frontiers | Research Topics

Inflammation and heart surgery

Edited by
Shahzad Raja, Nandor Marczin and Umberto Benedetto

Published in
Frontiers in Cardiovascular Medicine
Frontiers in Surgery

ISSN 1664-8714
ISBN 978-2-8325-5499-9
DOI 10.3389/978-2-8325-5499-9

About Frontiers

Frontiers is more than just an open access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers journal series

The Frontiers journal series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the *Frontiers journal series* operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews. Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the *Frontiers journals series*: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area.

Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers editorial office: frontiersin.org/about/contact

Inflammation and heart surgery

Topic editors

Shahzad Raja — Harefield Hospital, United Kingdom

Nandor Marczin — Imperial College London, United Kingdom

Umberto Benedetto — University of Studies G. d'Annunzio Chieti and Pescara, Italy

Citation

Raja, S., Marczin, N., Benedetto, U., eds. (2024). *Inflammation and heart surgery*. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-8325-5499-9

Table of contents

frontiers | Frontiers in Cardiovascular Medicine

TYPE Editorial
PUBLISHED 18 September 2024
DOI 10.3389/fcvm.2024.1493898

OPEN ACCESS

EDITED AND REVIEWED BY
Gianni Angelini,
University of Bristol, United Kingdom

*CORRESPONDENCE
Shahzad G. Raja
drsgraja@gmail.com

RECEIVED 09 September 2024
ACCEPTED 11 September 2024
PUBLISHED 18 September 2024

CITATION
Raja SG, Benedetto U and Marczin N (2024) Editorial: Inflammation and heart surgery. Front. Cardiovasc. Med. 11:1493898. doi: 10.3389/fcvm.2024.1493898

Editorial: Inflammation and heart surgery

Shahzad G. Raja[1]*, Umberto Benedetto[2] and Nandor Marczin[3,4]

[1]Department of Cardiac Surgery, Harefield Hospital, London, United Kingdom, [2]Department of Cardiac Surgery, University of Studies G. D'Annunzio Chieti and Pescara, Chieti, Italy, [3]Department of Anaesthesia and Critical Care, Harefield Hospital, London, United Kingdom, [4]Faculty of Medicine, Department of Surgery & Cancer, Imperial College, London, United Kingdom

KEYWORDS

cardiac surgery, inflammation, ischaemia and reperfusion injury, oxidative stress, systemic inflammatory response

Editorial on the Research Topic
Inflammation and heart surgery

Cardiac surgery involves some of the most intensive and at-risk surgical procedures. Various factors, such as surgical trauma, and the extracorporeal circulation, induce complex systemic inflammatory response as well as organ-specific perioperative morbidity and mortality. Despite advancements in surgical techniques and cardiopulmonary bypass (CPB) technologies over the previous decades, cardiac surgery continues to be associated with these significantly fatal complications (1). An analysis of the Society of Thoracic Surgeons database of >600,000 isolated coronary artery bypass graft procedures identified major postoperative complications including stroke, renal failure, re-operations, and prolonged ventilation in 10.3% patients undergoing this procedure, with a failure to rescue rate in 16%–22% of cases (2).

These complications are further aggravated in other complex procedures and affected by the increasingly aging population presenting for cardiac surgery. The major knowledge gaps in the prevention of these complications as well as monitoring and recovery of these at-risk patients necessitate the need for further research and innovation within these areas.

Various theories regarding the causes of these complications have emerged. The most common of these, identify inflammation, oxidative stress and ischaemia-reperfusion injury as the fundamental mechanisms underlying the pathogenesis of adverse events (3). The contribution of the inflammatory response to post-operative outcomes is increasingly being recognised as an arena for further research.

Tremendous progress has been made in this field; from understanding molecular mechanisms to discovering effective interventions in the laboratory and pre-clinical set up. However, limited data exists on the translation of these insights into everyday clinical practice. We have launched this article collection with an aim to provide a broad overview of the current and emerging research on the role of inflammation in cardiac surgery as well as the definition of best practices in this field.

This special issue on Inflammation and Heart Surgery comprises 11 papers, including 9 original articles, 1 systematic review, and 1 narrative review. The papers cover a broad range of topics focusing on current and emerging research on the role of inflammation in cardiac surgery. The original articles evaluate role of inflammation in perioperative neurocognitive decline, acute kidney injury, endothelial dysfunction, acute type A aortic dissection (ATAAD), mediastinitis, and acute lung injury (ALI) after cardiac surgical

interventions. Two original articles evaluate devices while the narrative review gives a comprehensive overview of inflammation after cardiac surgery on cardiopulmonary bypass and strategies to attenuate it. Finally, the systematic review evaluates all currently available infective endocarditis (IE) risk scores to establish effectiveness of existing scores.

Ma et al. tested the hypothesis that the Nod-like receptor family pyrin domain containing 3 (NLRP3) inflammasome, a macromolecular protein complex linked to disorders of the central nervous system, contributes to perioperative neurocognitive decline (PND) and cardiac surgery related PND occurrence is associated with an increase in NLRP3 level. This first in-human study validated the hypothesis and has implications for future research. Krűger et al. in a translational study investigated evolution of wingless-related integration site (WNT) plasma concentration over time and proposed WNT antagonism as a target for further investigation. Yu et al. and Feng et al. complemented existing literature by evaluating role of lymphocyte neutrophil ratio as a predictor of off-pump coronary artery bypass grafting-associated acute kidney injury and neutrophil count as an effective inflammatory index and independent risk factor for in-hospital mortality in patients with ATAAD. On a similar theme, Li et al. make a case for inclusion of interleukin-6 (IL-6) in predictive model for ALI after surgery for thoracic aortic disease. Laudanski et al. investigated impact of cardiac surgery on complement activation and its protective elements (apolipoprotein J and complement factor H) and reported prolonged alterations in complement milieu up to 3 months after cardiac surgery. Risnes et al. investigated the relationship between mediastinitis and troponin T (TnT) and N-terminal pro-brain natriuretic peptide (NT-proBNP) and concluded that raised levels of TnT and NT-proBNP in patients with mediastinitis are surrogates of myocardial injury and cardiac dysfunction. The SedLine device has been recently launched for monitoring of processed electroencephalography (pEEG). Belletti et al. investigated, the previously unknown, impact of temperature on SedLine derived changes in pEEG and provide new insights for clinicians using SedLine for monitoring depth of anesthesia. Geisler et al. evaluated the efficacy of a hemadsorption device, CytoSorb®, and failed to show a significant impact on reduction of IL-6 or periprocedural mortality in patients undergoing complex cardiac surgery. The narrative review by Banerjee et al. provides an expert overview of systemic inflammation after cardiac surgery on CPB and focuses on pharmacological and non-pharmacological strategies to mitigate CPB-related maladaptive inflammatory response. Lastly, the systematic review by Rizzo et al. evaluates all currently available IE scores and concludes that all scores have inherent limitations with lack of external validation, restricting their global utilization.

The spectrum of manuscripts in this special issue clearly suggests that a lot of research is going on in the arena of inflammation and heart surgery in different research avenues, all of them equally exhilarating and pertinent. However, they also highlight that we are at crossroads advancing the theory, expanding the translational evaluation, and leveraging fundamental basic science into clinical patient benefit.

The spectrum of basic science or translational research manuscripts in this collection demonstrates some exciting progress but we are still far from a comprehensive understanding of the principle dilemmas. There is no doubt, that all patients experience a degree of systemic inflammatory response with oxidative stress, leukocyte, and mediator activation but we still have not identified the global transcriptomic, proteomic and metabolic shifts that can differentiate mild and transient responses from those contributing significantly to postoperative complications. Similarly, we do not have a clear handle of patients' perioperative phenotypes that underpin susceptibility and predisposition of some patients for a higher inflammatory response with pathogenic contribution. While a multimodality approach is advocated targeting multiple steps in the inflammatory response, such an effort will only be successful if the component contribution is relevant.

Thus, in the era of global genetics, personal phenotyping, unparallelled bioinformatics repertoire, and artificial intelligence, there is an urgent need to fully uncover the precise interactions between genetic predisposition and the multiple environmental exposure modulating the patients' biochemical and immunological responses to perioperative trauma and operative procedural factors in causing clinical harm in high risk patients. We are of the opinion that this hugely important topic has not yet mobilised the greatest partnership between basic scientists and clinicians at national and international levels to place systemic inflammatory response syndrome (SIRS) on top of the agenda of surgical sciences. We advocate bringing together the leading force of basic scientists in the field of oxidative stress, immunology, and inflammation and clinical academics covering the full perioperative spectrum of cardiac surgery to fully define what needs to be done to decode the mechanisms, mediators, effector pathways, and the most sophisticated means to target these for clinical benefit. This now needs leading international co-operation between academia, clinicians, and industry under the auspices of major international professional societies.

Lots of work has been done and we are at the crossroads of SIRS research. This special issue collates some of these research activities focusing on new research lines and potential therapeutic targets emphasising the need for international consensus development.

Author contributions

SR: Conceptualization, Writing – original draft, Writing – review & editing. UB: Writing – review & editing. NM: Writing – original draft, Writing – review & editing.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

The handling editor GA declared past co-authorships with the author UB.

Publisher's note

References

1. Raja SG, Dreyfus GD. Modulation of systemic inflammatory response after cardiac surgery. *Asian Cardiovasc Thorac Ann*. (2005) 13(4):382–95. doi: 10.1177/021849230501300422

2. Edwards FH, Ferraris VA, Kurlansky PA, Lobdell KW, He X, O'Brien SM, et al. Failure to rescue rates after coronary artery bypass grafting: an analysis from the society of thoracic surgeons adult cardiac surgery database. *Ann Thorac Surg*. (2016) 102(2):458–64. doi: 10.1016/j.athoracsur.2016.04.051

3. Landis C. Why the inflammatory response is important to the cardiac surgical patient. *J Extra Corpor Technol*. (2007) 39(4):281–4.

frontiers | Frontiers in Surgery

TYPE Original Research
PUBLISHED 02 November 2022
DOI 10.3389/fsurg.2022.992769

OPEN ACCESS

EDITED BY
Shahzad Raja,
Harefield Hospital, United Kingdom

REVIEWED BY
Antonino S. Rubino,
University of Campania Luigi Vanvitelli, Italy
Manfred Seeberger,
University of Basel, Switzerland

*CORRESPONDENCE
Xiangkun Meng
mxkp000512@163.com

†These authors share first authorship

SPECIALTY SECTION
This article was submitted to Heart Surgery, a section of the journal Frontiers in Surgery

RECEIVED 13 July 2022
ACCEPTED 13 October 2022
PUBLISHED 02 November 2022

CITATION
Ma G, Sun P, Chen Y, Jiang X, Zhang C, Qu B and Meng X (2022) NLRP3 inflammasome activation contributes to the cognitive decline after cardiac surgery.
Front. Surg. 9:992769.
doi: 10.3389/fsurg.2022.992769

NLRP3 inflammasome activation contributes to the cognitive decline after cardiac surgery

Gang Ma[1†], Ping Sun[1†], Yi Chen[1], Xin Jiang[2], Caixia Zhang[2], Baofu Qu[2] and Xiangkun Meng[3*]

[1]Department of Anaesthesiology, General Hospital of Ningxia Medical University, Yinchuan, China, [2]Department of Anaesthesiology, Ningxia Medical University, Yinchuan, China, [3]Department of Gastroenterology, General Hospital of Ningxia Medical University, Yinchuan, China

Background: Perioperative neurocognitive disorders (PND) are a common complication of cardiac surgery in elderly patients. The etiopathogenesis of PND is not clear. Nod-like receptor family pyrin domain containing 3 (NLRP3) inflammasome, a macromolecular protein complex, regulates inflammation by inducing the release of proinflammatory cytokines interleukin (IL)-1β and IL-18. Studies have demonstrated a close link between the NLRP3 inflammasome and central nervous system diseases. Nevertheless, the involvement of NLRP3 inflammasome in the causation of PND occurring after cardiac surgery is unclear. This study aimed to investigate the association of serum NLRP3 level with PND.

Methods: We performed a retrospective study, enrolled 75 patients undergoing elective cardiac surgery and evaluated their cognitive functions one day before and 7 days after surgery. PND were determined according to the International Study of Postoperative Cognitive Dysfunction studies. Demographics and perioperative parameters were recorded. Perioperative serum NLRP3 protein, IL-1β, and IL-18 levels were monitored.

Results: The PND incidence in our cohort was 33.33%. NLRP3 protein levels were significantly increased in all patients at each postoperative time-point after general anesthesia and cardiac surgery under cardiopulmonary bypass. Patients showing cognitive dysfunction had higher serum NLRP3 protein, caspase-1, IL-1β, and IL-18 levels immediately after the operation. Variables associated with the incidence of early PND were included in the regression models. After adjusting for confounding variables, high serum NLRP3 protein level at the end of the operation and old age were identified as independent predictors of PND.

Conclusions: High serum NLRP3 protein level at the completion of cardiac surgery was associated with a higher risk of PND seven days after surgery.

Trial registration: The study was registered at Clinicaltrials.gov (registration number: NCT04191642).

Abbreviations

NLRP3, Nod-like receptor family pyrin domain containing 3; PND, Perioperative neurocognitive disorders; CPB, Cardiopulmonary bypass; IL, interleukin; MMSE, Mini-mental state examination; LVEF, left ventricular ejection fraction; MAP, mean arterial pressure; ICU, intensive care unit; BMI, body mass index; WDSST, WAIS Digit Symbol Substitution Test, RAVLT, Rey Auditory Verbal Learning Test (immediate and delayed recall); BVMT-R, Brief visuospatial memory test-revised, COWAT, Controlled Oral Word Association Test, and WAIS Digit Span Test (forward and backward); VAS, Visual Analog Scale; SD, standard deviation; ELISA, enzyme-linked immunosorbent assay measurements; CI, confidence interval; OR: odds ratio.

KEYWORDS

NLRP3 inflammasome, perioperative neurocognitive disorders (PND), inflammation, interleukin -1β, interleukin

Background

Cardiopulmonary bypass (CPB) during cardiovascular surgery facilitates the operation and helps sustain the patient's life. However, systemic inflammatory response induced by CPB contributes to a number of complications (1), including perioperative neurocognitive disorders (PND). PND are characterized by short-term or long-term decline in cognitive performance after surgery affecting different aspects of cognition (e.g., impaired visual or verbal memory, attention, language understanding, concentration), and thus increasing mortality and impairing the quality of life (2). Because of the major physiological impact of cardiac surgery, the incidence of PND is particularly high after cardiac surgery, with a reported incidence of 25%–50% within one week after coronary artery bypass grafting (3, 4). Although the pathogenetic mechanisms of PND are not well characterized, inflammation is believed to play a potential role in its causation. Surgical trauma provokes central nervous system and systemic inflammation, triggering the release of inflammatory mediators such as interleukin (IL)-1β. Neuroinflammation induced by surgical trauma can occur due to passage of inflammatory mediators across the blood–brain barrier or due to trauma-induced secretion of inflammatory cytokines within the brain, leading to cognitive decline. However, the pathogenesis of PND is a complex phenomenon that likely involves the interaction between multiple factors. Therefore, investigation of the specific role of inflammation in the pathogenesis of PND is a key imperative.

The inflammasome, a key element of the innate immune system, is a macromolecular protein complex that modulates inflammation. The Nod-like receptor family pyrin domain containing 3 (NLRP3) inflammasome, as the most-characterized inflammasome till date, is a platform where cysteine protease caspase-1 is activated. Caspase-1 initiates the production of proinflammatory cytokines IL-1β and IL-18. Increased level of NLRP3 has a high positive predictive value for diagnosing various disorders and is a determinant of short- and long-term prognoses (5, 6). Recent research has indicated a strong link between the NLRP3 inflammasome and central nervous system diseases (6, 7). Targeting the assembly and function of the NLRP3 inflammasome is a novel therapeutic strategy for inflammatory diseases including ischemic stroke concomitant with diabetes (7). The NLRP3 inflammasome has been identified as a potential therapeutic target and biomarker in the management of traumatic brain injury (6).

An animal experiment suggested the involvement of NLRP3 priming status in the brains of old mice in the causation of isoflurane-induced cognitive impairment and hippocampal inflammation (8). To the best of our knowledge, the involvement of the NLRP3 inflammasome in operation-induced PND is not well characterized. In our animal experiment, the upregulation of systemic and hippocampal NLRP3 expression after surgery in aged mice was found to be associated with memory and learning dysfunction (data not published). However, the relationship between cognitive dysfunction after surgical trauma and the NLRP3 expression in humans remains to be investigated.

Considering the above body of evidence, we designed this clinical study to test our hypotheses that the NLRP3 inflammasome participates in the incidence of PND and that the occurrence of PND after cardiac surgery is attributable to an increase in perioperative NLRP3 level. This study may provide evidence to develop specific drugs or therapies to reduce NLRP3-induced cognitive impairment-associated PND and to improve prognosis.

Materials and methods

Subjects

This study was approved by the Ethics Committee of the General Hospital of Ningxia Medical University (No. 2018-020). Written informed consent was obtained from all patients. The study was conducted in compliance with the principles of the *Declaration of Helsinki*. Patients receiving heart valve replacement at the General Hospital of Ningxia Medical University between May 2019 and March 2021were eligible for enrolment. The inclusion criteria were: patients aged ≥18 years who were scheduled for aortic or mitral valve replacement under moderate hypothermic CPB; minimum expected postoperative length of hospital: 7 days; American Society of Anesthesiologists physical status II or III. The exclusion criteria were: (1) incomplete cognitive assessment or a Mini-mental state examination (MMSE) score <18; (2) complications that may affect cognitive assessment, such as unstable mental status, mental illness, or language, visual or auditory dysfunction; (3) left ventricular ejection fraction (LVEF) <45%; (4) patients who received high-dose pharmacologic intervention (phenylephrine 100-μg bolus, and epinephrine >0.1 mg/kg/min) for hemodynamic stability [mean arterial pressure (MAP) >60 mmHg]. A nonsurgical control group (spouses who provided written informed consent and qualified the same inclusion/exclusion criteria) was established to determine the effects of repeated neuropsychological testing (practice effect). This study was registered at Clinicaltrials.gov (NCT04191642).

Anesthesia and surgery

Standard general anesthesia was applied throughout. Nasopharyngeal and rectal temperatures, 5-lead electrocardiogram, capnography, and pulse oximetry were routinely monitored. Systemic arterial blood pressure was detected by radial artery catheterization. Anesthesia was initiated using target-controlled infusion of propofol, and the bispectral index was maintained at 40–60. In the unconscious state, 0.2 mg/kg cisatracurium and 0.8–1.5 μg/kg sufentanil were infused. After tracheal intubation, the lungs were ventilated with O_2-rich air (0.6 of inspired O_2) calibrated to an end-tidal CO_2 partial pressure of 35–45 mmHg. Then, the central venous pressure and fluid control were monitored by inserting a central venous catheter. Intermittent IV boluses of sufentanil were administered (total dose, 3–5 μg/kg) according to the blood pressure and heart rate. All patients received infusion of 0.1 mg/kg/h cisatracurium throughout the surgery.

Standard median sternotomy was performed for surgical approach in all patients. The body temperature was reduced to 30–32°C during CPB. All patients were treated with an intermittent antegrade infusion of cooled high-potassium blood to induce cardioplegia during continuous aortic cross-clamping (ACC) using a non-pulsatile flow rate of 2.2–2.8 L/min/m^2. In a cell-saving device, the blood in the CPB circuit and from the surgical field was obtained, centrifuged, washed, and infused within 4 h after CPB. Hematocrit was maintained at >25%, with the addition of blood, if required. To normalize serum blood glucose level >150 mg/dl, insulin therapy was started perioperatively. Pre- or post-CPB hemodynamic modulation was targeted to maintain MAP at 60–100 mmHg. Hypertension was handled with sufentanil (bolus dose) or 0.1–0.5 mg/kg/min nitroglycerin, or both. Hypotension was treated with fluid intake (including crystalloids, colloids, and blood products) or use of vasoactive drugs (phenylephrine IV, 20–100 μg, or concomitant epinephrine, 0.01–0.1 mg/kg/min). The dosage of vasoactive drugs, fluid intake, and urine were recorded after the surgery.

Prior to skin closure, 0.08 mg/kg midazolam (bolus dose) was administered intravenously and the infusion of propofol was terminated. Postoperatively, all patients were admitted to the intensive care unit (ICU). Extubation was performed when a patient was able to maintain adequate spontaneous breathing and required the least oxygen support, which was indicated by normal arterial blood gas levels. When a patient did not require inotropic or oxygen and was hemodynamically stabilize with normal blood gas variables, he/she was discharged from the ICU. The length of the ICU stay was recorded.

Basic data collection included demographic variables obtained *via* questionnaire, including sex, age, years of education, and body mass index (BMI). Medical history data acquired from patient medical records included history of hypertension, hyperlipidemia, diabetes, aortic plaque, chronic renal dysfunction, cerebrovascular disease, and carotid artery stenosis. Hemodynamic measurements were performed before anesthesia initiation, before skin incision, after sternotomy, CBP-cessation, and at 1, 2 and 4 h after CPB (post-CPB 1, 2, 4 h respectively). At pre-incision, CBP-cessation, and post-CPB 2 h and 4 h, radial artery blood sample was collected for blood gas, lactic acid, and blood glucose examinations. Peripheral venous blood sample was collected to determine IL-1β, IL-18, and NLRP3 expressions at pre-induction, at the end of the operation, and post-CPB 3 d. Complications occurring within seven days postoperatively (i.e., infection, bleeding, and organ dysfunction) were recorded.

Neuropsychological assessment

Cognitive function assessments were performed 1 day before and 7 days after the operation in a quiet environment in the general ward. A widely-used test battery (9) was performed in Chinese. The specific tests conducted were: MMSE; Trail Making Test (A/B); WAIS Digit Symbol Substitution Test (WDSST); Rey Auditory Verbal Learning Test (RAVLT; immediate and delayed recall); brief visuospatial memory test-revised (BVMT-R); Controlled Oral Word Association Test (COWAT); and WAIS Digit Span Test (forward and backward). At 1 week post-operatively, the cognitive tests were repeated. Pain was assessed using the Visual Analog Scale (VAS) at 7 days postoperatively.

PND were defined using z scores as recommended by the International Study of Postoperative Cognitive Dysfunction studies (10). To examine the learning effect, we calculated the variations (including mean and standard deviation) from baseline in each test performance for controls. For the patients, we compared the scores before and at 1-week after the operation, subtracted the average learning impact, and divided the results by the standard deviation (SD) of the control group to determine the z score for each test. Then, a composite z score was computed as the sum of these z scores for any patient divided by the standard deviation of the corresponding sum in the controls. Then, to create a combined z score, the z scores of all tests in a patient were added and divided by the SD for this sum of z scores in the controls. PND was diagnosed if the z score was $\geq$1.96 in $\geq$2 separate cognitive tests or the composite z score was $\geq$1.96. Postoperative delirium was also considered as PND.

Serum NLRP3,IL-1β, and IL-18 levels

After 10 min of centrifugation at 2,000 *g*, serum was obtained and stored at −80°C until further processing. Enzyme-linked immunosorbent assay (ELISA) kits (CLOUD-

CLONE CORP., USA) were used to detect plasma levels of IL-18 (L200910330580), IL-1β (L200910324), and NLRP3 (L2009103305830), according to the manufacturer's protocols.

Endpoints and sample size

Our primary objective was to compare NLRP3 levels between patients with and without PND. The secondary objective was to compare serum IL-18 and IL-1β levels. In line with a previous study (11) and our pilot study, we assumed that 40% of the patients are affected by PND and that the postoperative NLRP3 levels of PND patients will be three times higher than those of non-PND patients. Factoring a two-tailed significance level of 0.05, a sample size of 71 was required to obtain a 90% statistical power to detect this difference. Given an estimated attrition rate of 10%, the final sample size was increased to a total of 81 patients.

Statistical analysis

Categorical variables were reported as frequency (percentage) and continuous variables as mean ± SD. Normality of distribution of continuous variables was assessed using the Kolmogorov–Smirnov test. Between-group differences with respect to normally-distributed variables were assessed using Student's *t*-test, while those with respect to non-normally distributed variables were assessed using the non-parametric Mann-Whitney test. Categorical variables were analyzed using Chi-squared test and Fisher's exact test. Differences in NLRP3, IL-1β, IL-18 levels, and other vital parameters over time between the PND and the non-PND groups were assessed using repeated measures analysis of variance (ANOVA) with *post hoc* Bonferroni correction.

Association of serum NLRP3 level with the onset of early PND was assessed using multivariate logistic regression analysis and the results presented as odds ratio (OR) and 95% confidence interval (CI). First, association of each baseline or perioperative variable with PND was tested. Variables associated with P values ≤0.05 were included in multivariate logistic regression to identify the risk-adjusted predictors of PND. Relationships of NLRP3 with IL-1β and IL-18 was assessed using Pearson correlation analysis. Two-tailed P values <0.05 were considered indicative of statistical significance. All statistical analyses were conducted using SPSS 22.0 (SPSS Inc, Chicago, Illinois).

Results

Ninety-three of the initial 100 patients qualified the selection criteria, of whom 10 opted out of the study. The remaining 83 patients provided informed consent and were enrolled. Of them, 8 patients did not complete all follow-up tests because of: withdrawal of consent due to no postoperative NLRP3 value or no postoperative cognitive testing ($n = 4$), or hemodynamic instability ($n = 3$), and one death caused by intractable ventricular fibrillation on the second day after surgery. Finally, 75 patients were included (**Figure 1**), and their characteristics are summarized in **Table 1**. To estimate the size of the practice effect for neuropsychological tests, we enrolled 21 control subjects during the same period, who were matched with the patients with respect to many parameters, such as age, proportion of females, education level, and BMI. Descriptive details of the changes in each cognitive test are shown in **Table 2**.

Patient characteristics

Two patients suffered delirium after the surgery, according to their clinical features and the CAM–ICU scores, their conditions were promptly resolved after drug treatment. According to the neuropsychological assessment at seventh day postoperatively (**Table 2**), cognitive impairment was identified in 25 patients (including the two patients with delirium), but in none of the control subjects. Therefore, the incidence of PND in our cohort was 33.33% (25/75). There were no significant differences between patients with and without PND with respect to physiological parameters or laboratory markers (e.g., MBP, HR, SPO_2, body temperature, lactic acid, blood glucose). Several indices, including the sex distribution, hypertension, BMI, occurrence of postoperative complications, and preoperative LVEF, were comparable in the PND and non-PND groups ($P > 0.05$; **Table 1**). Patients with PND were significantly older (57.33 ± 7.65 vs. 62.65 ± 6.94 years, $P = 0.004$) and less educated (7.43 ± 3.39 vs. 4.54 ± 3.93 years, $P = 0.001$) than the non-PND subjects. Regarding perioperative parameters, the PND group relative to the non-PND group had longer ACC time (111.42 ± 44.96 vs. 89.90 ± 32.28 h, $P = 0.019$) and postoperative ICU stay (3.85 ± 1.87 vs. 2.47 ± 1.12 days, $P < 0.001$).

Serum NLRP3, caspase-1, IL-1β and IL-18 profiles

We compared the NLRP3 inflammasome-related proteins between the two groups. Serum NLRP3 levels were low before surgery. General anesthesia and cardiac surgery under CPB significantly increased NLRP3 protein levels at each postoperative time-point in both PND group (1.69 ± 0.41, 1.82 ± 0.47 vs.1.21 ± 0.36 ng/ml, $P < 0.001$) and non-PND

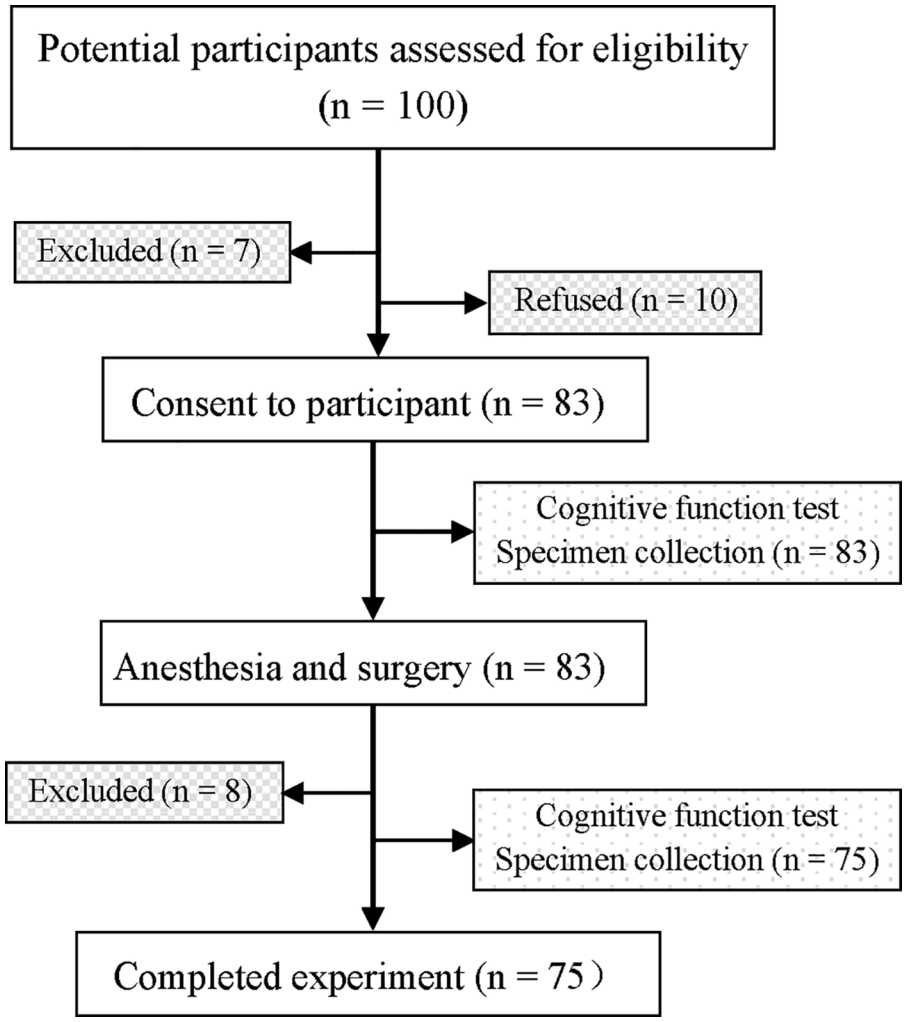


FIGURE 1
Flow chart of the study. overall, 100 patients were recruited, and 75 patients completed all the tests.

group (1.38 ± 0.36, 1.65 ± 0.39 vs. 1.19 ± 0.30 ng/ml, $P < 0.001$, Figure 2A).

There were no significant between-group differences with respect to preoperative serum NLRP3 levels (Figure 2A). However, immediately after the operation, the levels were significantly higher in early PND patients than in the non-PND patients (1.69 ± 0.41 vs. 1.38 ± 0.36 ng/ml, $P = 0.001$, Figure 2A).

Corresponding to the rising level of NLRP3, serum caspase-1, IL-1β, and IL-18 levels were elevated postoperatively and reached peak level immediately after the operation, and then decreased at day 3 postoperatively (Figures 2B–D), but remained higher than the baseline levels. Compared with the non-PND patients, PND patients had higher levels of caspase-1, IL-1β, and IL-18 immediately after the operation ($P = 0.009$, 0.021, and 0.001, respectively, Figures 2C,D).

Variables associated with occurrence of early PND

Variables associated with the occurrence of early PND were included in the regression models. After adjusting for potential confounding factors, high serum NLRP3 protein level at the end of the operation was found to be an independent predictor of cognitive dysfunction (OR 0.128, 95% CI, 0.021–0.763, $P = 0.024$; Table 3). Old age was another independent predictor of early PND (OR 0.910, 95% CI, 0.021–0.763, $P = 0.040$; Table 3).

Receiver operating characteristic (ROC) analysis

On ROC curve analysis, NLRP3 protein levels at the end of the operation showed a good predictive accuracy for PND (area

under the curve = 0.723; 95% CI, 0.603–0.843; $P = 0.002$; Figure 4).

Discussion

There were two main findings of our study. (1) Serum NLRP3 protein, IL-18, and IL-1β levels increased after cardiac surgery; and (2) elevated NLRP3 and old age were associated with higher risk of PND after cardiac surgery. These results seem to confirm our hypothesis that the NLRP3 inflammasome plays an instrumental role in the development of neurocognitive deficit after surgery and may be a predictor of PND following cardiac surgery.

The neuropsychological test battery and the definition of PND applied in this study have been widely used in previous studies (9, 12), including the assessment of attention, concentration, executive function, memory, psychomotor speed, and visuospatial ability. Though these disorders may also occur after noncardiac surgeries, they are a special concern after cardiac surgery because of perturbations, such as CPB, median sternotomy, and long surgical and anesthetic time (13). Elimination of some factors (e.g., poor cardiac function, history of mental illness) that may have influenced the results helped improve the credibility of our results. In our study, the incidence of new PND within 7 days after surgery was 33.33%. These statistics, despite being higher than the incidence rate after noncardiac surgery, are consistent with other research in cardiac surgery (14).

Surgical trauma can initiate a systemic inflammatory response, and inflammatory cytokines can cause neuroinflammation by triggering the release of inflammatory cytokines in the brain or by directly crossing the blood–brain barrier, contributing to cognitive deterioration. To verify the pro-inflammatory characteristics of NLRP3, we focused on the role of the NLRP3 inflammasome in PND occurrence. As evidenced by the increased levels of IL-1β and IL-18 and significant upregulation of the NLRP3 protein, our findings clearly demonstrate that anesthesia and/or cardiac surgery can induce severe inflammatory response.

Based on the behavioral performance after surgery, we divided the patients into PND and non-PND groups. PND were clearly identified as a multi-factor condition. Our results indicate higher risk factors in the PND group, including ACC time, poor education, postoperative ICU stay, surgery time, and serum NLRP3 and IL-1β levels at the end of the

TABLE 1 Characteristics of the study population.

Variables	Non-PND ($n = 50$)	PND ($n = 25$)	*P*-value
Male/Female	28/22	12/13	0.341
Age (years)	57.33 ± 7.65	62.65 ± 6.94	0.004
BMI (kg/m^2)	23.35 ± 2.60	23.83 ± 3.08	0.480
Education (years)	7.43 ± 3.39	4.54 ± 3.93	0.001
Hypertension	17/33	13/12	0.134
Diabetes	3/47	3/22	0.652
Preoperative LVEF (%)	61.08 ± 6.56	60.58 ± 7.11	0.76
CPB time (min)	131.67 ± 40.61	151.81 ± 48.08	0.059
ACC time (min)	89.90 ± 32.28	111.42 ± 44.96	0.019
Surgery time (min)	243.35 ± 51.75	268.96 ± 55.11	0.050
Extubation time after surgery (h)	11 ± 2	10 ± 2	0.78
Postoperative ICU stay (days)	2.47 ± 1.12	3.85 ± 1.87	0.000
VAS score	2.35 ± 1.28	2.31 ± 1.12	0.896
Occurrence of postoperative complications (within 7 days)	2 (pulmonary infection)	1 (wound infection)	0.998

Data presented as mean ± standard deviation or ratio (the same in other tables).
PND, perioperative neurocognitive disorders; BMI, body mass index; LVEF, left ventricular ejection fraction; CBP, cardiopulmonary bypass; ACC, aortic cross-clamping; VAS, visual analog scale.

TABLE 2 Cognitive performance of patients and control subjects.

Cognitive tests	Control subjects ($n = 21$)		Non-PND ($n = 50$)		PND ($n = 25$)	
	Baseline	After 7 days	Baseline	Postoperative	Baseline	Postoperative
MMSE (score)	23.14 ± 2.73	23.38 ± 2.73	24.18 ± 2.58	23.51 ± 2.72	22.96 ± 2.25	19.85 ± 2.17
RAVLT (immediate)	14.86 ± 4.36	15.19 ± 4.20	14.43 ± 4.51	15.08 ± 4.07	14.69 ± 3.52	11.92 ± 3.33
RAVLT (delayed)	27.86 ± 2.71	28.90 ± 2.56	28.55 ± 2.44	28.41 ± 2.49	27.19 ± 2.10	23.46 ± 2.02
BVMT-R	9.86 ± 1.35	10.48 ± 1.40	9.57 ± 1.53	10.20 ± 1.50	10.96 ± 1.37	8.8 ± 1.03
Trail Making A	74.38 ± 23.67	68.00 ± 22.93	69.00 ± 21.98	71.14 ± 22.25	91.31 ± 36.13	115.04 ± 41.32
Trail Making B	93.43 ± 19.31	85.05 ± 18.04	91.98 ± 26.02	90.43 ± 25.55	116.08 ± 26.83	139.50 ± 27.76
WAIS Digit Span	8.00 ± 1.41	8.10 ± 1.04	8.57 ± 1.34	8.49 ± 1.16	7.54 ± 0.99	6.46 ± 0.86
WDSST	15.33 ± 2.69	16.29 ± 2.78	16.96 ± 3.03	16.02 ± 3.31	13.81 ± 1.92	10.46 ± 1.92
COWAT	39.24 ± 5.24	40.29 ± 4.82	41.20 ± 4.90	41.43 ± 4.83	35.58 ± 4.66	29.42 ± 3.92

Data are shown as mean ± standard deviation.
PND, perioperative neurocognitive disorders; MMSE, mini-mental state examination; WDSST, WAIS digit symbol substitution test; RAVLT, Rey auditory verbal learning test; BVMT-R, brief visuospatial memory test-revised; COWAT, controlled oral word association test.

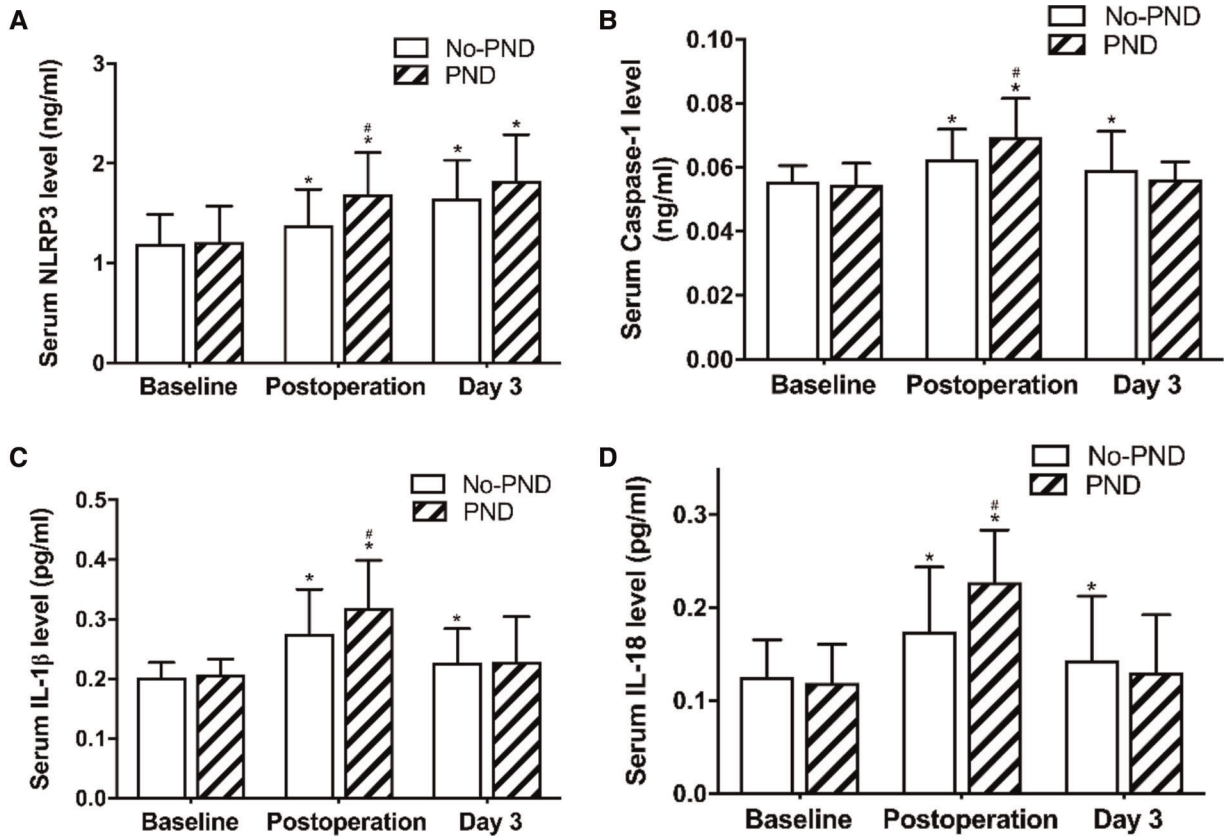


FIGURE 2
Serum NLRP3, caspase-3, IL-18, and IL-1β in non-PND and PND groups. * $P < 0.05$ vs. baseline; # $P < 0.05$ vs. the non-PND group at same time-point. A moderate positive association between NLRP3 and IL-1β ($r = 0.330$, $P = 0.004$) levels and a weak positive association of increase in NLRP3 with increase in IL-18 ($r = 0.265$, $P = 0.021$) and caspase-1 ($r = 0.241$, $P = 0.038$) levels was observed at the end of the surgery (Figure 3). Abbreviations: NLRP3, Nod-like receptor family pyrin domain containing 3; PND, Perioperative neurocognitive disorders; IL, interleukin.

TABLE 3 Predictors of postoperative cognitive dysfunction at 1 week after surgery.

