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Original Article

Methylprednisolone Does Not Enhance Paraoxonase 1 Activity During Cardiopulmonary Bypass Surgery—A Randomized, Controlled Clinical Trial

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Objectives: Cardiopulmonary bypass (CPB) is linked to systemic inflammatory responses and oxidative stress. Paraoxonase 1 (PON1) is an antioxidant enzyme with a cardioprotective role whose activity is decreased in systemic inflammation and in patients with acute myocardial and global ischemia. Glucocorticoids counteract the effect of oxidative stress by upregulating PON1 gene expression. The authors aimed to determine the effect of methylprednisolone on PON1 activity during cardiac surgery on CPB.
Design: Prospective, randomized, controlled clinical trial.
Setting: The University Medical Center Ljubljana, Slovenia.
Participants: Forty adult patients who underwent complex cardiac surgery on CPB between February 2016 and December 2017 were randomized into methylprednisolone and control groups (n = 20 each).
Interventions: Patients in the methylprednisolone group received 1 g of methylprednisolone in the CPB priming solution, whereas patients in the control group were not given methylprednisolone during CPB.
Measurements and Main Results: The effect of methylprednisolone from the CPB priming solution was compared with standard care during CPB on PON1 activity until postoperative day 5. Correlations of PON1 activity with lipid status, mediators of inflammation, and hemodynamics were analyzed also. No significant differences were found between study groups for PON1 activity, high-density lipoprotein, and low-density lipoprotein in any of the measurement intervals ($p > 0.016$). The methylprednisolone group had significantly lower tumor necrosis factor alpha ($p < 0.001$) and interleukin-6 ($p < 0.001$), as well as C-reactive protein and procalcitonin ($p < 0.016$) after surgery. No significant difference was found between groups for hemodynamic parameters. A positive correlation existed between PON1 and lipid status, whereas a negative correlation was found between PON1 activity and tumor necrosis factor alpha, interleukin-6, and CPB duration.
Conclusions: Methylprednisolone does not influence PON1 activity during cardiac surgery on CPB.

Key Words: paraoxonase 1; cardiopulmonary bypass; oxidative stress; inflammation; glucocorticoids; methylprednisolone

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CARDIOPULMONARY BYPASS (CPB) is an unavoidable part of most cardiac surgical procedures; however, endothelial injury and organ dysfunction are associated with its application, which has been directly linked to systemic inflammatory responses and oxidative stress caused by this technique.[1] Indeed, it has been demonstrated that the on-pump procedures

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give rise to a more pronounced systemic inflammation and oxidative stress than the off-pump cardiac surgery.[2,3]

Paraoxonase-1 (PON1) is a 44-kDa, calcium-dependent protein that remains associated with apolipoproteins apo-AI and apo-J on the particle of high-density lipoprotein (HDL). The enzyme is synthesized in the liver and secreted into the blood.[4] It was first studied for its organophosphate activity, but it has several other enzymatic activities, including peroxidase and aryl esterase-like activities, which act to detoxify reactive oxygen species (ROS). Paraoxonase-1 hydrolyzes the proinflammatory lipid peroxides generated by oxidized low-density lipoprotein (LDL), reducing oxidative stress.[5,6] Moreover, PON1 was shown to decrease monocyte chemotaxis and adhesion to endothelial cells,[7] and inhibit monocyte-to-macrophage differentiation,[8] whereas its absence was associated with overexpression of adhesion molecules.[9] PON1 directly suppresses macrophage proinflammatory responses, suggesting that it decreases sustained proinflammatory reactions.[10] These observations suggest an antiinflammatory role of PON1.

Serum PON1 activity reportedly decreases during inflammation.[11] It was shown that interleukin-1b (IL-1b) and tumor necrosis factor alpha (TNF-a) downregulate, but IL-6 upregulates the transcriptional regulatory activity of the PON1 gene in HepG2 cells. This difference in the regulation of the PON1 gene in HepG2 cells in response to various cytokines may contribute to the alteration of serum PON1 concentration according to the disease reflected by the cytokine networks.[12] The paraoxonase1 plasma activity is markedly reduced during coronary artery bypass grafting surgery.[13]

Glucocorticoids, potent antiinflammatory agents, have been administered traditionally to patients undergoing cardiac surgery with CPB to ward off detrimental physiologic alterations associated with the activation of the systemic inflammatory response. Yet, few well-controlled investigations exist, and the use of these drugs in this setting remains controversial.[14] From the available evidence, the 2004 American Heart Association guidelines for coronary artery bypass grafting "support liberal prophylactic use in patients undergoing extracorporeal circulation."[15] However, the newest evidence-based data[16] do not support the routine use of steroids in cardiac surgery, as their administration did not decrease the risk of death, myocardial infarction, stroke, renal failure, new atrial fibrillation, or transfusion, although they lowered the risk of respiratory failure and infection, and reduced lengths of intensive care unit (ICU) and hospital stay. However, a big part of the world still uses glucocorticoids during CPB surgery.

It may be speculated that some of the antiinflammatory effects of glucocorticoids could be mediated by interference with ROS. In addition, they stimulate the expression of antioxidant enzyme–related genes to suppress oxidative processes.[17–20] Dexamethasone counteracts the effect of oxidative stress by upregulating PON1 gene expression.[21,22]

The possible effect of glucocorticoids on PON1 during CPB is not yet known. Consequently, this study aimed to examine the effect of methylprednisolone in CPB priming solution on PON1 levels during complex cardiac surgery. The authors hypothesized that methylprednisolone increases PON1 activity during cardiac surgery on CPB.

