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# GLYCOGALYX DEGRADATION AND INFLAMMATION IN CARDIAC SURGERY

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**Abstract**

*Objective.* Experimental inflammation induces degradation of glycocalyx. We hypothesized that inflammation is an important determinant of glycocalyx degradation in patients undergoing cardiac surgery with cardiopulmonary bypass (CPB).

*Design.* A prospective observational study.

*Setting.* Operation theatre and intensive care unit of a university hospital.

*Participants.* Two separate prospective patient cohorts.

*Interventions.* Blood samples were collected at five perioperative time points in "the trial cohort" (30 patients), and only preoperatively in "the preoperative cohort" (35 patients). Plasma syndecan-1 (biomarker of glycocalyx degradation), interleukin-6 (IL-6), IL-8 and IL-10 were measured.

*Measurements and Main Results.* In the trial cohort, preoperative ranges were as follows: 0.8-198.0 ng/ml for syndecan-1; 0-902.0 pg/ml for IL-6; 0-314.9 pg/ml for IL-8 and 0-2909.0 pg/ml for IL-10. Seven out of 30 patients were outliers in terms of plasma concentrations of syndecan-1 and all cytokines preoperatively. The increase of syndecan-1 was 2.7-fold and those of IL-6 and IL-8 were both 2.5-fold. The increase of IL-10 was modest. Plasma syndecan-1 correlated with all cytokines preoperatively (IL-6: R=0.66, p<0.001; IL-8: R=0.67, p=0.001; IL-10: R=0.73, p<0.001) as well as at six hours postoperatively (IL-6: R=0.49, p=0.006; IL-8: R=0.43, p=0.02; IL-10: R=0.41, p=0.03) and on the postoperative morning (IL-6: R=0.57, p=0.001; IL-8: R=0.37, p=0.06; IL-10: R=0.51, p=0.005) but not intraoperatively. The preoperative findings of the trial cohort could be confirmed in the preoperative cohort.

*Conclusions.* In patient undergoing cardiac surgery with CPB, inflammation in terms of pro-inflammatory cytokines IL-6 and IL-8 and anti-inflammatory cytokine IL-10 is associated with glycocalyx degradation measured as plasma syndecan-1 concentrations.

**Key words:** glycocalyx, inflammation, interleukin 6, interleukin 8, interleukin 10, syndecan-1, cardiopulmonary bypass

**Introduction**

Endothelial cells are covered by a 50 µm thick layer of the glycocalyx, composed of glycoproteins and proteoglycans.[1] The glycocalyx has a regulatory role in adhesion of leukocytes and platelets as well as coagulation and hemostasis on the endothelial surface.[2-4] In an experimental setting, the pro-inflammatory cytokine TNFα mediates shedding of heparan sulfate, a major constituent of proteoglycans, and of syndecan-1, the backbone of the glycoprotein meshwork, from the glycocalyx.[5] This suggests that systemic inflammation may significantly threaten integrity of the endothelial glycocalyx. Hydrocortisone decreases glycocalyx degradation in an ex vivo model of cardiac reperfusion.[6,7]



Cardiopulmonary bypass (CPB) results in prompt degradation of the glycocalyx, which coinsides the CPB-induced systemic inflammatory response.[8,9] There is wide variation even in baseline plasma concentrations of degradation molecules of the glycocalyx in cardiac surgical patients.[10] Therefore, mathematical expression of relative changes of glycocalyx degradation markers as multiples of the respective baseline values has been applied in previous studies.[8,9,11] Thus, the interrelationship between glycocalyx degradation and activation of inflammation in previous studies is difficult to interpret. In the present study we hypothesized that CPB-induced inflammation is associated with glycocalyx degradation, as measured by syndecan-1, in patients undergoing cardiac surgery with CPB.