Risk factor	Univariate analyses[a], *P*	Multivariate logistic regression analysis[b]	
		OR (95% CI)	*P*
Age	0.017	0.910 (0.021, 0.763)	0.042
Education	0.011	1.198 (0.997, 1.440)	0.054
ACC Time	0.033	0.982 (0.964, 1.001)	0.070
ICU stay	0.030	0.737 (0.47, 1.163)	0.184
Serum NLRP3 level (Immediately after the operation)	0.024	0.128 (0.021 0.763)	0.024
Serum IL-18 level (Immediately after the operation)	0.034	0.042 (0.000, 40.526)	0.533

NLRP3, Nod-like receptor family pyrin domain containing 3; ICU, intensive care unit; CI, confidence interval; OR, odds ratio; ACC, aortic cross-clamping; IL, interleukin.
[a,b]Occurrence of postoperative cognitive dysfunction was modeled as a function of a single predictor and as a function of all significant predictors in the univariate analyses ($P \leq 0.05$) respectively.

operation. The increased NLRP3, IL-1β, and IL-18 levels in the PND patients are consistent with previous reports which showed that inflammation, particularly increased IL-1β level, is instrumental in PND occurrence (2, 15). More importantly, multiple logistic regression analyses and correlation analysis identified high serum NLRP3 level at the end of surgery was associated with a higher risk of cognitive dysfunction seven days after heart surgery. This indicates the involvement of NLRP3 inflammasome in the pathogenesis of PND in a clinically complex environment. These findings are consistent with another study in which NLRP3 inflammasome activation was found to be linked with inflammation-induced cognitive dysfunction and neuropathological variations with aging (16). In another study, patients with acute coronary syndrome showed increased peripheral blood monocyte NLRP3 protein level, which showed a correlation with the severity of coronary atherosclerosis. This suggested its potential prognostic relevance in predicting major adverse cardiac

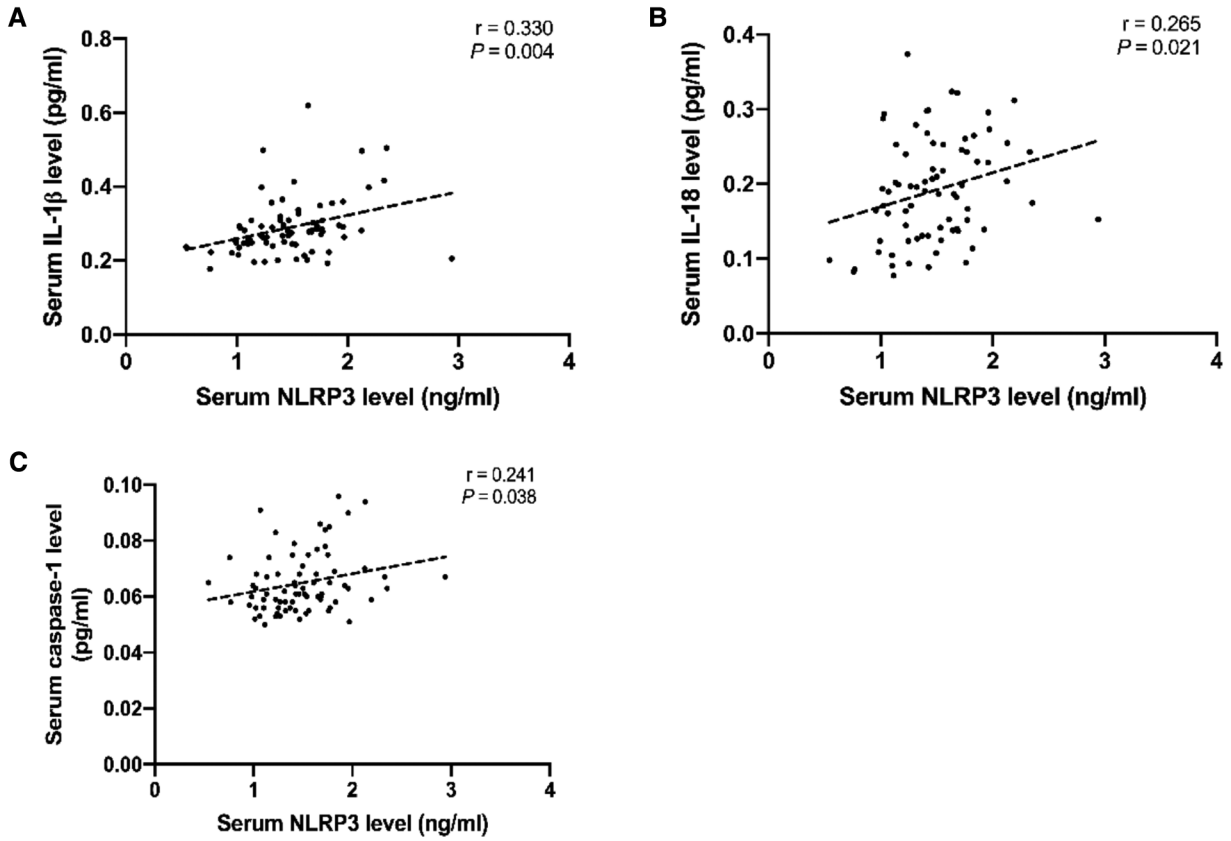


FIGURE 3
Scatter plots showing the positive relationship of NLRP3 with IL-1β (A), IL-18 (B), and caspase-1 (C). Dotted lines present the trend lines. Abbreviations: NLRP3, Nod-like receptor family pyrin domain containing 3; IL, interleukin.

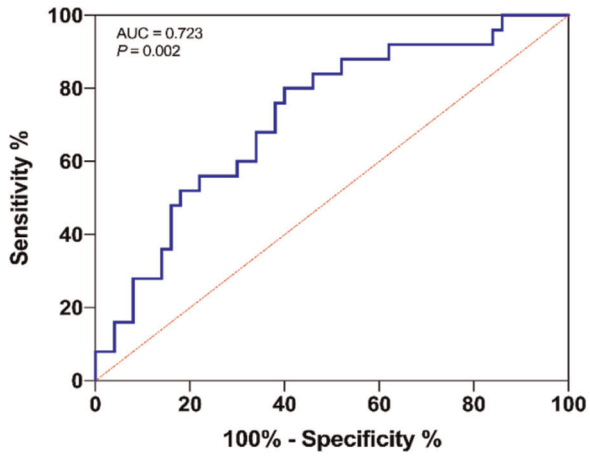


FIGURE 4
ROC curve showing the predictive ability of NLRP3 for PND in cardiac operation patients.

events (5). These studies indicate that NLRP3 can be a predictor of inflammation-related diseases. Our ROC curve analysis also suggested that serum NLRP3 level is a good predictor of PND. In addition, since the ablation of each part of the NLRP3 inflammasome helps prevent age-related cognitive decline caused by neuroinflammation and neurodegeneration, such as in Alzheimer's disease (16–18), we deduce that the NLRP3 inflammasome can be immensely valuable as a potential therapeutic target for PND.

Surgery can activate immune cells, thus amplifying the immune response. In this process, the NLRP3 inflammasome modulates caspase-1 activation, which determines the maturation and production of IL-1β and IL-18. IL-1β is known to play an important role in the pathological process of PND, and IL-18 can influence the integrity of neurons and increase neuroinflammation in the brain (19), thus leading to cognitive deterioration in Alzheimer's disease (15). Although we observed elevated levels of systemic IL-1β and IL-18 post-operatively, and a significant correlation was found between NLRP3 and IL-1β, IL-18, but after adjusting for confounding factors, IL-1β and IL-18 were not identified as risk factors for PND on multiple logistic regression analyses. This is inconsistent with the results of a previous study (2). The discrepancy can be ascribed to the relatively small sample size of our study, and the differences in populations and

neurocognitive testing modalities between studies. Thus, further investigations in this regard are required.

Three days postoperatively, however, we found no difference in IL-1β or NLRP3 levels between PND and non-PND groups. This is because the elimination of some pathogenic factors resulted in a postoperative decline in the expressions of inflammatory cytokines. Nevertheless, the ways in which memory and cognition are affected by an inflammatory event may be modulated by time-dependent mechanisms. Probably within hours to days after inflammation, the cytokine-dependent signaling directly interacts with the mechanisms of memory events (20). The memory and cognitive deficits found in the weeks to months after anesthesia and surgery can be attributed to these primary events that initiate long-lasting neuronal changes through neurogenetic and epigenetic alterations (20).

Our results also revealed old age as a significant risk factor for PND. Older patients often have more neurovascular disease risk factors, more severe cerebral white matter injury, and less cognitive reserve than younger patients, and thus are at a higher risk of cognitive disorders after anesthesia and surgery (21). Multiple studies have identified diabetes, lower educational level, duration of surgery, and duration of ICU stay as significant risk factors for PND (21–23), but no correlation was noted in the present study. This discrepancy may be attributable to the relatively small sample size and narrow differences in the duration of ICU stay, education background, and other aspects. Hence, future research with a larger sample size is warranted. The difference in the risk factors identified among studies can also be explained by differences in the diagnosis of PND, subjective factors, and diverse analytic procedures, which may mask the effects of some risk factors.

This study has at least two limitations. First, despite the strict inclusion criteria, our study had a relatively small sample size. In addition, the relationship between the NLRP3 level and the occurrence of PND after non-cardiac surgeries is unknown. Second, our observation ended one week after the surgery because of difficulties in sampling blood and performing cognitive tests, and therefore, the temporal change in NLRP3 after surgery and the long-term relationship between NLRP3 and PND remain unknown.

Conclusions

In this study, high serum NLRP3 level at the end of surgery was associated with a higher risk of cognitive dysfunction seven days after heart surgery. Larger clinical studies are required to provide more robust evidence of the value of NLRP3 as a predictive biomarker for PND.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author/s.

Ethics statement

The studies involving human participants were reviewed and approved by The ethics committee of the General Hospital of Ningxia Medical University (approval number 2018-020). The patients/participants provided their written informed consent to participate in this study.

Author contributions

GM and PS carried out the studies, participated in data collection, and drafted the manuscript. YC and XJ performed statistical analysis and participated in its design. XJ, CZ, and BQ participated in data collection. XM carried out the studies, performed the statistical analysis and participated in its design, and drafted the manuscript. All authors contributed to the article and approved the submitted version.

Funding

This study was supported by National Natural Science Foundation of China (No. 81860213), Natural Science Foundation of Ningxia Province (No. 2021AAC03402) and Natural Science Foundation of Ningxia Province (No. 2022AAC02061).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Fujii Y. Evaluation of inflammation caused by cardiopulmonary bypass in a small animal model. *Biology (Basel)*. (2020) 9(4):81. doi: 10.3390/biology9040081

2. Ganna A, Roland K, Georg W, Reinhard S. Biomarkers of postoperative delirium and cognitive dysfunction. *Front Aging Neurosci*. (2015) 7:1–17. doi: 10.3389/fnagi.2015.00112

3. Xiao QX, Cheng CX, Deng R, Liu Q, Zhang Y. Lncrna-Myl2–2 and mir-124-3p are associated with perioperative neurocognitive disorders in patients after cardiac surgery. *J Invest Surg*. (2020) 3:1–7. doi: 10.1080/08941939.2020.1797949

4. Norkienė I, Samalavičius R, Misiūrienė I, Paulauskienė K, Budrys V, Ivaškevičius J. Incidence and risk factors for early postoperative cognitive decline after coronary artery bypass grafting. *Medicina (B Aires)*. (2010) 46 (7):460–4. doi: 10.3390/medicina46070066

5. Afrasyab A, Qu P, Zhao Y, Peng K, Wang H, Lou D, et al. Correlation of Nlrp3 with severity and prognosis of coronary atherosclerosis in acute coronary syndrome patients. *Heart Vessels*. (2016) 31(8):1218–29. doi: 10.1007/s00380-015-0723-8

6. O'Brien WT, Pham L, Symons GF, Monif M, Mcdonald SJ. The Nlrp3 inflammasome in traumatic brain injury: potential as a biomarker and therapeutic target. *J Neuroinflammation*. (2020) 17(1):104. doi: 10.1186/s12974-020-01778-5

7. Hong P, Gu RN, Li FX, Xiong XX, Zhang HF. Nlrp3 inflammasome as a potential treatment in ischemic stroke concomitant with diabetes. *J Neuroinflammation*. (2019) 16(1):121. doi: 10.1186/s12974-019-1498-0

8. Wang Z, Meng S, Cao L, Chen Y, Zuo Z, Peng S. Critical role of Nlrp3-caspase-1 pathway in age-dependent isoflurane-induced microglial inflammatory response and cognitive impairment. *J Neuroinflammation*. (2018) 15(1):109. doi: 10.1186/s12974-018-1137-1

9. Lin GX, Wang T, Chen MH, Hu ZH, Ouyang W. Serum high-mobility group box 1 protein correlates with cognitive decline after gastrointestinal surgery. *Acta Anaesthesiol Scand*. (2014) 58(6):668–74. doi: 10.1111/aas.12320

10. Steinmetz J. Long term consequences of postoperative cognitive dysfunction. *Acta Anaesthesiol Scand*. (2009) 53:30–2. doi: 10.1111/j.1399-6576.2009.02055.x

11. Mu DL, Li LH, Wang DX, Li N, Shan GJ, Li J, et al. High postoperative serum cortisol level is associated with increased risk of cognitive dysfunction early after coronary artery bypass graft surgery: a prospective cohort study. *PLoS One*. (2013) 8(10):e77637-e. doi: 10.1371/journal.pone.0077637

12. Eckenhoff RG, Maze M, Xie Z, Culley DJ, Goodlin SJ, Zuo Z, et al. Perioperative neurocognitive disorder: state of the preclinical science. *Anesthesiology*. (2020) 132(1):55–68. doi: 10.1097/ALN.0000000000002956

13. Miles B, Niccolò T, Kendall S, Jeffrey NB, Mark FN, Joseph PM. Neurocognitive function after cardiac surgery: from phenotypes to mechanisms. *Anesthesiology*. (2018) 129(4):829–51. doi: 10.1097/ALN.0000000000002194

14. Kapoor MC. Neurological dysfunction after cardiac surgery and cardiac intensive care admission: a narrative review part 1: the problem; nomenclature; delirium and postoperative neurocognitive disorder; and the role of cardiac surgery and anesthesia. *Ann Card Anaesth*. (2020) 23(4):383–90. doi: 10.4103/aca.ACA_138_19

15. Bossù P, Ciaramella A, Salani F, Bizzoni F, Varsi E, Iulio FD, et al. Interleukin-18 produced by peripheral blood cells is increased in Alzheimer's disease and correlates with cognitive impairment. *Brain Behav Immun*. (2008) 22(4):487–92. doi: 10.1016/j.bbi.2007.10.001

16. Youm YH, Grant RW, Mccabe LR, Albarado D, Dixit VD. Canonical Nlrp3 inflammasome links systemic low-grade inflammation to functional decline in aging. *Cell Metab*. (2013) 18(4):519–32. doi: 10.1016/j.cmet.2013.09.010

17. Heneka MT, Kummer MP, Stutz A, Delekate A, Schwartz S, Vieira-Saecker A, et al. Nlrp3 is activated in Alzheimer's disease and contributes to pathology in App/Ps1 mice. *Nature*. (2013) 493(7434):674–8. doi: 10.1038/nature11729

18. Goldmann T, Lengtay T, Prinz M. Love and death: microglia, Nlrp3 and the Alzheimer's brain. *Cell Res*. (2013) 5:595–6. doi: 10.1038/cr.2013.24

19. Sutinen EM, Pirttil T, Anderson G, Salminen A, Ojala JO. Pro-Inflammatory interleukin-18 increases Alzheimer's disease-associated amyloid-*B* production in human neuron-like cells. *J Neuroinflammation*. (2012) 9(1):199. doi: 10.1186/1742-2094-9-199

20. Donzis EJ, Tronson NC. Modulation of learning and memory by cytokines: signaling mechanisms and long term consequences. *Neurobiol Learn Mem*. (2014) 115:68–77. doi: 10.1016/j.nlm.2014.08.008

21. Krzych LJ, Pluta MP, Putowski Z, Czok M. Investigating association between intraoperative hypotension and postoperative neurocognitive disorders in non-cardiac surgery: a comprehensive review. *J Clin Med*. (2020) 9(10):3183. doi: 10.3390/jcm9103183

22. Majewski P, Zegan-Barańska M, Karolak I, Kaim K, Żukowski M, Kotfis K. Current evidence regarding biomarkers used to aid postoperative delirium diagnosis in the field of cardiac surgery-review. *Medicina (Kaunas)*. (2020) 56 (10):493. doi: 10.3390/medicina56100493

23. Ordóñez-Velasco LM, Hernández-Leiva E. Factors associated with delirium after cardiac surgery: a prospective cohort study. *Ann Card Anaesth*. (2021) 24 (2):183–9. doi: 10.4103/aca.ACA_43_20

frontiers | Frontiers in Surgery

TYPE Original Research
PUBLISHED 08 November 2022
DOI 10.3389/fsurg.2022.1047050

OPEN ACCESS

EDITED BY
Shahzad Raja,
Harefield Hospital, United Kingdom

REVIEWED BY
Birkan Bozkurt,
Başakşehir Çam & Sakura City Hospital Organ Transplant Center, Turkey
Yang Yu,
Capital Medical University, China

*CORRESPONDENCE
Xiangbin Meng
henry2008meng@hotmail.com

SPECIALTY SECTION
This article was submitted to Heart Surgery, a section of the journal Frontiers in Surgery

RECEIVED 17 September 2022
ACCEPTED 18 October 2022
PUBLISHED 08 November 2022

CITATION
Yu R, Song H, Bi Y and Meng X (2022) Predictive role of the neutrophil: lymphocyte ratio in acute kidney injury associated with off-pump coronary artery bypass grafting.
Front. Surg. 9:1047050.
doi: 10.3389/fsurg.2022.1047050

Predictive role of the neutrophil: lymphocyte ratio in acute kidney injury associated with off-pump coronary artery bypass grafting

Ruiming Yu, Han Song, Yanwen Bi and Xiangbin Meng*

Department of Cardiovascular Surgery, Qilu Hospital of Shandong University, Jinan, China

Objectives: This study aims to investigate whether the ratios of cell types in peripheral blood could be used as reliable predictors of off-pump coronary artery bypass grafting (CABG)-associated acute kidney injury (AKI).

Materials and methods: We retrospectively reviewed patients ($n = 420$) undergoing off-pump CABG from January 1, 2021 to January 1, 2022 in Qilu Hospital of Shandong University. We used logistic regression analysis to identify the potential predictors of off-pump CABG-associated AKI and construct a predictive model. Receiver operating characteristic (ROC) curve analysis was used to evaluate the predictive ability of predictors and prediction models.

Results: The prevalence of AKI associated with off-pump CABG was 20.95%. Patients in the AKI group had significantly higher ratios of peripheral blood cells on postoperative day (POD)1 than patients in the non-AKI group ($P < 0.01$). The area under the ROC curve (AUC) of the neutrophil:lymphocyte ratio (NLR) on POD1 for predicting off-pump CABG-associated AKI was 0.780 and the cutoff value was 20.07. Patients with high NLR on POD1 had a poor short-term prognosis. The AUC of the predictive model constructed by logistic regression analysis was 0.882. The sensitivity was 68.2% and the specificity was 93.1%.

Conclusion: The NLR on POD1 was a reliable predictive biomarker of off-pump CABG-associated AKI. And we successfully construct a prediction model, which contribute to the early recognition and management of off-pump CABG-associated AKI.

KEYWORDS

coronary artery bypass grafting, acute kidney injury, risk factors, predictive model, cardiopulmonary bypass

Introduction

Acute kidney injury (AKI) involves a sudden decline in renal function. Approximately 20% of adult patients develop AKI during hospitalization, 10% of whom require dialysis (1). Studies have indicated that even mild AKI is associated with a significantly increased risk of death, and the mortality in patients requiring renal replacement therapy (RRT) is 50%, which poses a huge challenge for medical professionals (1–4).

AKI development is heterogenous, and several mechanisms may be involved (5). Patients undergoing cardiac surgery are more likely to develop AKI due to hemodynamic changes, an increased inflammatory response, and use of nephrotoxic medications (6). Moreover, cardiac surgery-associated AKI (CSA-AKI) is associated independently with short-term and long-term mortality (7–9). Considering the high prevalence (≤42%) and severe effects of CSA-AKI, early recognition and intervention are very important (10).

The inflammatory cascade is considered to be a major event that aggravates injury to tubular epithelial cells and reduces the glomerular filtration rate (GFR) during the extension phase: this represents the most promising phase for successful treatment and intervention of AKI (11). Therefore, the predictive role of inflammatory response-related biomarkers in CSA-AKI has been studied extensively.

The relevant ratios of different cell types in peripheral blood are able to reflect the inflammation and have been found to be potential predictors of AKI after acute type-A aortic dissection, on-pump coronary artery bypass grafting (CABG), and transcatheter implantation of aortic valves (12–17). However, studies on the relationship between off-pump CABG-associated AKI and the inflammatory response are lacking.

Off-pump CABG-associated AKI also significantly increases the risk of renal replacement therapy and death in patients (18–20). The risk factors for the development of AKI behind off-pump CABG are not well understood. We investigated whether the ratios of cell types in peripheral blood could be predictors of off-pump CABG-associated AKI. We look forward to providing guidance for the early recognition and treatment of off-pump CABG-associated AKI.

Materials and methods

Ethical approval of the study protocol

This study protocol was approved by the Medical Ethics Committee of Qilu Hospital of Shandong University (Jinan, China). Written informed consent was waived and all patient information was stored anonymously.

TABLE 1 Stage of off-pump CABG-associated AKI following KDIGO criteria.

Stage	Serum creatinine	Urine volume
I	Increase ≥0.3 mg/dl (≥26.5 μmol/L) within 48 h or increase to 1.5–1.9-times baseline levels	<0.5 ml/kg/h for 6–12 h
II	Increase to 2.0–2.9-times baseline levels	<0.5 ml/kg/h for ≥12 h
III	Increase to ≥4.0 mg/dl (≥353 μmol/L) or increase to ≥3-times baseline levels or RRT initiation	<0.3 ml/kg/h for ≥24 h or anuria

AKI, acute kidney injury; KDIGO, Kidney Disease: Improving Global Outcome; RRT, renal replacement therapy.

Study design

We retrospectively reviewed patients undergoing off-pump CABG from January 2021 to January 2022 at the Department of Cardiovascular Surgery within Qilu Hospital of Shandong University.

Exclusion criteria

Patients were excluded if: (i) they needed intraoperative CPB; (ii) they had preoperative severe chronic kidney disease necessitating RRT; (iii) their postoperative serum creatinine (SCr) data were incomplete.

Surgical procedures

Off-pump CABG was undertaken in patients with severe coronary artery disease [left main disease, three-vessel disease, combined with diabetes mellitus (DM)] or failed stenting. After the induction of general anesthesia with endotracheal intubation, a median sternal incision was made. The left internal mammary artery and great saphenous vein were freed as bridge vessels simultaneously. After heparinization, the anastomotic site was secured using a stabilizer. The anastomosis was undertaken with an intra-coronary shunt and deep pericardial suture. The operating surgeon measured the flow of vein grafts after the anastomosis to ensure the patency of grafted vascular bridges.

Definition

We selected the most recent SCr value before the surgical procedure as the baseline level. We applied the Chronic Kidney Disease Epidemiology Collaboration equation to obtain the estimated glomerular filtration rate (eGFR) (21). The diagnosis and staging of AKI followed the criteria of the Kidney Disease: Improving Global Outcome (KDIGO) guideline (22) (**Table 1**).

We calculated the neutrophil:lymphocyte ratio (NLR), monocyte:lymphocyte ratio (MLR), and platelet:lymphocyte ratio (PLR) as biomarkers associated with the inflammatory response.

Data collection

We documented the perioperative variables of patients. These were: age, gender, body mass index (BMI), tobacco

smoking, hypertension, DM, hyperlipemia, history of cerebral diseases, kidney disease without RRT, chronic obstructive pulmonary disease (COPD), percutaneous coronary intervention (PCI), New York Heart Association (NYHA) functional classification, preoperative left ventricular ejection fraction (LVEF), preoperative peripheral blood counts, preoperative blood biochemistry, preoperative renal function, intraoperative erythrocyte transfusion, intraoperative urine volume, intraoperative fluid replacement, central venous pressure (CVP) and mean arterial pressure (MAP) at intensive care unit (ICU) admission, use of an intra-aortic balloon pump (IABP), low cardiac output syndrome (LCOS), RRT, application of vasoactive agents, duration of mechanical ventilation, peripheral blood counts on postoperative day (POD)1, postoperative renal function, postoperative erythrocyte transfusion, complications, duration of hospital stay, duration of ICU stay, death.

Statistical analyses

We used SPSS 25.0 (IBM, Armonk, NY, United States) for statistical analyses. Measurement data were tested to see if they had a normal distribution. Variables with a normal distribution are expressed as the mean ± SD and were analyzed by the Student's *t*-test. Variables with a non-normal distribution are expressed as medians and quartiles and were analyzed by the Mann–Whitney *U*-test. Categorical data are expressed as frequencies and percentages and were compared by the chi-square test or Fisher's exact test. $P < 0.05$ (two-sided) was considered significant. Multivariate analysis incorporated variables with significant differences in univariate analysis. The results of multivariate logistic regression analysis are expressed as odds ratio (OR) and 95% confidence interval (CI). A receiver operating characteristic (ROC) curve and Hosmer–Lemeshow goodness of fit test were applied to evaluate the ability of predictive models. The maximum value of the Youden index was used to determine the cutoff value.

Results

Characteristics of the study cohort

From January 1, 2021 to January 1, 2022, 485 patients underwent off-pump CABG in the Department of Cardiovascular Surgery within Qilu Hospital of Shandong University. We excluded 65 patients according to our exclusion criteria (**Figure 1**). Finally, the data of 420 patients were analyzed and their baseline characteristics are shown in **Table 2**.

Eighty-eight patients (20.95%) developed AKI after off-pump CABG (67 patients with stage-I, 7 patients with stage-II, and 14 patients with stage-III AKI). Sixteen patients were diagnosed with AKI on POD1. The peak value of SCr occurred on POD2. Patients in the AKI group had a longer stay in the ICU and hospital. Seven patients received RRT after off-pump CABG, and 10 patients died within 28 days after CABG.

Inflammation-related biomarkers and off-pump CABG-associated AKI

We measured the levels of inflammation-related biomarkers before and on POD1 (**Table 3**). The counts for leukocytes, neutrophils, monocytes, and the levels of procalcitonin (PCT), which are correlated positively with inflammation, increased significantly on POD1. The lymphocyte count and platelet count, which are correlated negatively with the inflammatory response, decreased significantly on POD1. These results demonstrated that patients undergoing off-pump CABG experienced a dramatic inflammatory response. Moreover, there were significant differences in the ratios of cell types in peripheral blood on POD1 between patients in the AKI group and non-AKI group ($P < 0.01$), which implied a more pronounced inflammatory response in the AKI group. Patients in the AKI group had significantly higher levels of interleukin (IL)-6 than patients in the non-AKI group on POD1 (**Supplementary Table S1** and **Supplementary Figure S1**).

In addition, we found that ten of the patients who were diagnosed with AKI on POD1 developed more severe AKI in the following days. We divided them into the deteriorate and stable group based on their development of AKI. Patients in the deteriorate group had higher NLR on POD1 than those in the stable group, which indicated that NLR on POD1 may be used as predictors for more severe AKI in these patients (**Supplementary Table S2** and **Supplementary Figure S2**).

Independent risk factors of off-pump CABG-associated AKI

The results of univariate analysis exhibited that there were statistical differences between AKI and no-AKI groups regarding older than 65, female gender, DM, kidney disease without RRT, NYHA score greater than 2, hemoglobin, hematocrit, triglyceride, preoperative cystatin C, preoperative blood urea nitrogen, preoperative SCr, eGFR below 60%, LVEF below 50%, application of vasoactive agents, erythrocyte transfusion, duration of mechanical ventilation, LCOS, use of an IABP, preoperative NLR, NLR

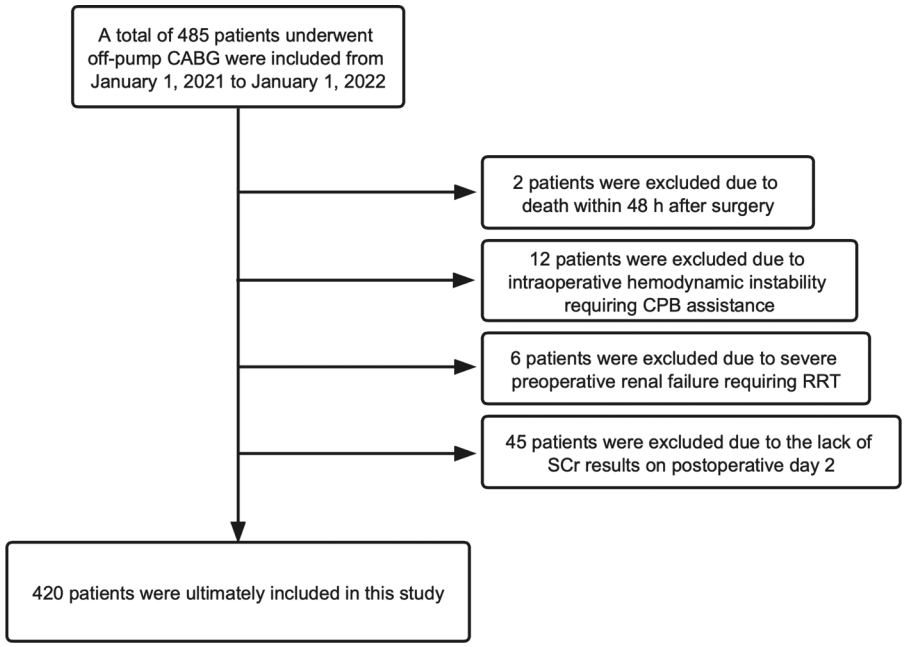


FIGURE 1
Flowchart of our study.

on POD1, MLR on POD1, PLR on POD1, PCT on POD1 (Table 2 and Table 3).

We included variables described above into a multivariate logistics regression model. Being female (OR = 3.200, 95%CI = 1.118–9.115), total erythrocyte transfusion (1.157, 1.019–1.31), NLR on POD1 (1.149, 1.071–1.232), PCT level on POD1 (1.061, 1.021–1.102), and duration of mechanical ventilation (1.027, 1.009–1.045) were independent risk factors of off-pump CABG-associated AKI (Table 4).

Predictive model of off-pump CABG-associated AKI

We used ROC-curve analysis to calculate the predictive ability of the NLR on POD1. The area under the ROC curve (AUC) of the NLR for predicting off-pump CABG-associated AKI was 0.780 (Figure 2A). The sensitivity was 84.1% and the specificity was 63.6%. When the Youden index reached a maximum, the cutoff value of the NLR was 20.07. Next, we included all the independent risk factors obtained by multivariate analysis into a predictive model for ROC-curve analysis. The AUC of the new predictive model was 0.882 (Figure 2B), which exhibited a better predictive ability. The sensitivity was 68.2% and the specificity was 93.1%. And the *P* value of Hosmer–Lemeshow goodness of fit test equals 0.074 ($P > 0.05$).

Correlation between the NLR and postoperative complications

We divided patients into a high-NLR group and low-NLR group according to the cutoff value (20.07) of the NLR on POD1. A high NLR on POD1 was closely associated with more severe AKI, pulmonary infection, hydrothorax, severe respiratory failure, and malignant arrhythmia (Table 5). Moreover, postoperative 28-day mortality was significantly higher in patients with a high NLR than in those with a low NLR ($P < 0.05$). These results demonstrated that patients with a high NLR on POD1 had poor short-term outcomes.

Discussion

Ischemic AKI is the most prevalent type of CSA-AKI. According to the change in the GFR, the development of ischemic AKI can be divided into four phases: initiation, extension, maintenance, and recovery (23). In the initiation phase, ischemia-induced damage to tubular epithelial cells and endothelial cells leads to the release of chemokines and cytokines that activate inflammatory cascades (24). The inflammatory response aggravates tubular-cell injury in the extension phase, which leads to a continued reduction in the GFR. Significant infiltration of inflammatory cells in the renal outer medulla occurs as early as 24 h after ischemia (25), and leukocytes may appear as early as 2 h after ischemia (26).

TABLE 2 Characteristics of the study population.

Variable	All patients ($n = 420$)	Non-AKI ($n = 332$)	AKI ($n = 88$)	*P*
Preoperative				
Age (years)	65 (58, 69)	64 (58, 68)	67 (62, 72)	<0.01
Age ≥65	216 (51.4%)	159 (47.9%)	57 (64.8%)	<0.01
55 < age < 65	136 (32.4%)	116 (34.9%)	20 (22.7%)	0.029
Age ≤55	68 (16.2%)	57 (17.2%)	11 (12.5%)	0.290
Female	137 (32.6%)	100 (30.1%)	37 (42.0%)	0.034
BMI (kg/m^2)	25.1 (23.2, 27.3)	25.2 (23.2, 27.3)	24.5 (22.8, 26.6)	0.171
Tobacco smoking	187 (44.5%)	151 (45.5%)	36 (40.9%)	0.443
Hypertension	257 (61.2%)	198 (59.6%)	59 (67.0%)	0.205
DM	174 (41.4%)	129 (38.9%)	45 (51.1%)	0.038
History of cerebral diseases	82 (19.5%)	63 (19.0%)	19 (21.6%)	0.582
Kidney disease without RRT	6 (1.4%)	2 (0.6%)	4 (4.5%)	<0.01
COPD	8 (1.9%)	6 (1.8%)	2 (2.3%)	0.776
PCI	50 (11.9%)	39 (11.7%)	11 (12.5%)	0.846
NYHA grade	—	—	—	<0.01
NYHA grade >2	152 (36.2%)	107 (32.2%)	45 (51.1%)	<0.01
NYHA grade ≤2	268 (63.8%)	225 (67.8%)	43 (48.9%)	<0.01
Hemoglobin (g/L)	137 (126, 147)	138 (127, 146)	133 (118, 148)	0.016
HCT (%)	41.10 (37.90, 43.50)	41.20 (38.45, 43.50)	39.10 (36.30, 43.35)	<0.01
Albumin (g/L)	42.30 (40.10, 44.30)	42.40 (40.40, 44.35)	41.80 (38.85, 44.05)	0.055
LDL (mmol/L)	1.97 (1.58, 2.49)	1.98 (1.57, 2.49)	1.97 (1.58, 2.49)	0.796
HDL (mmol/L)	0.99 (0.85, 1.13)	1.00 (0.85, 1.14)	0.96 (0.83, 1.09)	0.174
TG (mmol/L)	1.28 (0.96, 1.72)	1.24 (0.93, 1.69)	1.41 (1.08, 1.95)	0.017
Cys-C (mg/L)	1.01 (0.89, 1.15)	0.99 (0.88, 1.10)	1.14 (0.98, 1.37)	<0.01
BUN (mmol/L)	5.50 (4.52, 6.55)	5.40 (4.50, 6.40)	6.00 (5.00, 7.15)	<0.01
SCr (μmol/L)	76.0 (64.0, 86.0)	74.5 (64.0, 85.0)	82.5 (65.0, 97.0)	<0.01
eGFR (ml/min/ 1.73 m^2)	93 (81, 101)	95 (85, 102)	87 (67, 97)	<0.01
eGFR ≥90	252 (60.0%)	215 (64.8%)	37 (42%)	<0.01
60 < eGFR < 90	144 (34.3%)	109 (32.8%)	35 (39.8%)	0.223
eGFR ≤60	24 (5.7%)	8 (2.4%)	16 (18.2%)	<0.01
LVEF (%)	60 (51, 65)	60 (53, 65)	57 (43, 62)	<0.01
LVEF ≥60	212 (50.5%)	177 (53.3%)	35 (39.8%)	0.024
50 < LVEF < 60	106 (25.2%)	84 (25.3%)	22 (25.0%)	0.954
LVEF ≤50	102 (24.3%)	71 (21.4%)	31 (35.2%)	<0.01
Intraoperative				
Emergency surgery	33 (7.9%)	23 (6.9%)	10 (11.4%)	0.169
Operation time (min)	270 (240, 300)	265 (240, 295)	273 (250, 295)	0.074
Erythrocyte transfusion (U)	0 (0, 2)	0 (0, 2)	1 (0, 4)	<0.01
Urine volume (ml)	700 (500, 1,000)	750 (500, 1,000)	700 (450, 1,000)	0.917
Fluid replacement (ml)	2,700 (2,500, 3,000)	2,700 (2,500, 3,000)	2,700 (2,500, 3,500)	0.189
Postoperative				
CVP (cmH_2O)	8 (6, 10)	8 (6, 10)	8 (7, 11)	0.223
MAP (mmHg)	88 (76, 98)	88 (78, 98)	84 (72, 99)	0.259
Medicine application	160 (38.1%)	108 (32.5%)	52 (59.1%)	<0.01
Erythrocyte transfusion (U)	0 (0, 2)	0 (0, 2)	2 (0, 4)	<0.01
Mechanical ventilation (min)	780 (541, 1,140)	720 (513, 1,029)	1,200 (792, 3,390)	<0.01
LCOS	43 (10.2%)	14 (4.2%)	29 (33.0%)	<0.01
IABP	41 (9.8%)	14 (4.2%)	27 (30.7%)	<0.01
RRT	7 (1.7%)	0	7 (8.0%)	<0.01
Duration of hospital stay (day)	12 (10, 14)	12 (10, 14)	14 (12, 19)	<0.01
Duration of ICU stay (day)	3 (2, 4)	2 (2, 3)	4 (3, 7)	<0.01
28-day mortality	10 (2.4%)	1 (0.3%)	9 (10.2%)	<0.01

AKI, acute kidney injury; BMI, body mass index; BUN, blood urea nitrogen; COPD, chronic obstructive pulmonary disease; CVP, central venous pressure; Cys-C, cystatin C; DM, diabetes mellitus; eGFR, estimated glomerular filtration rate; HCT, hematocrit; HDL, high density lipoprotein; IABP, intra-aortic balloon pump; ICU, intensive care unit; LCOS, low cardiac output syndrome; LDL, low density lipoprotein; LVEF, left ventricular ejection fraction; MAP, mean arterial pressure; NYHA, New York Heart Association; PCI, percutaneous coronary intervention; RRT, renal replacement therapy; SCr, Serum creatinine; TG, triglyceride.

Therefore, early identification and interrupting the amplification of the inflammatory response in the extension phase is very important.

The SCr level peaked on POD2 and the infiltration of inflammatory cells would appear early, so we chose the cell ratios in peripheral blood on POD1 as biomarkers. Our study found that the NLR on POD1 was a reliable biomarker for the early prediction of off-pump CABG-associated AKI (AUC = 0.780, cutoff = 20.07). The NLR was derived from the hematological observation that the neutrophil count is associated positively with cardiovascular events and the lymphocyte count is associated negatively with cardiovascular events (27, 28). Neutrophil activation is an important sign of acute inflammatory response, and lymphopenia is a marker of poor health condition and physiological stress. Compared with C-reactive protein, IL-6, and other inflammation-specific biomarkers, the NLR can be obtained more readily and in an inexpensive manner, so it has attracted the attention of researchers. A meta-analysis involving five randomized clinical

TABLE 3 Inflammation-related biomarkers in the non-AKI and AKI group.

Variables	All patients ($n = 420$)	Non-AKI ($n = 332$)	AKI ($n = 88$)	*P*
Preoperative inflammation-related biomarkers				
WBC (10^9/L)	6.37 (5.26, 7.45)	6.33 (5.27, 7.49)	6.50 (5.14, 7.33)	0.685
NEU (10^9/L)	3.96 (3.08, 4.86)	3.93 (3.05, 4.82)	4.19 (3.17, 4.94)	0.264
LYM (10^9/L)	1.60 (1.31, 1.96)	1.60 (1.32, 1.97)	1.53 (1.27, 1.83)	0.160
MON (10^9/L)	0.46 (0.37, 0.57)	0.46 (0.37, 0.57)	0.45 (0.39, 0.57)	0.841
PLT (10^9/L)	224 (186, 265)	224 (186, 263)	230 (197, 284)	0.347
NLR	2.47 (1.84, 3.15)	2.40 (1.80, 3.07)	2.64 (2.13, 3.25)	0.034
MLR	0.28 (0.22, 0.37)	0.28 (0.22, 0.37)	0.30 (0.23, 0.37)	0.200
PLR	138 (111, 173)	135 (110, 172)	145 (123, 179)	0.058
LDH (U/L)	210 (187, 241)	211 (188, 240)	210 (185, 247)	0.752
Inflammation-related biomarkers on POD1				
WBC (10^9/L)	12.22 ± 3.89	12.20 ± 3.90	12.25 ± 3.88	0.915
NEU (10^9/L)	10.78 ± 3.54	10.70 ± 3.52	11.09 ± 3.58	0.359
LYM (10^9/L)	0.56 (0.41, 0.73)	0.60 (0.44, 0.78)	0.41 (0.31, 0.50)	<0.01
MON (10^9/L)	0.72 (0.51, 0.98)	0.74 (0.52, 0.99)	0.68 (0.48, 0.92)	0.102
PLT (10^9/L)	166 (133, 206)	166 (134, 204)	166 (129, 219)	0.908
NLR	19.13 (13.53, 26.34)	17.18 (12.63, 23.62)	26.40 (21.04, 33.93)	<0.01
MLR	1.30 (0.92, 1.74)	1.24 (0.89, 1.61)	1.74 (1.22, 2.44)	<0.01
PLR	302 (212, 415)	271 (203, 393)	382 (300, 526)	<0.01
PCT (ng/ml)	1.31 (0.49, 3.16)	1.13 (0.44, 2.59)	2.32 (0.68, 5.61)	<0.01
LDH (U/L)	211 (175, 259)	209 (173, 254)	219 (181, 302)	0.071

AKI, acute kidney injury; LDH, lactate dehydrogenase; LYM, lymphocyte; MLR, monocyte:lymphocyte ratio; MON, monocyte; NEU, neutrophil; NLR, neutrophil:lymphocyte ratio; PCT, procalcitonin; PLR, platelet:lymphocyte ratio; PLT, platelet; POD, postoperative day; WBC, white blood cell.

trials with large cohorts showed that the NLR at baseline independently predicted the risk of cardiovascular events and all-cause mortality in patients (29). Kim and colleagues revealed a high NLR on POD1 to be closely associated with an increased risk of CSA-AKI and 1-year mortality (30).

The high NLR level on POD1 may indicate the early inflammatory response after off-pump CABG, which is one of the most important mechanisms of AKI. Many animal experiments have proved the important role of inflammatory response in the development of AKI. Kelly and colleagues found that anti-intercellular adhesion molecule-1 therapy prevented ischemic AKI in mice *via* neutrophil-dependent pathway (31, 32). Rabb and coworkers confirmed that the adhesion molecules CD11 and CD18 on the surface of leukocytes play an important role in ischemic AKI in rats (33). In addition, selectin ligand inhibitors also successfully attenuated renal ischemia-reperfusion injury in rat and pig models (34, 35). Besides the adhesion molecules described above, chemokines, proinflammatory cytokines, reactive oxygen species, C-reactive protein and danger-associated molecular patterns also participate in the inflammatory response leading to unacceptable AKI.