Methods

Study subjects were selected from the subjects of the **I**mmuno**m**odulatory **E**ffect of **E**xtra**c**orporeal **C**ytokine **A**dsorption in **C**ardiac **S**urgery (IMEECCACS) trial. IMEECCACS was a prospective, randomized, blinded, interventional, single-center controlled clinical trial, and was carried out at the Clinical Department of Anesthesiology and Perioperative Intensive Therapy, Division of Cardiovascular Anesthesia and Intensive Therapy, and Clinical Department of Cardiovascular Surgery at the University Medical Centre in Ljubljana (Slovenia), between February 2016 and December 2017. Approval for the trial was obtained from the National Medical Ethics Committee (Affiliation: Ministry of Health of Republic of Slovenia; approval No. 118/02/15), and was accompanied by written informed consent from each patient. The trial was conducted following the Helsinki Declaration and registered at ClinicalTrials.gov (NCT02666703) before patient recruitment began.[23]

The present study included 2 of 3 groups in the IMEECCACS trial: the methylprednisolone group (MP; n = 20; 1 g of methylprednisolone added in CPB priming solution), and the control group, CO (n = 20; usual care; without methylprednisolone during CPB). These 40 patients were assigned for elective complex cardiac surgery with CPB, and previously were allocated randomly into different study groups. Randomization was carried out by one of the members of the study team a day before surgery, and achieved using identical sealed envelopes, whereby each patient selected an envelope that assigned them to 1 of the 2 treatment groups. Randomization allocation numbers were generated by Research Randomizer (https://www.randomizer.org/). Patients, ICU, ward personnel, and laboratory staff who participated in the trial were "blinded" for assigned treatment throughout the study. Exception from being blinded was for personnel in the operating room, who were not included in data collection and analysis.

Based on previous literature,[24] the power of study calculation for the IMEECCACS trial[23] was based on the assumption that a change in the mean difference of 1 standard deviation would suffice as a clinically relevant effect. To achieve 80% statistical power with a significance level of 5%, the calculation defined the need for 17 patients per group, which indicated 34 patients across the 2 treatment groups. The power analysis for this trial was based on the data of a previously published experiment[13] on PON1 activity during cardiac surgery on CPB (Supplementary Table S1). Data in all stages of the experiment, regardless of whether paraoxonase activity toward paraoxon or paraoxonase activity toward phenyl acetate, were considered, provided the same sample size. Considering an estimated 15% dropout rate, 20 patients per group were included in the final analysis to avoid the risk of low power.

Inclusion and Exclusion Criteria

This study included patients >18 years old who were admitted for elective complex cardiac surgery with an expected CPB duration of >90 minutes. The surgery thus included combined valve and coronary bypass grafting surgery, concomitant surgery of 2 or more valves, surgery of the ascending aorta and aortic arch, and reoperations of the same type.

Exclusion criteria included refusal to participate in the study; age <18 years; pregnant women; emergency procedures; heart transplantation; implantation of left ventricular assist device, right ventricular assist device, or total artificial heart; treatment with chemo/immunosuppressive therapy; treatment with antileukocyte drugs or TNF-a blockers; immunocompromised patients (eg, with AIDS); leucopenia (<4.0 × 109 cells L-1); clinical and/or laboratory signs of infection (ie, C-reactive protein [CRP] >2 mg dL-1 [20 mg L-1]; procalcitonin >0.5 mg L-1; leukocytes >10.0 × 109 cells L-1); serum creatinine >2 mg dL-1 (176 mmol L-1); bilirubin >2 mg dL-1 (34.2 mmol L-1); history of stroke; malnourished patients; and body mass index <18 kg m^2-1.

Procedure

After preoperative assessment and premedication with benzodiazepines orally (alprazolam 0.25 mg or 0.5 mg the night before surgery; 1 hour before surgery: pantoprazole 40 mg and diazepam 2 mg or 5 mg orally, as per department's protocol), patients underwent surgery under general anesthesia. Before induction of general anesthesia, each patient had an arterial cannula inserted into their radial artery for hemodynamic measurements (FloTrac System; Edwards Lifesciences, Irvine, CA) and for drawing blood samples for analysis. Induction of general anesthesia was intravenous, using fentanyl 5-to-10 mg/kg, propofol 1-to-2 mg/kg, and rocuronium bromide 0.6 mg/kg for tracheal intubation. Patients were ventilated with volume-controlled ventilation with a tidal volume (Vt) of 6-to-8 mL/kg ideal body weight, to maintain normocarbia. After induction of general anesthesia, a central venous catheter was inserted (PreSep Central Venous Oximetry catheter; Edwards Lifesciences) for hemodynamic measurements, as well as a high-flow device (AVA; Edwards Lifesciences) for fluid replacement therapy (both in the jugular veins). Total intravenous anesthesia was maintained throughout the whole surgical procedure using continuous infusions of propofol (dose according to the measurement of the depth of anesthesia with bispectral index) and remifentanil (0.3-0.5 μg/kg/min).

Standard and extended hemodynamic monitoring was maintained intraoperatively, including for electrocardiogram, invasive arterial blood pressure, central venous pressure, cardiac index, systemic vascular resistance index, and central venous oxygen saturation (EV 1000 Platform; Edwards Lifesciences). Hemoglobin oxygen saturation, capnography (end-tidal CO_2), cerebral oxygen saturation (near-infrared spectroscopy; INVOS Cerebral/Somatic Oximeter; Medtronic, Minneapolis, MN), depth of anesthesia (bispectral index; BIS VISTA Brain Monitoring System; Medtronic), body temperature, urine output, and acid/base status with blood gas analysis were also monitored. The type and duration of surgery were recorded, along with the duration of CPB and aortic cross-clamp, as well as blood loss and the quantity of administered crystalloids, colloids, blood components, procoagulant factors, and inotropic/vasoactive drug and insulin consumption.

The CPB used was standard, mildly hypothermic (32°C to 34°C), with a nonpulsatile flow of 2.2-to-2.4 L m^2 and a −1 body surface area. Two types of oxygenators were used (at the discretion of the leading perfusionist): Inspire system (Sorin, Milan, Italy), coated with a phosphorylcholine inert surface, and Fusion system (Medtronic), coated with a hydrophilic polymer. Priming solutions included 1,200 mL of lactated ringer, 250 mL of 20% mannitol, and 100 mg of heparin. For the MP group, methylprednisolone 1 g was added to the priming solution and used to fill the CPB machine. For the CO group, no methylprednisolone was used with CPB.

Norepinephrine was used as the main vasoactive drug, and its administration was mean arterial pressure–guided, aiming for 70-to-75 mmHg throughout the perioperative course. Blood transfusion was performed at the discretion of the leading anesthesiologist and by institutional guidelines. Coagulation factor administration was guided predominantly by rotational thromboelastometry. Transesophageal echocardiography was used during each surgical procedure to determine global cardiac function, regional wall motion abnormalities, valvular function, volume status, and deaeration before unclamping the aorta.