**Methods**

The study consists of two separate patient series, both of which were approved by the local ethics committee. All patients of both series gave written informed consent to participate in the study. The patients of the first patient cohort ("trial cohort") originated from a previous trial comparing 6% HES 130/0.42 solution (Tetraspan®, B. Braun Medical, Helsinki, Finland) and Ringer's acetate solution (Ringer-acetat®; Baxter, Helsinki, Finland) for CPB priming in cardiac surgery (ClinicalTrials.gov; NCT00797589).[12] The CPB priming fluid comprised of either 2000 ml of Ringer's acetate solution only (n=18) or 20 ml/kg of HES solution with additional Ringer's acetate solution up to 2000 ml (n=12). Otherwise, only Ringer's acetate solution was administered to all patients intraoperatively. Postoperatively, all patients received Ringer's acetate solution as a background infusion of 30-50 ml/kg and, if needed, 4% albumin solution (Albunorm 40 g/l, Octapharm, Stockholm, Sweden) for hypovolemia. Anesthesia was induced with alfentanil and etomidate and maintained with sevoflurane and alfentanil infusion. Fluid and blood product administration, management of hemodynamics as well as heparin and protamine dosing were protocolized. Mean arterial pressure was targeted to 70 mmHg when off CPB and to 50-60 mmHg during CPB. CPB was performed using non-pulsatile pump (2-2.4 l/min/$m^2$) and membrane oxygenator with mild hypothermia (nasopharyngeal temperature of 30-33 °C)[12] Plasma samples of five patients were missing. Thus, the trial cohort consisted of thirty patients. Patients with preoperative coagulation disorders, renal or hepatic failure, preoperative left ventricular ejection fraction lower than 40%, or treated with warfarin, heparin, low molecular weight heparin or clopidogrel within previous 5 days were excluded.[12]

Blood samples were drawn into polypropylene tubes containing 3.2% buffered citrate (BD Vacutainer®, BD Diagnostics, Plymouth, UK) via a non-heparinized radial arterial catheter at the following time points: 1) before induction of anesthesia, 2) after protamine administration, 3) immediately before leaving the operation theatre, 4) six hours postoperatively, and 5) at the first postoperative morning. In order to repeat the preoperative findings of the trial cohort, in the second patient series ("preoperative cohort"), a preoperative blood sample was drawn before the anesthesia induction into polypropylene tubes containing 3.2% buffered citrate (BD Vacutainer®, BD Diagnostics, Plymouth, UK) via a non-heparinized radial arterial catheter in 35 consecutive patients undergoing cardiac surgery. The blood sample tubes were immediately centrifuged and separated plasma was stored at- 70 °C until analysis of plasma concentrations of interleukin-6 (IL-6), interleukin-8 (IL-8), interleukin-10 (IL-10) (Quantikine, R&D Systems, Abington, UK) and syndecan-1 (Diaclone SAS, Besancone, France). The analyses were preformed using unthawed samples within 6 months after end of patient recruitment of each trial. C-reactive protein (CRP) and leukocyte count were measured preoperatively as part of standard clinical routine in the clinical laboratory of the hospital. The detection limit of CRP was 3 mg/l.



Data were analysed with SPSS Version 24 for Windows program (IBM Corp, Armonk, New York, USA). Kolmogorov-Smirnov test and visual inspection of histograms were used for assessing data distributions. Plasma concentrations of syndecan-1 and all cytokines did not show normal distribution. After logarithmic transformation, plasma concentrations of the trial cohort study groups were normally distributed. There were no differences of syndecan-1 or any cytokines between the HES and Ringer acetate groups in trial cohort in analysis of variance (ANOVA) for repeated measures. Thus, patients of the trial cohort were pooled as a single group in further statistical analyses. In further analyses, non-parametric tests were used without logarithmic transformation. Friedman test was used for testing differences as a function of time and Spearman's test for bivariate correlations. P-values less than 0.05 were considered statistically significant. Data are expressed as medians and interquartile ranges (IQRs) or depicted as box plots or as individual values in scatter plots.