CPB during cardiac surgery activates the immune system significantly: a large number of cytokines and chemokines are released, which increases the risk of AKI (36). Parlar and coworkers found that the postoperative NLR was an independent predictor of AKI after on-pump CABG (37). However, we found that the leukocyte count, PCT level, and IL-6 level were also significantly higher in patients after off-pump CABG, which reflected a marked inflammatory response. Studies correlating inflammation with off-pump CABG-associated AKI are lacking. Our findings demonstrated this association and revealed the predictive value of the NLR on POD1.

We also discovered four other independent risk factors of off-pump CABG-associated AKI according to multivariate analysis: gender, total erythrocyte transfusion, PCT level on POD1, and duration of mechanical ventilation. In our study, female patients accounted for 32.6% of the study cohort, but the prevalence of AKI was much higher than that in male patients ($P < 0.05$). After adjustment for other variables, being female remained an independent risk factor of off-pump CABG-associated AKI. This observation is consistent with findings in some studies (38, 39) but not in other studies (40, 41).

The free hemoglobin and free iron released from an erythrocyte transfusion would cause oxidative stress, which aggravates inflammation and ischemia–reperfusion injury in the kidney (42, 43). Therefore, many researchers expect to reduce the occurrence of CSA-AKI by restricting erythrocyte transfusion (44, 45). The association between mechanical ventilation and AKI was documented first by Drury and coworkers in 1947. They found that continuous-pressure ventilation caused a decline in renal function (46). Afterwards, Kuiperet and colleagues proposed that mechanical ventilation may affect renal function due to hemodynamic alterations and ventilator-induced lung injury which activates a systemic inflammatory response (47). We also found a correlation between higher levels of inflammatory biomarkers on POD1 and pulmonary complications.

PCT is a commonly used indicator for the diagnosis of sepsis in the ICU. The PCT level can be used to assess bacterial infection in the body. Studies of PCT and AKI have focused mainly on patients with sepsis, with fewer studies concentrating on patients undergoing cardiac surgery. Heredia-Rodríguez and colleagues enrolled patients with a systemic inflammatory response or sepsis after cardiac surgery. They showed that the PCT level was significantly higher in patients with CSA-AKI (48). Our results also confirmed this correlation, and we found that a high PCT level on POD1 was an independent risk factor of off-pump CABG-associated AKI. We hypothesize that patients on POD1 did not develop a bacterial infection, but the high PCT

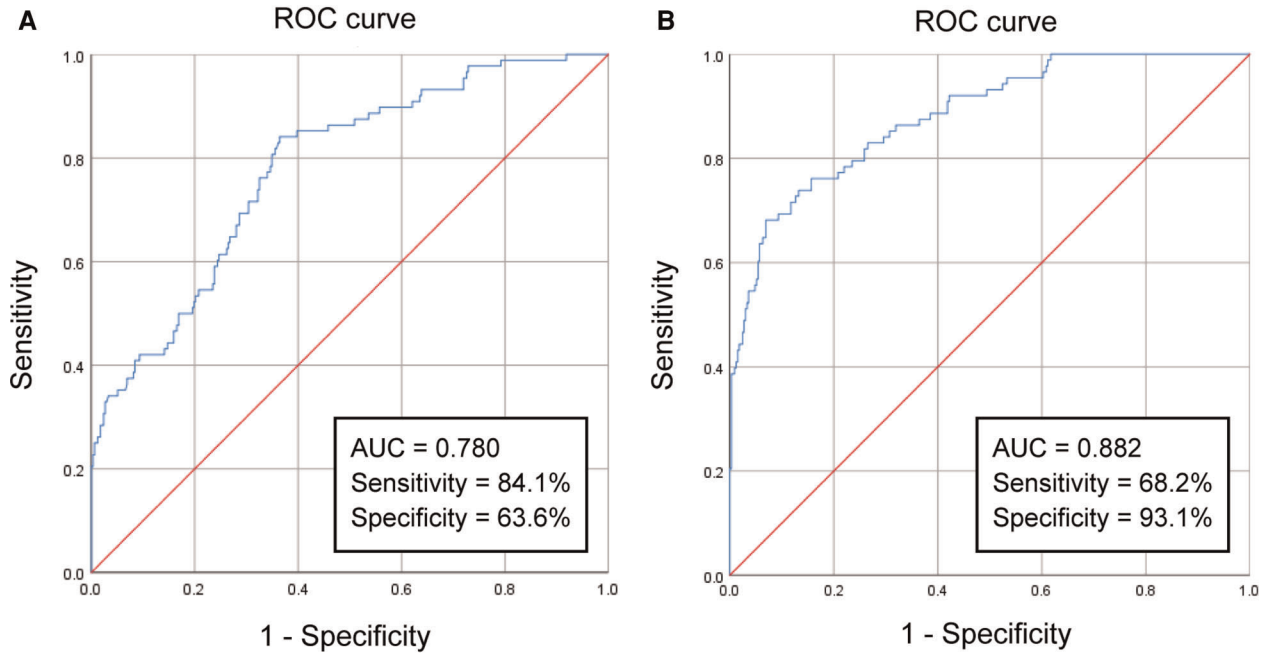


FIGURE 2
The results of ROC-curve analysis: (A) the ROC curve of the NLR for predicting off-pump CABG-associated AKI. (B) The ROC curve of the predictive model.

level may suggest an inflammatory reaction occurring *in vivo* and a higher risk of infection.

We included the independent risk factors stated above into a prediction model and obtained a high predictive ability (AUC = 0.882). Our study provides a predictive biomarker and predictive model for the early recognition and timely intervention of off-pump CABG-associated AKI. We aim to improve the prognosis of patients undergoing off-pump CABG.

Our study had three main limitations. First, this was a single-center retrospective study. The predictors obtained and prediction model created must undergo external validation. Second, we assessed only the short-term outcomes of patients after off-pump CABG. Third, the predictive value of NLR on POD1 was attenuated in patients who have had AKI on POD1. In the future, we will dedicate ourselves to improving the intraoperative management of off-pump CABG and carrying out a prospective study that documents the prevalence of postoperative AKI in patients undergoing different treatments.

TABLE 4 Independent risk factors of off-pump CABG-associated AKI.

Variables	*B*	*P*	OR	95%CI
Female	1.163	0.030	3.200	1.118–9.155
Total erythrocyte transfusion	0.145	0.024	1.157	1.019–1.313
NLR on POD1	0.139	<0.01	1.149	1.071–1.232
PCT on POD1	0.059	<0.01	1.061	1.021–1.102
Mechanical ventilation	0.026	<0.01	1.027	1.009–1.045

AKI, acute kidney injury; NLR, neutrophil:lymphocyte ratio; PCT, procalcitonin; POD, postoperative day.

TABLE 5 Correlation between the NLR and postoperative complications.

Complications	All patients (*n* = 420)	Low-NLR group (*n* = 225)	High-NLR group (*n* = 195)	*P*
Stage-I AKI	67 (16.0%)	13 (5.8%)	54 (27.7%)	<0.01
Stage-II AKI	7 (1.7%)	0	7 (3.6%)	<0.01
Stage-III AKI	14 (3.3%)	1 (0.4%)	13 (6.7%)	<0.01
Cerebral infarction	14 (3.3%)	5 (2.2%)	9 (4.6%)	0.173
Pulmonary infection	130 (31.0%)	59 (26.2%)	71 (36.4%)	0.024
Incision infection	5 (1.2%)	1 (0.4%)	4 (2.1%)	0.130
Multiple operations	6 (1.4%)	1 (0.4%)	5 (2.6%)	0.068
Hydrothorax	110 (26.2%)	44 (19.6%)	66 (33.8%)	<0.01
Severe respiratory failure	36 (8.6%)	11 (4.9%)	25 (12.8%)	<0.01
Atrial fibrillation	107 (25.5%)	52 (23.1%)	55 (28.2%)	0.232
Malignant arrhythmia	16 (3.8%)	2 (0.9%)	14 (7.2%)	<0.01
28-day mortality	10 (2.4%)	2 (0.9%)	8 (4.1%)	0.031

AKI, acute kidney injury; NLR, neutrophil:lymphocyte ratio.

Conclusion

We demonstrated the NLR on POD1 to be a reliable biomarker for predicting off-pump CABG-associated AKI. Also, we constructed a prediction model that may contribute to the early recognition and management of off-pump CABG-associated AKI.

Data availability statement

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

Ethics statement

The studies involving human participants were reviewed and approved by Medical Ethics Committee of Qilu Hospital of Shandong University. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

RY performed data analysis, statistics, and draft writing. HS performed data collection. XM and YB designed the study and critically revised the manuscript. All authors contributed to the article and approved the submitted version.

Acknowledgments

The authors would like to thank Dr. Tingyi Liang for her assistance with the research.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fsurg.2022.1047050/full#supplementary-material.

References

1. Susantitaphong P, Cruz DN, Cerda J, Abulfaraj M, Alqahtani F, Koulouridis I, et al. World incidence of aki: a meta-analysis. *Clin J Am Soc Nephrol.* (2013) 8 (9):1482–93. doi: 10.2215/cjn.00710113

2. Lassnigg A, Schmidlin D, Mouhieddine M, Bachmann LM, Druml W, Bauer P, et al. Minimal changes of Serum creatinine predict prognosis in patients after cardiothoracic surgery: a prospective cohort study. *J Am Soc Nephrol.* (2004) 15 (6):1597–605. doi: 10.1097/01.asn.0000130340.93930.dd

3. Lassnigg A, Schmid ER, Hiesmayr M, Falk C, Druml W, Bauer P, et al. Impact of minimal increases in Serum creatinine on outcome in patients after cardiothoracic surgery: do we have to revise current definitions of acute renal failure? *Crit Care Med.* (2008) 36(4):1129–37. doi: 10.1097/CCM.0b013e318169181a

4. Lameire NH, Bagga A, Cruz D, De Maeseneer J, Endre Z, Kellum JA, et al. Acute kidney injury: an increasing global concern. *Lancet.* (2013) 382 (9887):170–9. doi: 10.1016/s0140-6736(13)60647-9

5. Ronco C, Bellomo R, Kellum JA. Acute kidney injury. *Lancet.* (2019) 394 (10212):1949–64. doi: 10.1016/s0140-6736(19)32563-2

6. Nadim MK, Forni LG, Bihorac A, Hobson C, Koyner JL, Shaw A, et al. Cardiac and vascular surgery-associated acute kidney injury: the 20th international consensus conference of the adqi (acute disease quality initiative) group. *J Am Heart Assoc.* (2018) 7(11):e008834. doi: 10.1161/jaha.118.008834

7. Hobson CE, Yavas S, Segal MS, Schold JD, Tribble CG, Layon AJ, et al. Acute kidney injury is associated with increased long-term mortality after cardiothoracic surgery. *Circulation.* (2009) 119(18):2444–53. doi: 10.1161/circulationaha.108.800011

8. Lopez-Delgado JC, Esteve F, Torrado H, Rodríguez-Castro D, Carrio ML, Farrero E, et al. Influence of acute kidney injury on short- and long-term outcomes in patients undergoing cardiac surgery: risk factors and prognostic value of a modified rifle classification. *Crit Care.* (2013) 17(6):R293. doi: 10.1186/cc13159

9. Machado MN, Nakazone MA, Maia LN. Prognostic value of acute kidney injury after cardiac surgery according to kidney disease: improving global outcomes definition and staging (kdigo) criteria. *PLoS One.* (2014) 9(5):e98028. doi: 10.1371/journal.pone.0098028

10. Wang Y, Bellomo R. Cardiac surgery-associated acute kidney injury: risk factors, pathophysiology and treatment. *Nat Rev Nephrol.* (2017) 13 (11):697–711. doi: 10.1038/nrneph.2017.119

11. Basile DP, Anderson MD, Sutton TA. Pathophysiology of acute kidney injury. *Compr Physiol.* (2012) 2(2):1303–53. doi: 10.1002/cphy.c110041

12. Ma X, Chen S, Yun Y, Zhao D, Li J, Wu Z, et al. The predictive role of lymphocyte-to-monocyte ratio in acute kidney injury in acute debakey type I aortic dissection. *Front Surg.* (2021) 8:704345. doi: 10.3389/fsurg.2021.704345

13. Chen W, Song X, Hong L, Xu H, Qian Y, Zhang W, et al. The association between lymphocyte-monocyte ratio and postoperative acute kidney injury in patients with acute type a aortic dissection. *J Cardiothorac Surg.* (2022) 17 (1):60. doi: 10.1186/s13019-022-01813-x

14. Fisher LA, Stephenson S, Reid MT, Anderson SG. Acute kidney injury following cardiopulmonary bypass in Jamaica. *JTCVS Open*. (2022) 36(11): 161–75. doi: 10.1016/j.xjon.2022.05.012

15. Olasińska-Wiśniewska A, Perek B, Grygier M, Urbanowicz T, Misterski M, Puślecki M, et al. Increased neutrophil-to-lymphocyte ratio is associated with higher incidence of acute kidney injury and worse survival after transcatheter aortic valve implantation. *Cardiol J*. (2021). doi: 10.5603/CJ.a2021.0149. [Epub ahead of print]

16. Koo CH, Eun Jung D, Park YS, Bae J, Cho YJ, Kim WH, et al. Neutrophil, lymphocyte, and platelet counts and acute kidney injury after cardiovascular surgery. *J Cardiothorac Vasc Anesth*. (2018) 32(1):212–22. doi: 10.1053/j.jvca.2017.08.033

17. Parlar H, Şaşkın H. Are Pre and postoperative platelet to lymphocyte ratio and neutrophil to lymphocyte ratio associated with early postoperative aki following cabg? *Braz J Cardiovasc Surg*. (2018) 33(3):233–41. doi: 10.21470/1678-9741-2017-0164

18. Lamy A, Devereaux PJ, Prabhakaran D, Taggart DP, Hu S, Paolasso E, et al. Off-pump or on-pump coronary-artery bypass grafting at 30 days. *N Engl J Med*. (2012) 366(16):1489–97. doi: 10.1056/NEJMoa1200388

19. Lamy A, Devereaux PJ, Prabhakaran D, Taggart DP, Hu S, Paolasso E, et al. Effects of off-pump and on-pump coronary-artery bypass grafting at 1 year. *N Engl J Med*. (2013) 368(13):1179–88. doi: 10.1056/NEJMoa1301228

20. Cheungpasitporn W, Thongprayoon C, Kittanamongkolchai W, Srivali N, O'Corragain OA, Edmonds PJ, et al. Comparison of renal outcomes in off-pump versus on-pump coronary artery bypass grafting: a systematic review and meta-analysis of randomized controlled trials. *Nephrology (Carlton)*. (2015) 20 (10):727–35. doi: 10.1111/nep.12506

21. Inker LA, Eneanya ND, Coresh J, Tighiouart H, Wang D, Sang Y, et al. New creatinine- and cystatin C-based equations to estimate gfr without race. *N Engl J Med*. (2021) 385(19):1737–49. doi: 10.1056/NEJMoa2102953

22. Khwaja A. Kdigo clinical practice guidelines for acute kidney injury. *Nephron Clin Pract*. (2012) 120(4):c179–84. doi: 10.1159/000339789

23. Sutton TA, Fisher CJ, Molitoris BA. Microvascular endothelial injury and dysfunction during ischemic acute renal failure. *Kidney Int*. (2002) 62 (5):1539–49. doi: 10.1046/j.1523-1755.2002.00631.x

24. Rabb H, O'Meara YM, Maderna P, Coleman P, Brady HR. Leukocytes, cell adhesion molecules and ischemic acute renal failure. *Kidney Int*. (1997) 51 (5):1463–8. doi: 10.1038/ki.1997.200

25. Ysebaert DK, De Greef KE, Vercauteren SR, Ghielli M, Verpooten GA, Eyskens EJ, et al. Identification and kinetics of leukocytes after severe ischaemia/reperfusion renal injury. *Nephrol Dial Transplant*. (2000) 15 (10):1562–74. doi: 10.1093/ndt/15.10.1562

26. Willinger CC, Schramek H, Pfaller K, Pfaller W. Tissue distribution of neutrophils in postischemic acute renal failure. *Virchows Arch B Cell Pathol Incl Mol Pathol*. (1992) 62(4):237–43. doi: 10.1007/bf02899687

27. Friedman GD, Klatsky AL, Siegelaub AB. The leukocyte count as a predictor of myocardial infarction. *N Engl J Med*. (1974) 290(23):1275–8. doi: 10.1056/nejm197406062902302

28. Horne BD, Anderson JL, John JM, Weaver A, Bair TL, Jensen KR, et al. Which white blood cell subtypes predict increased cardiovascular risk? *J Am Coll Cardiol*. (2005) 45(10):1638–43. doi: 10.1016/j.jacc.2005.02.054

29. Adamstein NH, MacFadyen JG, Rose LM, Glynn RJ, Dey AK, Libby P, et al. The neutrophil-lymphocyte ratio and incident atherosclerotic events: analyses from five contemporary randomized trials. *Eur Heart J*. (2021) 42(9):896–903. doi: 10.1093/eurheartj/ehaa1034

30. Kim WH, Park JY, Ok SH, Shin IW, Sohn JT. Association between the neutrophil/lymphocyte ratio and acute kidney injury after cardiovascular surgery: a retrospective observational study. *Medicine (Baltimore)*. (2015) 94 (43):e1867. doi: 10.1097/md.0000000000001867

31. Kelly KJ, Williams Jr. WW, Colvin RB, Bonventre JV. Antibody to intercellular adhesion molecule 1 protects the kidney against ischemic injury. *Proc Natl Acad Sci U S A*. (1994) 91(2):812–6. doi: 10.1073/pnas.91.2.812

32. Kelly KJ, Williams Jr. WW, Colvin RB, Meehan SM, Springer TA, Gutierrez-Ramos JC, et al. Intercellular adhesion molecule-1-deficient mice are protected against ischemic renal injury. *J Clin Invest*. (1996) 97(4):1056–63. doi: 10.1172/jci118498

33. Rabb H, Mendiola CC, Dietz J, Saba SR, Issekutz TB, Abanilla F, et al. Role of Cd11a and Cd11b in ischemic acute renal failure in rats. *Am J Physiol*. (1994) 267(6 Pt 2):F1052–8. doi: 10.1152/ajprenal.1994.267.6.F1052

34. Nemoto T, Burne MJ, Daniels F, O'Donnell MP, Crosson J, Berens K, et al. Small molecule selectin ligand inhibition improves outcome in ischemic acute renal failure. *Kidney Int*. (2001) 60(6):2205–14. doi: 10.1046/j.1523-1755.2001.00054.x

35. Jayle C, Milinkevitch S, Favreau F, Doucet C, Richer JP, Deretz S, et al. Protective role of selectin ligand inhibition in a large animal model of kidney ischemia-reperfusion injury. *Kidney Int*. (2006) 69(10):1749–55. doi: 10.1038/sj.ki.5000335

36. Zhang WR, Garg AX, Coca SG, Devereaux PJ, Eikelboom J, Kavsak P, et al. Plasma il-6 and il-10 concentrations predict aki and long-term mortality in adults after cardiac surgery. *J Am Soc Nephrol*. (2015) 26(12):3123–32. doi: 10.1681/asn.2014080764

37. Parlar H, Arıkan AA, Önmez A. Dynamic changes in perioperative cellular inflammation and acute kidney injury after coronary artery bypass Grafting. *Braz J Cardiovasc Surg*. (2021) 36(3):354–64. doi: 10.21470/1678-9741-2020-0163

38. Thakar CV, Liangos O, Yared JP, Nelson D, Piedmonte MR, Hariachar S, et al. Arf after open-heart surgery: influence of gender and race. *Am J Kidney Dis*. (2003) 41(4):742–51. doi: 10.1016/s0272-6386(03)00021-0

39. Birnie K, Verheyden V, Pagano D, Bhabra M, Tilling K, Sterne JA, et al. Predictive models for kidney disease: improving global outcomes (kdigo) defined acute kidney injury in UK cardiac surgery. *Crit Care*. (2014) 18(6):606. doi: 10.1186/s13054-014-0606-x

40. Fang Y, Teng J, Ding X. Acute kidney injury in China. *Hemodial Int*. (2015) 19(1):2–10. doi: 10.1111/hdi.12193

41. Jiang W, Teng J, Xu J, Shen B, Wang Y, Fang Y, et al. Dynamic predictive scores for cardiac surgery-associated acute kidney injury. *J Am Heart Assoc*. (2016) 5(8):e003754. doi: 10.1161/jaha.116.003754

42. Billings FTT, Ball SK, Roberts 2nd LJ, Pretorius M. Postoperative acute kidney injury is associated with hemoglobinemia and an enhanced oxidative stress response. *Free Radic Biol Med*. (2011) 50(11):1480–7. doi: 10.1016/j.freeradbiomed.2011.02.011

43. Haase M, Bellomo R, Haase-Fielitz A. Novel biomarkers, oxidative stress, and the role of labile iron toxicity in cardiopulmonary bypass-associated acute kidney injury. *J Am Coll Cardiol*. (2010) 55(19):2024–33. doi: 10.1016/j.jacc.2009.12.046

44. Mazer CD, Whitlock RP, Fergusson DA, Hall J, Belley-Cote E, Connolly K, et al. Restrictive or liberal red-cell transfusion for cardiac surgery. *N Engl J Med*. (2017) 377(22):2133–44. doi: 10.1056/NEJMoa1711818

45. Murphy GJ, Pike K, Rogers CA, Wordsworth S, Stokes EA, Angelini GD, et al. Liberal or restrictive transfusion after cardiac surgery. *N Engl J Med*. (2015) 372(11):997–1008. doi: 10.1056/NEJMoa1403612

46. Drury DR, Henry JP, Goodman J. The effects of continuous pressure breathing on kidney function. *J Clin Invest*. (1947) 26(5):945–51. doi: 10.1172/jci101889

47. Kuiper JW, Groeneveld AB, Slutsky AS, Plötz FB. Mechanical ventilation and acute renal failure. *Crit Care Med*. (2005) 33(6):1408–15. doi: 10.1097/01.ccm.0000165808.30416.ef

48. Heredia-Rodríguez M, Bustamante-Munguira J, Fierro I, Lorenzo M, Jorge-Monjas P, Gómez-Sánchez E, et al. Procalcitonin cannot be used as a biomarker of infection in heart surgery patients with acute kidney injury. *J Crit Care*. (2016) 33:233–9. doi: 10.1016/j.jcrc.2016.01.015

frontiers | Frontiers in Cardiovascular Medicine

TYPE Original Research
PUBLISHED 11 November 2022
DOI 10.3389/fcvm.2022.997350

OPEN ACCESS

EDITED BY
Shahzad Raja,
Harefield Hospital, United Kingdom

REVIEWED BY
Kareem Salhiyyah,
Middle East University, Jordan
Ronaldo Arkader,
University of São Paulo, Brazil

*CORRESPONDENCE
Bernard D. Krüger
Bernard.Krueger@usz.ch

†These authors have contributed equally to this work and share first authorship

‡These authors have contributed equally to this work and share last authorship

SPECIALTY SECTION
This article was submitted to Heart Surgery, a section of the journal Frontiers in Cardiovascular Medicine

RECEIVED 18 July 2022
ACCEPTED 18 October 2022
PUBLISHED 11 November 2022

CITATION
Krüger BD, Hofer GE, Rudiger A, Spahn GH, Braun J, Bettex D, Schoedon G and Spahn DR (2022) Wingless-related integration site (WNT) signaling is activated during the inflammatory response upon cardiac surgery: A translational study. *Front. Cardiovasc. Med.* 9:997350. doi: 10.3389/fcvm.2022.997350

Wingless-related integration site (WNT) signaling is activated during the inflammatory response upon cardiac surgery: A translational study

Bernard D. Krüger[1,2*†], Gilles E. Hofer[1,2,3†], Alain Rudiger[2,3], Gabriela H. Spahn[1], Julia Braun[2,4,5], Dominique Bettex[1,2], Gabriele Schoedon[2,6‡] and Donat R. Spahn[1,2‡]

[1]Institute of Anesthesiology, University Hospital Zurich, Zurich, Switzerland, [2]University of Zurich, Zurich, Switzerland, [3]Department of Medicine, Limmattal Hospital, Schlieren, Switzerland, [4]Department of Epidemiology, Epidemiology, Biostatistics and Prevention Institute, University of Zurich, Zurich, Switzerland, [5]Department of Biostatistics, Epidemiology, Biostatistics and Prevention Institute, University of Zurich, Zurich, Switzerland, [6]Inflammation Research Unit, Department of Internal Medicine, University Hospital Zurich, University of Zurich, Zurich, Switzerland

Objective: Cardiac surgery and the use of cardiopulmonary bypass initiate a systemic inflammatory response. Wingless-related integration site (WNT) signaling is part of the innate immunity and has been attributed a major role in the regulation of inflammation. In preclinical research, WNT-5a may sustain an inflammatory response and cause endothelial dysfunction. Our aim was to investigate WNT signaling after cardiac surgery and its association with postoperative inflammation (Clinicaltrials.gov, NCT04058496).

Methods: In this prospective, single-center, observational study, 64 consecutive patients for coronary artery bypass grafting (CABG) ± valve surgery were assigned into three groups: off-pump CABG (n = 28), on-pump CABG (n = 16) and combined valve-CABG surgery (n = 20). Blood samples were acquired before surgery, at intensive care unit (ICU) admission and 4, 8, and 48 h thereafter. Plasma concentrations of WNT-5a and its antagonists Secreted frizzled-related protein 1 (sFRP-1), Secreted frizzled-related protein 5 (sFRP-5), and WNT inhibitory factor 1 (WIF-1) were determined by enzyme-linked immunosorbent assay. In addition, plasma concentrations of six inflammatory cytokines were measured by multiplex immunoassay. Parameters were analyzed for evolution of plasma concentration over time, interactions, intergroup differences, and association with clinical outcome parameters.

Results: At baseline, WNT-5a, sFRP-1, and WIF-1 were present in a minimal concentration, while sFRP-5 was elevated. A higher baseline value of WNT-5a, sFRP-5, and WIF-1 resulted in higher subsequent values of the respective parameter. At ICU admission, WNT-5a and sFRP-5 reached their maximum and minimum value, respectively. WIF-1 decreased over time and was lowest

8 h after surgery. sFRP-1 changed minimally over time. While WNT-5a returned to the baseline within 48 h, sFRP-5 and WIF-1 did not reach their baseline value at 48 h. Of the investigated WNT system components, only WIF-1 partially reflected the severity of surgery. WNT-5a and WIF-1 had an impact on postoperative fluid balance and noradrenaline requirement.

Conclusion: WNT-5a, sFRP-5, and WIF-1 are part of the systemic inflammatory response after cardiac surgery. WNT-5a peaks immediately after cardiac surgery and returns to baseline within 48 h, presumably modulated by its antagonist sFRP-5. Based on this translational study, WNT-5a antagonism may be further investigated to assess potentially beneficial effects in patients with a dysregulated inflammation after cardiac surgery.

KEYWORDS

inflammation, cardiac surgery, cardiopulmonary bypass, systemic inflammatory response syndrome, SIRS, WNT signaling, WNT-5a, inflammatory biomarkers

Introduction

During cardiac surgery, a sterile inflammation is initiated by the surgical trauma, blood contact with the artificial surfaces of a cardiopulmonary bypass (CPB) and ischemia-reperfusion injury predominantly of the heart and the lung (1). Damage associated molecular patterns activate the mononuclear phagocyte system, an essential component of the innate immunity, to mount an inflammatory response (2). While a localized inflammation is important for tissue repair and wound healing, a systemic inflammatory response may ensue when cytokines are released into the blood circulation, thereby alerting the jeopardized host and activating defense mechanisms (3). In some patients, the systemic inflammatory response is dysregulated thereby causing a distributive shock which may lead to organ dysfunctions and in the most severe cases even death (4). Microcirculatory dysfunction with vasodilation and vascular leakage are common and clinically important consequences of a systemic inflammation, requiring prompt treatment with vasopressors and intravenous fluids (5).

In addition to its involvement in cell development and tissue homeostasis, Wingless-related integration site (WNT) signaling is an integral part of the innate immunity (6). WNT ligands, frizzled receptors and their signaling pathways are profoundly involved in the regulation of proinflammatory mediators and are the subject of ongoing research (7). In particular, WNT-5a may be a valid candidate to readily identify and quantify a systemic inflammation (8). In preclinical research, WNT-5a may sustain the inflammatory response in activated macrophages independently from the original stimulus by a positive feedback loop (9). This autocrine action of WNT-5a is antagonized by the endogenous antagonist Secreted frizzled-related protein 1 (sFRP-1) (10). In cell cultures, WNT-5a diminished the barrier function of vascular endothelial cells (VEC), potentially leading to microvascular leakage. This paracrine action of WNT-5a on VEC is antagonized by WNT inhibitory factor 1 (WIF-1) (11). The preclinical data is graphically summarized in **Figure 1**. However, it remains unclear if WNT signaling may be utilized to predict and characterize a postoperative systemic inflammation in patients after cardiac surgery.

The aims of this study were (1) to investigate the blood plasma concentration course of the WNT signaling components WNT-5a, sFRP-1, sFRP-5 and WIF-1 after cardiac surgery and to analyze their interaction, (2) to explore the integration of the WNT signaling components during the inflammatory response, and (3) to examine the influence of WNT signaling components on clinical effects associated with postoperative inflammation. Emphasis was given to consider the severity of the surgical trauma and the use of CPB during surgery. We hypothesized that the WNT-5a plasma concentration would increase according to the complexity of cardiac surgery and that a higher WNT-5a plasma value would be associated with a longer period of postoperative hemodynamic instability requiring more vasopressors and a higher fluid administration.

Abbreviations: CABG, coronary artery bypass grafting; CPB, cardiopulmonary bypass; CRP, C-reactive protein; GRO, growth-regulated oncogene; ICU, intensive care unit; IL, interleukin; MCP, monocyte chemoattractant protein; MIP, macrophage inflammatory protein; OPCAB, off-pump coronary aortic bypass; sFRP, secreted frizzled-related protein; TNF, tumor necrosis factor; WBC, white cell blood count; WIF, WNT inhibitory factor; WNT, wingless-related integration site.

Materials and methods

The investigation conformed to the principles outlined in the Declaration of Helsinki. Data are reported following the STROBE guidelines (12).

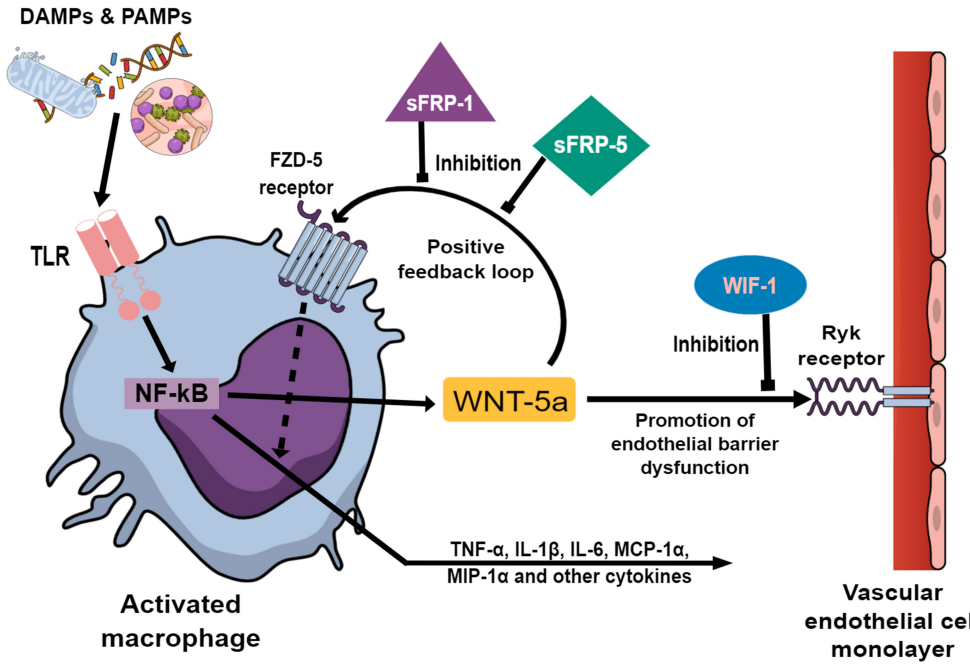


FIGURE 1
Preclinical studies investigating Wingless-related integration site (WNT) signaling components (9–11, 30) are graphically summarized to elucidate the background of this translational study. Damage and pathogen associated molecular patterns (DAMPs and PAMPs) activate macrophages *via* Toll-like receptors (TLR) which in turn promote transcription factor NF-κB to upregulate the expression of WNT-5a and a wide array of inflammatory cytokines. After secretion, WNT-5a binds to the membrane bound Frizzled-5 (FZD-5) receptor on the activated macrophages leading to an augmentation of the inflammatory cytokine production. This autocrine action enables WNT-5a to sustain the inflammatory response unrelated to the original stimulus. The positive feedback loop of WNT-5a is antagonized by Secreted frizzled-related protein 1 (sFRP-1) and Secreted frizzled-related protein 5 (sFRP-5) at the FZD-5 receptor. In vascular endothelial cells (VEC), WNT-5a promotes the loosening of intercellular tight junctions leading to increased paracellular permeability. This paracrine effect of WNT-5a is antagonized by WNT inhibitory factor 1 (WIF-1) at the membrane bound Ryk-receptor on VEC.

Study design

The study was designed as a prospective, single-center, observational study. Consecutive patients for coronary artery bypass grafting (CABG) with or without valve surgery were assigned into one of three groups according to the scheduled cardio-surgical procedure: (1) off-pump CABG (OPCAB group), (2) CABG using CPB (on-pump CABG group) and (3) combined CABG and valve surgery (valve-CABG group). It was assumed that the inflammatory response would increase with rising severity of the surgical trauma and the intraoperative use of CPB. In view of the limited pre-existing clinical research investigating WNT signaling in the setting of cardiac surgery, we targeted a sample size of 20 patients per group to reasonably use our resources.

To assess the study's feasibility, a pilot study comprising ten patients for on-pump CABG surgery was conducted first. We assumed that the on-pump CABG group would produce an intermediate level of systemic inflammation compared to the other groups (13). The study was conducted in the operating theater and the intensive care unit (ICU) for cardiovascular surgery at the University Hospital Zurich, Switzerland. The recruitment period for the pilot study was 06/2018 – 07/2018, and for the main study 11/2018 – 02/2020. Patients were followed up until being discharged from hospital.

In the pilot study, blood was sampled at nine time points to characterize the inflammatory response over time. Emphasis was placed on the acquisition of a baseline value (T1) before surgery as a reference. After a detailed look at the data acquired in the pilot study, we observed the peak of WNT-5a at the time of ICU admission (T2). At later time points, WNT-5a declined in all pilot study patients. To minimize the amount of blood loss for the patients and to optimize the analysis procedures, we reduced the blood sampling from nine to five time points for the main study. **Figure 2** depicts a timeline indicating all blood sampling time points used in the pilot and in the main study.

As the soluble WNT antagonists sFRP-1, sFRP-5 and WIF-1 are considered to bind to WNT-5a forming a complex (10, 11, 14), we assumed these parameters to co-exist in a relationship. To express the balance between WNT-5a and the other WNT parameters as a single variable we calculated ratios between WNT-5a and its antagonists. Ratios were calculated for every patient at all blood sampling time points.

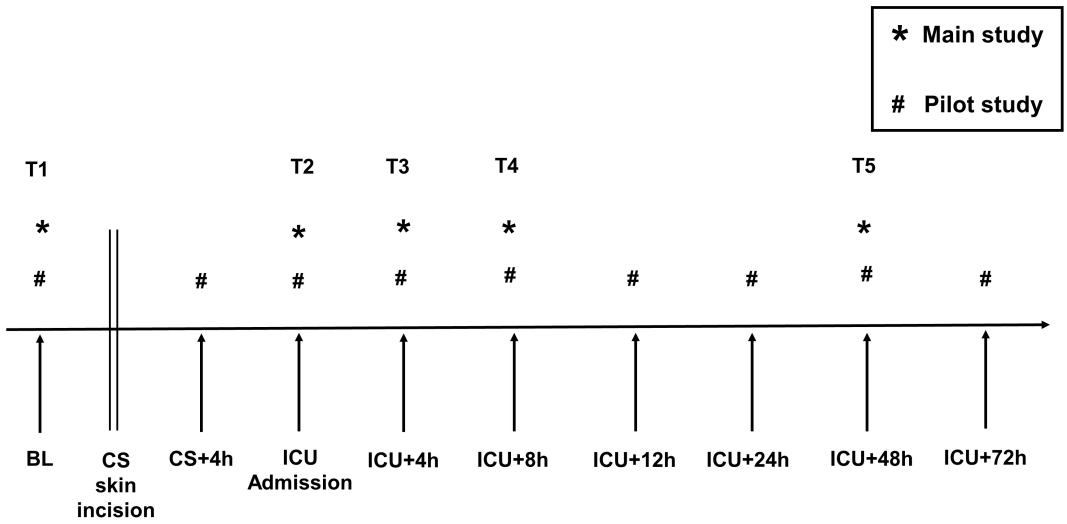


FIGURE 2
Blood sampling timeline. T1–T5, blood sampling time points used for statistical analysis. BL, Baseline; CS, cardiac surgery; CS + 4 h, 4 h after skin incision; ICU, intensive care unit. *, main study; #, pilot study.

To investigate the role WNT signaling components play in the inflammatory response and to explore potential interactions, we determined the plasma concentrations of inflammatory cytokines. These have been used previously to explore an inflammatory reaction to cardiac surgery (15, 16). In this study, plasma concentrations of interleukin 1β (IL-1β), interleukin 6 (IL-6), tumor necrosis factor α (TNF-α), growth-regulated oncogene α (GRO-α), macrophage inflammatory protein 1α (MIP-1α), and monocyte chemoattractant protein 1α (MCP-1α) were measured together with the WNT signaling components at all time points. More commonly used inflammatory parameters such as the C-reactive protein (CRP) and white cell blood count (WBC) were determined only at the baseline (T1) and at the time of ICU admission (T2) to minimize blood loss for the patients.

An inflammatory response to cardiac surgery may be influenced by additional factors. Blood product exposure has been shown experimentally to act pro-inflammatory in (pre-stimulated) endothelial cells (17). A perioperative glucocorticoid administration may modulate the immune response after cardiac surgery by inducing anti-inflammatory effects (18). To assess an influence of blood product transfusions and steroid use on the change of WNT signaling components upon cardiac surgery, we collected (1) details of red blood cell, platelet, and fresh frozen plasma transfusions intraoperatively and during the first 24 h after surgery and (2) details of a steroid stress dose administration intraoperatively and during the first 8 h after surgery.

A systemic inflammatory response to surgery may cause an endothelial dysfunction leading to a microvascular leakage and a reduced vasomotor tone potentially culminating in a distributive shock (19). In this study, we used three clinical outcome parameters as surrogates to quantify the clinical effects of a postoperative inflammation in our patients: (1) the fluid balance of the ICU admission day, (2) the noradrenaline dosage at the time of ICU admission, and (3) the time taken for noradrenaline to decline below <0.1 μg/kg/min with no concomitant inotropic drug therapy after ICU admission, which was defined as the "duration of hemodynamic instability."

Participants

Inclusion criteria were age >18 years, cardiac surgery *via* sternotomy, CABG with or without valve surgery and postoperative admittance to the cardio-surgical ICU. Exclusion criteria were the presence of preoperative infection, preexisting immunosuppressive therapy and the withdrawal of informed consent. Patients requiring a mass transfusion (>10 red blood cell concentrates within 24 h) and/or extracorporeal life support after cardiac surgery were excluded from the analysis.

Blood sample acquisition, handling, storage, and processing for laboratory analysis

Particular care was taken to keep the time interval between the acquisition and the laboratory analysis of the blood samples as short as possible. At each time point (T1–T9), venous blood was collected in a ethylenediaminetetraacetic acid (EDTA) containing vacutainer. Every blood sample was

promptly delivered to the in-hospital laboratory of the Institute of Clinical Chemistry for timely processing. The blood samples were centrifuged at 15,000 rpm for 3 min and the resulting plasma aliquots were filled into microtubes, frozen and stored at −20°C for up to 3 days. Thereafter, all samples were transferred frozen for long term storage at −80°C. An analysis of blood samples was subsequently performed when samples of a batch of up to 16 patients were acquired. For laboratory analysis the aliquots were thawed at 4°C. The blood plasma was centrifuged at 15,000 rpm for 5 min to separate the plasma from any residual sediment. During the preparation of the laboratory kits the aliquots were kept at maximum 4°C. Freeze-thaw cycles were avoided.

Blood samples were blinded for laboratory analysis. The laboratory kits were ordered from the manufacturer shortly before being used to avoid long-term storage associated quality issues. No cooling chain interruption occurred. All laboratory analyses were conducted in accordance with the manufacturer's recommendations. Free plasma concentrations of WNT-5a, sFRP-1, sFRP-5, and WIF-1 were determined by enzyme-linked immunosorbent assay (LifeSpan BioSciences, Inc., Seattle, WA, USA). Free plasma concentrations of IL-1β, IL-6, TNF-α, GRO-α, MIP-1α, and MCP-1α were measured by multiplex immunoassay (Bio-Plex Pro Human Inflammation Assays 6-plex and Biorad 200 Reader, Bio-Rad Laboratories AG, Cressier, Switzerland). Sensitivity levels were as follows: WNT-5a: 0.094 ng/ml, sFRP-1: 0.094 ng/ml, sFRP-5: 0.64 ng/ml, WIF-1: 5.9 pg/ml, IL-1β: 0.24 pg/ml, IL-6: 0.34 pg/ml, TNF-α: 1.13 pg/ml, GRO-α: 13.45 pg/ml, MIP-1α: 0.06 pg/ml, and MCP-1α: 0.44 pg/ml.