After surgery, patients were transferred to a cardiovascular ICU, intubated, sedated, and mechanically ventilated. They were awakened and extubated when the extubating criteria were fulfilled, such as an awake, cooperative patient with completely reversed neuromuscular function, hemoglobin oxygen saturation >96%, with a fraction of inspired O_2 of ≤0.4, end-tidal CO_2 of 4-to-6 kPa, stable hemodynamics, and normal core temperature, with retrosternal drainage <100 mL/h. Postoperative pain was treated by continuous intravenous infusion of morphine, as well as intravenous paracetamol. Patients were transferred to the ward when they met the following criteria: hemoglobin oxygen saturation of ≥94% at a fraction of inspired O_2 of ≤0.4; hemodynamic stability with no hemodynamically significant arrhythmias, without intravenous inotropic or vasopressor therapy, with diuresis >0.5 mL/kg/h, with neither delirium nor epileptic activity, and without signs of infection.

Patient follow-up after discharge from the hospital was done by telephone call 30 days after surgery, with the focus being put on late postoperative morbidity and mortality.

Data Collection and Measurement Time Frame

Patient preoperative data (ie, demographic characteristics, preoperative medical status, and patient assessment according to the European System for Cardiac Operative Risk Evaluation II [EuroSCORE II] for risk of death [http://www.euroscore.org/calc.html] and according to the American Society of Anesthesiologists classification) were documented, in addition to

intraoperative data (procedural times, blood loss, quantity of administered crystalloids, colloids, blood/blood components, procoagulant factors, inotropic /vasoactive drugs, and insulin). Furthermore, the following postoperative data were documented: duration of mechanical ventilation in the ICU; length of ICU and in-hospital stays; postoperative consumption of inotropic/vasoactive drugs and insulin, along with fluids, blood, and blood components; postoperative complications (eg, bleeding, hemodynamic instability, impaired respiratory function, worsening of renal, liver and brain functions, infections); and 30-day mortality.

Outcome Measures

Primary outcome measures were an evolution of PON1 activity, HDL, LDL, cytokine levels (TNF-a, IL-1b, IL-6, IL-8, IL-10), hs-CRP, and procalcitonin. Secondary outcome measures were changes in hemodynamic parameters (ie, cardiac index, systemic vascular resistance index, and central venous oxygen saturation). Other prespecified outcome measures included duration of postoperative mechanical ventilation, length of ICU stay, use of inotropic/vasoactive drugs, use of fluid/blood products and insulin, length of in-hospital stay, and 30-day mortality.

Blood Sampling

Determination of PON1 Activity

For serum activity determination of PON1, 3-to-5 mL of arterial blood were collected from all patients enrolled in the study into tubes without anticoagulants. After the blood had coagulated, the clot was removed from the serum by centrifugation. The prepared serum samples were then frozen at −20° C for 1 hour, waiting for analysis. PON1 activity in serum samples was determined spectrophotometrically by measuring the absorbance over time in the presence of phenylacetate substrate.[25]

Cytokine and Complement Analysis

For all laboratory analyses, blood samples were collected without any additives. The serum was separated from the clotted blood by centrifugation (1,500 g for 10 minutes), and aliquots were stored at −20°C until analyzed. Serum TNF-a, IL-1b, IL-6, IL-8, and IL-10 were measured using chemiluminescent immunometric assays on an automated analyzer (reagent and analyzer: Immulite; Siemens Healthcare, Erlangen, Germany). The analytical sensitivity was 0.1 ng/L for IL-1b and 1 ng/L for the other parameters.

Statistical Analysis

Demographic and clinical baseline data were summarized according to mean and SD, or median and range, as expressed through minimum and maximum values for metric variables, or with absolute frequencies for categorical variables.

As Kolmogorov–Smirnov tests for normal distributions rejected the null hypothesis that most variables were normally distributed, nonparametric Kruskal–Wallis tests were applied for independent samples and median tests for independent samples. Both these tests indicated that there were significant overall differences between the groups for several of the variables (with p values; Kruskal–Wallis's test). To analyze differences in greater detail, Mann–Whitney tests were applied for testing each pair of patient groups for different distributions. Variables with repeated measurements were analyzed using the Friedman test for related samples. Differences between individual pairs of measurements were further analyzed using the Wilcoxon signed-rank test. Spearman correlation test was used to test the correlation between variables. The analyses were performed using the SPSS v.25.0 software package (IBM SPSS, Inc, Chicago, IL). Before determining significance, the Bonferroni correction for multiple comparisons was applied with an initially stated significance threshold of $p < 0.05$.

Results

In total, 67 patients were assessed for eligibility, and 12 were excluded afterward (10 did not meet inclusion criteria, and 2 declined to participate). The remaining 55 patients were randomized in the MP or CO group, with 15 patients dropping out of the study after randomization. In 1 patient, it was impossible to start CPB (heavily calcified aorta and big arteries); 6 had shorter CPB than planned; 5 patients had surgical complications with redo thoracotomy and secondary chest closure; and 3 patients were lost to follow-up (Fig 1).

Forty patients (67.5% male, 32.5% female) were included in the final analysis, with 20 patients in each group. Baseline and perioperative characteristics are detailed in Table 1. There were no significant differences between groups for patient age, sex, EuroSCORE II, American Society of Anesthesiologists classification, left ventricular ejection fraction percentage, type of surgery, duration of surgery, duration of CPB, and aortic cross-clamping. There were also no significant differences between the groups for the 2 different types of oxygenators used for CPB.

PON1 after CPB recorded a decrease in its activity, which was greatest immediately after CPB in the MP group, and on postoperative day (POD) 5 in the CO group. That is, depleted PON1, after a temporary increase 24 hours after surgery, started to fall again until POD 5 because of oxidative stress during cardiac surgery and CPB (Fig 2, Supplementary Table S2, and Table S5). Otherwise, there was no statistically significant difference between the groups in terms of PON1 activity over each period.