**Results**

*Trial cohort*

Patient characteristics and procedure data of the trial cohort are presented in the Table 1. There were no major complications in any patient. In the trial cohort, seven patients were outliers in terms of plasma concentrations of syndecan-1 and all cytokines preoperatively (Fig. 1). Plasma concentrations of syndecan-1 ($p<0.001$) and all cytokines (IL-6: $p<0.001$; IL-8: $p<0.001$; IL-10: $p<0.03$) increased significantly as a function of time (Fig. 2). Perioperative plasma concentrations of syndecan-1 and all cytokines followed a level that was pre-defined by the preoperative level. In other words, preoperative plasma concentrations of syndecan-1 and all cytokines correlated with the corresponding concentrations at all later time points (data not shown). These correlations were strongest at the postoperative morning (preoperative vs. postoperative morning: syndecan-1: $R=0.71$, $p=0.004$; IL-6: $R=0.58$, $p=0.001$; IL-8: $R=0.65$, $p<0.001$; IL-10: $R=0.76$, $p<0.001$). Plasma syndecan-1 correlated significantly with all cytokines preoperatively (Fig.1) and again at six hours postoperatively (syndecan-1 vs. IL-6: $R=0.49$, $p=0.006$; syndecan-1 vs. IL-8: $R=0.43$, $p=0.02$;

syndecan-1 vs. IL-10: R=0.41, p=0.03) and on the postoperative morning (syndecan-1 vs. IL-6: R=0.57, p=0.001; syndecan-1 vs. IL-8: R=0.37, p=0.06; sydnecan-1 vs. IL-10: R=0.51, p=0.005) but not intraoperatively.

In the trial cohort, preoperative CRP was over the detection limit of 3 mg/l in only five patients and ranged between 4-13 mg/l. Preoperative leukocyte count was 7.0 (5.9-8.0) E9/l and the highest value was 10.4 E9/l. Preoperative CRP or leukocyte count did not correlate with either preoperative syndecan-1 or cytokine concentrations (data not shown).

*Preoperative cohort*

In order to repeat the above mentioned preoperative findings, we measured preoperative plasma syndecan-1 and cytokine concentrations in series of 35 patients independent of the trial cohort. Patient characteristics and procedure data of this preoperative cohort are presented in the Table 1. In this series, the range of plasma concentrations of syndecan-1 was 0-122 ng/ml and those of IL-6 0-1165 pg/ml, IL-8 0-399 pg/ml and IL-10 0-2762 pg/ml. Three patients were outliers in terms of plasma cytokine concentrations. Syndecan-1 levels correlated with all cytokines (IL-6: R=0.71, p<0.001; IL-8: R=0.70, p<0.001; IL-10: R=0.64, p<0.001). Preoperative CRP was over the detection limit of 3 mg/l in only eight patients and ranged between 4-23 mg/l. Preoperative leukocyte count was 6.8 (6.0-8.0) E9/l and the highest value was 12.2 E9/l. Preoperative CRP or leukocyte count did not correlate with either preoperative syndecan-1 or cytokine concentrations (data not shown).

**Discussion**

CPB induces a systemic inflammatory reaction.[13] Degradation of the glycocalyx in terms of plasma syndecan-1 concentrations has been well documented in both adult and pediatric cardiac surgery.[8-11,14-18] There were significant correlations between plasma cytokine and syndecan-1 concentrations both pre- and postoperatively. In experimental condition, TNFα-mediated syndecan-1 shedding could be prevented with hydrocortisone pretreatment.[5,6] Hydrocortisone reduced glycocalyx breakdown also in *ex vivo* model of cardiac ischemia-reperfusion.[7] Likewise, we have recently shown, that high-dose methylprednisolone conserved glycocalyx in neonates undergoing correction of complex congenital heart defects.[11]

Preoperatively in the trial cohort, there were seven outlying patients with high plasma concentrations of both syndecan-1 and all cytokines. As preoperative plasma concentrations of all these biomarkers correlated with the corresponding concentrations at all later time points, perioperative plasma concentrations of syndecan-1 and all cytokines were significantly pre-defined by the preoperative level. A wide distribution of preoperative syndecan-1 concentrations has been observed previously.[10] In the present study, the minimum and maximum preoperative syndecan-1 concentrations were 0.8 pg/ml and 198.0 pg/ml, respectively. This resulted in up to 247-fold difference of plasma syndecan-1 between the patients already preoperatively. As comparison, the median peak increase of syndecan-1 in the trial cohort as a function of time was only 2.7-fold. This indicates that syndecan-1 concentrations during cardiac surgery vary substantially more between patients than within patients, i.e. within different time points. This makes plasma syndecan-1 difficult to use as a biomarker. Indeed, in the pioneering study by Rehm and coworkers and in later studies both in adults and children, expression of fold-increase as multiples of the baseline value has been applied instead of absolute values.[8,9,11]