Perioperative patient management

After the application of standard monitoring, the patients were anesthetized for surgery. General anesthesia was induced with propofol and maintained with propofol or sevoflurane. Fentanyl and continuous sufentanil were used for analgesia and rocuronium for muscle relaxation. Perioperative cardiac function was evaluated by transesophageal echocardiography. Cardiac surgery was performed *via* median sternotomy. For cardiac bypass grafting, OPCAB patients received an initial heparin bolus of 250 IU/kg iv followed by the repeated administration of heparin 5,000 IU iv to obtain an activated clotting time of >350 s. Before CPB commencement for on-pump CABG with or without valve surgery, patients received heparin 300 IU/kg plus tranexamic acid 15 mg/kg iv followed by the repeated administration of heparin 5,000 IU iv to establish an activated clotting time of >500 s. The CPB system (Stöckert S5, LivaNova, UK; CAPIOX® FX25-Oxygenator, Terumo, Japan) was equipped with heparin coated tubes and primed with Ringerfundin®, tranexamic acid 500 mg and heparin 10,000 IU. During CPB, a blood flow according to a cardiac index of 2.4 l/min/m^2 was targeted. The patient's body temperature was lowered as specified by the attending surgeon. After completion of cardiac bypass grafting in OPCAB patients or weaning from CPB in patients receiving on-pump CABG with or without valve surgery, the initial heparin bolus was antagonized with protamine 1:1 iv. Blood product transfusion and treatment of coagulopathy were guided according to published guidelines of the University Hospital Zurich, Switzerland (20). An autologous retransfusion device (Cell Saver® Elite®, Haemonetics S.A., Signy Centre, Switzerland) was used to reinfuse shed mediastinal blood and residual blood from the CPB circuit. Postoperatively, all patients were admitted to the cardio-surgical ICU. Treatment guidelines for this ICU have been described earlier (21, 22). Patients were continuously assessed for shock and occurrence of postoperative complications. Sedation and mechanical ventilation were discontinued in all patients as soon as possible.

The administration of intravenous fluids and the use of vasoactive drugs were at the discretion of the attending anesthesiologist or intensivist. Intravascular volume was restored by infusion of balanced crystalloid and/or colloid solutions (Ringerfundin® and Physiogel®, respectively; B. Braun Medical AG, Sempach, CH). In patients with renal insufficiency albumin 5% (CSL Behring AG, Bern, Switzerland) was used as colloidal fluid replacement in the ICU. Noradrenaline was used as the first line vasopressor drug. If the noradrenaline dosage exceeded 0.3 μg/kg/min perioperatively, an empirical steroid stress dose of hydrocortisone was administered with an initial bolus of 100 mg iv followed by 50 mg iv qid. Hydrocortisone treatment was tapered off within 5 days.

Data sources and database

Clinical data were collected from the hospital's electronic patient data management systems KISIM (CISTEC AG, Zürich, Switzerland) and Metavision (iMDsoft®, Tel Aviv, Israel). All clinical and laboratory data were entered in a web based, good clinical practice compliant electronic data capture system (secuTrial®, interActive Systems GmbH, Berlin, Germany). The database was closed in 09/2020.

Statistical analysis

Statistical analyses were conducted using R (Version 4.0.5). Descriptive statistics are given as numbers (percentages) for categorical data and as median (interquartile range) or mean (±SD) for continuous data. The effect of cardiac surgery on WNT signaling components and inflammatory cytokines was investigated firstly by combining all surgical procedures and secondly by dividing the population into the three groups with increasing surgical complexity.

Differences of WNT signaling components and inflammatory cytokines between the baseline (T1) and the time of ICU admission (T2) were analyzed using Wilcoxon tests for paired data. Note that we performed Wilcoxon tests for

paired data only for the total dataset and stuck to a descriptive comparison per group to avoid a multiple testing problem. To further analyze the data, linear models were calculated to analyze data at a single time point, and linear mixed models with random intercept per patient to analyze repeated measurements over time. We did not analyze sFRP-1 in depth because this parameter changed only minimally over time.

Intergroup differences of WNT signaling components and inflammatory cytokines between the OPCAB, on-pump CABG and valve-CABG groups were investigated stepwise. The OPCAB group was used as the reference group. In a first step, differences between the three groups at the time of ICU admission (T2) were evaluated using a linear model. In a second step, differences over all time points after surgery (T2–T5) were examined using a linear mixed model adjusted for the respective baseline value (T1) and the respective time point. The different time points were included as a categorical variable to capture a possibly non-linear behavior of the outcome variables over time. We did not adjust for the severity of surgery (e.g., its duration) because this was covered by dividing patients into the three groups. Adding a surgery related variable would lead to multicollinearity and render the models non-interpretable.

To explore the event of a blood product transfusion or a perioperative steroid stress dose administration as potential confounders, these parameters were included in the mixed models for the WNT signaling components as a sensitivity analysis. Concerning blood products, only the event of an intraoperative transfusion was used to account for the temporal relationship between cause and effect.

Relations between WNT signaling components and inflammatory cytokines were explored stepwise. First, Spearman's rank correlation coefficient was calculated at the baseline (T1), the time of ICU admission (T2) and 48 h after surgery (T5). We chose this correlation coefficient because it is not sensitive to outliers. Second, the influence of WNT-5a on other WNT parameters and inflammatory cytokines over all time points (T1–T5) was assessed using linear mixed models.

The influence of WNT signaling components and inflammatory cytokines at the time of ICU admission (T2) on the three clinical outcome parameters (1) fluid balance of the ICU admission day, (2) norepinephrine dosage at the time of ICU admission (T2), and (3) duration of hemodynamic instability was explored using linear models. As covariates the values of WNT-5a, sFRP-5, WIF-1, IL-6, and MCP-1α at T2 were used.

Handling of missing data

Only in one patient of the valve-CABG group, data from blood sampling at one single time point, 48 h after surgery (T5), were missing. This was not expected to change the results. In the linear models we compared only values at the time of ICU admission (T2) for which the dataset was complete. The linear mixed models applied to analyze data over multiple time points used all available values without excluding a whole patient because of missing values. For these reasons and because of the otherwise complete dataset, the missing data did not necessitate any action in this study.

Presentation of data

Box plots show the box with the median, representing 25th, 50th, and 75th percentiles. Their whiskers represent values within 1.5× IQR. Values outside this range are outliers and extremes. WNT-5a, sFRP-5, and IL-6 are presented in figures with a zoomed scale to enable a reasonable graphical presentation. To comprehensively report our data, values of WNT signaling components including ratios, and inflammatory cytokines at all time points are presented in detail as **Supplementary material**.

Results

Patient population, surgery, and clinical outcome

A total of 73 consecutive patients were assigned to one of the three groups. Of these, nine patients were excluded because of cancellation of surgery ($n = 2$), study consent withdrawal ($n = 1$), a preoperative cardiac event with ICU admission ($n = 1$), pre-existing immunosuppressive therapy ($n = 1$), ECLS implantation ($n = 2$), and erroneous blood sampling ($n = 2$). Finally, 64 patients were included in the analysis. Follow-up was completed in 100% of included patients. Median follow-up time was 8 (7–12) days.

Patient and surgery characteristics are presented in **Table 1**. As four patients of the on-pump group were operated in off-pump technique after group assignment, the number of patients differed between the three groups. Patients had a median age of 70 years, were predominantly male and pre-obese. Approximately half of the patients were active or former smokers. Two-thirds of the operations were elective. The valve-CABG group included patients with aortic valve replacement ($n = 13$) or reconstruction ($n = 2$), mitral valve replacement ($n = 2$) or reconstruction ($n = 5$) and tricuspid valve reconstruction ($n = 2$). Three patients had interventions in up to three valves, and two patients underwent isolated valve surgery. Overall, a median of 3 (2–4) bypass grafts were performed. The highest intraoperative fluid administration was found in the OPCAB group. The three groups differed considering the type and the duration of surgery, use of CPB and aortic cross clamp time, with highest values in the valve-CABG group, intermediate values in the on-pump CABG group and lowest values in the

10.3389/fcvm.2022.997350

TABLE 1 Patient characteristics and details of cardiac surgery.

	Total (*n* = 64)	OPCAB (*n* = 28)	On-pump CABG (*n* = 16)	Valve-CABG (*n* = 20)
Patient population				
Age (years)	70 (62–74)	70 (62–77)	69 (61–72)	71 (67–76)
Male gender (*n*)	51 (80%)	24 (86%)	11 (68%)	16 (80%)
Body mass index (kg/m^2)	27 (25–31)	27 (26–30)	27 (25–31)	27 (23–30)
Tobacco smoking				
Pack years (years)	4 (0–34) *n* = 34	23 (0–45) *n* = 19	0.5 (0–36) *n* = 8	0 (0–6) *n* = 7
Preoperative blood laboratory values				
Hemoglobin (g/L)	140 (131–149)	140 (134–151)	141 (126–148)	141 (134–149)
Platelet count (g/L)	223 (179–258)	224 (182–255)	223 (190–266)	218 (173–265)
hsTroponin (ng/L)	14 (8–27)	14 (8–34)	17 (12–39)	13 (8–17)
Creatinine (μmol/L)	84 (72–98)	89 (71–99)	80 (72–93)	83 (77–93)
Cardiac surgery details				
EuroSCORE II (points)	1.9 (1.1–2.9)	1.2 (0.9–2.2)	1.9 (1.1–3.8)	2.8 (1.9–4.0)
Type of surgery				
Elective (*n*)	43 (67%)	16 (57%)	10 (63%)	17 (85%)
Urgent (*n*)	21 (33%)	12 (43%)	6 (38%)	3 (15%)
Surgery duration (min)	270 (237–326)	254 (200–276)	257 (245–298)	321 (265–360)
CPB duration (min)	97 (0–138)	n.a.	115 (105–123)	153 (136–185)
Aortic cross clamp duration (min)	61 (0–95)	n.a.	71 (61–82)	111 (92–135)
Bypass grafts (*n*)	3 (2–4)	3 (3–4)	3 (3–4)	2 (1–3)
Lowest body temperature (°C)	35.0 (34.0–35.6)	35.6 (35.2–36.3)	34.7 (34.0–35.3)	33.8 (32.7–34.2)
Intraoperative fluid administration (L)	4.2 (2.6–5.4)	5.1 (4.1–6.6)	2.9 (2.5–3.3)	3.3 (2.3–4.6)
Echocardiography				
Preoperative LVEF (%)	60 (51–62)	55 (50–60)	57 (50–61)	60 (55–65)
Postoperative LVEF (%)	55 (50–65)	55 (50–61)	58 (50–65)	58 (49–65)
Intraoperative blood product transfusions				
Patients with RBC transfusions (*n*)	6 (9%)	1 (4%)	1 (6%)	4 (20%)
RBC units (*n*)	0.2 (±0.7)	0.1 (±0.8)	0.2 (±0.8)	0.2 (±0.4)
Patients with platelet transfusions (*n*)	11 (17%)	3 (11%)	2 (13%)	6 (30%)
Platelet units (*n*)	0.2 (±0.5)	0.1 (±0.3)	0.2 (±0.5)	0.4 (±0.7)
Intraoperative steroid stress dose administration				
Hydrocortisone (*n*)	7 (11%)	2 (7%)	2 (13%)	3 (15%)

CABG, coronary artery bypass grafting; CBP, cardiopulmonary bypass; CRP, C-reactive protein; LVEF, left ventricular ejection fraction; n.a., not applicable; OPCAB, off-pump coronary artery bypass grafting; RBC, red blood cells. Values are presented as number (percentage), median (interquartile range), or mean (±SD).

OPCAB group. Overall, the lowest body temperature during surgery was 35.0 (34.0–35.6) °C.

Clinical outcome data are presented in Table 2. ICU and hospital survival were 100 and 97%, respectively. Two patients in the on-pump CABG group died postoperatively while in the hospital. One of these patients suffered a major cerebral stroke after cardiac surgery and died on postoperative day 20. The other patient died after an in-hospital cardiac arrest on postoperative day 3. Patients of the valve-CABG group tended to stay longer in the ICU, although the length of ICU stay was overall short. Duration of hemodynamic instability and daily fluid balance were highest in the valve-CABG group, intermediate in the on-pump CABG group and lowest in the OPCAB group. The highest noradrenaline dosage in the ICU was observed in the valve-CABG group, while the other groups had comparable values. Perioperatively, hydrocortisone was administered empirically as a steroid stress dose treatment in a total of 11 (17%) patients. One of these patients received hydrocortisone as a onetime bolus only during surgery and in four patients, hydrocortisone treatment was commenced within 8 h after surgery. Tables 1, 2 summarize patients with a steroid stress dose treatment according to the timing of hydrocortisone administration. A pneumonia was postulated in three patients 24 h, 48 h and 6 days after surgery, respectively. In these patients, an empirical antibiotic therapy with piperacillin–tazobactam was initiated. Perioperatively,

10.3389/fcvm.2022.997350

TABLE 2 Intensive care unit parameters and patient outcome.

	Total (n = 64)	OPCAB (n = 28)	On-pump CABG (n = 16)	Valve-CABG (n = 20)
Length of stay and patient survival				
Length of ICU stay (days)	1 (1–2)	1 (1–1)	1 (1–2)	2 (1–4)
Length of hospital stay (days)	8 (7–12)	7 (7–11)	8 (7–12)	9 (8–13)
Survival ICU (n)	64 (100%)	28 (100%)	16 (100%)	20 (100%)
Survival hospital (n)	62 (97%)	28 (100%)	14 (88%)	20 (100%)
ICU scores				
SAPS II (points)	28 (24–34)	28 (26–33)	26 (24–28)	32 (26–35)
SOFA ICU admission day (points)	6 (6–8)	6 (6–7)	6 (5–8)	8 (6–9)
SOFA POD 1 (points)	7 (4–8) n = 23	6 (4–8) n = 7	7 (5–8) n = 5	7 (5–9) n = 11
Neurologic system				
Postoperative delirium (n)	11 (17%)	2 (7%)	3 (19%)	6 (30%)
Blood laboratory values at ICU admission				
Hemoglobin (g/L)	112 (95–124)	115 (102–131)	104 (93–120)	105 (94–121)
Platelet count (G/L)	137 (110–172)	147 (122–178)	148 (118–176)	115 (80–140)
hsTroponin (ng/L)	566 (297–1,635)	289 (212–434)	840 (489–1,083)	2,415 (1,569–3,956)
Creatinine (μmol/L)	77 (59–86)	80 (66–89)	76 (57–84)	77 (63–84)
Cardiovascular system				
Duration of hemodynamic instability[1] (h)	9 (0–19)	6 (0–16)	12 (0–19)	15 (5–35)
Drug support at ICU admission				
Norepinephrine (n)	63 (98%)	27 (96%)	16 (100%)	20 (100%)
Norepinephrine dose (μg/min)	8 (5–14)	8 (5–14)	7 (5–10)	10 (7–16)
Inotropes (n)	13 (20%)	3 (11%)	4 (25%)	6 (30%)
Drug support at ICU admission + 8 h				
Norepinephrine (n)	50 (78%)	20 (71%)	11 (69%)	19 (95%)
Norepinephrine dose (μg/min)	5 (1–12)	4 (0–12)	3 (0–11)	7 (4–11)
Inotropes (n)	12 (19%)	3 (11%)	4 (25%)	5 (25%)
Postoperative steroid stress dose administration (0–8 h of ICU)				
Hydrocortisone (n)	10 (16%)	4 (14%)	2 (13%)	4 (20%)
Fluid balance				
ICU admission day (L)	2.9 (1.9–4.4)	2.5 (1.9–4.0)	2.8 (2.0–4.4)	3.4 (2.2–4.9)
Postoperative day 1 (L)	0.7 (−0.2 to 1.5) n = 24	0.5 (−1.5 to 0.8) n = 8	1.1 (0.8–1.3) n = 5	0.5 (−0.4 to 2.3) n = 11
Postoperative blood product transfusions (0–24 h of ICU)				
Patients with RBC transfusions (n)	14 (22%)	7 (25%)	3 (19%)	4 (20%)
RBC units (n)	0.5 (±1.4)	0.3 (±0.5)	0.3 (±0.8)	0.8 (±2.3)
Patients with FFP transfusions (n)	3 (5%)	0 (0%)	0 (0%)	3 (15%)
FFP units (n)	0.2 (±1.2)	0 (±0)	0 (±0)	0.8 (±2)
Patients with platelet transfusions (n)	6 (9%)	1 (4%)	1 (6%)	4 (20%)
Platelet units (n)	0.2 (±0.7)	0 (±0.2)	0.1 (±0.2)	0.4 (±1.1)

FFP, fresh frozen plasma; ICU, intensive care unit; RBC, red blood cells; SAPS, simplified acute physiology score; SOFA, sequential organ failure assessment; POD, postoperative day. "[1]" defined as time from ICU admission until iv norepinephrine dosage <0.1 μg/kg/min and no inotropic drug therapy. Values are presented as number (percentage), median (interquartile range), or mean (±SD).

blood products were administered in a total of 21 (33%) patients. Red blood cells were transfused in 18 (28%) patients and platelets in 13 (20%) patients. Regarding the three groups, a transfusion of red blood cells and platelets occurred mostly in patients in the valve-CABG group (n = 7, 35%, for both blood products), followed by the OPCAB group (n = 8, 29% and n = 4, 14%, respectively) and the on pump-CABG group (n = 3, 19% and n = 2, 13%, respectively). A transfusion of fresh frozen plasma occurred only postoperatively in 3 (15%) patients in the valve-CABG group. Tables 1, 2 summarize the details of blood product transfusions according to the timing of the administration.

Wingless-related integration site signaling components

The evolution of WNT-5a, sFRP-5, and WIF-1 over time is displayed in **Figure 3**. For the overall population, WNT-5a, sFRP-1, and WIF-1 were present in a minimal concentration at baseline, while sFRP-5 was elevated. WNT-5a was highest at the time of ICU admission and thereafter progressively returned to baseline within 48 h. sFRP-5 was lowest at the time of ICU admission and, rising thereafter but without reaching the baseline value at 48 h after surgery. WIF-1 showed a low kinetic profile and was lowest at 8 h after surgery. sFRP-1 changed only minimally over time in all samples throughout the study (not shown).

The baseline (T1) and time of ICU admission (T2) values of the WNT signaling components are presented in **Table 3**. For the overall population, we found very strong evidence for a difference in all parameters except for WIF-1 when comparing T2 to T1. A higher median value of WNT-5a, and a lower median value of sFRP-5 were found, whereas the change in sFRP-1 was (albeit statistically significant) numerically minimal. According to a linear mixed model, for every ng/ml (for WNT-5a and sFRP-5) or pg/ml (for WIF-1) increase at T1 the estimated values of T2–T5 increased for WNT-5a by 0.53 ng/ml (0.47–0.58, $p < 0.0001$), for sFRP-5 by 0.57 ng/ml (0.43–0.71, $p < 0.0001$) and for WIF-1 by 0.9 pg/ml (0.79–1.0, $p < 0.0001$), respectively.

The sensitivity analysis for the event of a perioperative steroid stress dose administration, and the event of an intraoperative transfusion of either red blood cell concentrates, or platelets did not change the mixed model estimates for WNT-5a, sFRP-5, and WIF-1.

Regarding intergroup differences, at T2 moderate evidence was found for WIF-1 to be on average 121 pg/ml (11–230, $p = 0.031$) higher in the valve-CABG group compared to the OPCAB group using a linear model. Over all time points after surgery (T2–T4), no evidence for an intergroup difference for any WNT signaling component was found in the linear mixed model.

With respect to the interaction between the WNT signaling components, Spearman's correlation at T1, T2, and T5 showed low coefficients between WNT-5a and sFRP-5 (0.19, 0.43, and 0.3, respectively), and WNT-5a and WIF-1 (0.15, 0.25, and 0.22, respectively). Accordingly, no evidence for an association between the WNT signaling components was found using a linear mixed model.

The evolution of the ratios between WNT-5a and sFRP5, and between WNT-5a and WIF-1 over time is displayed in **Figure 4**. For the overall population, the lowest median value of both ratios was found at the baseline (T1) and the highest median value at the time of ICU admission (T2). Thereafter, WNT-5a/sFRP-5 progressively declined but did not reach the baseline value within 48 h. WNT-5a/WIF-1 declined until 8 h after surgery (T4) and then tended to increase again at 48 h after surgery (T5). Using Wilcoxon tests for paired data, the median values of both ratios were higher at T2 compared to T1 (**Table 3**). No evidence for an intergroup difference was found for both ratios at T2 nor over all time points after surgery (T2–T5).

Cytokines and inflammatory parameters

For all investigated inflammatory cytokines a low baseline value was found. IL-1β was mostly below the detection limit at all time points (data not shown). For the overall population, TNF-α, GRO-α, and MIP-1α showed a kinetic profile with very low values over time.

The evolution of IL-6 and MCP-1α over time is displayed in **Figure 5**. For the overall population, both parameters were present in a minimal concentration at the baseline and were highest at the time of ICU admission. Thereafter, IL-6 decreased progressively without completely reaching the baseline value at 48 h. MCP-1α showed a rapid decline in the first 4 h after surgery and overall tended to decrease more rapidly than IL-6.

The baseline (T1) and time of ICU admission (T2) values of IL-6, MCP-1α, WBC, and CRP are summarized in **Table 3**. Compared to T1, higher median values were found for IL-6, MCP-1α, and WBC but not for CRP at T2 using Wilcoxon tests for paired data.

Compared to the OPCAB group, very strong evidence was found at the time of ICU admission (T2) for MCP-1α to be, on average, 0.92 ng/ml (0.46–1.38, $p = 0.0002$) higher in the valve-CABG group and moderate evidence of it being, on average, 0.58 ng/ml (0.09–1.07, $p = 0.0229$) higher in the on-pump CABG group. Considering all time points after surgery (T2–T5) in a linear mixed model, strong evidence was found for MCP-1α to be on average 0.27 ng/ml (0.12–0.42, $p = 0.001$) higher in the valve-CABG group than in the OPCAB group with no difference for the on-pump CABG group. For IL-6 no intergroup differences were found.

Between WNT-5a and IL-6, and WNT-5a and MCP-1α no correlation was found at single time points (T1, T2, and T5) nor an association over all time points after surgery (T2–T5).

Influence of Wingless-related integration site signaling components and inflammatory cytokines on postoperative clinical outcome parameters

For every ng/ml increase of WNT-5a at the time of ICU admission (T2), the fluid balance of the ICU admission day was reduced by −24.81 ml (−49 to −0.46, $p = 0.046$). For every

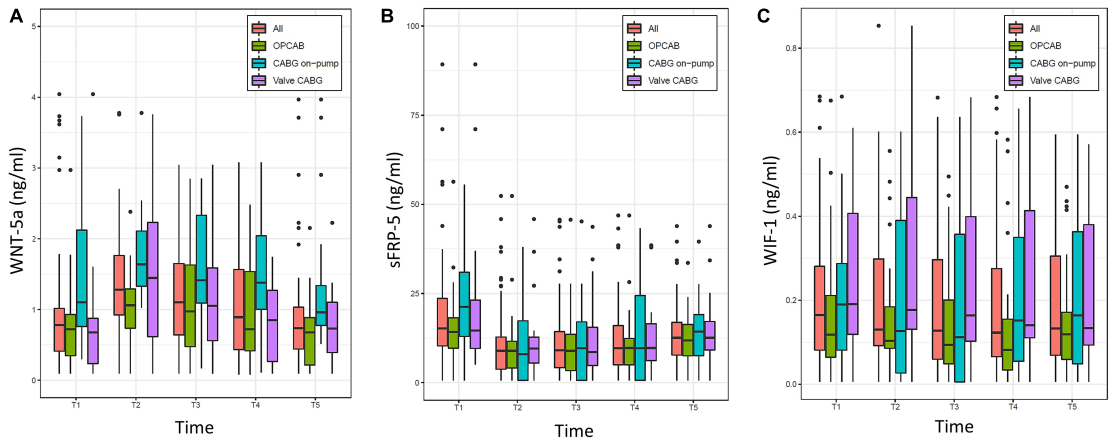


FIGURE 3
Evolution of **(A)** Wingless-related integration site 5a (WNT-5a), **(B)** Secreted frizzled-related protein 5 (sFRP-5), and **(C)** WNT inhibitory factor 1 (WIF-1) over time (T1–T5) in the overall population (All) and the three groups (OPCAB, CABG on-pump, and valve-CABG). Values are presented as median and interquartile range (IQR) (box), within 1.5× IQR (line) and outliers (dots). T1, baseline; T2, time of intensive care unit (ICU) admission; T3, 4 h after surgery; T4, 8 h after surgery; T5, 48 h after surgery; CABG, coronary artery bypass grafting; OPCAB, off-pump coronary artery bypass grafting.

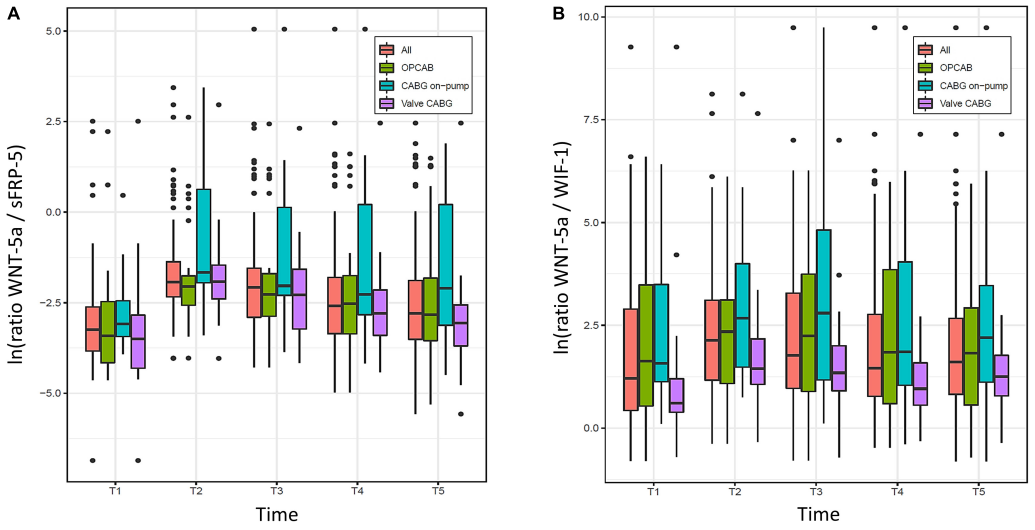


FIGURE 4
Evolution of ratios between **(A)** Wingless-related integration site 5a (WNT-5a) and Secreted frizzled-related protein 5 (sFRP-5), and **(B)** WNT-5a and WNT inhibitory factor 1 (WIF-1) over time (T1–T5) in the overall population (All) and the three groups (OPCAB, CABG on-pump and valve-CABG). Values are presented as median and interquartile range (IQR) (box), within 1.5× IQR (line) and outliers (dots). ln, natural logarithm; T1, baseline; T2, time of intensive care unit (ICU) admission; T3, 4 h after surgery; T4, 8 h after surgery; T5, 48 h after surgery; CABG, coronary artery bypass grafting; OPCAB, off-pump coronary artery bypass grafting.

pg/ml increase of WIF-1 at T2, the fluid balance of the ICU admission day increased by 3.81 ml (−0.09 to 7.71, $p = 0.056$), the noradrenaline requirement at the time of ICU admission (T2) increased by 0.017 µg/min (0.00–0.03, $p = 0.007$) and the duration of hemodynamic instability in the ICU increased by 0.9 h (0.03–0.14, $p = 0.002$). For sFRP-5, IL-6 and MCP-1α at T2 no evidence was found for an influence on postoperative hemodynamic outcome parameters.

Discussion

Main findings

Temporal changes of WNT-5a, sFRP-1, sFRP-5, and WIF-1 were investigated in the perioperative setting of cardiac surgery. This represents a very standardized and clinically relevant model of sterile inflammation. For the overall population, WNT-5a,

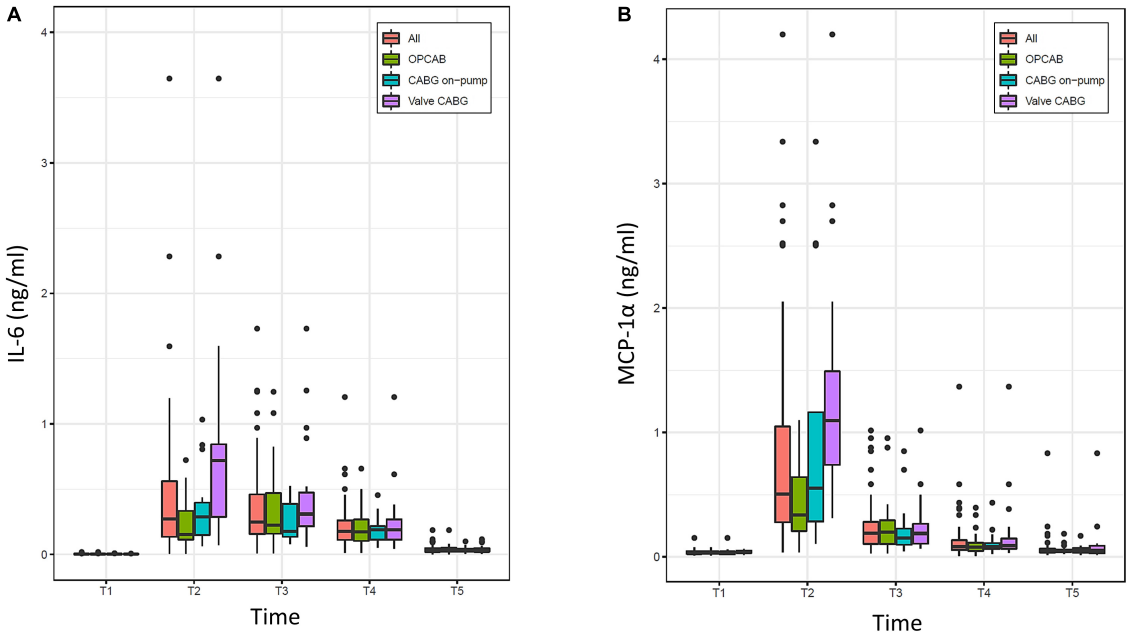


FIGURE 5
Evolution of **(A)** interleukin-6 (IL-6) and **(B)** monocyte chemoattractant protein 1α (MCP-1α) over time (T1–T5) in the overall population (All) and the three groups (OPCAB, CABG on-pump and valve-CABG). Values are presented as median and interquartile range (IQR) (box), within 1.5× IQR (line) and outliers (dots). T1, baseline; T2, time of intensive care unit (ICU) admission; T3, 4 h after surgery; T4, 8 h after surgery; T5, 48 h after surgery; CABG, coronary artery bypass grafting; OPCAB, off-pump coronary artery bypass grafting.

sFRP-1, and WIF-1 were present in low concentrations at the baseline, while sFRP-5 was elevated. The baseline values of WNT-5a, sFRP-5, and WIF-1 influenced the evolution of their follow-up values. WNT-5a reached its maximum and sFRP-5 its minimum value at the time of ICU admission with significant changes from the baseline. WNT-5a returned to the baseline within 48 h, while sFRP-5 remained below the baseline at 48 h. Values of sFRP-1 showed a minimal change over time. WIF-1 progressively declined to its minimum value at 8 h after surgery and increased thereafter without reaching the baseline within 48 h. The ratios of WNT-5a/sFRP-5 and WNT-5a/WIF-1 were higher at the time of ICU admission compared to the baseline. Of all investigated WNT signaling components, only WIF-1 partially reflected the severity of surgery at the time of ICU admission. Regarding the inflammatory cytokines, only IL-6 and MCP-1α were strongly expressed with highest values at ICU admission. For MCP-1α statistical differences between the three groups were found at the time of ICU admission. Finally, for WNT-5a and WIF-1 an influence on hemodynamic outcome parameters was found.

Patient population and clinical outcome

As intended, the three groups differed mainly regarding the complexity of surgery. The duration of postoperative hemodynamic instability and fluid balance after surgery numerically increased with rising surgical complexity across the groups. Accordingly, the valve-CABG-group had the highest noradrenaline dosage at the time of ICU admission. Despite having the highest fluid load intraoperatively, the OPCAB group had the lowest positive fluid balance at the end of the ICU admission day. This could result from less systemic inflammation with lower endothelial permeability compared to the other groups using CPB. The high intraoperative fluid load during OPCAB is explained by a temporary luxation of the heart causing a hemodynamic compromise (23). Considering ICU stay and survival rates, the postoperative course in most patients was favorable. Hence, we were able to compare three homogenous patient populations after cardiac surgery which developed a systemic inflammatory response. This response was sufficiently large to be measured but was not excessive or uncontrolled as clinical outcome was overall benign in our patients.

Evolution of Wingless-related integration site signaling components upon cardiac surgery

The mononuclear phagocyte system is an essential part of innate immunity and functions as first line defense to tissue damage and microbial infection. Activated macrophages produce WNT-5a and various cytokines that cause an inflammatory response (8). Macrophage activation may be

TABLE 3 Baseline (T1) and ICU admission values (T2) of Wingless-related integration site (WNT) signaling components, cytokines, and inflammatory parameters.

Parameter	Total ($n = 64$)			OPCAB ($n = 28$)		On-pump CABG ($n = 16$)		Valve-CABG ($n = 20$)	
	T1	T2	*P*-value	T1	T2	T1	T2	T1	T2
WNT-5a (ng/ml)	0.78 (0.43–1.1)	1.3 (0.93–1.9)	$p < 0.0001$	0.75 (0.36–0.98)	1.1 (0.74–1.3)	1.1 (0.76–2.1)	1.7 (1.4–2.2)	0.7 (0.27–0.90)	1.5 (0.65–2.3)
sFRP-1 (ng/ml)	0.09 (0.09–0.48)	0.09 (0.09–0.09)	$p = 0.007$	0.09 (0.09–0.09)	0.09 (0.09–0.09)	0.09 (0.09–1.1)	0.09 (0.09–0.17)	0.09 (0.09–6.2)	0.09 (0.09–0.26)
sFRP-5 (ng/ml)	15.2 (10.3–23.6)	8.9 (3.8–12.8)	$p < 0.0001$	14.3 (9.7–18.2)	8.9 (4.1–11.6)	21.3 (13–31)	8.0 (0.6–17.3)	14.6 (9.6–23.1)	9.6 (5.5–12.8)
WIF-1 (ng/ml)	0.17 (0.08–0.28)	0.13 (0.09–0.3)	$p = 0.84$	0.12 (0.06–0.21)	0.10 (0.09–0.18)	0.19 (0.08–0.29)	0.13 (0.03–0.39)	0.19 (0.12–0.41)	0.18 (0.13–0.44)
Ratio WNT-5a/sFRP-5	0.04 (0.02–0.07)	0.14 (0.10–0.26)	$p < 0.0001$	0.03 (0.02–0.08)	0.13 (0.08–0.17)	0.05 (0.03–0.10)	0.19 (0.03–1.9)	0.03 (0.00–0.06)	0.15 (0.02–0.23)
Ratio WNT-5a/WIF-1	3.4 (1.5–18.2)	8.4 (3.2–22.6)	$p = 0.004$	5.2 (1.7–34.1)	10.5 (0.69–22.9)	4.9 (3.1–33.4)	14.5 (4.4–83.0)	1.8 (1.5–3.3)	4.3 (2.9–8.8)
IL-6 (ng/ml)	0.00 (0.00–0.00)	0.29 (0.14–0.66)	$p < 0.0001$	0.00 (0.00–0.00)	0.15 (0.11–0.33)	0.00 (0.00–0.00)	0.29 (0.15–0.4)	0.00 (0.00–0.00)	0.76 (0.33–1.3)
MCP-1α (ng/ml)	0.04 (0.02–0.05)	0.50 (0.28–1.0)	$p < 0.0001$	0.03 (0.03–0.05)	0.34 (0.21–0.64)	0.03 (0.02–0.04)	0.55 (0.29–1.2)	0.04 (0.03–0.05)	1.1 (0.74–1.5)
CRP (mg/L)	3 (1–5)	2 (1–4)	$p = 0.045$	3 (1–6)	2 (1–4)	4 (1–6)	2 (1–4)	2 (1–4)	1 (1–4)
White blood cell count (g/L)	7.1 (5.6–8.2)	12.6 (10.4–15.8)	$p < 0.0001$	7.7 (6.5–8.4)	12.6 (11.5–17.0)	6.8 (5.8–7.6)	13.1 (8.2–15.1)	6.3 (5.4–7.6)	12.6 (10.1–14.2)

T1, baseline; T2, ICU admission; CABG, coronary artery bypass grafting; CRP, C-reactive protein; IL, interleukin; MCP, monocyte chemoattractant protein; OPCAB, off-pump coronary artery bypass grafting; sFRP, secreted frizzled-related protein; WNT, wingless-related integration site; WIF, WNT inhibitory factor. Data are presented as median (interquartile range). For statistical analysis Wilcoxon tests for paired data were used.

sustained and cytokine production augmented by autocrine binding of WNT-5a to the Frizzled-5 receptor on the macrophage cell membrane (9) and downstream activation of intracellular Ca^{2+}/calmodulin-dependent protein kinase 2 (10). Consecutively, WNT-5a signaling in macrophages plays an important role in inflammation because of its potential to upregulate an inflammatory response. However, in-depth knowledge about WNT signaling after cardiac surgery is scarce to date.

In this study, we present baseline plasma concentrations of WNT-5a, sFRP-1, sFRP-5, and WIF-1 in patients with coronary and/or valvular heart disease. These values represent an equilibrium state in a non- or low inflamed patient population as no patient with an active infection or inflammatory disease was included. Little is known about normal reference values of WNT parameters in cardiovascular disease. Elevated basal WNT-5a plasma concentrations have been described in patients with dilated cardiomyopathy and were associated with right ventricular dysfunction, especially in patients with more advanced disease (24). In patients undergoing cardiac surgery, elevated basal WNT-5a values in the serum and epicardial adipose tissue were associated with the presence of coronary artery disease (25). Inter-individual variations of WNT parameter basal plasma concentrations may be relevant for future research. We found very strong evidence that the individual baseline values of WNT-5a, sFRP-5, and WIF-1 had a measurable impact on subsequent values of the respective parameter after surgery. This is of interest, as the interaction between WNT signaling components may considerably influence the inflammatory response (6).

As hypothesized, WNT-5a increased after cardiac surgery but, contrary to our hypothesis, WNT-5a did not reflect the severity of surgery. Howsoever, WNT-5a seems to accurately describe the sterile systemic inflammatory response upon cardiac surgery considering its time-concentration course observed in the overall patient population in this study. It might be assumed that macrophages are maximally activated during surgery and produce a peak WNT-5a concentration to promote inflammation. Accordingly, we found the highest blood plasma concentration of WNT-5a directly after surgery. Thereafter, regulatory mechanisms seem to prevent an uncoupling of WNT-5a signaling from the inflammatory stimulus. In fact, the progressive clearance of WNT-5a within hours after cardiac surgery may indicate the resolution of the inflammatory response in patients with an uncomplicated postoperative course.

A rise of WNT-5a values continuing for hours beyond cardiac surgery may be a reason for concern. In the patient that suffered a perioperative stroke and died on postoperative day 20, we observed an increase of WNT-5a until 8 h after surgery and a return to the baseline value at 48 h. The kinetic profile of WNT-5a in this patient may be the result of an additional inflammatory response to the stroke that

occurred perioperatively. Indeed, experimental findings indicate that WNT signaling seems to be critically involved in the pathophysiology of stroke and WNT has been described to rise within hours in brain tissue after ischemic stroke (26). According to our study protocol, no blood sampling occurred between 8 and 48 h after surgery so the time point of the WNT-5a peak concentration in this patient remains at question. Another reason for concern may be the absent recovery of the WNT antagonist sFRP-5 towards the baseline value at 48 h after surgery which was also seen in this patient (data not shown).

Secreted frizzled related proteins (sFRPs) and WIF-1 are considered as soluble antagonists of WNT signaling. They prevent the binding of WNTs to their corresponding cellular receptors by binding themselves to WNT proteins forming a soluble complex that can no longer bind to the WNT receptor. By this way WNT signaling is inhibited by the antagonist (14). In preclinical research, sFRP-1 attenuated a macrophages inflammatory response by preventing the binding of WNT-5a to the membrane bound Frizzled-5 receptor (10). In our study, the sFRP-1 plasma concentration was low at baseline and did not increase after the surgical stimulus. Therefore, we assume that sFRP-1 may not play a relevant role in the sterile inflammatory response after cardiac surgery.

For sFRP-5 we found an elevated basal plasma concentration, which is in accordance with a previous study (25). This may indicate that the constitutional level of sFRP-5 is already higher at baseline compared to the baseline level of sFRP-1. The value of sFRP-5 decreased to approximately half of the initial concentration at the time of ICU admission and increased steadily thereafter. This may be due to the formation of a complex between WNT-5a and the soluble antagonist sFRP-5 resulting in a lower plasma concentration of the uncomplexed free form of both parameters. According to preclinical research (14), sFRP-5 may be in fact binding excess levels of WNT-5a and thereby limiting the inflammatory response after cardiac surgery. The effect of cardiac surgery and CPB on the blood plasma concentration of WNT-5a and sFRP-5 and their supposed interaction are graphically presented in **Figure 6**.

In the experimental setting, WIF-1 antagonized a WNT-5a induced tight junction disruption between VEC by interfering with the Rho-associated protein serine/threonine kinase/LIM kinase 2/Cofilin 1 pathway at the Ryk receptor (11). In our study, WIF-1 was detectable at low concentrations preoperatively and showed a tendency toward lower values until 8 h after surgery. It appears that WIF-1 is constitutionally present at a low plasma concentration and may be depleted from the plasma by complex formation with WNT-5a. Thereby, WIF-1 would prevent the binding of WNT-5a to the Ryk receptor on VEC and limit the extent of microvascular leakage during the inflammatory response after cardiac surgery.

In our study, the kinetic profile of WIF-1 showed some noticeable features in the valve-CABG group. In these patients, WIF-1 at baseline was highest. Furthermore, an intergroup difference was found at the time of ICU admission and the WIF-1 plasma concentration progressively declined until 48 h after surgery. Compared to other patients in this study, patients with combined valvular and coronary heart disease may be in a low inflammatory state. We speculate that basal WIF-1 production might be concomitantly upregulated to maintain a state of equilibrium with WNT-5a. This may explain the higher WIF-1 baseline value and intergroup difference at the time of ICU admission. Our assumption is based on previous findings that WNT-5a was found to be elevated in patients with advanced heart disease (24). It must be noted that the etiology of heart disease may differ considerably between patients with dilated cardiomyopathy and our study population. The decline of WIF-1 beyond 8 h after surgery may result from an ongoing WNT-5a antagonism by WIF-1.