Lipid status (Fig 2 and Supplementary Table S2) also showed dynamic changes after CPB. High-density lipoprotein concentrations decreased immediately after CPB; otherwise, they began to increase shortly after completion of surgery, and that increase continued in the following days, although POD 5 reported a slight decrease again. The LDL concentration also decreased after CPB and rose immediately after its end; it decreased again up to 48 hours after the intervention, although

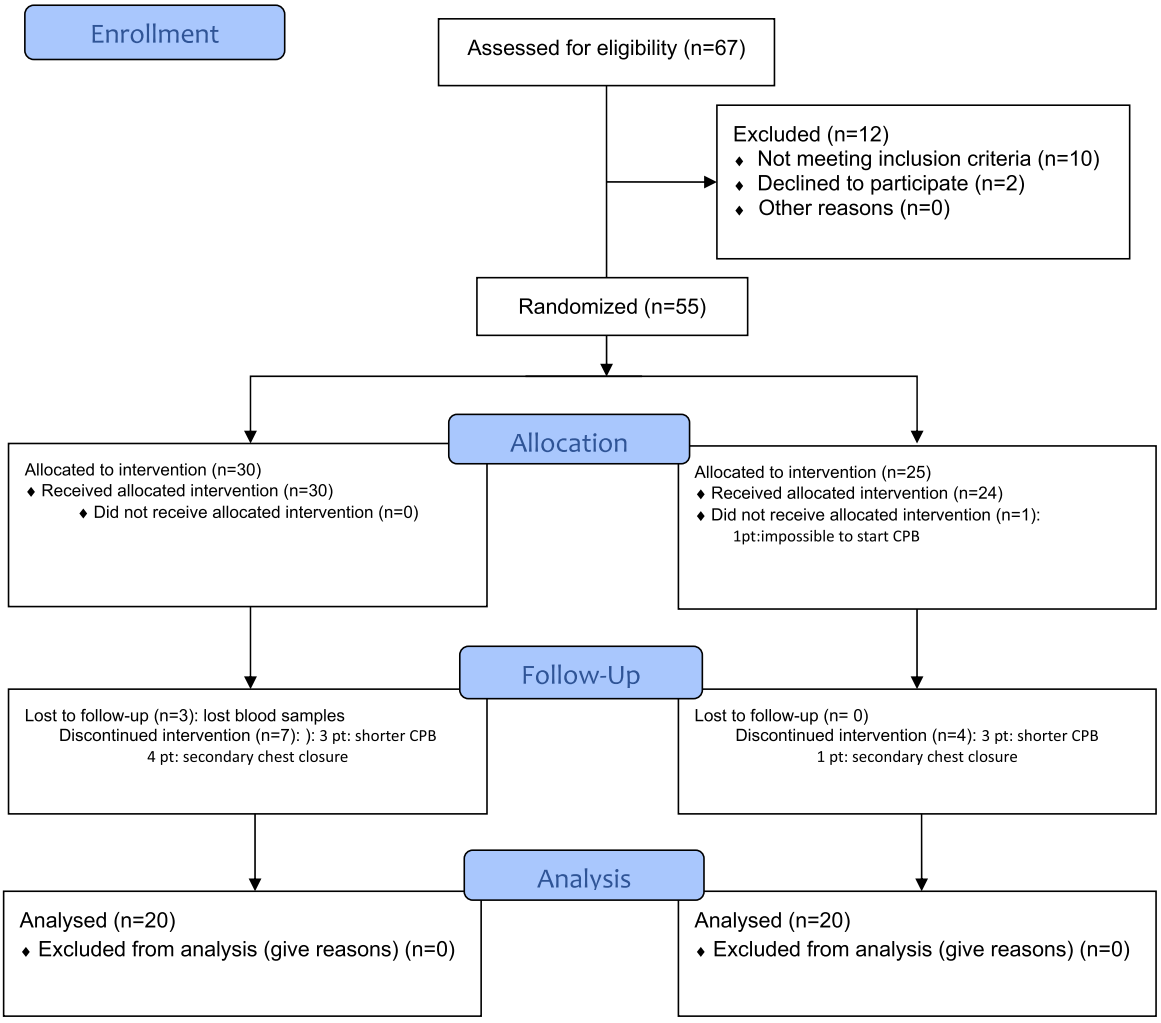

Fig 1. Consolidated Standards of Reporting Trials flow diagram. CPB, cardiopulmonary bypass.

the POD 5 values began to rise again. No significant difference existed between the groups for HDL and LDL ($p > 0.016$).

Inflammatory mediators (TNF-a, IL-1b, IL-6, IL-8, IL-10, hs-CRP, procalcitonin) (Fig 3 and Supplementary Table S2) primarily showed increased levels after CPB (except TNF-a in the MP group), which began to decrease down to pre-CPB levels mostly 24 hours after surgery; changes were more pronounced in the CO group (in addition to IL-10, which reached higher concentrations in the MP group). Statistical significance was seen for TNF-a, IL-8, and IL-10 after CPB and after surgery (on admission to the ICU) ($p < 0.016$), for IL-6 after surgery, 24 hours, and 48 hours after surgery ($p < 0.016$), and for hs-CRP and procalcitonin from 24 hours after surgery until POD 5 ($p < 0.016$).

No significant differences ($p > 0.016$) were reached between the groups for hemodynamic parameters (cardiac index, systemic vascular resistance index, central venous oxygen saturation, mean arterial pressure) during surgery and afterward, for the first 48 hours postoperatively (except for mean arterial pressure, 48 hours after surgery, which was higher in the CO group) (Fig 4 and Supplementary Table S3).

There was a negative correlation between PON1 levels (POD 5) and the duration of CPB ($p = 0.012$). On the other hand, PON1 correlated positively with HDL (24 and 48 hours after surgery; $p = 0.022$ and $p = 0.015$) and LDL (24 and 48 hours after surgery and POD 5; $p < 0.05$). The correlation between PON1 and proinflammatory mediators was seen with TNF-a and IL-6. Namely, the correlation between PON1 and TNF-a was 48 hours after surgery ($p = 0.01$); IL-6 levels correlated negatively with PON1 activity immediately after CPB, after surgery, and 24 hours afterward ($p = 0.039$, 0.031, and 0.032, respectively) (Supplementary Table S4).