Seven out of 30 patients in the trial cohort were in a pro-inflammatory state already preoperatively in terms of increased plasma concentrations of pro-inflammatory cytokines. The outlying patients presented preoperative IL-8 concentrations comparable to the concentrations in severe sepsis and higher than in severe acute pancreatitis.[19,20] Furthermore, in the same patients, preoperative IL-6 was at the level previously detected in cardiogenic shock and at the lower range of IL-6 observed in severe sepsis.[19,21] Direct comparison of cytokine levels between different studies should be done with caution, because different commercial ELISA kits may give slightly different results. Still, it can be concluded that the preoperative pro-inflammatory cytokine concentrations in some of our patients were high. In the trial cohort, preoperative cytokine concentrations correlated with the concentrations on the postoperative morning. This implies that when the perioperative pro-inflammatory insult of CPB has ceased, the patients returned to their preoperative state. Importantly, we were able to repeat the preoperative findings in a separate cohort of 35 patients. First, the upper ranges of all three cytokines were comparable to the ones in the trial cohort. Second, three out of 35 patients presented with substantially high cytokine and syndecan-1 concentrations. Third, syndecan-1 correlated significantly with all measured cytokines.



Although surprising, corresponding observations can actually be found in the existing literature. Marano et al. found that ten out of 36 patients undergoing elective coronary artery bypass grafting presented with high TNFα concentrations already before induction of anesthesia.[22] Furthermore, a wide distribution of preoperative IL-8 concentrations comparable to the present results has been reported in elective cardiac surgery.[13] The fact that the distribution of the latter reference study was rightward skewed (standard deviation 2.4 times as high as the mean) implies that, like in the present study, there must have been some outliers with especially high IL-8 concentrations.[13] Preoperative plasma syndecan-1 concentrations with outlying patients and distribution similar to the present study have been reported in cardiac surgery.[10] In both cohorts of the present study, preoperative CRP and leukocyte counts were within normal limits or in some patients only modestly increased and did not correlate with preoperative cytokine or syndecan-1 concentrations. Possibly due to small patient number, we could not find any association between available clinical data and syndecan-1 and cytokine concentrations, either. We cannot offer any pathophysiological mechanism for the subgroup of patients with substantially high pro-inflammatory cytokines and syndecan-1 already preoperatively.

As a weakness of this observational study, only association between inflammatory reaction and glycocalyx degradation was detected. This does not necessarily mean a causal relationship, although experimental and some clinical evidence for such a relationship exist.[5-7,11] Furthermore, two treatment arms of a previous clinical trial were pooled together for this study. However, the major findings, i.e. the preoperative pro-inflammatory state of a subgroup of patients and the association of inflammation to glycocalyx degradation, were mainly based on measurements at the preoperative time point. This time point was not hampered with differences of the treatment arms. As a strength, the preoperative findings of the trial cohorts were confirmed in a separate independent patient series.

As a conclusion, a subgroup of patients undergoing elective cardiac surgery presented an inflammatory state in terms of pro-inflammatory cytokines IL-6 and IL-8 and anti-inflammatory cytokine IL-10 already preoperatively. Furthermore, this inflammation was associated with glycocalyx degradation measured as plasma syndecan-1 concentrations. The clinical significance and mechanism behind these phenomena is to be studied.