Five measurements within 48 h allowed us to determine the course of the WNT signaling components over time and to extensively investigate their relation to each other. The concentration of WNT-5a evolved in the opposite direction compared to sFRP-5 and WIF-1. Since sFRP-5 and WIF-1 are considered as counterplayers of WNT-5a (14), we calculated the ratios between these parameters to investigate their interaction. The ratios show the balance between the free plasma concentrations of the respective parameters. The increased WNT-5a/sFRP-5 and the WNT-5a/WIF-1 ratios at the time of ICU admission indicate an excess of WNT-5a, which may actively promote the postoperative inflammatory response. Both ratios decreased within 48 h, which was paralleled by the clinical recovery of our patients. The approach of forming a ratio between the agonist and antagonist has been previously used for WNT-5a and sFRP-3 to show the grade of WNT-5a activity in a balanced system (24). Nevertheless, our assumptions on the interaction between WNT signaling components are challenged by the fact that, statistically, no correlation between WNT-5a and sFRP-5 or WIF-1 was found at any given time point in our study.

In this study, a blood product transfusion or steroid stress dose administration did not influence the evolution of WNT-5a, sFRP-5, and WIF-1 after cardiac surgery. In view of the scarcity of evidence on this topic and the small number of patients in our study, this result should be interpreted with caution. To assess the possible effect of an intraoperative blood product transfusion or perioperative hydrocortisone treatment, we included these variables in the mixed models for the WNT signaling components exclusively as a sensitivity analysis. An in-depth investigation of the influence of blood product transfusions or glucocorticoid administration on WNT signaling might be of interest for further research.

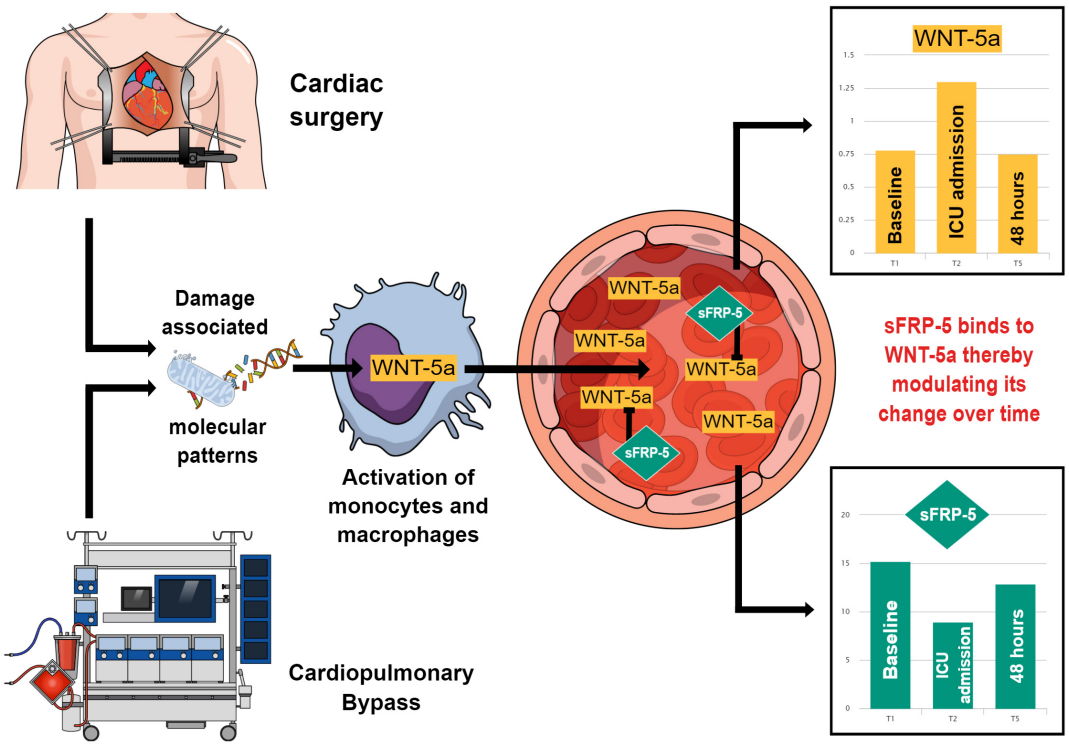


FIGURE 6
This figure summarizes how Wingless-related integration site 5a (WNT-5a) may come into play in a systemic inflammation caused by cardiac surgery and how its plasma concentration may be modulated by Secreted frizzled-related protein 5 (sFRP-5). Cardiac surgery and the use of cardiopulmonary bypass lead to the formation of damage associated molecular patterns which activate cells of the mononuclear phagocyte system. These cells produce and secrete WNT-5a which enters the circulatory system. In the blood stream, WNT-5a is bound by its antagonist sFRP-5 forming a soluble complex. Between the baseline (T1), the time of intensive care unit (ICU) admission (T2) and 48 h after surgery (T5), the blood plasma concentrations of WNT-5a and sFRP-5 move in the opposite direction compared to each other. This may demonstrate the modulatory effect of sFRP-5 on the WNT-5a plasma concentration leading to an attenuation of the WNT-5a peak value during the promotion of a systemic inflammation upon cardiac surgery and a timely return of WNT-5a to its baseline value during the resolution of the inflammatory response.

Interaction between Wingless-related integration site-5a and inflammatory cytokines after cardiac surgery

Inflammatory cytokines play a crucial role during a systemic inflammatory response and their kinetic profile in the setting of cardiac surgery has been studied previously (15). In this study, we obtained perioperative kinetic profiles of IL-1β, IL-6, TNF-α, GRO-α, MIP-1α, and MCP-1α. The low baseline values of inflammatory cytokines and more conventional inflammatory markers, CRP and leukocyte count, indicate that our patients were in a low or non-inflammatory state. A presumed upregulation of WNT signaling (particularly in patients with combined valve and coronary artery disease) may be too subtle to induce an upregulation of inflammatory cytokine production.

A particular function of IL-1β is to assist the immune system to defend the host against pathogens (27). In this study, no patient had clinical signs of an infection preoperatively. The low IL-1β values and the kinetic pattern of TNF-α, IL-6, and MCP-1α observed in our patients affirm that the inflammatory response investigated in this study represents a sterile inflammation resulting primarily from cardiac surgery and the use of CPB.

To assess an interaction between WNT-5a and inflammatory cytokines, we decided to use IL-6 and MCP-1α as these parameters have been in use for a long time to demonstrate and explore a systemic inflammation after cardiac surgery (16). WNT-5a, IL-6, and MCP-1α reached a peak concentration at the time of ICU admission and declined thereafter. This finding is in accordance with experimental data at the cellular level where WNT-5a and various cytokines are produced by macrophages upon activation (9). Although we were able to show a temporal relationship between WNT-5a, IL-6, and MCP-1α, no statistical correlation was found between these parameters. Interestingly, we found IL-6 and MCP-1α to increase by a multiple after cardiac surgery compared to WNT-5a barely doubling in plasma concentration. In theory, WNT-5a is considered to amplify the

production of inflammatory cytokines in macrophages (9). This may explain why we found a statistical difference for MCP-1α and a trend for IL-6 to reflect the severity of surgery but not for WNT-5a. Our findings are supported by a previous study describing higher blood plasma concentrations of MCP-1 and IL-6 in patients after valve surgery compared to CABG (28). It is WNTs supposed ability to amplify inflammatory cytokine production in macrophages that renders WNT-5a, in our opinion, a valid target to therapeutically influence a systemic inflammation after cardiac surgery by introducing WNT-5a antagonists.

Influence of Wingless-related integration site signaling components on clinical outcome parameters

One aim of the study was to investigate an impact of WNT signaling components, particularly of WNT-5a, on the postoperative clinical course after cardiac surgery. As clinical surrogates of the inflammatory response we used the postoperative fluid balance and the amount and duration of vasopressor requirement after surgery. Contrary to our study hypothesis, we found an inverse relationship between WNT-5a and WIF-1, and our clinical surrogate parameters for systemic inflammation. However, the associated numerical changes in the fluid balance of the ICU admission day, the noradrenaline requirement at ICU admission and the duration of hemodynamic instability were not in a clinically meaningful range. Referring to experimental data (29), we assumed that WNT-5a would induce a microvascular leakage leading to a higher fluid requirement and WIF-1 would reduce vascular barrier dysfunction resulting in enhanced hemodynamic stability. In the clinical setting, our findings seem to challenge WNT-5a and its associated WNT signaling components as meaningful regulators of an inflammatory response. But it must be stated that each of our three clinical outcome parameters only represent a part of the inflammatory response after cardiac surgery. Consecutively, a composite parameter may more adequately comprise and reflect clinical effects caused by inflammation. Yet, the accurate detection of an inflammation in a patient after cardiac surgery using clinical and laboratory criteria, has been reported as a delicate matter (4).

Limitations

The results of this study cannot be readily generalized. First, this is a single-center prospective study with a limited sample size leading to a comparably low power, so that significant differences may not have been detected. Second, we included only patients for CABG and valve surgery *via* sternotomy. The WNT signaling components may show a different time-concentration pattern in patients with more extensive cardiac surgery (e.g., type A aortic dissection) or with less tissue trauma if a minimal-invasive approach is used for surgery. Third, no patient in a critical condition, with pre-existing inflammatory disease or with an active infection (e.g., endocarditis) was included in this study. These patients as well may show a different WNT parameter profile after cardiac surgery. Fourth, although no effect of an intraoperative blood product transfusion on WNT signaling was found, this may not apply to patients with a mass transfusion of blood products as no such patient was included in this study. Fifth, WNT signaling components were followed up in this study only until 48 h after surgery. WNT parameter changes beyond this period may have gone unnoticed and, in addition, the point of return of WNT signaling to its preoperative equilibrium state remains uncertain. Sixth, CRP and WBC, two parameters commonly used to characterize an inflammatory state, were only collected at the baseline (T1) and after surgery at the time of ICU admission (T2). It would have been interesting to explore the evolution of CRP and WBC and their interaction with WNT-5a over time but to minimize the amount of blood loss for the patients we decided to include only two time points for these parameters.

Conclusion

Wingless-related integration site signaling is activated during the inflammatory response after cardiac surgery and its components show relevant plasma concentration changes over time. In patients with an uneventful postoperative course, the WNT-5a plasma concentration rises to a peak value immediately after surgery and returns to the baseline value within 48 h. The time-concentration course of WNT-5a is presumably modulated by the antagonistic action of sFRP-5. Further clinical studies are required to evaluate the significance of WNT-5a as an outcome marker of inflammation and to assess therapeutic options of WNT-5a antagonism to attenuate the effects of a dysregulated inflammatory response in patients after cardiac surgery.

Data availability statement

The original contributions presented in this study are included in the article/**Supplementary material**, further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving human participants were reviewed and approved by the Cantonal Ethics Committee, Zurich, Switzerland (BASEC 2017-01286). The patients/participants provided their written informed consent to participate in this study.

Author contributions

BK, GH, AR, GHS, DB, GS, and DS: conceptualization and investigation. BK, GH, and GHS: data curation. BK, GH, AR, JB, GS, and DS: formal analysis and methodology. GS and DS: funding acquisition and resources. BK, AR, GHS, GS, and DS: project administration. AR, GS, and DS: supervision. AR, DB, and GS: validation. BK and GH: visualization and writing—original draft. BK, GH, AR, GHS, JB, DB, GS, and DS: writing—review and editing. All authors contributed to the article and approved the submitted version.

Funding

This work was supported by the Institute of Anesthesiology, University Hospital Zurich, Switzerland (to DS) and by the "Stiftung für Klinisch–experimentelle Forschung," Department of Medicine, University Hospital Zurich, Switzerland (to GS).

Conflict of interest

The academic department of author DS has received grant support from Vifor SA and Vifor (International) AG. DS is co-chair of the ABC-Trauma Faculty, sponsored by unrestricted educational grants from Novo Nordisk Health Care AG, CSL Behring GmbH, LFB Biomédicaments, and Octapharma AG. DS received honoraria/travel support for consulting or lecturing from Alexion Pharmaceuticals Inc., AstraZeneca AG, Bayer AG, B. Braun Melsungen AG, CSL Behring GmbH, Celgene International II Sàrl, Daiichi Sankyo AG, Haemonetics, Instrumentation Laboratory (Werfen), LFB Biomédicaments, Merck Sharp & Dohme, Novo Nordisk Health Care AG, PAION Deutschland GmbH, Pharmacosmos A/S, Pfizer AG, Pierre Fabre Pharma, Portola Schweiz GmbH, Roche Diagnostics International Ltd, Sarstedt AG & Co., Shire Switzerland GmbH, Takeda, Tem International GmbH, Vifor Pharma, Vifor (International) AG, and Zuellig Pharma Holdings.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2022.997350/full#supplementary-material

References

1. Warren OJ, Smith AJ, Alexiou C, Rogers PL, Jawad N, Vincent C, et al. The inflammatory response to cardiopulmonary bypass: part 1–mechanisms of pathogenesis. *J Cardiothorac Vasc Anesth.* (2009) 23:223–31. doi: 10.1053/j.jvca.2008.08.007

2. Jentho E, Weis S. Damps and innate immune training. *Front Immunol.* (2021) 12:699563. doi: 10.3389/fimmu.2021.699563

3. Margraf A, Ludwig N, Zarbock A, Rossaint J. Systemic inflammatory response syndrome after surgery: mechanisms and protection. *Anesth Analg.* (2020) 131:1693–707. doi: 10.1213/ANE.0000000000005175

4. 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. *J Cardiothorac Vasc Anesth.* (2019) 33:1682–90. doi: 10.1053/j.jvca.2019.01.043

5. Andreis DT, Singer M. Catecholamines for inflammatory shock: a jekyll-and-hyde conundrum. *Intensive Care Med.* (2016) 42:1387–97. doi: 10.1007/s00134-016-4249-z

6. Ljungberg JK, Kling JC, Tran TT, Blumenthal A. Functions of the Wnt signaling network in shaping host responses to infection. *Front Immunol.* (2019) 10:2521. doi: 10.3389/fimmu.2019.02521

7. Jridi I, Cante-Barrett K, Pike-Overzet K, Staal FJT. Inflammation and Wnt signaling: target for immunomodulatory therapy? *Front Cell Dev Biol.* (2020) 8:615131. doi: 10.3389/fcell.2020.615131

8. Blumenthal A, Ehlers S, Lauber J, Buer J, Lange C, Goldmann T, et al. The wingless homolog wnt5a and its receptor frizzled-5 regulate inflammatory responses of human mononuclear cells induced by microbial stimulation. *Blood.* (2006) 108:965–73. doi: 10.1182/blood-2005-12-5046

9. Pereira CP, Bachli EB, Schoedon G. The Wnt pathway: a macrophage effector molecule that triggers inflammation. *Curr Atheroscler Rep.* (2009) 11:236–42. doi: 10.1007/s11883-009-0036-4

10. Pereira C, Schaer DJ, Bachli EB, Kurrer MO, Schoedon G. Wnt5a/Camkii signaling contributes to the inflammatory response of macrophages and is a target for the antiinflammatory action of activated protein C and interleukin-10. *Arterioscler Thromb Vasc Biol.* (2008) 28:504–10. doi: 10.1161/ATVBAHA.107.157438

11. Skaria T, Bachli E, Schoedon G. Wnt5a/Ryk signaling critically affects barrier function in human vascular endothelial cells. *Cell Adh Migr.* (2017) 11:24–38. doi: 10.1080/19336918.2016.1178449

12. von Elm E, Altman DG, Egger M, Pocock SJ, Gotzsche PC, Vandenbroucke JP, et al. The strengthening the reporting of observational studies in epidemiology (strobe) statement: guidelines for reporting observational studies. *Lancet.* (2007) 370:1453–7. doi: 10.1016/S0140-6736(07)61602-X

13. Jongman RM, Zijlstra JG, Kok WF, van Harten AE, Mariani MA, Moser J, et al. Off-Pump cabg surgery reduces systemic inflammation compared with on-pump surgery but does not change systemic endothelial responses: a prospective randomized study. *Shock.* (2014) 42:121–8. doi: 10.1097/SHK.0000000000000190

14. Kawano Y, Kypta R. Secreted antagonists of the wnt signalling pathway. *J Cell Sci.* (2003) 116:2627–34. doi: 10.1242/jcs.00623

15. Mitchell JD, Grocott HP, Phillips-Bute B, Mathew JP, Newman MF, Bar-Yosef S. Cytokine secretion after cardiac surgery and its relationship to postoperative fever. *Cytokine.* (2007) 38:37–42. doi: 10.1016/j.cyto.2007.04.009

16. de Mendonca-Filho HT, Pereira KC, Fontes M, Vieira DA, de Mendonca ML, Campos LA, et al. Circulating inflammatory mediators and organ dysfunction after cardiovascular surgery with cardiopulmonary bypass: a prospective observational study. *Critical Care.* (2006) 10:R46. doi: 10.1186/cc4857

17. Urner M, Herrmann IK, Buddeberg F, Schuppli C, Roth Z'graggen B, Hasler M, et al. Effects of blood products on inflammatory response in endothelial cells *in Vitro*. *PLoS One.* (2012) 7:e33403. doi: 10.1371/journal.pone.0033403

18. Weis F, Beiras-Fernandez A, Schelling G, Briegel J, Lang P, Hauer D, et al. Stress doses of hydrocortisone in high-risk patients undergoing cardiac surgery: effects on interleukin-6 to interleukin-10 ratio and early outcome. *Crit Care Med.* (2009) 37:1685–90. doi: 10.1097/CCM.0b013e31819fca77

19. Juffermans NP, van den Brom CE, Kleinveld DJB. Targeting endothelial dysfunction in acute critical illness to reduce organ failure. *Anesth Analg.* (2020) 131:1708–20. doi: 10.1213/ANE.0000000000005023

20. Spahn DR, Schoenrath F, Spahn GH, Seifert B, Stein P, Theusinger OM, et al. Effect of ultra-short-term treatment of patients with iron deficiency or anaemia undergoing cardiac surgery: a prospective randomised trial. *Lancet.* (2019) 393:2201–12. doi: 10.1016/s0140-6736(18)32555-8

21. Hauffe T, Kruger B, Bettex D, Rudiger A. Shock management for cardio-surgical icu patients - the golden hours. *Card Fail Rev.* (2015) 1:75–82. doi: 10.15420/cfr.2015.1.2.75

22. Hauffe T, Krüger B, Bettex D, Rudiger A. Shock management for cardio-surgical icu patient: the silver days. *Card Fail Rev.* (2016) 2:56–62.

23. Couture P, Denault A, Limoges P, Sheridan P, Babin D, Cartier R. Mechanisms of hemodynamic changes during off-pump coronary artery bypass surgery. *Can J Anaesth.* (2002) 49:835–49. doi: 10.1007/BF03017418

24. Abraityte A, Lunde IG, Askevold ET, Michelsen AE, Christensen G, Aukrust P, et al. Wnt5a is associated with right ventricular dysfunction and adverse outcome in dilated cardiomyopathy. *Sci Rep.* (2017) 7:3490. doi: 10.1038/s41598-017-03625-9

25. Tong S, Du Y, Ji Q, Dong R, Cao J, Wang Z, et al. Expression of Sfrp5/Wnt5a in human epicardial adipose tissue and their relationship with coronary artery disease. *Life Sci.* (2020) 245:117338. doi: 10.1016/j.lfs.2020.117338

26. Menet R, Lecordier S, ElAli A. Wnt pathway: an emerging player in vascular and traumatic mediated brain injuries. *Front Physiol.* (2020) 11:565667. doi: 10.3389/fphys.2020.565667

27. van der Meer JW, Barza M, Wolff SM, Dinarello CAA. Low dose of recombinant interleukin 1 protects granulocytopenic mice from lethal gram-negative infection. *Proc Natl Acad Sci U.S.A.* (1988) 85:1620–3. doi: 10.1073/pnas.85.5.1620

28. Kawahito K, Adachi H, Ino T. Influence of surgical procedures on interleukin-6 and monocyte chemotactic and activating factor responses: Cabg Vs Valvular Surgery. *J Interferon Cytokine Res.* (2000) 20:1–6. doi: 10.1089/107999000312676

29. Skaria T, Burgener J, Bachli E, Schoedon G. Il-4 causes hyperpermeability of vascular endothelial cells through Wnt5a signaling. *PLoS One.* (2016) 11:e0156002. doi: 10.1371/journal.pone.0156002

30. Skaria T, Bachli E, Schoedon G. Wif1 prevents Wnt5a Mediated Limk/Cfl phosphorylation and adherens junction disruption in human vascular endothelial cells. *J Inflamm.* (2017) 14:10. doi: 10.1186/s12950-017-0157-4

frontiers | Frontiers in Cardiovascular Medicine

TYPE Original Research
PUBLISHED 20 December 2022
DOI 10.3389/fcvm.2022.983617

OPEN ACCESS

EDITED BY
Shahzad Raja,
Harefield Hospital, United Kingdom

REVIEWED BY
Elmina Bach,
Ludwig-Maximilian-University, Germany
Weiyi Guo,
Capital Medical University, China

*CORRESPONDENCE
Krzysztof Laudanski
klaudanski@gmail.com

SPECIALTY SECTION
This article was submitted to Heart Surgery, a section of the journal Frontiers in Cardiovascular Medicine

RECEIVED 01 July 2022
ACCEPTED 23 November 2022
PUBLISHED 20 December 2022

CITATION
Laudanski K, Liu D, Gullipalli D, Song W-C, Okeke T and Szeto WY (2022) A decline of protective apolipoprotein J and complement factor H concomitant with increase in C5a 3 months after cardiac surgery—Evidence of long-term complement perturbations. *Front. Cardiovasc. Med.* 9:983617. doi: 10.3389/fcvm.2022.983617

A decline of protective apolipoprotein J and complement factor H concomitant with increase in C5a 3 months after cardiac surgery—Evidence of long-term complement perturbations

Krzysztof Laudanski[1,2,3]*, Da Liu[4], Damodar Gullipalli[5], Wen-Chao Song[5], Tony Okeke[6] and Wilson Y. Szeto[7]

[1]Department of Anesthesiology and Critical Care, The University of Pennsylvania, Philadelphia, PA, United States, [2]Department of Neurology, The University of Pennsylvania, Philadelphia, PA, United States, [3]Leonard Davis Institute for Health Economics, The University of Pennsylvania, Philadelphia, PA, United States, [4]Department of Obstetrics and Gynecology, Shengjing Hospital of China Medical University, Shenyang, China, [5]Department of Systems Pharmacology and Translational Therapeutics, The University of Pennsylvania, Philadelphia, PA, United States, [6]Department of Bioengineering, Drexel University, Philadelphia, PA, United States, [7]Division of Cardiovascular Surgery, Department of Surgery, University of Pennsylvania, Philadelphia, PA, United States

Background: Heart surgery results in complement activation with the potential for collateral end-organ damage, especially if the protective elements (complement factor H, Apolipoprotein J) are inadequate. Here, we have investigated if peri-operative stress results in an imbalance between complement activation and its protective mechanisms up to 3 months after heart surgery.

Methods: 101 patients scheduled for non-emergent cardiac surgery donated blood before the procedure ($t_{baseline}$), and 24 h (t_{24h}), 7 days (t_{7d}) and 3 months (t_{3m}) after. Complement activation was measured as a serum level of soluble activated component 5 (sC5a) and soluble terminal complement complex (sTCC). Simultaneously, protective complement factor H (CfH), and apolipoprotein J (ApoJ) were measured. Inflammatory responses were quantified using C-reactive protein (CRP) and interleukin-6 (IL-6). Details regarding anesthesia, intensive care unit (ICU) stay, pre-existing conditions, the incidence of postoperative complications, and mortality were collected from medical records.

Results: C5a declined at t_{24h} to rebound at t_{7d} and t_{3m}. sTCC was significantly depressed at t_{24h} and returned to baseline at later time points. In contrast, CfH and ApoJ were depressed at t_{3m}. Milieu of complement factors aligned along two longitudinal patterns:cluster#1 (C5a/sTTC continuously increasing and CfH/ApoJ preserved at $t_{baseline}$) and cluster#2 (transient

sC5a/sTTC increase and progressive decline of CfH). Most patients belonged to cluster #1 at t_{24h} (68%), t_{7d} (74%) and t_{3m} (72%). sTCC correlated with $APACHE_{1h}$ (r^2 =−0.25; $p < 0.031$) and $APACHE_{24h}$ (r^2 = 0.27; $p < 0.049$). IL-6 correlated with C5a (r^2 =−0.28; $p < 0.042$) and sTTC (r^2 =−0.28; $p < 0.015$). Peri-operative administration of acetaminophen and aspirin altered the complement elements. Prolonged hospital stay correlated with elevated C5a [t (78) = 2.03; p = 0.048] and sTTC serum levels [U (73) = 2.07; p = 0.037]. Patients with stroke had a decreased serum level of C5a at t_{7d} and t_{3m}.

Conclusion: There is a significant decrease in complement protective factors 3 months after cardiac surgery, while C5a seems to be slightly elevated, suggesting that cardiac surgery affects complement milieu long into recovery.

KEYWORDS

cardiac surgery, complement, terminal complement complex, complement component 5a, complement factor H, apolipoprotein J, clusterin

Introduction

Complement activation is triggered in three ways: classical, alternative and lectin-driven to activate an innate inflammation to augment pathogens and dead cell removal (1, 2). All pathways merge at C5a and C5b, followed by the formation of the terminal complement complex (TCC) as the common pathway. C5b-9 inserts itself into the cell membrane as one of the primary effector bactericidal mechanisms (3). However, TCC can be self-damaging *via* endothelial activation, cell death, and intravascular hemolysis. Several proteins keep the complement activation in check, with CD55, CD59, and CD46 being the most extensively studied (4, 5). In contrast, humoral factors such as complement factor H and clusterin/Apo J have experienced much less scrutiny despite their critical role in limiting collateral complement-mediated damage (6–19). Their depletion may exacerbate end-organ damage and contribute to an excess of morbidity (10, 13, 15, 16, 20).

During heart surgery, the complement system is activated *via* contact with artificial surfaces, immunoglobulins, or *via* a CRP-mediated pathway (18, 19). As a result, direct complement cytotoxicity is exacerbated by a reperfusion injury and the influx of inflammatory leukocytes (21). Subsequent vasoconstriction, thrombosis, and inflammation may result in hypoperfusion organ injuries. It is not surprising that imbalance in complement has been suggested as the target of therapeutic interventions. However, interference with effector components of complement failed to demonstrate favorable clinical outcomes, except in severely sick patients undergoing heart surgery (22–24). This lack of progress in effectively modulating complement activation may result from neglecting the post-surgical abnormalities in protective elements of complement (22–25). No study addressed the long-term changes in serum mechanisms moderating complement activation after cardiac surgery.

Here, we addressed the knowledge gap in temporal dynamics between cytoprotective (clusterin/ApoJ, complement factor H) vs. cytotoxic (C5a, TTC) elements after non-emergent cardiac surgery. We hypothesized that activation of complement and protective effectors would be synchronized to minimize end-organ damage (16, 17, 26). Specifically, patients with a misalignment between complement effector and protective would experience increased thrombotic events. Finally, we hypothesized that acute disturbances in complement components will resolve 3 months after surgery.

Abbreviations: APACHE, Acute Physiologic Assessment and Chronic Health Evaluation; ApoJ, Apolipoprotein J; BMI, Body Mass Index; C5a, Complement component 5a; CCI, Charlson Comorbidity Index; CfH, Complement Factor H; CRP, C Reactive Protein; CVA, Cerebrovascular accident; DVT, Deep Vein Thrombosis; EMR, Electronic Medical Review; ICU, Intensive Care Unit; IgG, Immunoglobulin G; IgM, Immunoglobulin M; IL-6, Interleukin 6; LOS, Length of Stay; PAMP, Pattern Activation of Molecular Patterns; PE, Pulmonary Embolism; SOFA, Sequential Organ Failure Assessment; sTCC, Soluble Terminal Complement Complex.

Patients and methods

Patients enrollment

Our study protocol was approved by the Institutional Review Board (IRB) of the University of Pennsylvania (#815686). All adult patients scheduled for non-emergent heart surgery were approached for consent. We excluded patients with pre-existing immunological aberrancies on immunosuppressant medications in the last 6 months (prednisone PO or IV more than 5 mg daily, αTNFα, αIL-6, αIL-3, αCD20 antibodies therapy, immunoglobulin, plasmapheresis, methotrexate, chemotherapy). The study did not include patients post-transplant.

Demographical and clinical data collections

Electronic medical records (EMR) were used to collect demographic and medical data for all enrolled participants. Charlson Comorbidity Index (CCI) measured the burden of chronic disease (27). EMR were extracted for description of surgical procedure and bundle as coronary artery graft bypass (CABG), aortic vavle surgery, mitral valve surgery, aortic arch surgery, and others (**Table 1**). Most patients have multiple procedure done during one surgery. The perioperative insult was gauged by the duration of anesthesia and surgery, estimated blood loss, and volume of crystalloid resuscitation. The usage of opioids, benzodiazepines, acetaminophen, ketorolac, and steroids during the perioperative 24 h was registered. Acute physiology and chronic health evaluation II (APACHE II) was calculated within 1 h ($APACHE_{1h}$), 24 h ($APACHE_{24h}$), and 48 h ($APACHE_{48h}$) after admission to the Intensive Care Unit (ICU) (28). The severity of the illness was determined by Sequential Organ Failure Assessment (SOFA) (29). Organ failure was defined according to MODS criteria or the Glue Grant framework (30, 31). Depp venous thrombosis (DVT), pulmonary embolism (PE), and stroke diagnoses were extracted from medical records. Survival was determined at 28 days and 3 months. Platelets count was extracted from routine lab from EHR.

The characteristics of the studied population are presented in **Table 1**.

TABLE 1 Demographical and clinical characteristic of the studied sample.

Demographics (101 patients)	
Age [X ± SD]	62.6 ± 12.44
Over 60 [%]	33.7%
Gender	
Male [%]	75.24%
Female [%]	24.75%
Not reported [%]	0%
Race	
Hispanic Latino [%]	1.98%
Black [%]	5.94%
White [%]	90.1%
Other/Asian/unknown [%]	3.96
Pre-existing conditions	
Weigh	84.6 ± 21.33
BMI	27.6 ± 5.47
Charleston comorbidity index [X ± SD]	3.9 ± 2.03
ACS/MI [%]	13.86%
CHF [%]	15.8%
PVD [%]	9.9%
CVA/TIA [%]	9.9%
Dementia [%]	0%
COPD [%]	5.94%
DM [%]	28.7%
Anesthesia and surgery data	
Duration of anesthesia; mean ± SD [min]	372.3 ± 105.89
Duration of surgery; mean ± SD [min]	266.8 ± 101.34
Duration of cardiopulmonary bypass; mean ± SD [min]	129.2 ± 65.04
Coronary artery bypass surgery; no.	51
Mitral valvuloplasty and replacement; no.	26
Aortic valvuloplasty and replacement; no.	40
Aortic aneurysm repair; no.	8
Others; no	4
Estimated blood loss [ml]	201.1 ± 283.89
Perioperative management	
Transfusions during surgery	
Packed red blood cells, mean (IQ25; IQ75) [mL]	115 [0; 1,200]
Fresh frozen plasma, mean (IQ25; IQ75) [mL]	93.4 [0; 1,750]
Total crystalloid during surgery[mL]	1,252 ± 552.38
Clinical care during 24 h post-surgery	
Packed red blood cells, mean; (IQ25; IQ75) [mL]	12.1 [0;600]
Fresh frozen plasma, mean; (IQ25; IQ75) [mL]	9.1 [0; 750]
Corticosteroid administration (% of all cases)	7.9%
Ketorolac administration (% of all cases)	7.9%
Acetaminophen administration (% of all cases)	29.7%
Acetylsalicylic acid administration	32.7%
Opioids administration	689.2 ± 221.89
BZD administration	0.38 + 2.34
ICU stay	
APACHE score at 1 h, mean ± SD	16.0 ± 5.55
APACHE score at 24 h, mean ± SD	8.8 ± 4.29
APACHE score at 48 h, mean ± SD	8.4 ± 4.11

(Continued)

Sample procurement

Blood was collected in sodium citrate tubes after collection from an arterial or central line. Plasma was isolated by centrifugation for 10 min at 1,200 ×g 4°C, aliquoted, and stored at −80°C. Blood was collected before non-emergent cardiac surgery ($t_{baseline}$) followed by 24 h (t_{24h}) and 7 days (t_{7d}) later, with final follow-ups at 3 months (t_{3m}).

Measures of complement effector activation

To detect human C5a levels in plasma samples, αC5a antibody neo-epitope (Biolegend, San Diego, CA) was utilized. Secondary detection was done with biotinylated anti-human C5a mAb (Biolegend, San Diego, CA) and avidin or streptavidin conjugated to horseradish peroxidase (BD, Franklin Lakes, NJ). Recombinant hC5a (Hycult, Wayne, PA) was used as the standard. An analogous process was utilized for the detection of sTCC by utilizing α human TCC mAb neoepitope (SantaCruz, San Diego, CA), biotinylated anti-human TTC mAb (QDC5,

TABLE 1 (Continued)

Demographics (101 patients)	
Outcome at 28 days	
LOS ICU	8.1 ± 40.23
LOS hospital	10.1 ± 21.08
DVT	0.99%
PE	2.97%
CVA	6.93%
Discharged/in the healthcare facility/expired	90.9%/5.94%/2.97%

in-house). sC5b-9 Complex (Complement Tech, Marlon, NJ) used as standard.

Assessment of the complement protective factors and inflammation markers

Complement factor H, apolipoprotein J, and C-reactive protein were measured with the multiplex kit (Thermofisher, Waltham MA). IL-6 in serum was determined *via* ELISA (Thermofisher, Waltham MA).

Statistical analysis

The Shapiro-Wilk W test and distribution plots tested the normality and distribution of variables. Parametric variables are expressed as mean ± SD and compared, using *t*-Student. For non-parametric variables, median (M_e) and interquartile ranges (IR) will be shown with the U-Mann-Whitney statistic, employed to compare such variables. ANOVA was calculated for parametric variables with multiple discrete values, with Shaffe's test as a *post hoc* test. When applicable, paired contrasts for longitudinal comparisons were used with $t_{baseline}$ as the reference point. Correlational momentum was calculated as r^2 Pearson. A regression analysis was done stepwise methods when appropriate. *k*-means cluster analyses and data normalization were calculated with *scikit-learn* package. A *p*-value less than 0.05 was considered statistically significant for all tests based on the hypothesis. Statistical analyses will be performed with SPSS 26 [IBM, Whalton, NY), and in R (32)].

Results

Longitudinal analysis of cytotoxic (sTTC, C5a) and protective humoral complement factors (clusterin/ApoJ, factor H) after cardiac surgery.

Age over 60, gender, and race did not significantly affect the baseline levels of studied factors (data not shown).

sC5a changed significantly over time with concentrations initially decreasing [U (85) =−3.17; $p = 0.0015$), rebounding to significantly higher values at 7 days [U (79) = 2.54; $p = 0.011$] and 3 months (U [69] = 3.34; $p = 0.00082$) (**Figure 1A**). When data were compared pairwise, the median changes from baseline were 83, 136, and 150% at t_{24h}, t_{7d}, and t_{3m}, respectively (**Figure 1B**). sTTC levels changed significantly at t_{24h} [U (63) =−4.31; $p = 0.00016$] followed by recovery to pre-surgical values (**Figure 1C**). When the data were compared pairwise, the median changes from baseline were 43, 101, and 83% at t_{24h}, t_{7d}, and t_{3m}, respectively (**Figure 1D**). However, significant sTTC variability at $t_{baseline}$ and t_{3m} was apparent. The correlation between sTTC and C5a was present only at t_{24h} ($r^2 = 0.37$; $p < 0.004$) (data not shown). A regression analysis revealed that the level of sC5a was the most significant contributor to sTTC levels at t_{24h}, accounting for 56% ($p = 0.0067$) and 26% ($p = 0.041$) of sTTC variance.

Significant changes over time were seen for both CfH and ApoJ. CfH levels were the lowest at t_{7d} [W (70) = 3.06; $p = 0.002$] and t_{3m} [W (56) = 3.82; $p = 0.00013$] (**Figure 2A**). When the data were compared pairwise, the median changes from baseline were 94, 84, and 64% at t_{24h}, t_{7d}, and t_{3m}, respectively, (**Figure 2B**). ApoJ was lowest at t_{24h} [W (86) = 2.56; $p = 0.01$] and at t_{3m} [W (60) = 3.46; $p = 0.00053$] (**Figure 2C**). The changes from baseline were 81, 82, and 58%, respectively (**Figure 2D**).

The interplay between complement protective and effector proteins.

There was a significant correlation between C5a and sTTC (**Figure 3A**) and CfH and ApoJ (**Figure 3B**) at t_{24h}. The unsupervised analysis identified two clusters with different time dynamics across all four factors studied (**Figures 3C,D**). Cluster #1 was a cluster with the gradual activation of C5a and sTTC over time while protective factors remained stable (CfH) or increased over time (ApoJ) (**Figures 3C,D**). Cluster #2 demonstrated an increase in sC5a, sTCC, and ApoJ at 7 days to decline at t_{3m} (**Figures 3C,D**). However, CfH rapidly declined and remained low at t_{3m} in the case of patients in complement cluster#2 (**Figures 3C,D**). Most patients belonged to complement cluster #1 at t_{24h} (68%), t_{7d} (74%), and t_{3m} (72%). Patients belonged to the original t_{24h} cluster, but the transition of individuals to other complement clusters was seen at t_{7d} and t_{3m} (**Figure 3E**).

Relationship of complement activation vs. severity of the peri-operative injury

There was no correlation between the time on cardiopulmonary bypass, aortic cross-clamp, or anesthesia and any complement markers (data not shown). sTCC correlated with $APACHE_{1h}$ ($r^2 = 0.25$; $p < 0.031$) and $APAPCHE_{24h}$ ($r^2 = 0.27$; $p < 0.049$). CfH ($r^2 = 0.32$; $p < 0.003$) correlated with the volume of transfused PRBC during t_{24hr}. Similar

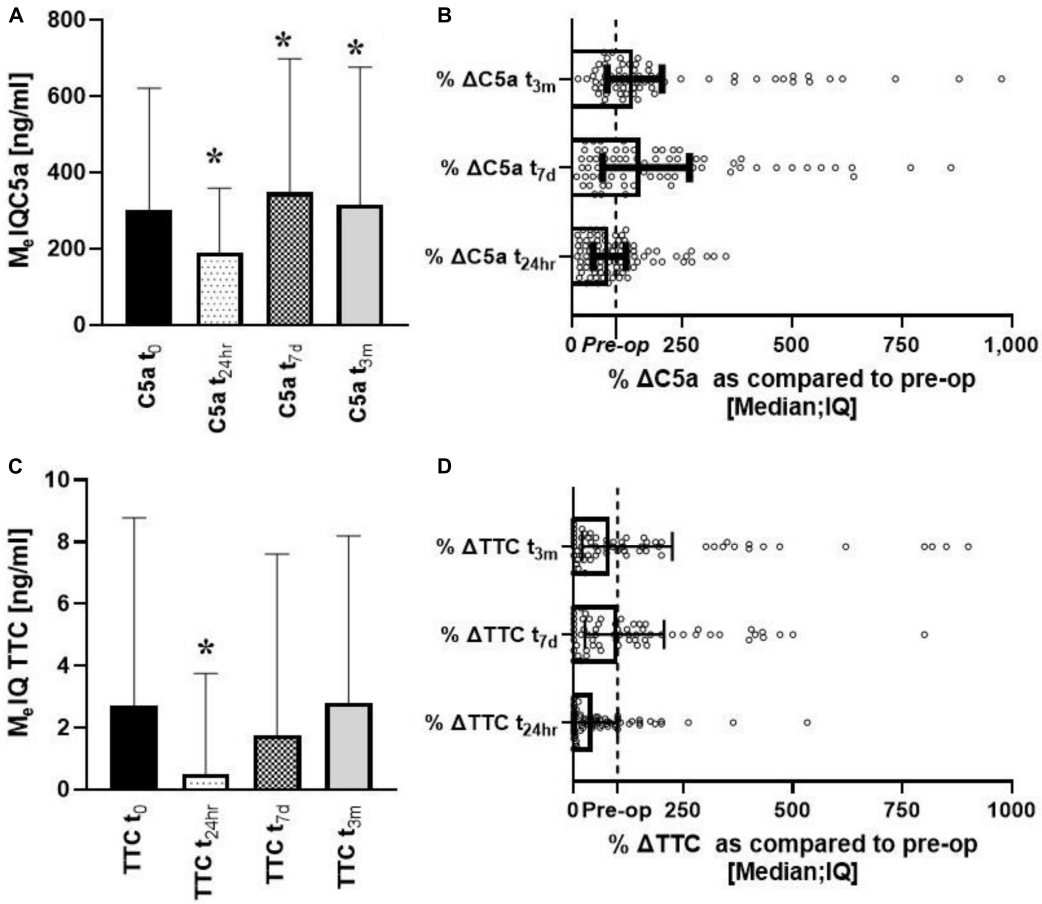


FIGURE 1
Longitudinal analysis of complement effectors (sC5a, sTCC) after heart surgery. Serum levels of C5a decreased early after surgery to increase up to 3 months of follow-ups **(A,B)**. In contrast, sTTC was depressed only at t_{24h} **(C,D)**. *Denotes the statistical significance of 0.05 or less.

correlations were seen between CfH (r^2 = 0.29; $p < 0.005$), and the volume of transfused fresh frozen plasma was transfused at the same time. Finally, the amount of crystalloid administered correlated weakly with CfH (r^2 = 0.28; $p < 0.008$) during surgery. C5a did not correlate with any measured variables of surgical insult severity.

Serum IL-6 inversely correlated with C5a (r^2 =−0.28; $p < 0.042$) and sTTC (r^2 =−0.28; $p < 0.015$) at t_{24h}. CRP correlated highly with CfH (r^2 =−0.42; $p = 0.029$) at t_{24h}.

Perioperative intake of acetaminophen resulted in diminished [21.5 (16.6; 52.3) vs. 41.5 (29.5; 87.9)] serum levels of CfHt_{24h} [U (91) = 2.84; $p = 0.0048$] while individuals receiving aspirin had lower serum sTTC at t_{24h} [3.5 (0; 6.7) vs. 3.5 (0; 1.7); U (75) = 1.97; $p = 0.049$]. The intake of ketorolac and steroids had no impact on the serum levels of C5a, sTTC, CfH, or ApoJ (data not shown). The number of benzodiazepines given in the first 24 h after surgery correlated significantly with CfH (r^2 =−0.28; $p < 0.008$), and ApoJ (r^2 =−0.23; $p < 0.028$) at t_{24h} but not with the perioperative intake of opioids (data not shown).