Discussion

In this study, the effect of CPB on serum PON1 activity was investigated as a "surrogate" of the antioxidant status of patients during cardiac surgery; its values were compared between patients who received methylprednisolone in CPB priming solution, with a group of "control" patients, who were not given glucocorticoids during surgery. The results of the trial rejected the hypothesis, as no significant differences in

Table 1
Patient and Surgery Characteristics at Baseline

Characteristic	Methylprednisolone Group (n = 20) Median (IQR) (Q1-Q3)	Control Group (n = 20) Median (IQR) (Q1-Q3)	p Value*
Age, Y	66.5 (37) (43-80)	71 (54) (31-85)	0.171
Male/female	13/7	14/6	0.739
Body surface area, m^2	1.81 (0.58) (1.55-2.13)	1.97 (0.61) (1.65-2.26)	0.068
ASA status	3 (1) (3-4)	3 (1) (3-4)	
EuroSCORE II	1.99 (12.02) (0.96-12.98)	2.78 (9.78) (0.96-10.74)	0.203
LVEF %	61.5 (58) (29-87)	65 (38) (39-77)	0.683
One valve surgery + CABG, M/F	5/3	6/3	0.749
More than 1 valve surgery + CABG, M/F	2/0	1/0	0.548
More than 1 valve surgery, M/F	1/3	1/2	0.677
Surgery of ascending aorta, M/F	1/1	4/0	0.376
Valve surgery + CABG + surgery of ascending aorta, M/F	1/0	0/0	0.311
Valve surgery + surgery of ascending aorta, M/F	2/0	1/1	1.000
Valve surgery + other procedures (ASD, RFA), M/F	1/0	1/0	1.000
Valve surgery + surgery of ascending aorta + other procedure (ASD, RFA), M/F	0/0	0/0	1.000
Anesthesia time, min	358 (314) (249-563)	335 (270) (258-528)	0.561
Surgery time, min	245.5 (295) (150-445)	237 (232) (161-393)	0.457
Cardiopulmonary bypass time, min	150.5 (150) (90-240)	127 (148) (90-238)	0.239
Aorta cross-clamping time, min	104 (140) (66-206)	102 (121) (63-184)	0.482
Packed red blood cells, mL	0 (585) (0-585)	0 (1,760) (0-1,760)	0.176
Fresh frozen plasma, mL	0 (1,322) (0-1,322)	503 (1,282) (0-1,282)	0.616
Platelets, mL	0 (350) (0-350)	0 (0) (0-0)	0.317
Cell saver, mM	580 (2,927) (0-2,927)	511 (1,000) (0-1,000)	0.285
Fibrinogen, g	0 (4) (0-4)	0 (3) (0-3)	0.482
Prothrombin complex concentrate, IE	0 (3,000) (0-3,000)	0 (0) (0-0)	0.076
Recombinant activated factor VII, mg	0 (7) (0-7)	0 (0) (0-0)	0.317
Crystalloids, mL	1,500 (1,000) (1,000-2,000)	1,000 (1,000) (1,000-2,000)	0.69
Colloids, mL	0 (1,000) (0-1,000)	0 (500) (0-500)	0.386
Postoperative mechanical ventilation, h	9.5 (1,254) (3-1,257)	8.5 (24) (5-29)	0.514
Length of stay (ICU), h	90.5 (1,874) (58-1,932)	117 (343) (64-407)	0.465
Length of stay (hospital), d	10 (76) (6-82)	11 (20) (6-26)	0.644
New-onset atrial fibrillation	4	3	0.677
Worsening of renal function	0	1	0.311
Postoperative myocardial infarction	0	1	0.311
Postoperative delirium	0	0	1.000
Dressler syndrome	3	2	0.633
Tamponade	3	1	0.292
Thrombocytopenia	1	0	0.311
Infection	2	4	0.376
Without postoperative complications	7	4	0.288
30-d mortality	0	0	1.000
30-d re-admission	2	3	0.633
1-y mortality	1	0	0.311

NOTE. Data are presented as median (IQR) (Q1-Q3).
Abbreviations: ASA, American Society of Anesthesiologists; ASD closure, atrial septal defect; CABG, coronary artery bypass grafting; EuroSCORE II, European System for Cardiac Operative Risk Evaluation II; ICU, intensive care unit; LVEF %, left ventricular ejection fraction percentage; M/F, male/female; RFA, Radiofrequency ablation.
* *P* value for comparisons of methylprednisolone group versus control group.

PON1 activity were found between groups, which demonstrated that methylprednisolone most likely does not enhance PON1 activity during CPB surgery. This was the opposite of the results of other authors who demonstrated upregulation of PON1 gene expression and increase of PON1 messenger RNA (mRNA) level with glucocorticoids in inflammatory bowel disease[21] and in mouse hepatoma cell lines[22]; however, they used dexamethasone instead of methylprednisolone, which is not supposed to be inferior to dexamethasone except for indications other than inflammation and oxidative stress. However, there is no existing report regarding the effect of glucocorticoids on PON1 activity in cardiac surgery with CPB.

The results of the study also confirmed the already known data that PON1 activity decreases after CPB. Time series indicate that differences between preinduction measurements and other individual measurements throughout the trial were statistically significant in both study groups. PON1 activity was lower with longer CPB duration (negative correlation). A negative correlation also was documented between PON1 activity and TNF-a, as well as IL-6, which agreed with data that inflammation and oxidative stress downregulate serum PON1 expression due to the