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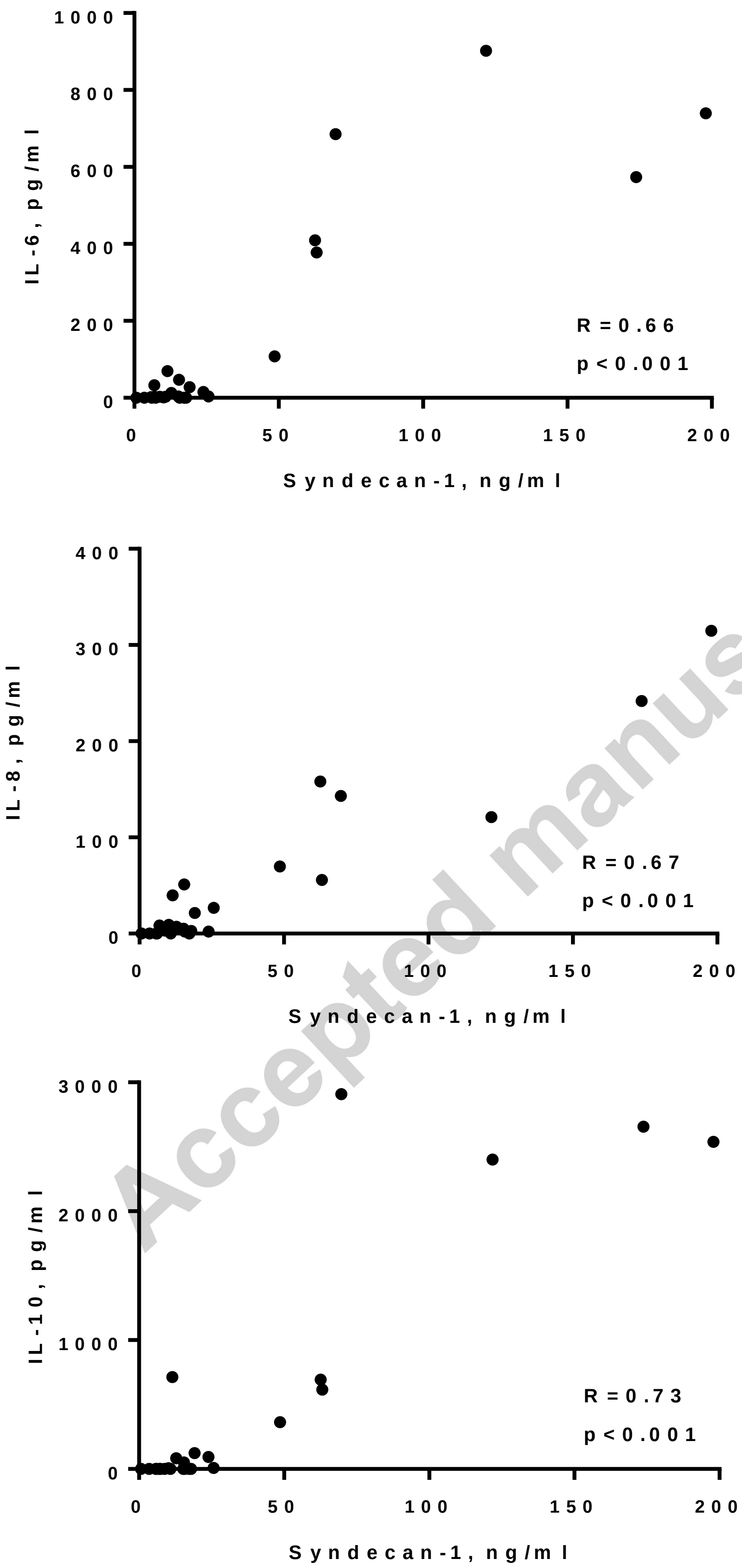
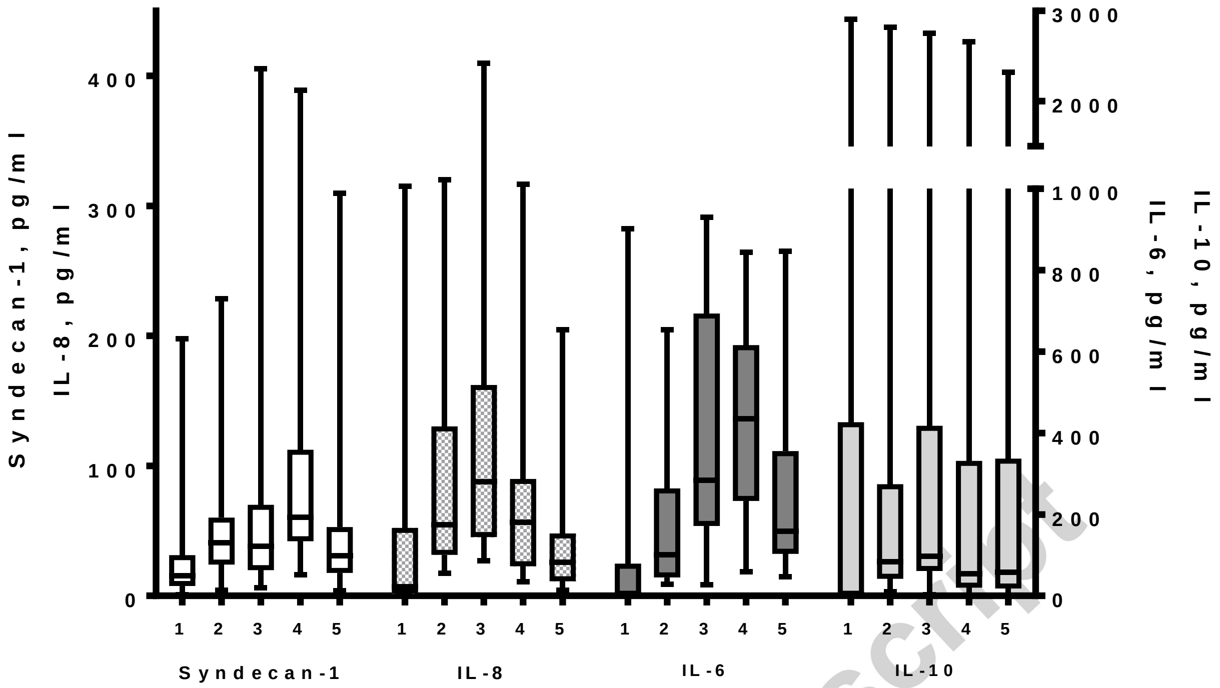
**Figure legends**