Correlations with clinical outcomes

Low mortality or the incidence of DVT or PE in our studied group precluded the comparison of studied complement factors. Patients who experienced an acute CVA significantly diminished levels of C5a at t_{7d} and t_{3m} (**Figure 4**). Emergence of AKI at 24 h or at the discharge was not related to changes in C5a, sTTC, CfH, or ApoJ at any time point (data not shown). However, only few patients (n = 5) experienced AKI at that time. Patients who were hospitalized at 28 days had significantly elevated serum C5a [186.2 ± 154.3 vs. 343.7 ± 327; t (78) = 2.03; $p = 0.048$] and sTTC serum levels [0.3 (0; 3.6) vs. 6 (4.35; 6.9); U (73) = 2.07; $p = 0.037$]. The length of stay (LOS) in the ICU or hospital did not correlate significantly with serum C5a, sTTC, CfH, or ApoJ.

Discussion

The first unique finding of this study is the observation of severe disruption in the complement milieu extending

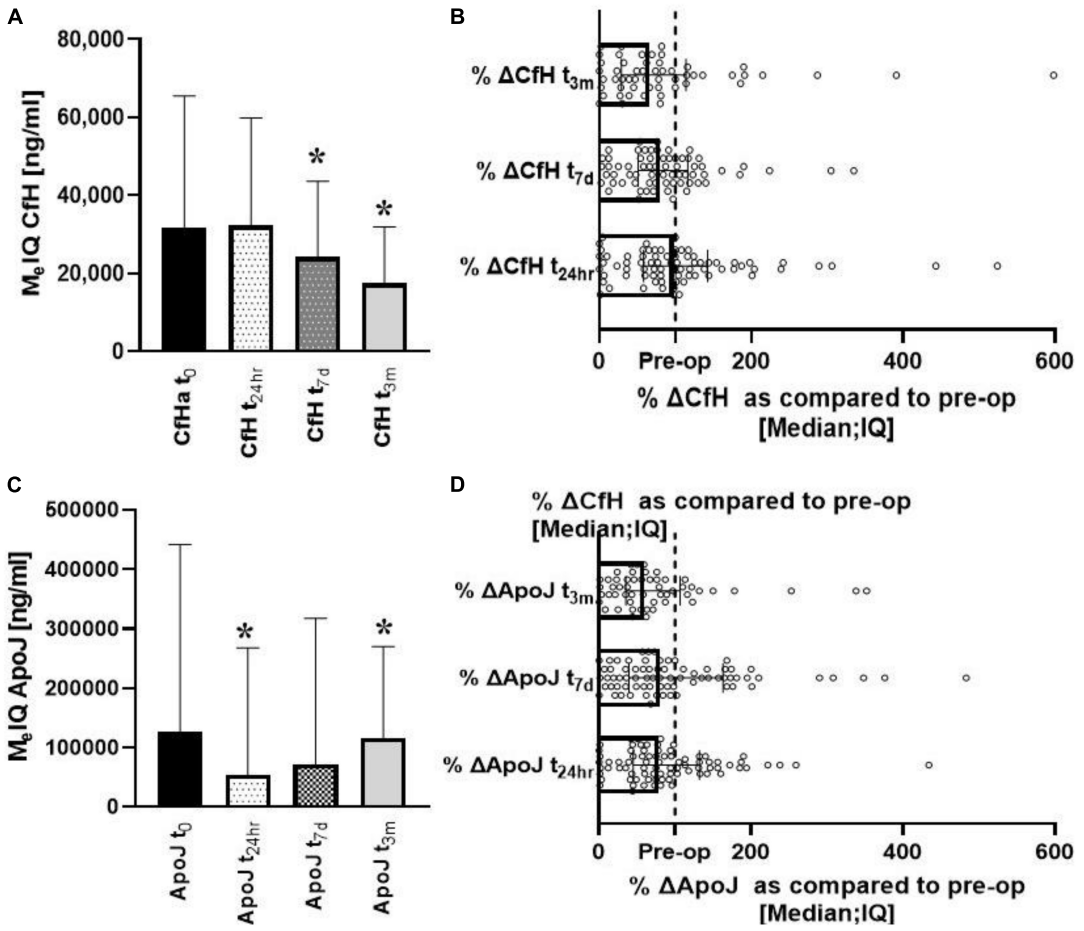


FIGURE 2
Longitudinal analysis of complement protective factors (CfH, ApoJ) after heart surgery. Progressive depletion of the CfH could be seen in the post-op period **(A,B)**. In contrast, ApoJ started to recover after initial depletion **(C,D)**. *Denotes the statistical significance of 0.05 or less.

into post-surgical recovery and outside the period typically considered for peri-operative inflammation. Prior data reported that the effector elements of complement were altered for up to 48 h after surgery (8, 18, 19, 33, 34). Our study focused on 3 months' performance of complement after heart surgery in adults. We demonstrated increased activity of C5a but no significant changes in serum sTTC in the wake of cardiac surgery and up to 3 months after. C5a plays a vital role in the chemotaxis of granulocytes and in coagulation activation (2, 3). More importantly, elevated C5a increases the risk of graft failure and coronary vasospasm and accelerates atherosclerosis (33, 35, 36). Here we observed decreased level of C5a connected to peri-operative stroke incidence, but the data should be considered a pilot for a more extensive study. The abnormal level of sC5a in patients experiencing a stroke may result from vasculitis consuming complement, as it has been seen in transplanted hearts (36). The etiology of elevated C5a is unclear. Acute inflammation measured by IL-6 seemed resolved. However, elevated mannose levels at the discharge of pediatric patients undergoing heart surgery suggest a potential mechanism of protracted C5a activation (18, 34, 37).

The second important and novel finding is that the long-term increase of sC5a is not counterbalanced by factors protecting from the overactivation of the complement system (6, 11–13, 15, 17, 20). As demonstrated both in time series and cluster analysis, both ApoJ and CfH were severely depressed in several patients if their C5a and sTCC were elevated. This is the first observation of this nature to date. Hemodilution is unlikely to be responsible for decline of ApoJ and CfH considering that 3 months after surgery patients fluid status should be balanced. Excessive consumption is potential reason but a generalized phenomenon like disseminated intravascular coagulation should resolve at 3 months. Also, the platelets count normalized at the time of discharge (data not shown). Interestingly, the milieu of patients' complement factors created two relatively uniform clusters initially right after surgery. However, over time, patients tended to group in cluster#1. This cluster sustained activation of C5a and preserved ApoJ and CfH,

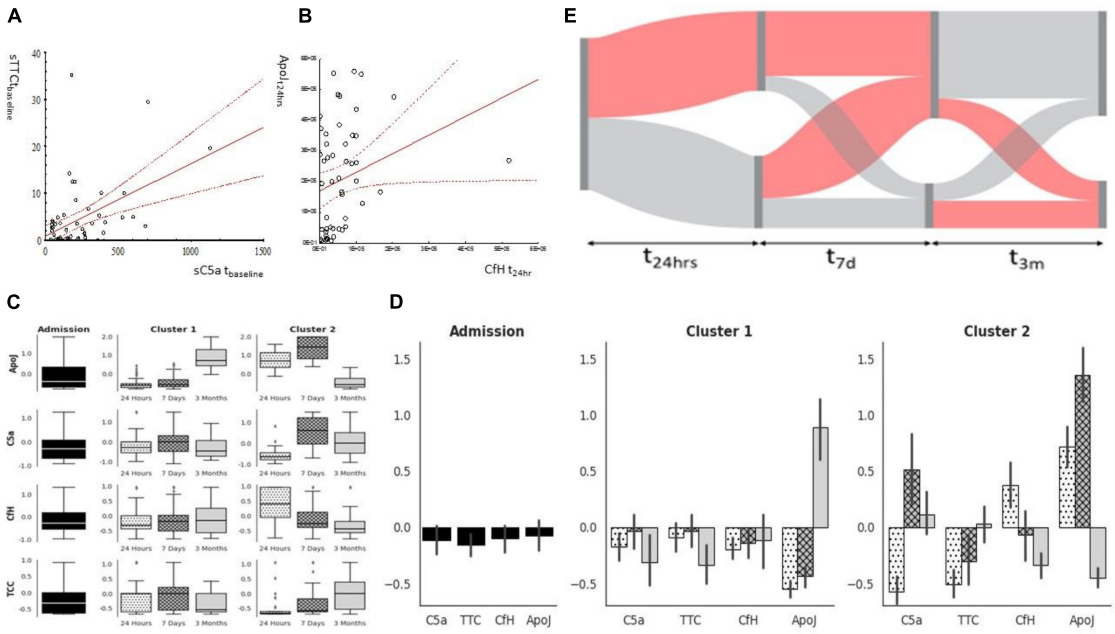


FIGURE 3
Complement factors milieu after cardiac surgery. C5a and TTC correlated strongly at the baseline **(A)**, while protective factors (ApoJ CfH) correlated at t_{24h} **(B)**. While factors were analyzed using clustering, two subgroups emerged: cluster#1 can be characterized by a significant increase in ApoJ at 3 months while cluster#2 is characterized by significant activation of sC5a later after surgery concomitant ApoJ **(C,D)**. Patients' complement milieu was not stagnant as complement activation characteristics moved between clusters **(E)**.

and resembles a natural resolution of complement activation where elevated levels of C5a and sTTC are counterbalanced by protective elements. The alternative cluster was different mainly due to the profound long-term depletion of CfH and ApoJ. CfH is critical as the alternative activation pathway of the complement system (2, 6, 11). Alternative and mannose-driven complement activation is predominantly affected during cardiopulmonary bypass surgery (6, 18, 35). The liver is one of the predominant producers of CfH and ApoJ, but incidence of liver failure was low in our studied population (38). CfH is activated by pentraxin, but this protein family was not in our study (17). Finally, acute inflammation was measured *via* IL-6 and normalized at 7 days so the ongoing inflammatory process cannot account for the decrease in ApoJ and CfH (8, 18).

Applying the multidimensional approach to data is an alternative to the prior studies. It aligns well with the current understanding of critical care illnesses, such as dysregulation failure. It offers a holistic assessment of complement where several biological components are considered as several regulatory components like CfH and ApoJ may be critical in restoring post-cardiac effector components imbalance (22, 23). It also offers a new approach for treating the post-cardiac surgery abnormalities as the prior clinical studies modulating complement failed to demonstrate widespread clinical benefit (25). Anti-complement modulators have been advocated for long time as potential drugs for patient undergoing heart surgery but clinical trial failed. The failure is often attributed to insufficient understanding of complement activation during heart surgery (39, 40). Our study suggested that complement performance is multidimensional and involved multiple pro- and anti-complement factors (2, 22). It is possible that modulating of C5a/sTTC vs. supplementing ApoJ or CfH needs to be precisely targeted in specific patients subpopulation as demonstrated in our cluster analysis. The clinical meaning of

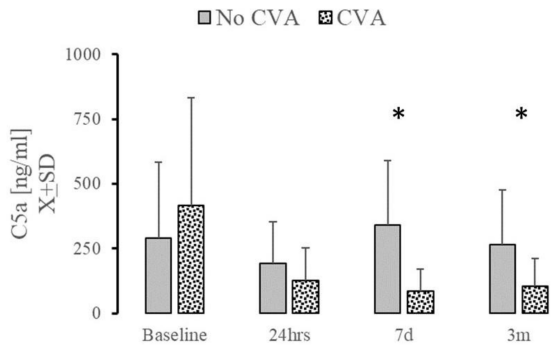


FIGURE 4
The effect of complement changes on the incidence of cerebrovascular accidents. Serum level of C5a at t_{7d} and t_{3m} was significantly depressed in patients (n = 7) who experienced stroke during 28 days after surgery. *Denotes the statistical significance of 0.05 or less.

our findings has yet to be determined as limited study samples, and incidence of stroke precluded statistical analysis.

Limitations of our study need to be acknowledged. First, extending the study to a different center and using commercially available kits is necessary to generalize the findings. The high standardization of care in our system may have increased the chance of this bias. Second, we only investigated the effector arm of the complement activation system while interference with the mannose/lectin activated pathway may be more dominant in cardiac surgery patients (34, 41). Other elements of the complement regulatory components were not measured while representing distinctive activation, inhibition, and regulation (4, 12, 17, 26, 37). Third, our study was not powered to look for the clinical impact of the complement milieu. No prior study has analyzed several complement factors simultaneously. Most of the correlations between complement factors and clinical measurements reflecting cardiac surgery severity were weak and, at best, suggested potential relationships. Most of these correlations were related to blood loss or perioperative transfusions. Consequently, changes in the complement system may be related to exogenous injections of CfH and ApoJ as they are abundant in serum (6, 7). Finally, CfH polymorphisms is rare and unlikely as the factor affecting results (14). Also, pre-existing condition leading to surgery were very ambiguous. Some patients had diagnosis of coronary artery disease, while others had pre-existing endocarditis leading to surgery. Several others pre-existing conditions could be extracted from chart. Several of these conditions may affect the baseline complement status at baseline with even less predictable effect for the peri-surgical fluctuations of factors (2, 22, 36).

Our pilot study offers several methodological advantages over prior studies. First, it provides a relatively large sample and, more importantly, a longitudinal analysis extending 3 months after surgery (18). The same heparinized circuit was used throughout the study's duration (19). We also account for the acetaminophen, ketorolac, aspirin, and steroids used during the surgery (19). High standardization of the care reduced post-surgery care variability. We accounted for inflammation levels by measuring IL-6. Several components of perioperative management were factored in more detail as compared to prior studies (18, 22, 25).

In summary, we demonstrated for the first time imbalance between C5a and CfH and ApoJ three months after non-emergent cardiac surgery. This imbalance was related to the longer LOS and emergence of cerebrovascular events.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by the University of Pennsylvania IRB. The patients/participants provided their written informed consent to participate in this study.

Author contributions

KL: conceptualization, methodology, investigation, and writing—original draft preparation. KL and TO: formal analysis, data curation, and visualization. KL, TO, W-CS, DG, and WS: writing—review and editing. KL and WS: project executions and funding acquisition. All authors have read and agreed to the published version of the manuscript.

Funding

This research was supported by the NIH NIGMS award (K23 GM120630) and KL's own funds.

Acknowledgments

We acknowledge the staff of the Heart and Vascular Intensive Care Unit at Penn Presbyterian Medical Center and our colleagues from the Department of Anesthesiology and Critical Care University of Pennsylvania. We thank Mariana Restrepo and Justin Wain for their help in the preparation of this manuscript.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Kang Y-H, Tan LA, Carroll MV, Gentle ME, Sim RB. Target pattern recognition by complement proteins of the classical and alternative pathways. *Adv Exp Med Biol.* (2009) 653:117–28. doi: 10.1007/978-1-4419-0901-5_8

2. Rawish E, Sauter M, Sauter R, Nording H, Langer HF. Complement, inflammation and thrombosis. *Br J Pharmacol.* (2021) 178:2892–904. doi: 10.1111/bph.15476

3. Bohnsack JF, Widjaja K, Ghazizadeh S, Rubens CE, Hillyard DR, Parker CJ, et al. A role for C5 and C5a-ase in the acute neutrophil response to group B streptococcal infections. *J Infect Dis.* (1997) 175:847–55. doi: 10.1086/513981

4. Yao X, Verkman AS. Complement regulator CD59 prevents peripheral organ injury in rats made seropositive for neuromyelitis optica immunoglobulin G. *Acta Neuropathol Commun.* (2017) 5:57. doi: 10.1186/s40478-017-0462-4

5. Coppo R, Peruzzi L, Loiacono E, Bergallo M, Krutova A, Russo ML, et al. Defective gene expression of the membrane complement inhibitor CD46 in patients with progressive immunoglobulin A nephropathy. *Nephrol Dial Transplant.* (2019) 34:587–96. doi: 10.1093/ndt/gfy064

6. Parente R, Clark SJ, Inforzato A, Day AJ. Complement factor H in host defense and immune evasion. *Cell Mol Life Sci.* (2017) 74:1605–24. doi: 10.1007/s00018-016-2418-4

7. Rodríguez-Rivera C, Garcia MM, Molina-Álvarez M, González-Martín C, Goicoechea C. Clusterin: always protecting. Synthesis, function and potential issues. *Biomed Pharmacother.* (2021) 134:111174. doi: 10.1016/j.biopha.2020.111174

8. Tárnok A, Hambsch J, Emmrich F, Sack U, van Son J, Bellinghausen W, et al. Complement activation, cytokines, and adhesion molecules in children undergoing cardiac surgery with or without cardiopulmonary bypass. *Pediatr Cardiol.* (1999) 20:113–25. doi: 10.1007/s002469900417

9. Michalski M, Pągowska-Klimek I, Thiel S, Świerzko AS, Hansen AG, Jensenius JC, et al. Factors involved in initiation and regulation of complement lectin pathway influence postoperative outcome after pediatric cardiac surgery involving cardiopulmonary bypass. *Sci Rep.* (2019) 9:2930. doi: 10.1038/s41598-019-39742-w

10. French LE, Tschopp J, Schifferli JA. Clusterin in renal tissue: preferential localization with the terminal complement complex and immunoglobulin deposits in glomeruli. *Clin Exp Immunol.* (1992) 88:389–93. doi: 10.1111/j.1365-2249.1992.tb06459.x

11. Kang YH, Urban BC, Sim RB, Kishore U. Human complement Factor H modulates C1q-mediated phagocytosis of apoptotic cells. *Immunobiology.* (2012) 217:455–64. doi: 10.1016/j.imbio.2011.10.008

12. Trouw LA, Bengtsson AA, Gelderman KA, Dahlbäck B, Sturfelt G, Blom AM. C4b-binding protein and factor H compensate for the loss of membrane-bound complement inhibitors to protect apoptotic cells against excessive complement attack. *J Biol Chem.* (2007) 282:28540–8. doi: 10.1074/jbc.AAC120.014609

13. Leffler J, Herbert AP, Norström E, Schmidt CQ, Barlow PN, Blom AM, et al. Annexin-II, DNA, and histones serve as factor H ligands on the surface of apoptotic cells. *J Biol Chem.* (2010) 285:3766–76. doi: 10.1074/jbc.M109.045427

14. Alic L, Papac-Milicevic N, Czamara D, Rudnick RB, Ozsvar-Kozma M, Hartmann A, et al. A genome-wide association study identifies key modulators of complement factor H binding to malondialdehyde-epitopes. *Proc Natl Acad Sci USA.* (2020) 117:9942–51. doi: 10.1073/pnas.1913970117

15. Kárpáti É, Papp A, Schneider AE, Hajnal D, Cserhalmi M, Csincsi ÁI, et al. Interaction of the Factor H Family Proteins FHR-1 and FHR-5 With DNA and dead cells: implications for the regulation of complement activation and opsonization. *Front Immunol.* (2020) 11:1297. doi: 10.3389/fimmu.2020.01297

16. Sjöberg AP, Trouw LA, Clark SJ, Sjölander J, Heinegård D, Sim RB, et al. The factor H variant associated with age-related macular degeneration (His-384) and the non-disease-associated form bind differentially to C-reactive protein, fibromodulin, DNA, and necrotic cells. *J Biol Chem.* (2007) 282:10894–900. doi: 10.1074/jbc.M610256200

17. Wang L, Cano M, Datta S, Wei H, Ebrahimi KB, Gorashi Y, et al. Pentraxin 3 recruits complement factor H to protect against oxidative stress-induced complement and inflammasome overactivation. *J Pathol.* (2016) 240:495–506. doi: 10.1002/path.4811

18. Bruins P, te Velthuis H, Yazdanbakhsh AP, Jansen PG, van Hardevelt FW, de Beaumont EM. Activation of the complement system during and after cardiopulmonary bypass surgery. *Circulation.* (1997) 96:3542–8. doi: 10.1161/01.cir.96.10.3542

19. Jansen PGM, te Velthuis H, Huybregts RAJM, Paulus R, Bulder ER, van der Spoel HI, et al. Reduced complement activation and improved postoperative performance after cardiopulmonary bypass with heparin-coated circuits. *J Thorac Cardiovasc Surg.* (1995) 110:829–34. doi: 10.1016/S0022-5223(95)70117-6

20. Jagdale A, Nguyen H, Li J, Burnette K, Ayares D, Cooper DKC, et al. Does expression of a human complement-regulatory protein on xenograft cells protect them from systemic complement activation? *Int J Surg.* (2020) 83:184–8. doi: 10.1016/j.ijsu.2020.09.034

21. Ehrnthaller C, Amara U, Weckbach S, Kalbitz M, Huber-Lang M, Bahrami S. Alteration of complement hemolytic activity in different trauma and sepsis models. *J Inflamm Res.* (2012) 5:59–66. doi: 10.2147/JIR.S31787

22. ClinicalTrials.gov. *Studying Complement Inhibition in Complex Cardiac Surgery (CARDIAC).* (2016). Available online at: https://clinicaltrials.gov/ct2/show/NCT02866825 (accessed February 15, 2017).

23. Carrier M, Ménasché P, Levy JH, Newman MF, Taylor KM, Haverich A, et al. Inhibition of complement activation by pexelizumab reduces death in patients undergoing combined aortic valve replacement and coronary artery bypass surgery. *J Thorac Cardiovasc Surg.* (2006) 131:352–6. doi: 10.1016/j.jtcvs.2005.10.011

24. Smith PK, Carrier M, Chen JC, Haverich A, Levy JH, Menasché P, et al. Effect of pexelizumab in coronary artery bypass graft surgery with extended aortic cross-clamp time. *Ann Thorac Surg.* (2006) 82:781–8. doi: 10.1016/j.athoracsur.2006.02.024

25. Smith PK, Shernan SK, Chen JC, Carrier M, Verrier ED, Adams PX, et al. Effects of C5 complement inhibitor pexelizumab on outcome in high-risk coronary artery bypass grafting: combined results from the PRIMO-CABG I and II trials. *J Thorac Cardiovasc Surg.* (2011) 142:89–98. doi: 10.1016/j.jtcvs.2010.08.035

26. Stravalaci M, Davi F, Parente R, Gobbi M, Bottazzi B, Mantovani A, et al. Control of complement activation by the long pentraxin PTX3: implications in age-related macular degeneration. *Front Pharmacol.* (2020) 11:591908. doi: 10.3389/fphar.2020.591908

27. Buntinx F, Niclaes L, Suetens C, Jans B, Mertens R, Van den Akker M. Evaluation of Charlson's comorbidity index in elderly living in nursing homes. *J Clin Epidemiol.* (2002) 55:1144–7. doi: 10.1016/s0895-4356(02)00485-7

28. Barie PS, Hydo LJ, Fischer E. Comparison of APACHE II and III scoring systems for mortality prediction in critical surgical illness. *Arch Surg.* (1995) 130:77–82. doi: 10.1001/archsurg.1995.01430010079016

29. Peres Bota D, Melot C, Lopes Ferreira F, Nguyen Ba V, Vincent JL. The Multiple Organ Dysfunction Score (MODS) versus the Sequential Organ Failure Assessment (SOFA) score in outcome prediction. *Intensive Care Med.* (2002) 28:1619–24. doi: 10.1007/s00134-002-1491-3

30. Cuenca AG, Maier RV, Cuschieri J, Moore EE, Moldawer LL, Tompkins RG, et al. The glue grant experience: characterizing the post injury genomic response. *Eur J Trauma Emerg Surg.* (2011) 37:549–58. doi: 10.1007/s00068-011-0148-8

31. Venkataraman R, Kellum JA. Defining acute renal failure: the RIFLE criteria. *J Intensive Care Med.* (2007) 22:187–93. doi: 10.1177/0885066607299510

32. R Core Team. R: a Language and Environment for Statistical Computing. Vienna: *R Foundation for Statistical Computing* (2021).

33. Speidl WS, Exner M, Amighi J, Kastl SP, Zorn G, Maurer G, et al. Complement component C5a predicts future cardiovascular events in patients with advanced atherosclerosis. *Eur Heart J.* (2005) 26:2294–9. doi: 10.1093/eurheartj/ehi339

34. Pągowska-Klimek I, Świerzko AS, Michalski M, Głowacka E, Szala-Poździej A, Sokołowska A, et al. Activation of the lectin pathway of complement by cardiopulmonary bypass contributes to the development of systemic inflammatory response syndrome after paediatric cardiac surgery. *Clin Exp Immunol.* (2016) 184:257–63. doi: 10.1111/cei.12763

35. Limnell V, Aittoniemi J, Vaarala O, Lehtimäki T, Laine S, Virtanen V, et al. Association of mannan-binding lectin deficiency with venous bypass graft occlusions in patients with coronary heart disease. *Cardiology.* (2002) 98:123–6. doi: 10.1159/000066313

36. Qian Z, Hu W, Liu J, Sanfilippo F, Hruban RH, Baldwin WMIII. Accelerated graft arteriosclerosis in cardiac transplants: complement activation promotes progression of lesions from medium to large arteries. *Transplantation.* (2001) 72:900–6. doi: 10.1097/00007890-200109150-00027

37. McGrath FDG, Brouwer MC, Arlaud GJ, Daha MR, Hack CE, Roos A. Evidence that complement protein C1q interacts with C-reactive protein through its globular head region. *J Immunol.* (2006) 176:2950–7. doi: 10.4049/jimmunol.176.5.2950

38. Schwaeble W, Zwirner J, Schulz TF, Linke RP, Dierich MP, Weiss EH. Human complement factor H: expression of an additional truncated gene product of 43 kDa in human liver. *Eur J Immunol.* (1987) 17:1485–9. doi: 10.1002/eji.1830171015

39. Patel JA, Ghatak SB. Pexelizumab and its role in the treatment of myocardial infarction and in coronary artery bypass graft surgery: a review. *Recent Pat Cardiovasc Drug Discov.* (2008) 3:145–52. doi: 10.2174/157489008784705322

40. Salameh A, Dhein S, Dähnert I, Klein N. Neuroprotective strategies during cardiac surgery with cardiopulmonary bypass. *Int J Mol Sci.* (2016) 17:1945. doi: 10.3390/ijms17111945

41. Chun N, Haddadin AS, Liu J, Hou Y, Wong KA, Lee D, et al. Activation of complement factor B contributes to murine and human myocardial ischemia/reperfusion injury. *PLoS One.* (2017) 12:e0179450. doi: 10.1371/journal.pone.0179450

frontiers | Frontiers in Cardiovascular Medicine

TYPE Original Research
PUBLISHED 07 February 2023
DOI 10.3389/fcvm.2023.1008825

OPEN ACCESS

EDITED BY
Shahzad Raja,
Harefield Hospital, United Kingdom

REVIEWED BY
Ramesh Daggubati,
West Virginia University, United States
Shiho Naito,
University Medical Center Hamburg-Eppendorf, Germany
Aveek Jayant,
Homi Bhabha Cancer Hospital and Research Centre, India

*CORRESPONDENCE
Ivar Risnes
✉ ivar.risnes@lovisenberg.no

SPECIALTY SECTION
This article was submitted to Heart Surgery, a section of the journal Frontiers in Cardiovascular Medicine

RECEIVED 01 August 2022
ACCEPTED 16 January 2023
PUBLISHED 07 February 2023

CITATION
Risnes I, Aukrust P, Lundblad R, Rødevand O, Ueland T, Rynning SE and Saeed S (2023) Increased levels of NT-proBNP and troponin T 2 years after coronary artery bypass grafting complicated by mediastinitis. *Front. Cardiovasc. Med.* 10:1008825. doi: 10.3389/fcvm.2023.1008825

Increased levels of NT-proBNP and troponin T 2 years after coronary artery bypass grafting complicated by mediastinitis

Ivar Risnes[1,2,3]*, Pål Aukrust[4,5,6], Runar Lundblad[1,2], Olaf Rødevand[1], Thor Ueland[4,5], Stein Erik Rynning[3] and Sahrai Saeed[3]

[1]Department of Cardiac Surgery, LHL Heart Clinic, Gardermoen, Norway, [2]Department of Thoracic and Cardiovascular Surgery, Oslo University Hospital, Rikshospitalet, Oslo, Norway, [3]Department of Heart Disease, Haukeland University Hospital, Bergen, Norway, [4]Research Institute of Internal Medicine, Oslo University Hospital, Rikshospitalet, Oslo, Norway, [5]Institute of Clinical Medicine, Faculty of Medicine, University of Oslo, Oslo, Norway, [6]Section of Clinical Immunology and Infectious Diseases, Oslo University Hospital, Rikshospitalet, Oslo, Norway

Background: Mediastinitis after coronary bypass grafting (CABG) increases the risk of the internal mammary artery (IMA) graft obstruction, and has a detrimental effect on long-term survival. The pathogenesis for this increased mortality is poorly understood. In the present study, we aimed to investigate the relationship between mediastinitis and persistently elevated cardiac-specific biomarkers [troponin T (TnT) and N-terminal pro-brain natriuretic peptide (NT-proBNP)] and C-reactive protein (CRP) at mid-term follow-up following CABG.

Material and methods: The epidemiologic design was of an exposed (mediastinitis, $n = 41$) vs. randomly selected non-exposed (non-mediastinitis) controls ($n = 41$) cohort. Serum samples for measurements of NT-proBNP, TnT, and CRP were obtained at a median follow up time of 2.7 (range 0.5–5.2) years after CABG surgery.

Results: NT-proBNP (mean 65.0 pg/ml vs. 34.8 pg/ml, $p = 0.007$) and TnT levels (mean 14.7 ng/L vs. 11.2 ng/L, $p = 0.004$) were significantly higher in the mediastinitis group than in the control group. Patients with mediastinitis had also higher body mass index (BMI) and were more likely to have diabetes and previous myocardial infarction. There was no difference in serum CRP level between the groups. After controlling for potential confounders (previous myocardial infarction, age, and BMI), the presence of mediastinitis was associated with higher levels of log NT-proBNP ($p = 0.02$) and log TnT ($p = 0.04$).

Conclusion: Mediastinitis increases the concentrations of cardiac-specific biomarkers NT-proBNP and TnT at mid-term follow-up, representing persistent myocardial injury and impaired cardiac function.

KEYWORDS

cardiac biomarkers in mediastinitis following coronary artery bypass grafting, coronary artery bypass grafting, C-reactive protein, mediastinitis, N-terminal probrain natriuretic peptide, troponin T

Introduction

The incidence of mediastinitis after coronary artery bypass grafting (CABG) is low; around 1%. However, this feared complication is associated with an increased risk of morbidity and decreased long-term survival (1–4). In addition, the risk of cardiac death is significantly higher in patients with mediastinitis compared to patients without mediastinitis (1). The mechanism for development of mediastinitis and its complications is multifactorial, and not fully understood

(1–5). Mediastinitis increases the risk of early internal mammary artery (IMA) graft obstruction (6), which may be due to inflammation or mechanical damage to the IMA. IMA graft failure is probably one of several factors that explains cardiac death after mediastinitis. N-terminal pro-brain natriuretic peptide (NT-proBNP) is synthesized and released from the myocardium in response to increased wall stress and injury (7, 8). The serum levels of NT-proBNP are increased in patients with heart failure (HF), and have been shown to provide important prognostic information in these patients (9, 10). Similarly, higher concentrations of troponin T (TnT), a specific marker of cardiomyocyte damage, have been shown to be prognostically important, not only in patients with myocardial infarction (MI) and unstable coronary artery disease (CAD), but also in patients with stable CAD and in patients with HF (11, 12).

C-reactive protein (CRP) is a stable and reliable marker of inflammation, and has been shown to give prognostic information in a wide number of atherosclerotic disorders, as well as HF, reflecting the involvement of inflammatory pathways in these disorders (13, 14). Numerous studies have shown that NT-proBNP, TnT and CRP provide important prognostic information in various cardiovascular disorders. However, whether these markers are regulated by mediastinitis following CABG, is not fully elucidated. Therefore, in the present study, we examined the levels of NT-proBNP, TnT and CRP following CABG in patients with and without complicating mediastinitis.

Materials and methods

Study population

Between September 2005 and April 2010, a total of 6,620 patients >18 years undergoing CABG surgery were considered as the source population for the present study. In this period, patients suffering from deep sternal wound infection were treated with vacuum-assisted closure (VAC). The diagnosis was based upon the criteria established by the Centres for Disease Control and Prevention (www.cdc.gov) (15, 16).

Epidemiological design

This is a cohort study of 82 patients undergoing CABG surgery. By epidemiological design, the study included 41 exposed (mediastinitis) and 41 non-exposed (non-mediastinitis), controls. Blood samples were collected and analyzed for; (1) NT-proBNP, (2) TnT and (3) CRP. Blood samples were obtained at routine examination at a median follow up time of 2.7 (range, 0.5–5.2) years after primary CABG surgery. All CABG patients who had developed post-operative mediastinitis during 4.7 years follow-up, from September 2005 to April 2010, were considered as exposed patients. The non-exposed cohort was a random control sample of 41 patients without mediastinitis (Figure 1), collected from the same source population and the same period of time (17). Evaluation of the laboratory markers was performed during a period of nine months, from April to December 2010. Median observation time from primary CABG to laboratory control and coronary CT angiography was 2.6 (range 0.5–5.2) years in the mediastinitis and 2.8 (range 0.7–4.6) years in the control group ($p = 0.87$) (Figure 1). Evaluation of the endpoints was performed *via* coronary CT angiography. The evaluation of the images was performed by two independent radiologists blinded to the status of the patients and exposition to mediastinitis. Surgical technique for the CABG operation, surgical revision of mediastinitis and anesthesia were performed as previous described (1, 6). All cases of cardiac surgery were performed with cardiopulmonary bypass machine and no Off-Pump method was used.

NT-proBNP, TnT, and CRP analyses

The levels of TnT were determined by a third-generation high-sensitivity assay on an Elecsys (Roche Diagnostic, Basel Switzerland), with a level of detection of 0.01 μg/L. CRP concentrations were measured with a chemiluminescent enzyme-labeled immunometric high-sensitivity assay (Immulite CRP; Diagnostic Products Corp), with a limit of detection of 0.1 mg/L. Serum NT-proBNP was determined with a sandwich immunoassay on an Elecsys 2010 (Roche Diagnostics).

Statistical methods

The IBM SPSS 20.0 software (IBM Corporation, Armonk, New York) was used for data management and analysis. Data are presented as mean ± SD for continuous variables and as percentages for categorical variables. NT-proBNP, CRP, and TnT were not normally distributed and were log-transformed. Differences in the level of NT-proBNP, CRP, and TnT between mediastinitis patients and controls were assessed using a non-parametric statistic (Mann Whitney U test) as the distribution of those markers were not Gaussian and very skewed (18). Association between the presence of mediastinits and the three markers was done *via* the multivariate linear regression models to control for the variables with confounding effect in the relationship between presence of mediastinitis and outcome. All markers were logged when considered as outcomes in the multivariate model (19). $P < 0.05$ (two-sided) were considered statistically significant.

Results

This was a cohort study of exposed (mediastinitis) and non-exposed (random controls without mediastinitis) patients chosen from the same source population. Baseline characteristics are presented in Table 1. There was no difference in age, gender, New York Heart Association (NYHA) functional class, and left ventricular ejection fraction (EF) between the groups, but body mass index (BMI) was significantly higher in patients with mediastinitis. Patients with mediastinitis had also higher burden of previous MI and diabetes (Table 1). The need for blood transfusion in relation to the CABG procedure was also significantly higher in patients with mediastinitis ($p = 0.03$). In contrast to these differences, there was no difference in number of stenotic coronary arteries with 2.7 (±0.5) in the mediastinitis group and 2.8 (±0.4) in the control group (mean ± SD, $p = 0.71$).

10.3389/fcvm.2023.1008825

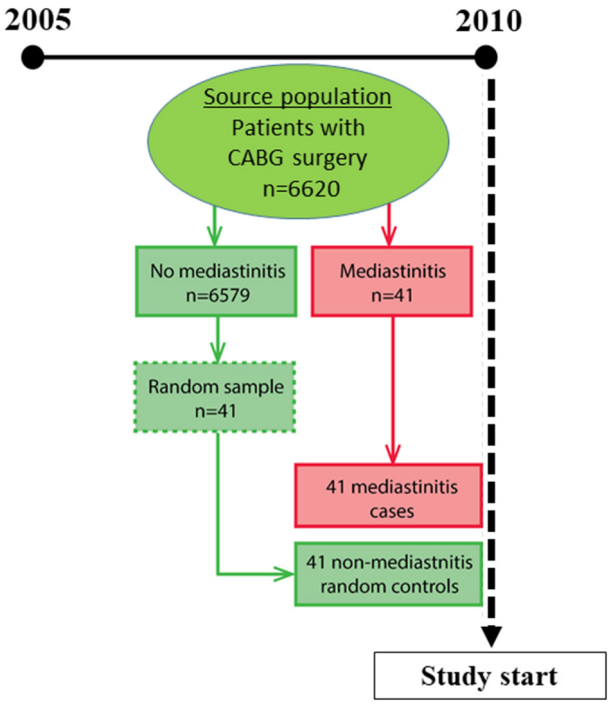


FIGURE 1
Study design.

TABLE 1 Clinical profile of patients with mediastinitis and random controls without mediastinitis.

	Mediastinitis ($n = 41$)	Controls ($n = 41$)	p-value
Preoperative			
Age (years)	67.0 (±9.9)	64.0 (±9.9)	0.30
Male gender (%)	87.8	78.0	0.13
Body mass index (kg/m^2)	30.1 (±4.0)	27.0 (±3.9)	<0.01
Hypertension (%)	64	61	0.60
NYHA (III–IV) (%)	68.2	49.1	0.09
Ejection fraction (%)	60 (±14.5)	66.2 (±9.9)	0.29
Left main stenosis (%)	31.7	26.8	0.28
Emergency surgery (%)	39.0	24.4	0.12
Unstable angina (%)	43.9	24.4	0.05
Previous myocardial infarction (%)	65.9	34.1	<0.01
Preoperative atrial fibrillation (%)	17.1	9.8	0.52
Chronic obstructive pulmonary disease (%)	22.0	14.6	0.28
Diabetes (%)	41.5	7.3	<0.01
Operative			
Aortic cross-clamp time (min)	36.1 (±15.6)	32.5 (±13.9)	0.32
Cardio-pulmonary bypass time (min)	60.8 (±28.5)	50.6 (±18.5)	0.12
Postoperative			
Mediastinal drainage (ml)	740 (±422)	695 (±278)	0.77
Blood transfusion (units)	1.2 (±2.6)	0.2 (±0.8)	0.03

Differences in NT-proBNP, TnT, and CRP level between the study groups

Patients with mediastinitis following CABG had significantly higher levels of NT-proBNP as compared with those without this complication 65.0 (±63.6) pmol/l vs. 34.8 (±34.8) pmol/l (mean ± SD, $p = 0.007$) (Figure 2). A similar pattern was seen for TnT with the highest levels in those with mediastinitis 14.7 (±6.8) ng/l vs. 11.2 (±2.7) ng/l (mean ± SD, $p = 0.004$), but not for CRP ($p = 0.14$) (Figure 2).

Multivariate analysis controlling for confounders

Table 2 summarizes the results of the univariate and multivariate linear regression models. In separate models after controlling for the potential confounders (i.e., previous MI, age, and BMI), the presence of mediastinitis was associated with higher log NT-proBNP ($p = 0.02$) and log TnT ($p = 0.04$).

Discussion

In the present study we showed that patients with VAC-treated mediastinitis after CABG, had significantly higher serum levels of NT-proBNP and TnT, but not of CRP, compared with patients without mediastinitis even 2.7 years (median) following CABG. Cardiac biomarkers like NT-proBNP and TnT are useful for prediction of cardiac events and mortality (7–14), and raised levels in the mediastinitis group may at least partly explain the observed decreased long-term survival in this group of patients.

Mediastinitis is a severe complication after CABG, and is associated with increased risk of early and late cardiovascular death (1). In a prospective study of 5,185 patients undergoing cardiac surgery, 41 patients had mediastinal infection. Among these, 16 patients had isolated CABG and 8 combined with valve intervention (20). Mediastinal infection after cardiac operation was associated with longer hospital stay, readmissions, and death. However, the frequency of graft obstruction and its association with prognosis was not studied. Based on our previous observations in this cohort (6), we hypothesized that inflammation pathway may be a major contributor to the pathogenesis of atherosclerotic obstruction of the IMA graft, and this might explain why postoperative mediastinitis is a major contributor of reduced long-term survival after CABG.

Levels of NT-proBNP rise with increasing degree of cardiac wall stress secondary to ventricular enlargement and maladaptive cardiac remodeling seen in patients with HF and after MI (21). Importantly, however, NT-proBNP is secreted in response to raised intra-cardiac pressure and wall stress irrespective of the underlying cause (7–10). TnT is a sensitive marker of cardiomyocyte damage following necrosis or apoptosis, and raised levels have been shown to

10.3389/fcvm.2023.1008825

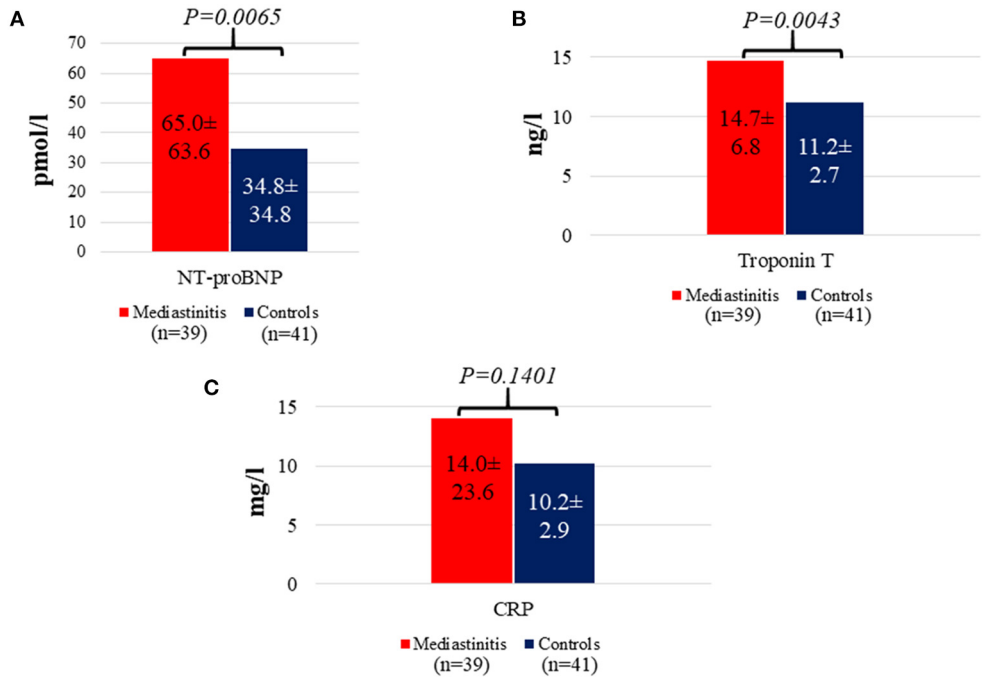


FIGURE 2
Distribution of NT-proBNP **(A)**, Troponin T **(B)**, and CRP concentrations **(C)** in patients with mediastinitis versus controls. Date are presented here as mean ± SD.

provide important prognostic information in various cardiovascular disorders including MI, stable CAD and HF (11, 12). In the present study, we show that patients with mediastinitis following CABG have elevated levels of NT-proBNP and TnT more than 2 years following CABG. These findings suggest that patients with mediastinitis may have persistent myocardial damage and impaired cardiac function, potentially contributing to the poorer long-term prognosis in these patients that also include increased occurrence of cardiac death (1).