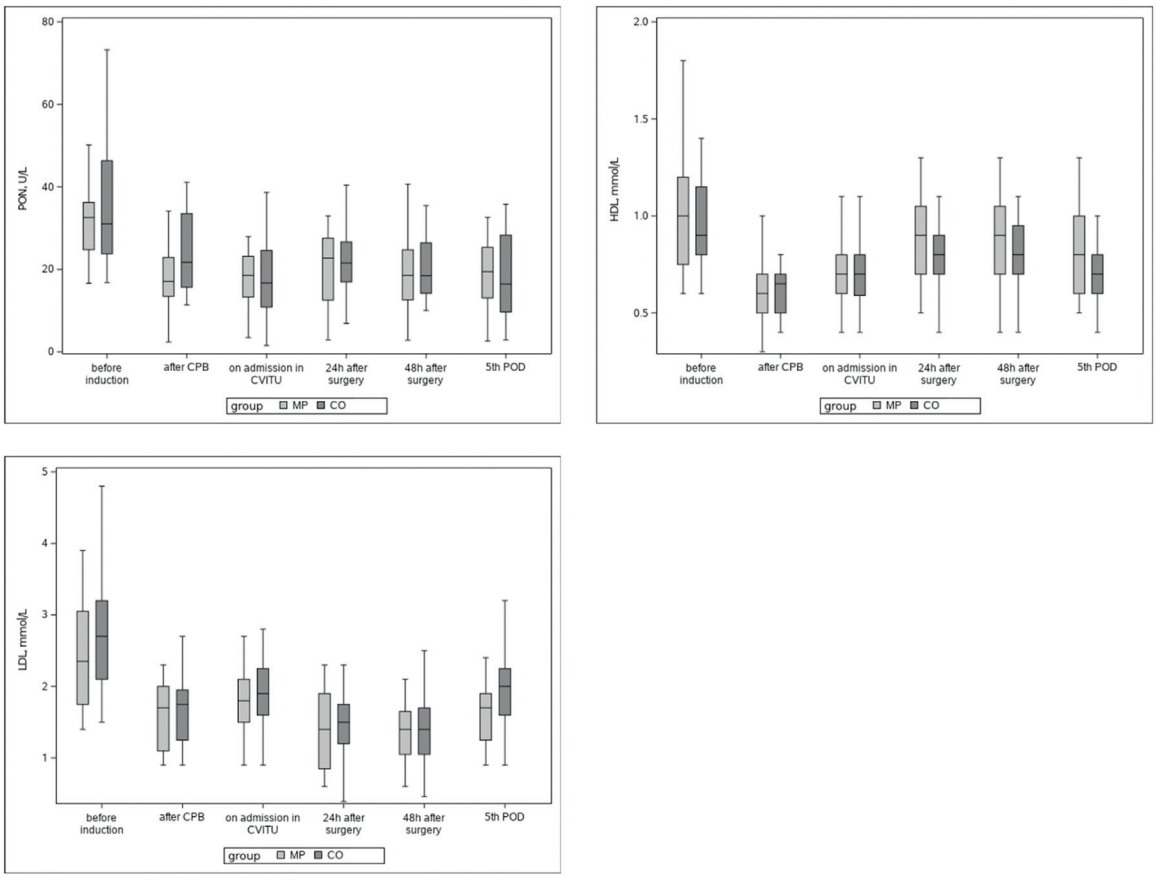

Fig 2. Paraoxonase 1 and lipid status. CO, control group; CVITU, cardiovascular intensive therapy unit; HDL, high-density lipoprotein; LDL, low-density lipoprotein; MP, methylprednisolone group; POD, postoperative day.

changes in the redox status.[28] On the other hand, PON1 correlated positively with patients' lipid status (HDL and LDL).

Oxidative stress arises when there is a marked imbalance between the production of ROS and their removal by antioxidants.[21] The paraoxonase family consists of 3 enzymes: PON1, PON2, and PON3. These enzymes are closely associated with the cellular antioxidant system; all 3 paraoxonases modulate oxidative stress and inflammation.[27] Among all of them, PON1 is the most studied.[21] Known functions of PON1 include atheroprotective, esterase, and peroxidase activity, cholesterol transport, antioxidant activity, antiplatelet activity, and lactonase and thiolactone activity. It is impressive that a single enzyme carries out so many reactions. It is, therefore, considered that PON1 is not a single enzyme but a multiple-enzyme system.

PON1 is a negative acute-phase protein: Plasma PON1 levels decrease rapidly in response to a systemic inflammatory response. Enzyme synthesis is regulated by changes in mRNA levels in the acute-phase reaction. PON1 mRNA levels in human hepatocytes have been observed to decrease after incubation with oxidatively modified phospholipids or with cytokines that stimulate acute-phase reactions such as IL-1b and TNF-a. Luyten et al.[1] observed that the use of CPB decreases the total antioxidant capacity of plasma. In circumstances of heightened oxidative stress, the PON1 supply may be depleted due to inhibition of PON1 expression and decreased protein synthesis in the liver, which may affect the prognosis after surgery.

It has been shown that tissue ischemia caused by CPB is associated with increased levels of oxidative stress. In addition, during reperfusion, tissue oxygen supply is restored rapidly, and free oxygen radicals are generated in quantities that exceed local antioxidant defenses. Under conditions of intense oxidative stress, the levels of plasma antioxidants are reduced. Total plasma antioxidant capacity in patients who undergo surgery on CPB is depleted gradually after surgery.[26] A study[13] found a significant decrease in PON1 activity after aortic ligation and then an increase after surgery; no correlation was observed between PON1 activity and aortic cross-clamp time. In another study,[29] however, the authors found a significant increase in oxidative stress measurements after reperfusion, and the relation of oxidation imbalance to aortic clamping time. These findings supported the idea that the intense oxidative stress that arises with CPB contributes to the depletion of the plasma supply of PON1. This phenomenon may be due to inhibition of PON1 gene expression and decreased protein synthesis in the liver. Differences in PON1 activity against phenyl acetate were observed if expressed as PON1 activity per gram of plasma protein. This may indicate that the hemodilution process is not the only reason for the change in PON1 activity.[13]

Over the years, a wide variety of antiinflammatory treatment options have been used in patients subjected to CPB in hopes of attenuating systemic inflammatory response syndrome (SIRS), including glucocorticoids. Despite numerous clinical trials in the setting of surgery and sepsis, it remains unclear whether there are benefits of glucocorticoids in modifying inflammation during CPB surgery.[30]

Some studies suggest that perioperative steroids have significant clinical benefits in patients undergoing surgery on CPB by decreasing the risk of new-onset atrial fibrillation, whereas