**Figure 1.** Preoperative correlations of plasma syndecan-1 concentrations with interleukin 6, interleukin 8 and interleukin 10 concentrations.

**Figure 2.** Plasma concentrations of syndecan-1, interleukin 6, interleukin 8 and interleukin 10 as a function of time. Time points: 1) before induction of anesthesia, 2) after protamine administration, 3) immediately before leaving the operation theater, 4) six hours postoperatively, and 5) at the first postoperative morning.



1000
800
600
400
200
0
IL-6, pg/ml
R = 0.66
p < 0.001
0
50
100
150
200
Syndecan-1, ng/ml
400
300
200
100
0
IL-8, pg/ml
R = 0.67
p < 0.001
0
50
100
150
200
Syndecan-1, ng/ml
3000
2000
1000
0
IL-10, pg/ml
R = 0.73
p < 0.001
0
50
100
150
200
Syndecan-1, ng/ml



Syndecan-1, pg/ml
IL-8, pg/ml
400
300
200
100
0
3000
2000
1000
800
600
400
200
0
IL-10, pg/ml
IL-6, pg/ml
1 2 3 4 5 1 2 3 4 5 1 2 3 4 5 1 2 3 4 5
Syndecan-1
IL-8
IL-6
IL-10

**Table 1.** Patient data of the trial and preoperative cohorts.

The data are presented as medians and interquartile ranges or as numbers.

| | Trial cohort | Preoperative cohort |
|---|---|---|
| **Age, yrs** | 73 (68-80) | 67 (56-73) |
| **Gender male/female, n** | 18/12 | 26/9 |
| **Surgery** | | |
| **CABG, n** | 0 | 16 |
| **Aortic reconstruction, n** | 2 | 3 |
| **AVR, n** | 0 | 5 |
| **AVR + CABG, n** | 24 | 5 |
| **MVR, n** | 0 | 3 |
| **MVR + CABG, n** | 3 | 3 |
| **AVR + MVR, n** | 1 | 0 |
| **CPB time, min** | 146 (125-159) | |
| **Aortic cross-clamping time, min** | 111 (99-127) | |
| **Intraoperative fluid balance, ml** | 3987 (2614-5813) | |
| **Postoperative fluid balance till the first postoperative morning, ml** | 1266 (536-2218) | |
| **Cumulative fluid balance till the first postoperative morning, ml** | 5601 (4209-6681) | |