Although the exact reasons for the increased levels of NT-proBNP and TnT in patients with mediastinitis remain unknown, a possible explanation may be the significantly higher prevalence of cardiometabolic risk factors in the mediastinitis group. It is well-established that metabolic syndrome leads to structural and functional left ventricular remodeling, preclinical or clinical systolic and/or diastolic dysfunction and fibrosis (22). However, elevated levels of TnT and NT-proBNP were also found after adjusting for BMI, suggesting that our findings may not merely reflect an increased prevalence of cardiometabolic risk in patients with mediastinitis. It is also important to note that NT-proBNP has been seen to be affected by other factors such as anemia (23) and renal failure (24) that was not adjusted for in the present study. Furthermore, the prevalence of prior MI was as twice as common in patients with mediastinitis compared with controls. Hence, silent/residual coronary ischemia, particularly due to microvascular disease, in patients with mediastinitis may also be a possible reason for increased TnT. However, all patients were clinically stable and asymptomatic, and underlying silent coronary ischemia was not assessed by stress echocardiography or other imaging modalities. Nonetheless, this highlights the importance of optimal cardioprotective medications including statin and anti-angina and antihypertensive medications following CABG surgery.

Persistent mediastinitis *per se* as an inflammatory condition could potentially be a contributing factor, but the lack of difference in CRP levels between the two groups, makes this possibility unlikely. However, low-grade myocardial inflammation that is not reflected by systemic CRP levels cannot be excluded. Moreover, mediastinitis may lead to an accelerated coronary ischemia and artery spasm, potentially contributing to ischemic cardiomyopathy. Also, the presence of mediastinitis in the early period following CABG, increases the risk of IMA-graft obstruction, but not saphenous vein grafts (SVG) (6). The mechanism of mediastinitis-induced IMA failure, which in turn could induce myocardial dysfunction, may be mechanical damage due to repetitive sternal revision or inflammatory reaction to the IMA with subsequent occlusion. Whatever the mechanisms, our findings suggest that mediastinitis following CABG may induce myocardial dysfunction that could be evident even more than 2 years following CABG surgery.

The present study has some limitations. First, our study groups are relatively small with some additional methodological limitations such as the retrospective design and lack of long-term outcome data; however we believe our results are of topical interest, hypothesis generating and should trigger lager prospective studies with longer follow-up to investigate the association of post-CABG mediastinitis with cardiac biomarkers as well as with serum biomarkers of inflammation. Second, serum biomarkers were retrospectively collected and the study lacks some relevant data. Therefore, our conclusions should be interpreted cautiously. Third, the lack of data on mortality and morbidity, including cardiac related hospitalizations and the incidence of recurrent

TABLE 2 Univariate and multivariable-adjusted effect of mediastinits on log NT-proBNP and log troponin-T.

	Coefficient	SE (coefficient)	Statistics-Z	*p*-value
Endpoint log NT-proBNP				
Univariate				
Mediastinitis (yes/no)	0.6845	0.2251	3.04	0.030
Multivariable				
Mediastinitis (yes/no)	0.5200	0.2180	2.39	0.020
Previous MI	0.4916	0.2028	2.42	0.018
Age	0.0465	0.0099	4.71	<0.001
BMI	−0.0403	0.0251	−1.61	0.090
Endpoint log troponin-T				
Univariate				
Mediastinitis (yes/no)	0.1962	0.6715	2.92	0.005
Multivariable				
Mediastinitis	0.1483	0.0710	2.09	0.040
Previous MI	0.1470	0.0661	2.22	0.029
Age	0.0090	0.0032	2.80	0.007
BMI	−0.0058	0.0816	−0.71	0.010

BMI, body mass index; MI, myocardial infarction.

angina. Fourth, only baseline left ventricular EF by echocardiography was included. Data on global longitudinal strain derived by speckle-tracking echocardiography or diastolic dysfunction were not available. Similarly, echocardiographic data during follow-up was not collected. Fifth, cardiac magnetic resonance (CMR) imaging is a gold standard for assessment of persistent myocardial inflammation, subendocardial injury and scar and fibrosis. Our findings suggest that mediastinitis following CABG surgery could induce a degree of myocardial dysfunction potentially contributing to the increase in short- and long-term mortality in these patients. However, CMR was not included in the present study. Larger prospective studies in the future that also include echocardiography and CMR should examine whether elevated TnT and NT-proBNP concentration could be used as prognostic markers for long term survival after CABG.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

The study was approved by the Regional Medical Ethics Committee, and represents a multi-center collaboration between Oslo University Hospital, Rikshospitalet, Feiring Heart Clinic, Oslo Heart Center and Akershus University Hospital. The institutions maintain the same diagnostic criteria and treatment with active and prospective epidemiologic surveillance of hospital infections. Written informed consent was obtained from all participants.

Author contributions

IR: data collection, project administration, and original draft. IR, PA, and SS: conceptualization, formal analysis, methodology, supervision, validation, visualization, and writing. IR and SS: literature search. RL, OR, TU, and SR: review and editing. PA and SS: critically revised the manuscript. All authors read and approved the final version before submission.

Acknowledgments

The authors would particularly like to thank Ulla Bella and Mona Bekken Vold at Feiring Heart Center for continuous support and for their contribution in organizing the data. We are indebted to the chief of Oslo Heart Center, Eivind Øvrum, MD, PhD, Nihal D. Perera, MSCI and Rolf Øystese, CCPP for computer and programming assistance.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated

organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

1. Risnes I, Abdelnoor M, Almdahl SM, Svennevig JL. Mediastinitis after coronary artery bypass grafting risk factors and long term survival. *Ann Thorac Surg.* (2010) 89:1502–10. doi: 10.1016/j.athoracsur.2010.02.038

2. Milano CA, Kesler K, Archbald N, Sexton DJ, Jones RH. Mediastinitis after coronary bypass graft surgery. Risk factors and long-term survival. *Circulation.* (1995) 92:2245–51. doi: 10.1161/01.CIR.92.8.2245

3. Abbod CS, Wey SB, Baltar VT. Risk factors for mediastinitis after cardiac surgery. *Ann Thorac Surg.* (2004) 77:676–83. doi: 10.1016/S0003-4975(03)01523-6

4. Loop FD, Lytle BW, Cosgrove DM, Stewart RW, Goormastic M, Williams GW, et al. Influence of the internal-mammary-artery graft on 10-year survival and other cardiac events. *N Engl J Med.* (1986) 314:1–6. doi: 10.1056/NEJM198601023140101

5. Ross R. Atherosclerosis—an inflammatory disease. *N Engl J Med.* (1999) 340:115–26. doi: 10.1056/NEJM199901143400207

6. Risnes I, Abdelnoor M, Ulimoen G, Rynning SE, Veel T, Svennevig JL, et al. Mediastinitis after coronary artery bypass grafting increases the incidence of left internal mammary artery obstruction. *Int Wound J.* (2014) 11:594–600. doi: 10.1111/iwj.12007

7. Bay M, Kirk V, Parner J, Hassager C, Nielsen H, Krogsgaard K, et al. NT-proBNP. A new diagnostic screening tool to differentiate between patients with normal and reduced left ventricular function. *Heart.* (2003) 89:150–4. doi: 10.1136/heart.89.2.150

8. Campbell DJ, Mitchelhill KI, Schlicht SM, Booth RJ. Plasma amino-terminal pro-brain natriuretic peptide: a novel approach to the diagnosis of cardiac dysfunction. *J Card Fail.* (2000) 6:130–9. doi: 10.1016/S1071-9164(00)90015-0

9. Omland T, Aakvaag A, Bonarjee VV, Caidahl K, Lie RT, Nilsen DW, et al. Plasma brain natriuretic peptide as an indicator of left ventricular function and long-term survival after acute myocardial infarction. Comparison with plasma atrial natriuretic peptide and N-terminal proatrial atriuretic peptide. *Circulation.* (1996) 93:1963–9. doi: 10.1161/01.CIR.93.11.1963

10. Richards AM, Nicholls MG, Yandle TG, Frampton C, Espiner EA, Turner JG, et al. Plasma N-termianl pro-brain natriuretic peptide and adrenomedullin: new neurohormonal predictors of left ventricular function and prognosis after myocardial infarction. *Circulation.* (1998) 97:1921–9. doi: 10.1161/01.CIR.97.19.1921

11. Saunders JT, Nambi V, de Lemos JA, Chambless LE, Virani SS, Boerwinkle E, et al. Cardiac troponin T measured by a highly sensitive assay predicts coronary heart disease, heart failure, and mortality in the atherosclerosis risk in communities study. *Circulation.* (2011) 123:1367–76. doi: 10.1161/CIRCULATIONAHA.110.005264

12. Lindahl B, Venge P, Wallentin L. Relation between troponin T and risk subsequent cardiac events in unstable coronary artery disease. The FRISC study group. *Circulation.* (1996) 93:1651–7. doi: 10.1161/01.CIR.93.9.1651

13. Roivainen M, Viik-Kajander M, Palosuo T, Toivanen P, Leinonen M, Saikku P, et al. Infection, inflammation, and the risk of coronary heart disease. *Circulation.* (2000) 101:252–7. doi: 10.1161/01.CIR.101.3.252

14. Libby P, Egan D, Skarlatos S. Roles of infectious agents in atherosclerosis and restenosis: an assessment of the evidence and need for future research. *Circulation.* (1997) 96:4095–103. doi: 10.1161/01.CIR.96.11.4095

15. Garner GS, Jarvis WR, Emori TG, Horan TC, Hughes JM. CDC definitions for nosocomial infections. *Am J Infect Control.* (1988) 16:128–40. doi: 10.1016/0196-6553(88)90053-3

16. El Oakley RM, Wright JE. Postoperative mediastinitis: classification and management. *Ann Thorac Surg.* (1996) 61:1030–6. doi: 10.1016/0003-4975(95)01035-1

17. Rothman K. *Modern Epidemiology, Volume 316.* Little Brown and Company: Boston, TN, 75776–4.

18. Henderson WG, Moritz T, Goldman S, Copeland J, Souchek J, Zadina K, et al. The statistical analysis of graft patency data in a clinical trial of antiplatelet agents following coronary artery bypass grafting. *Control Clin Trials.* (1988) 9:189–205. doi: 10.1016/0197-2456(88)90060-8

19. Kleinbaun DG, Mitchell K. *Logistic Regression. Statistics for Biology and Health.* Berlin: Springer (2010).

20. Perrault LP, Kirkwood KA, Chang HL, Mullen JC, Gulack BC, Argenziano M, et al. A prospective multi-institutional cohort study of mediastinal infections after cardiac operations. *Ann Thorac Surg.* (2018) 105:461–8. doi: 10.1016/j.athoracsur.2017.06.078

21. Bax JJ, Di Carli M, Narula J, Delgado V. Multimodality imaging in ischaemic heart failure. *Lancet.* (2019) 393:1056–70. doi: 10.1016/S0140-6736(18)33207-0

22. Saeed S, Waje-Andreassen U, Nilsson PM. The association of the metabolic syndrome with target organ damage: focus on the heart, brain, and central arteries. *Expert Rev Cardiovasc Ther.* (2020) 18:601–14. doi: 10.1080/14779072.2020.1807327

23. Karakoyun I, Colak A, Arslan FD, Hasturk AG, Duman C. Anemia considerations when assessing natriuretic peptide levels in ED patients. *Am J Emerg Med.* (2017) 35:1677–81. doi: 10.1016/j.ajem.2017.05.048

24. Yang WL, Fahim M, Johnson DW. Pathophysiology and significance of natriuretic peptides in patients with end-stage kidney disease. *Clin Biochem.* (2020) 83:1–11. doi: 10.1016/j.clinbiochem.2020.05.013

frontiers | Frontiers in Cardiovascular Medicine

TYPE Systematic Review
PUBLISHED 20 February 2023
DOI 10.3389/fcvm.2023.1093363

OPEN ACCESS

EDITED BY
Shahzad Raja,
Harefield Hospital, United Kingdom

REVIEWED BY
Emanuele Durante-Mangoni,
University of Campania Luigi Vanvitelli, Italy
Alberto Albertini,
Maria Cecilia Hospital, Italy

*CORRESPONDENCE
Victoria Rizzo
victoria.rizzo@nhs.net

SPECIALTY SECTION
This article was submitted to Heart Surgery, a section of the journal Frontiers in Cardiovascular Medicine

RECEIVED 08 November 2022
ACCEPTED 01 February 2023
PUBLISHED 20 February 2023

CITATION
Rizzo V, Salmasi MY, Sabetai M, Primus C, Sandoe J, Lewis M, Woldman S and Athanasiou T (2023) Infective endocarditis: Do we have an effective risk score model? A systematic review.
Front. Cardiovasc. Med. 10:1093363.
doi: 10.3389/fcvm.2023.1093363

Infective endocarditis: Do we have an effective risk score model? A systematic review

Victoria Rizzo[1]*, Mohammad Yousuf Salmasi[2], Michael Sabetai[1], Christopher Primus[3], Jonathan Sandoe[4], Michael Lewis[5], Simon Woldman[3] and Thanos Athanasiou[2]

[1]Cardiothoracic Surgery, St. Thomas Hospital, Guy's and St Thomas' NHS Foundation Trust, London, United Kingdom, [2]Department of Cardiothoracic Surgery, Hammersmith Hospital, Imperial College Healthcare NHS Trust, London, United Kingdom, [3]Specialised Cardiology, St Bartholomew's Hospital, Barts Health NHS Trust, London, United Kingdom, [4]Department of Microbiology, Leeds Teaching Hospitals NHS Trust, Leeds, United Kingdom, [5]Department of Cardiothoracic Surgery, Royal Sussex County Hospital, Brighton and Sussex University Hospitals NHS Trust, Brighton, United Kingdom

Background: Infective endocarditis (IE) is a rare, highly morbid condition with 17% in-hospital mortality. A total of 25–30% require surgery and there is ongoing debate with regard to markers predicting patient outcomes and guiding intervention. This systematic review aims to evaluate all IE risk scores currently available.

Methods: Standard methodology (PRISMA guideline) was used. Papers with risk score analysis for IE patients were included, with attention to studies reporting area under the receiver-operating characteristic curve (AUC/ROC). Qualitative analysis was carried out, including assessment of validation processes and comparison of these results to original derivation cohorts where available. Risk-of-bias analysis illustrated according to PROBAST guidelines.

Results: Of 75 articles initially identified, 32 papers were analyzed for a total of 20 proposed scores (range 66–13,000 patients), 14 of which were specific for IE. The number of variables per score ranged from 3 to 14 with only 50% including microbiological variables and 15% including biomarkers. The following scores had good performance (AUC $>$ 0.8) in studies proposing the score (often the derivation cohort); however fared poorly when applied to a new cohort: PALSUSE, DeFeo, ANCLA, RISK-E, EndoSCORE, MELD-XI, COSTA, and SHARPEN. DeFeo score demonstrated the largest discrepancy with initial AUC of 0.88, compared to 0.58 when applied to different cohorts. The inflammatory response in IE has been well documented and CRP has been found to be an independent predictor for worse outcomes. There is ongoing investigation on alternate inflammatory biomarkers which may assist in IE management. Of the scores identified in this review, only three have included a biomarker as a predictor.

10.3389/fcvm.2023.1093363

Conclusion: Despite the variety of available scores, their development has been limited by small sample size, retrospective collection of data and short-term outcomes, with lack of external validation, limiting their transportability. Future population studies and large comprehensive registries are required to address this unmet clinical need.

KEYWORDS

endocarditis, outcome assessment, risk score, risk factors, cardiac surgery

Introduction

Infective endocarditis (IE) is a rare, highly morbid condition, affecting 6.8 patients per 100,000 per year in the United Kingdom (UK) (1), with an in-hospital mortality rate of 17.1% (2). In the 2019 EURO-ENDO registry data, almost 70% of patients had a theoretical indication for surgery with 51% undergoing surgical intervention (2). The aim of surgery in this group of patients is removal of the vegetation/infection source and repair/replacement of the valve involved to restore function (3). Despite the advances in diagnostic testing, antibiotic therapy and surgical techniques, the incidence and mortality of IE has remained largely the same over the past 30 years (4).

By virtue of its pathophysiology, care of IE patients requires a multi-specialty approach, involving cardiologist, microbiologist, surgeon, intensivist and imaging specialist at the very least. The 2015 ESC (European Society of Cardiology) Guidelines for the management of IE emphasize this approach in the form of an "endocarditis team," recommending prognostic assessment based on clinical, microbiological and echocardiographic data (5). However, according to both literature and clinical practice, there is no prognostic tool available that encompasses these three levels of information, collected within 48–72 h from admission.

The need for a modern, comprehensive and widely applicable predictive score for risk stratification of this diverse patient group is essential for decision-making within the Endocarditis Team. A validated risk score encompassing the triad of clinical, microbiological and imaging characteristics (5) would be a useful tool to help define prognosis and management.

Published risk scores have been limited to small, very specific patient groups spanning a long period of time. In addition, many of the scores have been developed specifically for surgical cohorts, treated in tertiary centers, excluding patients with implantable cardiac devices or prosthetic valves.

This systematic review aims to synthesize the data on predictive models reported in the literature intended to guide management decisions during the acute care of adults with IE and assess their reported performance in the clinical setting. This data will highlight areas for development and improved data analysis for IE patients, as well as provide a framework for ongoing research in the identification of a comprehensive predictive score.

Materials and methods

Standard methodology for systematic review as per Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines which can be accessed on *prisma-statement.org*.

Search for existing literature in Medline (*via* PubMED) and EMBASE (*via* OVID) databases with the keywords: < "infective endocarditis" AND "risk score" > from inception until May 2021. Records were independently assessed by two separate reviewers and cross-referenced with the senior reviewer in order to reach concordance. Reference lists from relevant studies were also analyzed for suitable research titles.

Studies included involved scoring systems intended to guide treatment during the acute care of adults with IE, such as the need of surgical intervention. Publications which included risk score analysis and assessment were included. The area under the receiver operating characteristic curve (AUC/ROC) for each risk score with details of patient cohorts and their corresponding data were extracted for each paper. AUC compares the sensitivity (true positives) with the specificity (false positives), thus assessing performance and determining accuracy of the multi-factor risk scores (6). Studies describing development only, development and validation and validation only were all included in the review. Studies comparing different scores when applied to new populations were included. Studies reporting non-validated risk scores were also included.

Studies of pediatric or congenital populations, analysis of timing of surgery for IE, assessment of dental practice, case reports and literature reviews were excluded from the review. Studies of scoring systems designed to aid the diagnosis, investigation or prevention of IE were excluded. Research that was only represented by a conference abstract were excluded due to lack of detail for comparison purposes.

Data were extracted according to a structured protocol and included patient demographics, clinical covariates, microbiological results and imaging criteria. Reported outcomes were documented, including mortality and morbidity numerators. Details of risk score assessment, by means of AUC/ROC analysis, were collected and used to qualitatively compare score performance, including sensitivity and specificity where reported.

The scores extracted were individually assessed for risk of bias and applicability to our review using the Prediction model Risk Of Bias Assessment Tool (PROBAST) (7).

Confirmed IE was defined by modified Duke's Criteria as described by Li et al. (8). The definitions of variables included were the same as those described for the EuroSCORE II model and are elaborated in the tabulated results. Active IE was defined as patients undergoing antibiotic therapy at the time of analysis. Any instance where the definition varied from the above has been described.

Results

A total of 33 studies were included in the qualitative synthesis based on the inclusion criteria (PRISMA Diagram 1). There were a total of 20 relevant scores, with 14/20 being derived from and created specifically for IE populations: **Table 1** (3, 9–20). Non-specific scores are tabulated in **Table 2** (3, 9–12, 14, 21–27) and include EuroSCORE I and II, Society of Thoracic Surgery (STS) risk score, Ontario province risk (OPR) score, Charlson Co-Morbidity Index and Sequential Organ Failure Assessment (SOFA) scores. STS for IE score was considered with non-specific scores since although IE patients were considered, no IE specific characteristics (such as intra-cardiac abscess) were evaluated.

AEPEI, SHARPEN and Cystatin C scores, although made for IE populations, did not include specific variables pertaining to IE such as: micro-organism, embolic events, degree of myocardial/valve damage, abscess formation and large vegetations, although these were evaluated. Discrimination performance was classified depending on AUC: Excellent with AUC 0.9–1.0, good with AUC 0.8–0.9, fair with AUC 0.7–0.8, poor with AUC 0.6–0.7, and very poor with AUC 0.5–0.6 (28).

The scores assessed in this review and corresponding AUC results from different studies are tabulated in **Tables 1**, **2** (3, 9–28). Of these scores, only six have been externally validated in a separate cohort (3, 12, 14, 17, 18). EuroSCORE I was most often used for comparison, being analyzed in 11 separate studies with a consistently fair performance. EuroSCORE II was reviewed in 10 studies, STS-IE and PALSUSE score were reviewed in nine studies each. STS-IE had an overall fair discrimination performance across studies, with only 3/9 studies with an AUC < 0.7 (11, 21, 25). The PALSUSE score was frequently included in comparative studies but performed overall poorly with an AUC < 0.7 in five of these comparative studies (3, 11, 12, 21, 22).

Statistical methods and selection of variables

The majority of studies identified variables for inclusion in the risk score by multivariate logistic regression analysis. The exception was Park et al. in the development of the simplified risk (or ICE) score, where the variables considered were selected *a priori* by an experienced cardiologist (17) before analysis for significance. Martinez-Sellis et al. in the development of the PALSUSE score utilized stepwise logistic regression analysis (9).

The number of variables assigned to each score and the variables included have been divided into three broad categories: Clinical variables (patient demographics, co-morbid and acute physiological state); Imaging characteristics (mainly echocardiographic findings); Microorganisms. **Figures 1**, **2** are graphic representations of the scores within these categories. Variables included for each score are depicted in **Supplementary Table 1**.

Of the IE-specific scores, 7/14 included microbiology criteria: Four studies considered a positive blood culture and four considered the presence of *Staphylococcus aureus* within their score. ICE score included both, as well as the presence of "Viridans streptococci." The scores which did not include microorganisms in their model (De Feo, RISK-E, AEPEI I and II, COSTA, SHARPEN, MELD-XI, and CYSTATINC) had access to microorganism data for their patient set; however, the microorganism was not found to be a significant factor in univariate and multivariate analysis and was subsequently excluded from their model.

Discrimination performance

Good discrimination performance of the following scores PALSUSE, De Feo, ANCLA, RISK-E, EndoSCORE, COSTA, and SHARPEN, were only identified in the studies proposing the score and most often in the derivation cohort. In follow-up, validation and other comparative studies, this result was not replicated. The only score to repeatedly score an AUC > 0.8 was the ANCLA score which was included in only two studies by the same first author.

EuroSCORE I, II, and STS-IE were most frequently used for comparison purposes with a fair performance (AUC 0.7–0.8). Relative difference between the best and worst AUC estimates for each score ranged from 15 to 34%. Many of the scores performed fairly (AUC 0.7–0.8) when compared in other studies. As expected, the performance was below that described in the original derivation cohort studies for these scores (**Tables 1**, **2**).

Calibration and model performance

Inter-model comparisons were provided for 14 studies: Hosmer-Lemeshow Test Statistic was used in 10 reports, Calibration slope provided in two studies and U statistic in one study. In the majority of calibration studies, the risk scores analyzed were found to be adequately calibrated.

The EuroSCORE II was found to have inaccurate calibration in one study (24) which authors attributed to the lack of specific IE factors in the score, however; in the same study, EuroSCORE I (lacking the same IE-specific factors) had adequate calibration. The Brier score, analyzing the difference between prediction and actual outcome, with a result of 0 being perfect, has been utilized in only one study proposing the EndoSCORE, quoting a Brier score of 0.078 (13).

Outcome selection bias

Long-term outcome data for IE patients is often unavailable. The majority of studies considered in-hospital mortality or mortality within 30 days as the primary end-point (3, 9–14, 16, 19, 22–25, 28), some interchangeably. Other end-points included 6-month mortality (17), urgent surgery OR in-hospital mortality (18) and long-term mortality of 29 months (19) and 5 years (20).

TABLE 1 Comparison of Risk Scores and their Performance—Risk Scores made for IE populations.

SCORE	First proposed	Time Scale	Number of variables	Score designed to predict	Study type [Ref]	Patients (*n*)[Ref]	Populations considered[Ref]	AGE[Ref]	AUC[Ref]	External validation
PALSUSE (9)	2014	2008–2010	7	In-hospital mortality	**Prospective** (3, 9, 12), Retrospective (11, 28), Observational retrospective (23, 24)	361 (3) **437** (9) 138 (11) 671 (12) 324 (28) 107 (21) 192 (22) 180 (23, 24)	**Surgical patients** with definite IE as defined by modified Duke criteria: (11, 21, 28) **active IE** (9) left-sided active IE (12, 23) native and prosthetic valve ACTIVE IE (24) including implantable cardiac devices (3) MEDICAL patients with definite IE (Mod Duke's) not considered for surgery. Incl all valves and devices (22)	59.1 ± 15.4 (3) **61.4 ± 16.4** (9) 60 ± 8.5 (11) 61 ± 14 (12) 61.8 ± 14.6 (28) 58.1 ± 14.5 (21) 65.2 ± 15.2 (22) 63.4 ± 13.8 (23) 63.2 ± 1 (24)	0.684 (0.633–0.731) (3) **0.84 (CI 0.79–0.88)** (9) 0.694 (CI 0.610–0.770) (11) 0.64 (CI 0.58–0.68) (12) 0.703 (CI 0.650–0.752) (28) 0.68 (CI 0.57–0.79) (21) 0.695 (CI 0.598–0.792) (22) 0.73 (CI 0.66–0.79) (23) 0.73 (CI 0.66–0.79) (24)	No
De Feo Score (10)	2012	1980–2009	6	Post-operative mortality (in-hospital/30-day)	**Prospective** (3, 10) Retrospective (11, 22, 25, 28) Observational retrospective (23, 24)	361 (3) **440** (10) 138 (11) 324 (28) 192 (22) 180 (23, 24) 146 (25)	**Surgical patients** with definite IE as defined by modified Duke criteria: (11, 28) **native valve IE only** (10) left-sided active IE (23) native and prosthetic valve ACTIVE IE (24, 25) including implantable cardiac devices (3) MEDICAL patients with definite IE (Mod Duke's) not considered for surgery. Incl all valves and devices (22)	59.1 ± 15.4 (3) **49 ± 16** (10) 60 ± 8.5 (11) 61.8 ± 14.6 (28) 65.2 ± 15.2 (22) 63.4 ± 13.8 (23) 63.2 ± 1 (24) 48.8 ± 16.0 (25)	0.722 (CI 0.654–0.790) (3) **0.88 (CI 0.82–0.93)** (10) 0.695 (CI 0.611–0.771) (11) 0.615 (CI 0.559–0.668) (28) 0.584 (CI 0.489–0.680) (22) 0.68 (CI 0.58–0.76) (23) 0.68 (CI 0.58–0.76) (24) 0.744 (CI 0.590–0.899) (25)	No
ANCLA score (11)	2017	2000–2015	5	In-hospital mortality	**Retrospective** (11, 28)	**138** (11) 324 (28)	**Surgical patients with definite IE as defined by modified Duke criteria** (11, 28)	**60 ± 8.5** (11) 61.8 ± 14.6 (28)	**ANCLA pre-op 0.828 (CI 0.754–0.887)** **ANCLA combined 0.823 (CI 0.749–0.883)** (11) 0.842 (CI 0.798–0.880) (28)	No

(Continued)

10.3389/fcvm.2023.1093363

TABLE 1 (Continued)

SCORE	First proposed	Time Scale	Number of variables	Score designed to predict	Study type [Ref]	Patients (*n*)[Ref]	Populations considered[Ref]	AGE[Ref]	AUC[Ref]	External validation
Risk-E Endocarditis Score (12)	2017	1996–2014	8	In-hospital mortality	**Prospective** (12) Retrospective (21, 28) Observational retrospective (23)	**671** (12) 324 (28) 107 (21) 180 (23)	**Surgical patients** with definite IE as defined by modified Duke criteria: (21, 28) **left-sided active IE** (12, 23)	**61 ± 14** (12) 61.8 ± 14.6 (28) 58.1 ± 14.5 (21) 63.4 ± 13.8 (23)	**0.82 (CI 0.75–0.88)** (12) **0.76 (CI 0.64–0.88) *n* = 204, ext validation sample** (12) 0.669 (CI 0.615–0.720) (28) 0.71 (CI 0.60–0.81) (21) 0.76 (CI 0.78–0.82) (23)	**Yes** (12)
EndoSCORE (3)	2017	2000–2015	9	30-day mortality	**Retrospective** (13, 21, 22, 28)	**2,715** (13) 324 (28) 107 (21) 192 (22)	**Surgical patients** with definite IE as defined by modified Duke criteria: (21, 28) **native and prosthetic valves** (13) MEDICAL patients with definite IE (Mod Duke's) not considered for surgery. Incl all valves and devices (22)	**59.6 ± 15.1** (13) 61.8 ± 14.6 (28) 58.1 ± 14.5 (21) 65.2 ± 15.2 (22)	**0.851 (CI 0.845–0.858)** (13) 0.663 (CI 0.609–0.715) (28) 0.76 (CI 0.66–0.86) (21) 0.724 (CI 0.634–0.814) (22)	No
APORTEI score (14)	2020	2008–2018	11	In-hospital/30-day mortality	**Prospective registry** (14) Prospectiv (26)	**1,338** (14) 111 (26)	**Surgical patients** with definite IE as defined by modified Duke criteria (26) **native and prosthetic valve ACTIVE IE** (14)	**63.6 ± 13.1** (14) 58.9 ± 13.7 (26)	**0.75 (CI 0.72–0.77)** (14) 0.88 (CI 0.83–0.93) (26)	**Yes** (14)
AEPEI Score I (3)	2017	2000–2015	5	In-hospital mortality	**Prospective** (3) Retrospective (21, 22, 28)	**361** (3) 324 (28) 107 (21) 192 (22)	**Surgical patients** with definite IE as defined by modified Duke criteria: (21, 28) **including implantable cardiac devices** (3) MEDICAL patients with definite IE (Mod Duke's) not considered for surgery. Incl all valves and devices (22)	**59.1 ± 15.4** (3) 61.8 ± 14.6 (28) 58.1 ± 14.5 (21) 65.2 ± 15.2 (22)	**0.780 (CI 0.734–0.822)** (3) 0.787 (CI 0.738–0.830) (28) 0.65 (CI 0.53–0.77) (21) 0.654 (CI 0.552–0.756) (22)	**Yes** (3)
AEPEI Score II (alternate model) (3)	2017	2000–2015	3	In-hospital mortality	**Prospective** (3) Retrospective (22, 28)	**361** (3) 324 (28) 192 (22)	**Surgical patients** with definite IE as defined by modified Duke criteria: (28) **including implantable cardiac devices** (3) MEDICAL patients with definite IE (Mod Duke's) not considered for surgery. Incl all valves and devices (22)	**59.1 ± 15.4** (3) 61.8 ± 14.6 (28) 65.2 ± 15.2 (22)	**0.774 (CI 0.727–0.816)** (3) 0.771 (CI 0.722–0.816) (28) 0.633 (CI 0.527–0.739) (22)	**Yes** (3)

(Continued)

10.3389/fcvm.2023.1093363

TABLE 1 (Continued)

SCORE	First proposed	Time Scale	Number of variables	Score designed to predict	Study type [Ref]	Patients (*n*)[Ref]	Populations considered[Ref]	AGE[Ref]	AUC[Ref]	External validation
COSTA (15)	2007	1988–1999	6	Risk of death	**Retrospective** (15), Observational retrospective (24)	**186** (15), 180 (24)	**Medical and Surgical Patients** (15) Surgical patients with definite IE as defined by modified Duke criteria: native and prosthetic valve ACTIVE IE (24)	**33.9** (15) 63.2 ± 1 (24)	**0.872** (15) 0.65 (CI 0.57–0.72) (24)	No
SHARPEN (16)	2015	2001–2011	7	In-hospital mortality	**Retrospective** (16)	**233** (16)	**Medical and Surgical Patients** (16)	**50 ± 19** (16)	**0.86 (CI 0.80–0.91)** (16)	No
Simplified Risk Score (ICE) (17)	2016	2000–2006	14	6-month mortality	**Prospective** (17, 19) Retrospective (22)	**4,049** (17) 858 (19) 192 (22)	**Medical and Surgical Patients** (19) **prosthetic valve excluded (54.67% surgical)** (17) MEDICAL patients with definite IE (Mod Duke's) not considered for surgery. Incl all valves and devices (22)	**45–72** (17) 45 ± 15 (19) 65.2 ± 15.2 (22)	**Harrell's C statistic 0.715 (CI 0.62–0.89)** (17) 0.771 (19)–long-term mortality, 0.816 (19)–in-hospital death 0.682 for validation model 0.706 (CI 0.617–0.798) (22)	**Yes** (17)
LOPEZ (18)	2011	1996–2003	3	In-hospital mortality or urgent surgery	**Retrospective** (18)	Ext Validation 264 (18)	**Surgical patients** with definite IE as defined by modified Duke criteria: **left-sided active IE** (18)	**61 ± 16** (18)	**0.67 Sensitivity 79%, Specificity 57%** (18)	**Yes** (18)
Modified MELD-XI (19)	2018	2009–2015	5	In-hospital/Long-term mortality	**Prospective** (19)	858 (19)	**Medical and Surgical Patients** (19)	**45 ± 15** (19)	**0.823** (19) **in-hospital mortality 0.730 (CI 0.658–0.803)** (19) **long-term mortality**	No
Cystatin C (20)	2012	1999–2005	4	5-year mortality	**Retrospective** (20)	125 (20)	**Medical and Surgical Patients, including prosthetic valves and cardiac devices** (20)	**62.7 ± 16.9** (20)	**0.74 (CI 0.70–0.87)** (20)	No

The results for the IE specific scores (i.e., the scores created for IE populations) which are delineated in bold/underline denote the studies. In which the risk score in question was first proposed, with data in bold referring to the derivation cohort. All other data (not in bold) for each score, include studies where the score in question has been used in comparison to other scores. All scores which have been validated (either described in the same paper or in a separate paper) have been identified in the last column, with the paper reference indicated accordingly in the last column.

10.3389/fcvm.2023.1093363

TABLE 2 Comparison of Risk Scores and their Performance–Risk Scores not specific for IE populations.

SCORE	Number of variables	Score designed to predict	Study type[Ref]	Patients (*n*)[Ref]	Populations considered[Ref]	AGE[Ref]	AUC[Ref]
OPR–Ontario province risk	6	Mortality, prolonged ICU stay (>6 days), prolonged post-op stay (>17 days)	Prospective (3), Retrospective (11, 22)	361 (3), 138 (11), 192 (22)	Surgical patients with definite IE as defined by modified Duke criteria: (11) including implantable cardiac devices (3) MEDICAL patients with definite IE (Mod Duke's) not considered for surgery. Incl all valves and devices (22)	59.1 ± 15.4 (3) 60 ± 8.5 (11) 65.2 ± 15.2 (22)	0.698 (CI 0.647–0.745) (3) 0.637 (CI 0.661–0.717) (11) 0.669 (CI 0.573–0.765) (22)
The Society of Thoracic Surgery (STS) risk score OR STS for IE score	12	Post-operative mortality and morbidity	Prospective (3, 12), Retrospective (11, 21, 22, 25, 28), Observational retrospective (23, 24)	361 (3) 138 (11) 671 (12) 324 (28) 107 (21) 192 (22) 180 (23, 24) 146 (25)	Surgical patients with definite IE as defined by modified Duke criteria: (11, 21, 28) left-sided active IE (12, 23) native and prosthetic valve ACTIVE IE (24, 25) including implantable cardiac devices (3) MEDICAL patients with definite IE (Mod Duke's) not considered for surgery. Incl all valves and devices (22)	59.1 ± 15.4 (3) 60 ± 8.5 (11) 61 ± 14 (12) 61.8 ± 14.6 (28) 58.1 ± 14.5 (21) 65.2 ± 15.2 (22) 63.4 ± 13.8 (23) 63.2 ± 1 (24) 48.8 ± 16.0 (25)	0.709 (CI 0.659–0.756) (3) 0.540 (CI 0.453–0.625) (11) 0.74 (CI 0.68–0.79) (12) 0.742 (CI 0.691–0.789) (28) 0.67 (CI 0.56–0.79) (21) 0.757 (CI 0.676–0.837) (22) 0.76 (CI 0.68–0.82) (23) 0.76 (CI 0.68–0.82) (24) 0.699 (CI 0.534–0.865) (25)
EUROSCORE II	18	In-hospital mortality	Prospective (3, 12, 26), Retrospective (11, 20–22, 25), Observational retrospective (23, 24)	361 (3) 138 (11) 671 (12) 465 (20) 107 (21) 192 (22) 180 (24) 146 (25) 111 (26)	Surgical patients with definite IE as defined by modified Duke criteria: (11, 21, 26, 28) left-sided active IE (12) native and prosthetic valve ACTIVE IE (24, 25) including implantable cardiac devices (3) Surgical patients diagnosed with IE depending on blood culture and intra-op findings (20) MEDICAL patients with definite IE (Mod Duke's) not considered for surgery. Incl all valves and devices (22)	59.1 ± 15.4 (3) 60 ± 8.5 (11) 61 ± 14 (12) 50 ± 16.9 (20) 58.1 ± 14.5 (21) 65.2 ± 15.2 (22) 63.2 ± 1 (24) 48.8 ± 16.0 (25) 58.9 ± 13.7 (26)	0.751 (CI 0.704–0.795) (3) 0.733 (CI 0.683–0.831) (11) 0.76 (CI 0.70–0.82) (12) 0.816 (20) 0.69 (CI 0.58–0.8) (21) 0.773 (CI 0.704–0.843) (22) 0.74 (CI 0.66–0.82) (24) 0.656 (CI 0.466–0.846) (25) 0.74 (CI 0.69–0.79) (26)
EUROScore I	17	In-hospital mortality	Prospective (9, 10, 12, 26), Prospective registry (14), Retrospective (11, 21, 22, 25), Observational retrospective (23, 24)	437 (9) 440 (10) 138 (11) 671 (12) 1,338 (14) 107 (21) 192 (22) 180 (23, 24) 146 (25) 111 (26)	Surgical patients with definite IE as defined by modified Duke criteria: (11, 21, 26) active IE (9) left-sided active IE (12, 18, 23) native valve IE only (10) native and prosthetic valve ACTIVE IE (14, 24, 25) MEDICAL patients with definite IE (Mod Duke's) not considered for surgery. Incl all valves and devices (22)	61.4 ± 16.4 (9) 49 ± 16 (10) 60 ± 8.5 (11) 61 ± 14 (12) 63.6 ± 13.1 (14) 58.1 ± 14.5 (21) 65.2 ± 15.2 (22) 63.2 ± 1 (24) 48.8 ± 16.0 (25) 58.9 ± 13.7 (26)	0.73 (CI 0.70–0.77) (9) 0.84 (CI 0.77–0.91) (10) Additive 0.733 (CI 0.651–0.805) Logistic 0.658 (CI 0.572–0.736) (11) 0.76 (CI 0.71–0.82) (12) 0.72 (CI 0.69–0.75) (14) 0.77 (CI 0.66–0.86) (21) 0.777 (CI 0.710–0.844) (22) 0.74 (CI 0.66–0.82) (24) Additive 0.653 (CI 0.487–0.819) Logistic 0.645 (CI 0.487–0.803) (25) 0.77 (CI 0.72–0.82) (26)

(Continued)

10.3389/fcvm.2023.1093363

TABLE 2 (Continued)

SCORE	Number of variables	Score designed to predict	Study type[Ref]	Patients (*n*)[Ref]	Populations considered[Ref]	AGE[Ref]	AUC[Ref]
SOFA–Sequential Organ Failure Assessment	8	In-hospital/intensive care mortality	Retrospective (27)	66 (27)	Surgical patients with definite IE as defined by modified Duke criteria (27)	70 (19–88) (27)	0.915 (CI 0.845–0.986) (27)
Charlson Co-Morbidity Index	14	10-year mortality	Retrospective (27)	66 (27)	Surgical patients with definite IE as defined by modified Duke criteria (27)	70 (19–88) (27)	0.788 (CI 0.655–0.922) (27)

The different population cohorts assessed are specified in **Tables 1**, **2**. While all studies defined IE using the modified Duke's criteria, some opted to only include patients with active IE. Only seven studies included medically managed patients (15–17, 19, 20, 22, 27), with others including surgical patients only. ICE, SHARPEN, Modified MELD-XI, Cystatin C, and COSTA scores were developed from cohorts with both medical and surgical patients. The COSTA score performed poorly when later applied to a surgical cohort (24). The ICE score maintained fair discrimination performance when applied to an exclusively medically treated cohort (22). Furthermore, only three papers included patients with implantable cardiac devices (3, 20, 22).

Overall risk of bias

In the PROBAST assessment (**Supplementary Table 2**), the majority of studies were found to have high risk of bias in participant choice due to the specific populations considered (e.g., surgical patients only, native valve only etc.). This systematic review aims to encompass scores that incorporate the whole of the IE population if possible. The simplified risk score (ICE) is the score with the least risk of bias; however, it is one of the few scores assessing a long-term outcome of 6 month mortality rather than in-hospital or 30 day mortality, making it difficult to compare with the other scores available. Moreover, it has over double the variables of the other scores, making it less user-friendly.

Discussion

This systematic review has highlighted important limitations that preclude the transportability of published risk-scores to various IE groups in different healthcare settings and regions. The challenge with risk stratification and accurate prognostication in IE is largely due to the heterogenous patient population affected. The majority of scoring systems identified address the issue of surgical risk, therefore being unable to estimate mortality risk for medically treated populations.