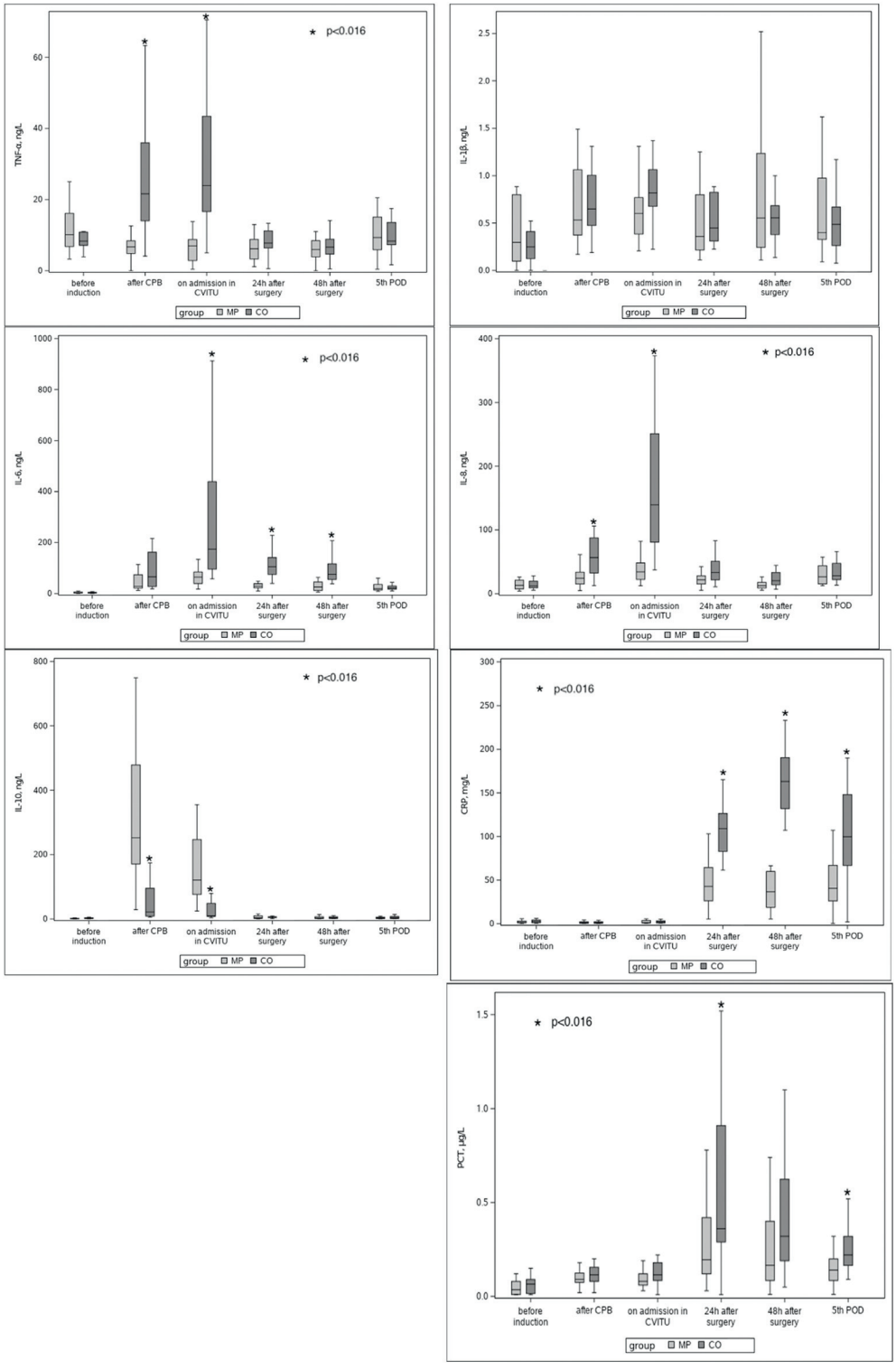

Fig 3. Inflammatory mediators. CO, control group; CPB, cardiopulmonary bypass; CRP, C-reactive protein; CV-ITU, cardiovascular intensive therapy unit; IL, interleukin; MP, methylprednisolone group; PCT, procalcitonin; POD, postoperative day; TNF, tumor necrosis factor.

