, Guida P, Visicchio G et al. Prevalence and Clinical Impact of Systemic Inflammatory Reaction After Cardiac Surgery. Journal of Cardiothoracic and Vascular Anesthesia. 2019;33(6):1682–90. DOI: 10.1053/j.jvca.2019.01.043
2. MacCallum NS, Finney SJ, Gordon SE, Quinlan GJ, Evans TW. Modified Criteria for the Systemic Inflammatory Response Syndrome Improves Their Utility Following Cardiac Surgery. Chest. 2014;145(6):1197–203. DOI: 10.1378/chest.13-1023
3. Warren OJ, Watret AL, De Wit KL, Alexiou C, Vincent C, Darzi AW et al. The Inflammatory Response to Cardiopulmonary Bypass: Part 2–Anti-Inflammatory Therapeutic Strategies. Journal of Cardiothoracic and Vascular Anesthesia. 2009;23(3):384–93. DOI: 10.1053/j.jvca.2008.09.007
4. Warren OJ, Smith AJ, Alexiou C, Rogers PLB, Jawad N, Vincent C et al. The Inflammatory Response to Cardiopulmonary Bypass: Part 1–Mechanisms of Pathogenesis. Journal of Cardiothoracic and Vascular Anesthesia. 2009;23(2):223–31. DOI: 10.1053/j.jvca.2008.08.007
5. Gao M, Xie B, Gu C, Li H, Zhang F, Yu Y. Targeting the proinflammatory cytokine tumor necrosis factor-α to alleviate cardiopulmonary bypass-induced lung injury (Review). Molecular Medicine Reports. 2015;11(4):2373–8. DOI: 10.3892/mmr.2014.3050
6. Ayıkgöz Y, Salih Aydın M, Kankılıç N, Temiz E. Nuclear factor erythroid 2-related factor 2 (Nrf2), tumor necrosis factor alpha protein (TNF-α), heme oxygenase-1 (HO-1) gene expressions during cardiopulmonary bypass. Gene. 2021;790:145690. DOI: 10.1016/j.gene.2021.145690
7. Trop S, Marshall JC, Mazer CD, Gupta M, Dumont DJ, Bourdeau A et al. Perioperative cardiovascular system failure in South Asians undergoing cardiopulmonary bypass is associated with prolonged inflammation and increased Toll-like receptor signaling in inflammatory monocytes. Journal of Surgical Research. 2014;187(1):43–52. DOI: 10.1016/j.jss.2013.09.035
8. Zhao P, Lieu T, Barlow N, Sostegni S, Haerteis S, Korbmacher C et al. Neutrophil Elastase Activates Protease-activated Receptor-2 (PAR2) and Transient Receptor Potential Vanilloid 4 (TRPV4) to Cause Inflammation and Pain. Journal of Biological Chemistry. 2015;290(22):13875–87. DOI: 10.1074/jbc.M115.642736
9. Kotani N, Hashimoto H, Sessler DI, Muraoka M, Wang J-S, O'Connor MF et al. Neutrophil Number and Interleukin-8 and Elastase Concentrations in Bronchoalveolar Lavage Fluid Correlate with Decreased Arterial Oxygenation After Cardiopulmonary Bypass. Anesthesia & Analgesia. 2000;90(5):1046–51. DOI: 10.1097/00000539-200005000-00009
10. Jarczak D, Nierhaus A. Cytokine Storm–Definition, Causes, and Implications. International Journal of Molecular Sciences. 2022;23(19):11740. DOI: 10.3390/ijms231911740
11. Becker S, Lang H, Vollmer Barbosa C, Tian Z, Melk A, Schmidt BMW. Efficacy of CytoSorb®: a systematic review and meta-analysis. Critical Care. 2023;27(1):215. DOI: 10.1186/s13054-023-04492-9
12. Liu M-H, Yu H, Zhou R-H. Application of Adsorptive Blood Purification Techniques during Cardiopulmonary Bypass in Cardiac Surgery. Oxidative Medicine and Cellular Longevity. 2022;2022:6584631. DOI: 10.1155/2022/6584631
13. Magoon R, Loona M, Kaur Kohli J, Kashav R. Cytokine Adsorption in Cardiac Surgery: where do we stand? Brazilian Journal of Cardiovascular Surgery. 2020;35(3):XV–XVI. DOI: 10.21470/1678-9741-2019-0480
14. Baumann A, Buchwald D, Annecke T, Hellmich M, Zahn PK, Hohn A. RECCAS - REmoval of Cytokines during CArdiac Surgery: study protocol for a randomised controlled trial. Trials. 2016;17(1):137. DOI: 10.1186/s13063-016-1265-9
15. Garau I, März A, Sehner S, Reuter DA, Reichenspurner H, Zöllner C et al. Hemadsorption during cardiopulmonary bypass reduces interleukin 8 and tumor necrosis factor α serum levels in cardiac surgery: a randomized controlled trial. Minerva Anestesiologica. 2019;85(7):715–23. DOI: 10.23736/S0375-9393.18.12898-7
16. Liu C, Yang Y, Du L, Chen S, Zhang J, Zhang C et al. Platelet-leukocyte aggregate is associated with adverse events after surgical intervention for rheumatic heart disease. Scientific Reports. 2019;9(1):13069. DOI: 10.1038/s41598-019-49253-3
17. Yang S, Huang X, Liao J, Li Q, Chen S, Liu C et al. Platelet-leukocyte aggregates – a predictor for acute kidney injury after cardiac surgery. Renal Failure. 2021;43(1):1155–62. DOI: 10.1080/0886022X.2021.1948864
18. Khalil AA, Hall JC, Aziz FA, Price P. Tumour necrosis factor: implications for surgical patients. ANZ Journal of Surgery. 2006;76(11):1010–6. DOI: 10.1111/j.1445-2197.2006.03921.x
19. Neuschäfer-Rube F, Pathe-Neuschäfer-Rube A, Hippenstiel S, Püschel GP. PGE2 enhanced TNFα-mediated IL-8 induction in monocytic cell lines and PBMC. Cytokine. 2019;113:105–16. DOI: 10.1016/j.cyto.2018.06.020
20. Ghasemi H, Ghazanfari T, Yaraee R, Faghihzadeh S, Hassan ZM. Roles of IL-8 in Ocular Inflammations: A Review. Ocular Immunology and Inflammation. 2011;19(6):401–12. DOI: 10.3109/09273948.2011.618902
21. Márquez EJ, Chung C, Marches R, Rossi RJ, Nehar-Belaid D, Eroglu A et al. Sexual-dimorphism in human immune system aging. Nature Communications. 2020;11(1):751. DOI: 10.1038/s41467-020-14396-9
22. Huang Z, Chen B, Liu X, Li H, Xie L, Gao Y et al. Effects of sex and aging on the immune cell landscape as assessed by single-cell transcriptomic analysis. Proceedings of the National Academy of Sciences. 2021;118(33):e2023216118. DOI: 10.1073/pnas.2023216118