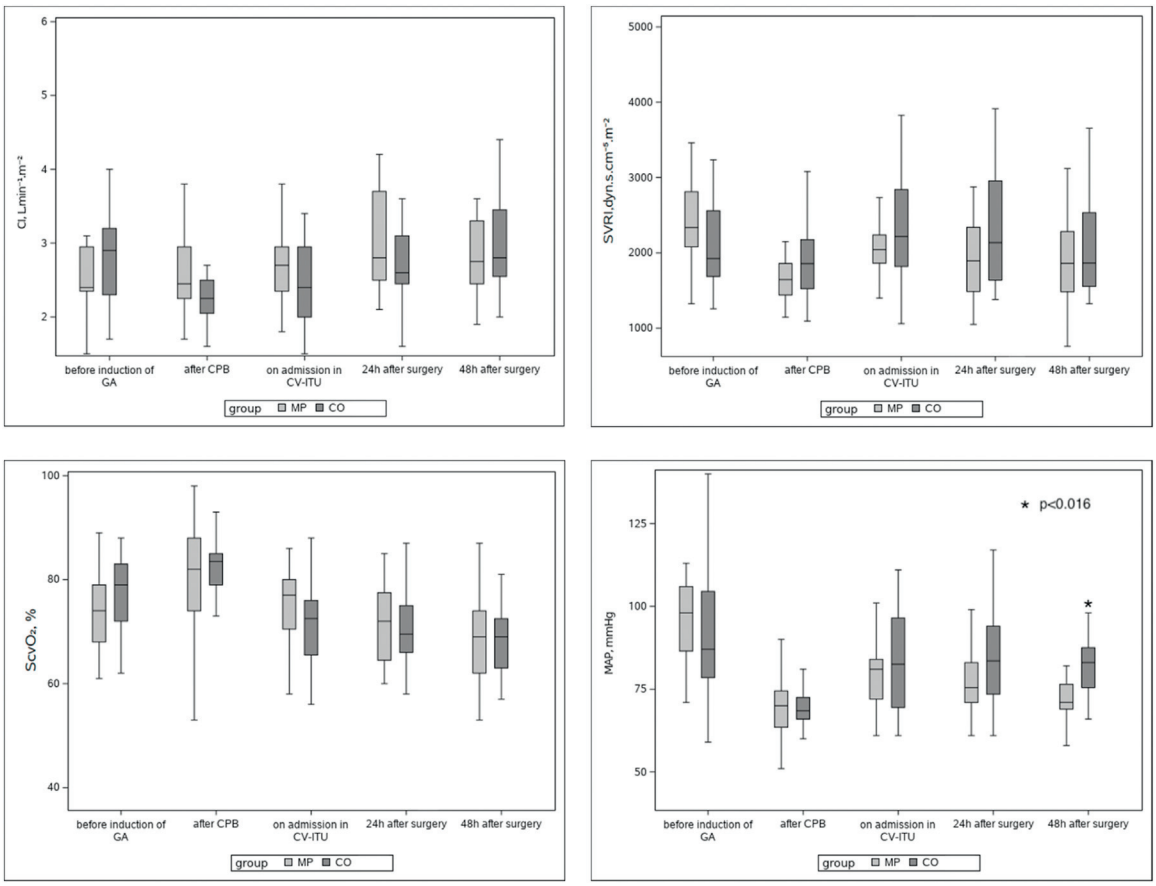

Fig 4. Hemodynamic measurements. CI, cardiac index; CO, control group; CPB, cardiopulmonary bypass; CV-ITU, cardiovascular intensive therapy unit; GA, general anesthesia; IL, interleukin; MAP, mean arterial pressure; MP, methylprednisolone group; $ScvO_2$, central venous oxygen saturation; SVRI, systemic vascular resistance index; TNF, tumor necrosis factor.

the results are encouraging for reducing bleeding, length of stay, and mortality.[31] On the other hand, their use is linked with the concern of wound infections and gastrointestinal complications. However, complications of steroid use are known to be dose-dependent. Although the doses of steroids used in this field have traditionally been very high (30 mg/kg methylprednisolone), nowadays, the trend is to use protocols with low pulse-dose steroids, which help for better hemodynamics, shorter mechanical ventilation times, less blood loss and required less time in the ICU compared with those receiving placebo.[32]

Yet, newer studies advise against the use of glucocorticoids in cardiac surgery.[16] The meta-analysis of Whitlock et al. from 2020 of patient-level data from the SIRS and Dexamethasone for Cardiac Surgery (DECS) trials did not support prophylactic steroid administration during cardiac surgery. The authors concluded that steroids did not decrease the risk of death nor myocardial infarction based on the third universal definition, stroke, renal failure, new atrial fibrillation, or transfusion. Perioperative steroids did increase the risk of myocardial injury as defined in the SIRS trial (more release of creatine kinase-MB)—a finding consistent in both the SIRS and DECS trials, independently. However, perioperative steroids lowered the risk of respiratory failure and infection and reduced the length of ICU and hospital stays.

Another newer systematic review and meta-analysis of the use of prophylactic corticosteroids in cardiac surgery with CPB also could not demonstrate an effect on mortality.[33] This finding supports the recommendation of the 2017 EACTS Guidelines on Perioperative Medication in Adult Cardiac Surgery, in which the routine use of prophylactic corticosteroids is not indicated for adults undergoing cardiac surgery (class of recommendation III and level of evidence A).[34] The meta-analysis included the 2 famous RCTs as well (DECS and SIRS trials), both of which had much larger sample sizes compared with earlier published RCTs.[35,36] Both trials included primarily "high-risk" patients for cardiac surgery. As these 2 trials dominated the results of the meta-analysis, the authors deduced that evidence from their systematic review and meta-analysis could be considered generalizable to the current population undergoing cardiac surgery, which commonly consists of older patients with multiple comorbidities. Still, this meta-analysis did not achieve the required population sample size to detect a 20% reduction in mortality based on a 5% risk of type 1 error (2-sided) and 80% power. Thus, the findings of this meta-analysis cannot reliably exclude that corticosteroids may influence mortality in patients undergoing cardiac surgery with CPB. The higher incidence of myocardial adverse events in patients receiving corticosteroids needs to be interpreted with caution, as different definitions of myocardial infarction

were used across different RCTs. In the present systematic review and meta-analysis, the reduction of pulmonary complications, atrial fibrillation, and surgical site infections, along with shorter durations of ICU and hospital stay in patients receiving corticosteroids, may indicate the limited value of mortality as an outcome in which the disease-specific benefit is likely to be in other clinical outcomes.[33] There remains scope for further investigation of patient recovery outcomes and inflammation-specific outcomes in future trials. Future research designs need to be further refined and primarily revolve around administration regimens and patient selection, specifically targeting patients at higher perioperative risk.[30] An ongoing RCT (DECS-II study, NCT03002259) has been designed with an "efficient trial design" to evaluate whether high-dose dexamethasone has a patient-centered benefit of enhancing recovery and increasing the number of days at home after cardiac surgery.[37]

As far as PON1 is concerned, steroids counteract the effect of oxidative stress by upregulating PON-1 gene expression, and this effect was proven in several studies in various pathologic conditions.[21,22,38–40]

Nonetheless, no data exist in the literature about the effect of glucocorticoids on PON1 activity during CPB surgery. To the best of the authors' knowledge, this was the first study that reported methylprednisolone's effect on PON1 activity during cardiac surgery for CPB. Whether intense oxidative stress that arises with CPB being used contributes to the depletion of the plasma supply of PON1, so that a possible enhancing effect of glucocorticoids in this setting is diminished, is a question for future research.

There were some major limitations to the present study, the biggest being that it was a single-center trial that was part of a large study. The sample size was small, and although sufficient for assessing primary and secondary outcomes, it did not allow definitive conclusions on the effects of study treatment on postoperative complications and patient clinical outcomes. Another limitation could be the dilution during CPB due to excessive fluid therapy, which may dilute the concentration of inflammatory biomarkers, as well as the situation of cytokines being measured from serum samples that were stored at −20° C, which may have had an effect on the exact concentrations. The limitation of multiple testing in a relatively low number of patients was compensated for by doing the Bonferroni correction for multiple comparisons. Future prospective randomized studies are needed to address the potential limitations discussed here.

Conclusions

Methylprednisolone in CPB priming solution does not have any significant effect on PON1 activity during and after complex cardiac surgery.

Declaration of competing interest

None.

CRediT authorship contribution statement

Gordana Taleska Štupica: Conceptualization, Data curation, Investigation, Methodology, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **Maja Šoštarič:** Conceptualization, Investigation, Methodology, Project administration, Supervision, Validation, Visualization, Writing – review & editing. **Matej Jenko:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Visualization, Writing – review & editing. **Matej Podbregar:** Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

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Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1053/j.jvca.2023.12.035.

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