The IE patient is now wholly different from the one 30 years ago. Percutaneous vascular interventions have become more commonplace, as have the number of cardiac implantable devices. IE associated with cardiac devices has been reported in up to 7% of cases (29), coinciding with a rise in staphylococcal infections (4). There is also an increase in prosthetic valve endocarditis. These under-represented groups of patients are often excluded from the outset in the development cohorts for predictive scoring.

The prevalence of intravenous drug users presenting with IE is also on the rise, with cases doubling between 2008 and 2014 (30). They tend to be younger, more acutely unwell patients, with infection caused by gram-positive pathogens (31).

Clinical impact of IE risk-scoring

Infective endocarditis remains a highly morbid and highly fatal condition, in spite of advances in imaging, improvements in microbiological testing, antibiotic therapy and surgical treatment.

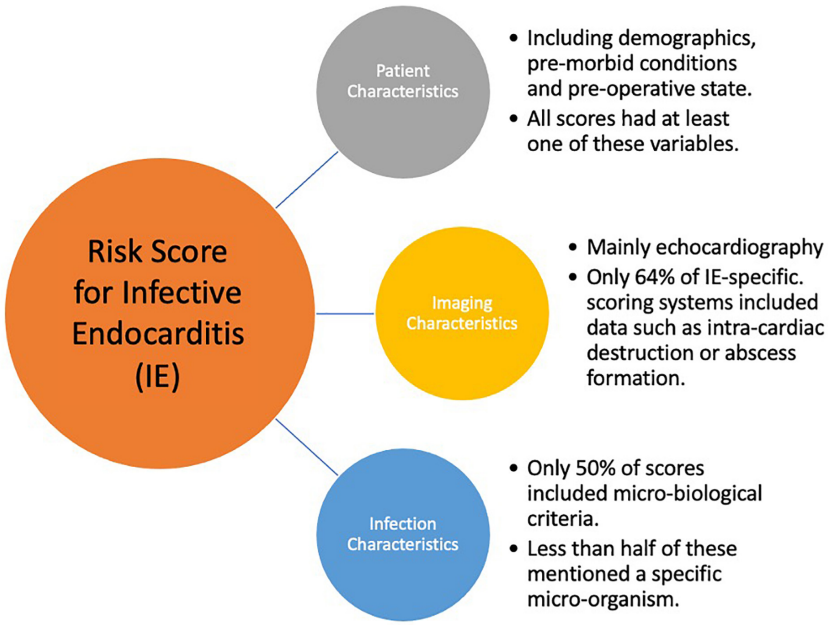


FIGURE 1
Key components for an Infective Endocarditis Risk Score.

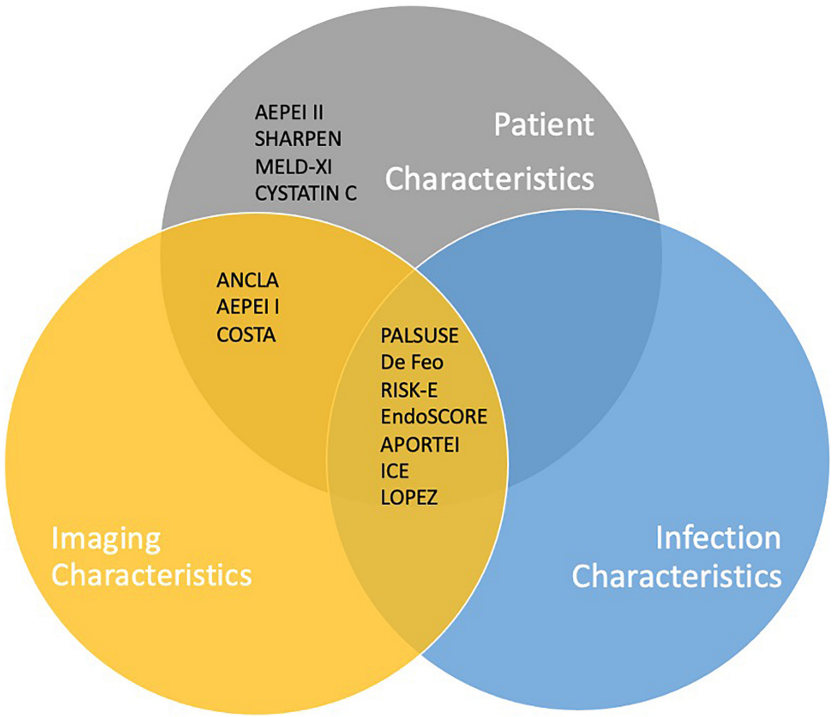


FIGURE 2
Inclusion of key criteria in IE-specific risk scores currently available.

Ideally scores should be available within 48–72 h of patient admission into hospital, to guide early management decisions. Lopez et al. only examined variables available within 72 h of admission (18). Possible routes of infection are multiple, with data available at different time-points and not necessarily standardized for all patients. Number and frequency of blood cultures taken may vary, as well as access to trans-thoracic/trans-oesophageal echocardiography (TTE/TOE).

Recent EURO-ENDO registry data showed that for patients in which surgical intervention was found to be necessary, 22.5% died before surgery could be performed (2). This highlights the need for quick and effective decision making which would be

significantly easier with a reliable risk tool. Pooling of different IE groups may allow differentiation of risk between the groups within the tool.

Surgical intervention is often carried out as an emergency or urgent procedure after evidence of embolization, heart failure or in the presence of uncontrolled infection (32). This is a complex decision with surgery in the active phase often associated with significant risk. For example, patients with new neurology may experience peri-operative cerebral bleeding with early cardiac surgery intervention. There is variation between studies in the definition of "early surgery" and the results are inconclusive (33). The heart team meeting is essential in making decisions about timing of intervention and while it is beyond the scope of this review, risk-scoring has the potential role in guiding a more accurate selection process toward optimal timing of surgery.

Validity of risk scores

The ideal risk score should have easily measurable parameters which are comparable across centers (13), clear definitions of predictive parameters and outcomes to ensure widespread use, as well as generalisability to future patients and transportability to other data-sets/patients, determined through a robust validation process (34, 35). Predictors should be easy to collect and the result of cheap and non-invasive testing (36).

Only 6 of the 14 IE-specific scores proposed have undergone formal external validation, limiting their transportability. The absence of externally validated scores has been highlighted multiple times in the literature and analysis of the European IE-Registry (EURO-ENDO) was proposed to achieve this aim (28). Despite the lack of external validation, many scores have been frequently re-assessed in separate studies with different cohorts.

Comparison of the AUC/ROC for the same score between studies allows for understanding of model performance in different IE groups. De Feo score performed poorly in most comparative studies (11, 22–24, 28); however, this score was derived from a small specific cohort of patients with left-side only native valve endocarditis (10), which may explain the inaccurate results when applied to different populations. In addition, it was developed for patients treated from 1980 to 2009. As can be expected, the management of patients in 1980 would have differed significantly from that in 2009, as has the nature of the disease.

EuroSCORE I and II had the least favorable performance in the study by Wang et al. (25), potentially due to the very young average age of their study group (48.8 $\pm$ 16 years). There is likely to be an increased significance of the specific IE variables in this young age bracket, which are not included in the EuroSCOREs.

Less than 50% of studies carried out model calibration or performance assessment. Model calibration assesses congruence between model prediction and observed outcome (37). The power of Hosmer-Lemeshow "goodness-of-fit" test increases with sample size and its interpretation in small cohorts such as these, may be inaccurate (38). The use of newer, more advanced methods of performance assessment, such as Brier scoring are known to support risk score use in the clinical setting (37). This should be emphasized going forward, to allow for detailed comparative studies between available scores.

Prioritization of variables

A fundamental drawback in 7/14 IE specific risk scores is the absence of microbiology from the predictive models. In contrast, the literature demonstrates *Staphylococcus aureus* to be the most common causative microorganism in IE worldwide (39) with strong evidence to suggest its association with worsened morbidity/mortality. ESC guidelines highlight positive blood cultures at 3 days of antimicrobial treatment as an independent risk factor for in-hospital death (5). Investigations vary in different centers and risk scores may standardize this process (e.g., frequency of blood cultures).

Two scores missing microbiology predictors (AEPEI, COSTA) included patients with non-active IE (not undergoing antibiotic treatment at the time of analysis/surgery) at rates of 28.5 and 36%, respectively. The effect of the causative microorganism in patients outside the active phase of IE may be less relevant to outcome and may be the reason for lack of significance in these patient groups.

The PALSUSE score includes EuroSCORE II >10 as a variable. This is a potential confounding factor due to age, gender and urgency of surgery being variables in both PALSUSE and EuroSCORE II, therefore doubling the effect of these variables (9).

Biomarkers feature in only three scores in this review (16, 19, 20), with the most commonly used being C-reactive protein (CRP) of different values. CRP has been found to be an independent predictor for worse outcomes in IE, including an increased risk of embolic events (40), surgical intervention (41), and in-hospital mortality (42). In addition, improvement in CRP was a good predictor of long term outcomes (41).

Furthermore, biomarkers such as sensitive troponin I, interleukin-15 and C-C-chemokine-ligand-4 have been shown in separate studies to predict mortality in IE patients; however, this data is limited to small cohorts (43, 44). The inflammatory response in IE is well documented and is different to other infections (43). Mapping of pro-inflammatory cytokines may be key in risk stratification models to guide early decisions for more aggressive treatment, including surgical intervention.

The effect of novel diagnostic/treatment on risk-scoring

Developing surgical techniques may have a significant impact on prognosis. Destruction of both the aortic and mitral valves is one of the more challenging presentations of IE; however the "commando" procedure with reconstruction of the aortic-mitral curtain and replacement of both valves has been performed with good results (45). Due to small patient samples for major surgical reconstruction, it is difficult to assess the impact of novel procedures on risk.

Improved patient outcomes have been repeatedly shown for specialized high-volume centers; however, this has not yet been explored for IE patients. Involvement of multiple valves has nonetheless been reflected in some models (13, 14).

Echocardiography is a key tool for prognostication in IE, as reflected in multiple guidelines. The advent of 4D-echocardiography and TOE (pre and intra-operatively) has allowed for detailed understanding of intra-cardiac damage secondary to

infection (46). Destructive valve lesions, abscesses and vegetations (with embolization risk) can be identified and are crucial for surgical planning (46).

The use of computed tomography (CT) and F-fluorodeoxyglucose positron emission tomography (PET) has increased particularly for prosthetic or device-related IE; however, regional differences are evident with their use being more common in Western Europe (2). Novel imaging techniques may be incorporated into risk scoring systems for IE and recommendations for use may be found in the ESC guidelines (5).

Population bias and other limitations in available risk scoring models

Patients with IE undergoing surgery (vis-à-vis most published risk scores) may have a survival advantage as they are already deemed fit for surgery and/or have survived to surgery. There was a particularly high mortality rate reported in the EURO-ENDO registry for patients with indications for surgery who did not undergo surgery (2).

On the flip side, patients may be "too-well" to require surgery due to minimal intra-cardiac destruction and effective response to medical management. Published risk scores fail to capture "antibiotic responders," especially since they are often managed outside tertiary centers. This effect has been highlighted previously and many risk scores, when tested on medical IE patients NOT considered for surgery, fared quite poorly (22).

Advanced model performance assessment is often missing and the majority of scores have not been externally validated. The limitation of these scores are an extension of the constraints within the studies that propose them. The recurring stumbling blocks include small groups of patients, collected over long time-spans; heterogeneous populations (e.g., left-sided IE, exclusion of cardiac devices, and medical patients) with a focus on tertiary centers, creating a referral bias; single center or regional studies which may not be applicable elsewhere; retrospective analysis (including of prospectively collected data) with certain variables often missing; definition of variables inconsistent across studies and analysis of short-term outcomes with a consistent lack of long-term data.

The lack of long-term data is a significant draw-back, with the majority of the papers reviewed here considering 30 day or in-hospital mortality as the end-point or primary outcome. There is limited data to understand what survival and morbidity, or even quality of life, is like beyond this date. The MDT is still unable to guide patients toward reasonable expectations of what their recovery might involve.

Moreover, it has proved difficult to capture the impact on patient outcomes as a result of delay in diagnosis, time to initiation of treatment and time of referral to specialized care in tertiary centers. The point of referral to an MDT is heterogenous across populations and difficult to assess. This emphasizes the need for a standardized prospective registry encompassing data from the initial clinical presentation to the end of the patient journey and recovery. The implementation of artificial intelligence has not yet been explored in endocarditis patients. This may identify critical negative prognostic signs through imaging and cytokine response, creating personalized risk models.

Conclusion

In conclusion, despite the multitude of available IE risk-scores, the lack of adequate score validity limits their clinical utility and widespread applicability in this important group of patients. Being a highly morbid condition with a multifactorial pathophysiology and a heterogenous patient population, the accumulation of large sets of real-world data from future coordinated registries including novel biomarkers will produce more robust prediction models. Future registries should also encompass populations with much wider inclusion criteria and more refined classification systems, thus improving patient-specific prognostication. Improved risk scores will have the potential to empower MDTs with an objective stratification tool to guide management in patients with IE, as well as allow for key comparative studies and improved management strategies for IE.

Data availability statement

The original contributions presented in this study are included in this article/**Supplementary material**, further inquiries can be directed to the corresponding author.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work, and approved it for publication.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2023.1093363/full#supplementary-material

References

1. Quan T, Muller-Pebody B, Fawcett N, Fawcett N, Young B, Minaji M, et al. Investigation of the impact of the NICE guidelines regarding antibiotic prophylaxis during invasive dental procedures on the incidence of infective endocarditis in England: an electronic health records study. *BMC Med.* (2020) 18:84. doi: 10.1186/s12916-020-01531-y

2. Habib G, Erba P, Lung B, Donal E, Cosyns B, Laroche C, et al. Clinical presentation, aetiology and outcome of infective endocarditis. results of the ESC-EORP EURO-ENDO (European IE) registry: a prospective cohort study. *Eur Heart J.* (2019) 40:3222–32.

3. Gatti G, Perrotti A, Obadia J, Duval X, Iung B, Alla F, et al. Simple Scoring System to predict in-hospital mortality after surgery for infective endocarditis. *J Am Heart Assoc.* (2017) 6:e004806.

4. Beynon R, Bahl V, Prendergast B. Infective endocarditis. *BMJ.* (2006) 333:334.

5. Habib G, Lancellotti P, Antunes M, Bongiorni M, Casalta J, Del Zotti F, et al. 2015 ESC Guidelines for the management of infective endocarditis: the task force for the management of infective endocarditis of the European Society of Cardiology(ESC). endorsed by: European Association for Cardio-Thoracic Surgery(EACTS), the European Association of Nuclear Medicine(EANM). *Eur Heart J.* (2015) 36:3075–128. doi: 10.1093/eurheartj/ehv319

6. Florkowski C. Sensitivity, specificity, receiver-operating characteristic (ROC) curves and likelihood ratios: communicating the performance of diagnostic tests. *Clin Biochem Rev.* (2008) 29 Suppl1:S83–7.

7. MoonsK G, Wolff R, Riley R, Whiting P, Westwood M, Collins G, et al. PROBAST: a tool to assess risk of bias and applicability of prediction model studies: explanation and elaboration. *Ann Intern Med.* (2019) 170:W1–33. doi: 10.7326/M18-1377

8. Li J, Sexton D, Mick N, Nettles R, Fowler V Jr, Ryan T, et al. Proposed modifications to the Duke criteria for the diagnosis of infective endocarditis. *Clin Infect Dis.* (2000) 30:633–8. doi: 10.1086/313753

9. Martinez-Sellís M, Muínoz P, Arnaiz A, Moreno M, Gálvez J, Rodríguez-Roda J, et al. Valve surgery in active infective endocarditis: a simple score to predict in-hospital prognosis. *Int J Cardiol.* (2014) 175:133–7.

10. DeFeo M, Cotrufo M, Carozza A, De Santo L, Amendolara F, Giordano S, et al. The need for a specific risk prediction system in native valve infective endocarditis surgery. *ScientificWorldJournal.* (2012) 2012:307571. doi: 10.1100/2012/307571

11. Gatti G, Benussi B, Gripshi F, Della Mattia A, Proclemer A, Cannatà A, et al. A risk factor analysis for in-hospital mortality after surgery for infective endocarditis and a proposal of a new predictive scoring system. *Infection.* (2017) 45:413–23. doi: 10.1007/s15010-016-0977-9

12. Olmos C, Vilacosta I, Habib G, Maroto L, Maroto L, Fernández C, et al. Risk score for cardiac surgery in active left-sided infective endocarditis. *Heart.* (2017) 103:1435–42.

13. Di Mauro M, Dato G, Barili F, Gelsomino S, Santè P, Corte A, et al. A predictive model for early mortality after surgical treatment of heart valve or prosthesis infective endocarditis. the EndoSCORE. *Int J Cardiol.* (2017) 241:97–102.

14. Varela-Barca L, Fernandez-Felix B, Navas-Elorza E, Mestres C, Muñoz P, Cuerpo-Caballero G, et al. Prognostic assessment of valvular surgery in active infective endocarditis: multicentric nationwide validation of a new score developed from a meta-analysis. *Eur J Cardiothorac Surg.* (2020) 57:724–31. doi: 10.1093/ejcts/ezz328

15. Costa M, Wollman D, Campos A, Della Mattia A, Proclemer A, Cannatà A, et al. Risk index for death by infective endocarditis: a multivariate logistic model. *Braz J Cardiovasc Surg.* (2007) 22:192–200.

16. Chee O, Tan Y, Ngiam J, Win M, Shen X, Choo J, et al. The SHARPEN clinical risk score predicts mortality in patients with infective endocarditis: an 11-year study. *Int J Cardiol.* (2015) 191:273–6. doi: 10.1016/j.ijcard.2015.04.236

17. Park L, Chu V, Peterson G, Skoutelis A, Lejko-Zupa T, Bouza E, et al. Validated risk score for predicting 6-month mortality in infective endocarditis. *J Am Heart Assoc.* (2016) 5:e003016. doi: 10.1161/JAHA.115.003016

18. Lopez J, Fernandez-Hidalgo N, Revilla A, Vilacosta I, Tornos P, Almirante B, et al. Internal and external validation of a model to predict adverse outcomes in patients with left-sided infective endocarditis. *Heart.* (2011) 97:1138–42. doi: 10.1136/hrt.2010.200295

19. He P, Wei X, Luo S, Chen X, Ke Z, Yu D, et al. Risk prediction in infective endocarditis by modified MELD-XI score. *Eur J Clin Microbiol Infect Dis.* (2018) 37:1243–50. doi: 10.1007/s10096-018-3240-8

20. Bjurman C, Snygg-Martin U, Olaison L, Fu M, Hammarsten O. Cystatin C in a composite risk score for mortality in patients with infective endocarditis: a cohort study. *BMJ Open.* (2012) 2:e000856. doi: 10.1136/bmjopen-2012-000856

21. Pivatto F, Bellagamba C, Pianca E, Fernandes F, Butzke M, Busato S. Analysis of risk scores to predict mortality in patients undergoing cardiac surgery for endocarditis. *Arq Bras Cardiol.* (2020) 114:518–24.

22. Gatti G, Chocron S, Obadia J, Duval X, Iung B, Alla F, et al. Using surgical risk scores in nonsurgically treated infective endocarditis patients. *Hellenic J Cardiol.* (2019) 61:246–52.

23. Varela Barca L, Lopez Menendez J, Rodriguez-Roda Stuart J. Response: risk score for cardiac surgery in active left-sided infective endocarditis. *Heart.* (2017) 103:1923. doi: 10.1136/heartjnl-2017-311943

24. Varela L, Lopez-Menendez J, Redondo A, Fajardo E, Miguelena J, Centella T, et al. Mortality risk prediction in infective endocarditis surgery: reliability analysis of specific scores. *Eur J Cardiothorac Surg.* (2018) 53:1049–54. doi: 10.1093/ejcts/ezx428

25. Wang T, Oh T, Voss J, Gamble G, Kang N, Pemberton J. Comparison of contemporary risk scores for predicting outcomes after surgery for active infective endocarditis. *Heart Vessels.* (2015) 30:227–34. doi: 10.1007/s00380-014-0472-0

26. Urso S, Tena MÁ, Horcajada I, Paredes F, González-Barbeito M, Portela F. Prediction of surgical risk in patients with endocarditis: comparison of logistic EuroSCORE, EuroSCORE II and APORTEI Score. *Enferm Infecc Microbiol Clin.* (2020) 39:244–7. doi: 10.1016/j.eimc.2020.05.012

27. Asai N, Shiota A, Ohashi W, Watanabe H, Shibata Y, Kato H, et al. The SOFA score could predict the severity and prognosis of infective endocarditis. *J Infect Chemother.* (2019) 25:9650–971. doi: 10.1016/j.jiac.2019.05.014

28. Gatti G, Sponga S, Peghin M, Givone F, Ferrara V, Benussi B, et al. Risk scores and surgery for infective endocarditis: in search of a good predictive score. *Scand Cardiovasc J.* (2019) 53:117–24. doi: 10.1080/14017431.2019.1610188

29. Edelstein S, Yahalom M. Cardiac device-related endocarditis: epidemiology, pathogenesis, diagnosis and treatment – a review. *Int J Angiol.* (2009) 18:167–72.

30. Deo S, Raza S, Kalra A, Deo V, Altarabsheh S, Zia A, et al. Admissions for infective endocarditis in intravenous drug users. *J Am Coll Cardiol.* (2018) 71:1596–7.

31. Jordan M, Tiako N, Mori M, Hioda K, Mangi A, Yun J, et al. Recidivism is the leading cause of death among intravenous drug users who underwent cardiac surgery for infective endocarditis. *Semin Thorac Cardiovasc Surg.* (2019) 31:40–5.

32. Hoen B, Duval X. Infective endocarditis. *N Engl J Med.* (2013) 368:1425–33.

33. Kang D. Timing of surgery in infective endocarditis. *Heart.* (2015) 101:1786–91.

34. Altman D, Vergouwe Y, Royston P, Moons K. Prognosis and prognostic research: validating a prognostic model. *BMJ.* (2009) 338:b605.

35. Royston P, Moons K, Altman D, Vergouwe Y. Prognosis and prognostic research: developing a prognostic model. *BMJ.* (2009) 338:b604.

36. Eryu X, Yiqin Y, Enliang X, Jing M, Wen S. From risk prediction models to risk assessment service: a formulation of development paradigm. *arXiv* [Preprint]. (2019) doi: 10.48550/arXiv.1903.07551

37. Steyerberg E, Vickers A, Cook N, Gerds T, Gonen M, Obuchowski N, et al. Assessing the performance of prediction models: a framework for some traditional and novel measures. *Epidemiology.* (2010) 21:128–38.

38. Bertolini G, D'Amico R, Nardi D, Tinazzi A, Apolone G. One model, several results: the paradox of the Hosmer-Lemeshow goodness-of-fit test for the logistic regression model. *J Epidemiol Biostat.* (2000) 5:251–3.

39. Murdoch D, Corey G, Hoen B, Miró J, Fowler V Jr, Bayer A, et al. Clinical presentation, etiology, and outcome of infective endocarditis in the 21st century: the international collaboration on endocarditis–prospective cohort study. *Arch Intern Med.* (2009) 169:463–73. doi: 10.1001/archinternmed.2008.603

40. Durante Mangoni E, Adinolfi L, Tripodi M, Andreana A, Gambardella M, Ragone E. Risk factors for "major" embolic events in hospitalized patients with infective endocarditis. *Am Heart J.* (2003) 146:311–6.

41. Heiro M, Hans Helenius H, Sundell J, Koskinen P, Engblom E, Nikoskelainen J. Utility of serum C-reactive protein in assessing the outcome of infective endocarditis. *Eur Heart J.* (2005) 25:1873–81.

42. Nunes M, Guimarães-Júnior M, Murta Pinto P, Coelho R, Souza Barros T, Faleiro Maia N. Outcomes of infective endocarditis in the current era: early predictors of a poor prognosis. *Int J Infect Dis.* (2018) 68:102–7. doi: 10.1016/j.ijid.2018.01.016

43. Ris T, Teixeira-Carvalho A, Coelho R, Brandao-de-Resende C, Gomes M, Amaral L. Inflammatory biomarkers in infective endocarditis: machine learning to predict mortality. *Clin Exp Immunol.* (2019) 196:374–82. doi: 10.1111/cei.13266

44. Siciliano R, Gualandro D, Bittencourt M, Paixão M, Marcondes-Braga F, Soeiro A. Biomarkers for prediction of mortality in left-sided infective endocarditis. *Int J Infect Dis.* (2020) 96:25–30.

45. Jiang X, Liu J, Khan F, Tang R, Zhang Y, Gu T. Aortic and mitral valve surgery for infective endocarditis with reconstruction of the intervalvular fibrous body: an analysis of clinical outcomes. *J Thorac Dis.* (2020) 12:1427–36.

46. Habib G, Badano L, Tribouilloy C, Vilacosta I, Zamorano J, Galderisi M, et al. On behalf of the European Association of Echocardiography. recommendations for the practice of echocardiography in infective endocarditis. *Eur J Echocardiogr.* (2010) 11:202–21. doi: 10.1093/ejechocard/jeq004

frontiers | Frontiers in Cardiovascular Medicine

TYPE Original Research
PUBLISHED 03 March 2023
DOI 10.3389/fcvm.2023.1095646

OPEN ACCESS

EDITED BY
Shahzad Raja,
Harefield Hospital, United Kingdom

REVIEWED BY
Habib Khan,
King's College London, United Kingdom
Francesco Bianco,
Azienda Ospedaliero Universitaria Ospedali Riuniti, Italy

*CORRESPONDENCE
Ruixin Fan
✉ fanruixin@163.com

†These authors have contributed equally to this work

SPECIALTY SECTION
This article was submitted to Heart Surgery, a section of the journal Frontiers in Cardiovascular Medicine

RECEIVED 11 November 2022
ACCEPTED 16 February 2023
PUBLISHED 03 March 2023

CITATION
Feng W, Li H, Wang Q, Li C, Wu J, Yang J and Fan R (2023) Prognostic significance of neutrophil count on in-hospital mortality in patients with acute type A aortic dissection. Front. Cardiovasc. Med. 10:1095646. doi: 10.3389/fcvm.2023.1095646

Prognostic significance of neutrophil count on in-hospital mortality in patients with acute type A aortic dissection

Weiqi Feng[1,2†], Huili Li[1,2†], Qiuji Wang[1,3†], Chenxi Li[4], Jinlin Wu[1], Jue Yang[1] and Ruixin Fan[1*]

[1]Department of Cardiovascular Surgery, Guangdong Provincial Cardiovascular Institute, Guangdong Provincial People's Hospital (Guangdong Academy of Medical Sciences), Southern Medical University, Guangzhou, China, [2]School of Medicine, South China University of Technology, Guangzhou, China, [3]The Second School of Clinical Medicine, Southern Medical University, Guangzhou, China, [4]Department of Cardiac Surgery Intensive Care Unit, Guangdong Cardiovascular Institute, Guangdong Provincial People's Hospital (Guangdong Academy of Medical Sciences), Southern Medical University, Guangzhou, China

Backgrounds: The goal of this study was to assess the impact of neutrophil count, in patients with acute type A aortic dissection (ATAAD).

Methods: This study retrospectively collected data from patients between September 2017 and June 2021. Youden's index was used to determine the optimal cut-off value for the neutrophil count and patients were divided into two subgroups. A restricted cubic spline (RCS) was used to model the relationship between variables and in-hospital mortality. The least absolute shrinkage and selection operator (LASSO) method and multivariate logistic regression analyses were used to investigate the independent prognostic factors for in-hospital mortality in patients with ATAAD.

Results: A total of 467 patients were enrolled in this study. In-hospital mortality was 7.28%. The group with elevated neutrophil counts had significantly higher mortality than the group with decreased neutrophil counts (10.8% vs. 3.2%, $P = 0.02$). This data shows that elevated neutrophil count was significantly associated with in-hospital mortality (OR 3.07, 95% CI 1.22–7.62, $P = 0.02$).

Conclusions: Neutrophil count is an independent risk factor for in-hospital mortality in patients with ATAAD. It is an effective inflammatory index, which can be individualized for patients.

KEYWORDS

inflammation, neutrophil, mortality, type A aortic dissection, NLR

Introduction

Aortic dissection, with its high mortality rate, is a rare and lethal disease. The dissection of the aorta allows blood to flow between the layers of the aortic wall, forcing the layers apart (1). Acute type A aortic dissection (ATAAD) accounts for 58%–62% of aortic diseases (2).

Inflammation plays a crucial role in the pathogenesis and progression of cardiovascular disease (3). White blood cell (WBC) count and C-reactive protein (CRP), which are useful and easily available through routine blood tests, are commonly used biomarkers in cardiovascular disease, including ATAAD. Neutrophils make up 50%–70% of circulating WBCs and are an important inflammation factor. With the aggravation of inflammation, the number of neutrophils circulating in the blood rapidly increases. Recent findings indicated that neutrophil accelerated atherosclerosis promoted atherosclerotic plaque

instability, and aggravated ischemic stroke (4, 5). The abundance of neutrophils destroys the aneurysmal vessel wall, thereby promoting progressive enlargement and rupture (6).

Despite recent findings, the specific association between neutrophils and the endpoint of patients with aortic dissection remains unclear. This study aimed to assess the impact of neutrophil count in patients with acute type A aortic dissection and focus on whether neutrophil counts have potential value for early detection in patients with ATAAD, and determine if they can aid in identifying patients at increased risk of mortality.

Methods

Study setting

A series of consecutive patients enrolled in this retrospective study were admitted to the hospital from September 2017 to June 2021. The inclusion criteria were as follows: 1. All the patients were diagnosed with ATAAD by computer tomography; 2. Patients were ≥ 18 years of age; 3. Routine blood tests were evaluated within 2 h of admission; 4. All patients underwent surgery within 4 days of admission.

This study was approved by the Ethics Committee of Guangdong Provincial People's hospital.

Data collection

All clinical information, including demographics, admission laboratory results, operative information, and clinical results, were collected from the hospital's medical record system. Admission laboratory tests, including routine blood tests and metabolic profiles, were run within 2 h of arrival to the emergency room. Operative information was collected including cardiopulmonary bypass time (CPB), coronary artery bypass graft (CABG), aortic cross-clamp time (ACC) and whether patients underwent a Bentall procedure. All data from our study were used without *a priori* sample size calculations. And all null value data in the data set were deleted to ensure validity.

Endpoint

All-cause deaths during hospitalization were defined as the primary endpoint of study. Gastrointestinal bleeding, paraplegia, acute kidney failure, chest reopening, low cardiac output syndrome, cerebrovascular accident, multiple organ dysfunction syndrome (MODS) were also included as the secondary endpoint.

Acute kidney failure was defined as Serum creatinine increased by >3 times the baseline values, GFR decreased by >75%, oliguria: urine output <0.3 ml · kg−1 · h−1 for 24 h, or anuria >12 h or requiring temporary hemodialysis support for resolution (7). Low cardiac output syndrome was defined as large doses of vasoactive drugs with signs of hypoperfusion of tissues, requiring intra-aortic balloon pump insertion or requiring extracorporeal membrane oxygenation support.

Statistical analysis

Continuous variables are summarized as the mean ± standard deviation and median (inter-quartile range). Categorical variables are summarized as frequency rates and percentages. The differences between the two groups were compared using Student's *t*-test, Mann-Whitney *U* test, and $\chi 2$ tests.

The Kaplan-Meier method was used to construct the survival curve. Receiver operating characteristic (ROC) curves were constructed, and the area under the curve (AUC) was assessed for analyzing the prognostic value. The continuous variables of laboratory results are determined by Youden's index, which is helpful to determine the optimal cutoff values for dividing subjects into subgroups. A restricted cubic spline (RCS), with three knots at 25th, 50th and 75th, was used to assess the non-linear relationship between neutrophil count and in-hospital mortality.

The least absolute shrinkage and selection operator (LASSO) method was performed. LASSO regression is known to be able to remove unimportant variables *via* the regression coefficients penalizing the size of the parameters (8). Tuning parameter (λ) selection in the LASSO model used 10-fold cross-validation *via* minimum criteria. And then, variables with non-zero coefficients in the LASSO-logistic analysis were selected for further stepwise logistic regression analysis. Calibration plot was used to represent perfect prediction that model-predicted probability matches actually observed probability. Clinical utility was estimated by decision curve analysis (DCA). In our study, a two-sided *P*-value of < 0.05 was considered to be significant. Stata software (StataCorp, United States) and R software were used for statistical analysis.

Results

From September 2017 to June 2021, a total of 477 consecutive patients were included in our study. 10 patients were excluded by the inclusion criteria. The clinical characteristics of all patients are presented in **Table 1**. 394 (84.37%) patients were male, and the mean age of all patients was 52.0 ± 10.59.

Clinical characteristics of patients

Patients were divided into a survivor group ($n = 433$) and a non-survivor group ($n = 34$). The age in the survivor group was younger than in the non-survivor group (51.74 ± 10.49 vs. 56.09 ± 11.12, $P = 0.02$). Hypertension ($P = 0.03$), and CABG procedures ($P < 0.01$) were more commonly found in the non-survivor group. Patients had longer CPB and ACC time ($P < 0.01$), higher neutrophil to lymphocyte ratio (NLR; $P < 0.01$), and higher neutrophil counts (10.12 ± 3.67 vs. 11.70 ± 2.98, $P = 0.01$) in the non-survivor group. There were no significant differences between the two groups in other measured variables (**Table 1**).

TABLE 1 Baseline characteristics of clinical data in patients.

	Overall ($n = 467$)	Survivor ($n = 433$)	Non-survivor ($n = 34$)	*P*-value
Demographics				
Age (years)	52.06 ± 10.59	51.74 ± 10.49	56.09 ± 11.12	0.02
Gender/male	394 (84.37%)	367 (84.75%)	27 (79.41%)	0.41
BMI(kg/m^2)	24.75 ± 3.95	24.73 ± 3.74	24.91 ± 6.12	0.79
Smoking[a]	161 (34.48%)	154 (35.57%)	7 (20.59%)	0.08
Medical history				
Hypertension	320 (68.52%)	291 (67.21%)	29 (85.3%)	0.03
Diabetes	8 (1.71%)	7 (1.62%)	1 (2.95)	0.57
CAD	46 (9.85%)	40 (9.24%)	6 (17.65%)	0.11
Known history of cardiovascular surgery	38 (8.14%)	36 (8.31%)	2 (5.9%)	0.62
MFS	24 (5.14%)	24 (5.54%)	0 (0.0%)	0.16
Aortic regurgitation				0.03
Non/mild		293 (69.1%)	18 (52.9%)	
Moderate		74 (17.5%)	13 (38.2%)	
Severe		57 (13.4%)	3 (8.8%)	
Laboratory results				
White blood cell count ($\times 10^9$)	12.80 ± 3.79	12.74 ± 3.86	13.59 ± 2.75	0.21
Neutrophil ($\times 10^9$)	10.23 ± 3.65	10.12 ± 3.67	11.70 ± 2.98	0.01
Lymphocyte ($\times 10^9$)	1.07 (0.83–1.44)	1.08 (0.85–1.44)	1.02 (0.69–1.47)	0.30
Platelets ($\times 10^9$)	184 (152–228)	185 (152–228.5)	164 (146.5–211)	0.07
NLR	9.22 (6.13–13.45)	8.86 (6.03–13.18)	11.42 (8.00–16.80)	<0.01
Serum creatinine (g/dl)	90.00 (71.10–115.33)	89.39 (70.83–111.89)	103.12 (82.73–137.64)	0.73
AST	24.0 (19.0–34.0)	24.0 (18.0–34.0)	28.5 (21.8–36.8)	0.67
ALT	21.0 (14.8–32.0)	21.0 (14.0–32.0)	24.0 (17.5–30.0)	0.64
Operative information				
CPB time (minute)	240.5 (210.0–279.0)	236.5 (208.0–274.0)	298.5 (259.5–365.3)	<0.01
CABG	31 (6.64%)	18 (4.16%)	13 (38.23%)	<0.01
ACC time (minute)	134.3 ± 41.1	131.3 ± 37.8	172.0 ± 59.5	<0.01
Bentall	107 (22.91%)	100 (23.09%)	7 (20.59%)	0.74
Total arch replacement	449 (96.15%)	416 (96.07%)	33 (97.06%)	0.77

[a]Smoking is defined as current smoking (smoking more than 100 cigarettes and having smoked in the last 1 month) and ex-smoking.
BMI: body mass index; CAD: coronary arterial disease; MFS: Marfan syndrome; NLR: neutrophil to lymphocyte ratio; AST: aspartate aminotransferase; ALT: Alanine aminotransferase; CPB: cardiopulmonary bypass; ACC: aortic cross-clamp time; CABG: coronary artery bypass graft.

Neutrophil count and endpoints

Youden's index was used to determine the optimal cut-off value for the neutrophil count, and then, patients were divided into two subgroups: the decreased neutrophil group ($\leq 9.59 \times 10^9$/L), and the elevated neutrophil group ($>9.59 \times 10^9$/L). As shown in Table 2, the elevated neutrophil group ($>9.59 \times 10^9$/L) patients were more likely to present with death ($P < 0.01$), gastrointestinal bleeding ($P = 0.04$) and low cardiac output syndrome ($P = 0.02$) than the decreased neutrophil group patients. There were no significant differences between two groups in other endpoints.

Total in-hospital mortality was 7.28%. The in-hospital mortality rate was 10.8% in the elevated neutrophil group and 3.2% in the decreased neutrophil group. Figure 1 shows that the cumulative probability of the overall survival between the two groups was statistically significant, with patients in the elevated neutrophil group having a higher rate of in-hospital mortality. A restricted cubic spline was used to model the non-linear relationship between the neutrophil count and in-hospital mortality. As shown in Figure 2, the neutrophil count was the risk factor for in-hospital mortality when 9.9–16.4 $\times 10^9$/L.

Logistic regression methods

LASSO analyses were performed to evaluate the risk factor of in-hospital mortality in patients with ATAAD using 10-fold cross-validation (Table 3). LASSO regression showed log (λ) =

TABLE 2 The endpoints of the different neutrophil groups.

Endpoints	Neutrophil ≤ 9.59 × 109/L ($n = 217$)	Neutrophil > 9.59 × 109/L ($n = 250$)	*P*-value
Mortality	7 (3.2%)	27 (10.8%)	<0.01
Gastrointestinal bleeding	2 (0.9%)	10 (4.0%)	0.04
Paraplegia	8 (3.7%)	12 (4.8%)	0.55
Acute kidney failure	34 (15.7%)	56 (22.4%)	0.07
Reopen the chest	2 (0.9%)	5 (2.0%)	0.34
Low cardiac output syndrome	6 (2.8%)	19 (7.6%)	0.02
Cerebrovascular accident	8 (3.7%)	18 (7.2%)	0.10
MODS	3 (1.4%)	9 (3.6%)	0.13

MODS: multiple organ dysfunction syndrome.

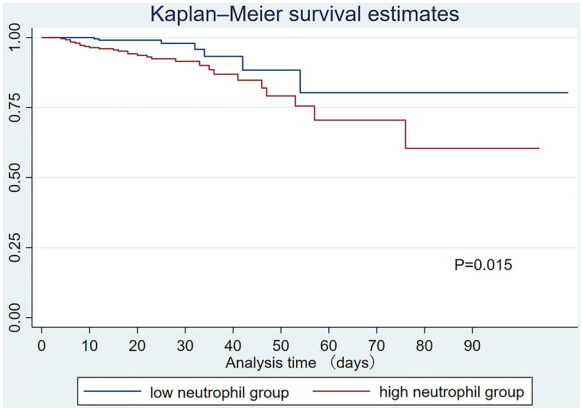


FIGURE 1
Kaplan-Meier analysis for in-hospital mortality by different neutrophil counts. The survival difference was significant ($P = 0.015$).

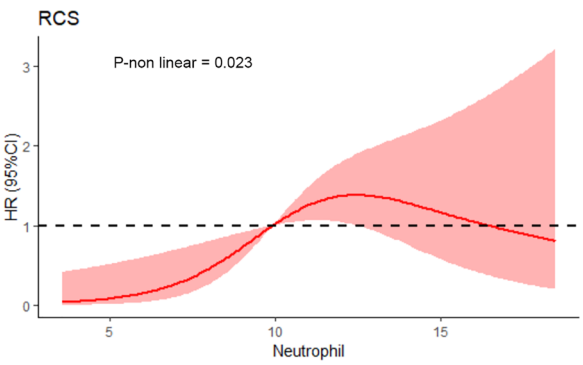


FIGURE 2
Restricted cubic splines of neutrophil count and in-hospital mortality. *(P non-linear = 0.023)*.

TABLE 3 Risk factors selected by the LASSO-logistic regression model.

Variables	Coefficient
Gender/male	0.127
Age	0.055
Smoke/no	0.188
Hypertension/no	−0.219
Diabetes/no	−0.090
CABG/no	−0.424
CPB	0.170
ACC	0.328
Neutrophil ≤ 9.59	−0.370
NLR ≤9.18	−0.029
Creatinine ≤92.24	−0.324

CABG: coronary artery bypass graft; CPB: cardiopulmonary bypass; ACC: aortic cross-clamp time; NLR: neutrophil to lymphocyte ratio; AST: aspartate aminotransferase.

0.0077 when the error of the model is minimized, and 12 variables (gender, age, smoking, hypertension, diabetes, CABG, CPB, ACC, neutrophil count, NLR, creatinine) were selected with non-zero coefficients for further analyses (Figure 3).

Multivariate logistic regression analysis then incorporated factors that were selected in the LASSO analyses (Table 4). In the model, elevated neutrophil counts (OR 3.07, 95% CI 1.22–7.62, $P = 0.02$), CABG (OR 9.54, 95% CI 3.47–26.20, $P < 0.01$), elevated serum creatinine (OR3.06, 1.23–7.62, $P = 0.02$) and ACC time (OR 1.01, 95% CI 1.00–1.02, $P < 0.01$) were significant independent risk factors for in-hospital mortality in patients with ATAAD.

The ROC curve analysis of model was shown in Figure 4, and the AUC is 0.824. Calibration plot of the model that represents perfect prediction that model-predicted probability matches actually observed probability was shown in Figure 5. As shown in Figure 6, the DCA for mortality in the nomogram of the model was built. When the threshold probability of occurrence of in-hospital mortality was around 0.02 to 0.62, the net benefit

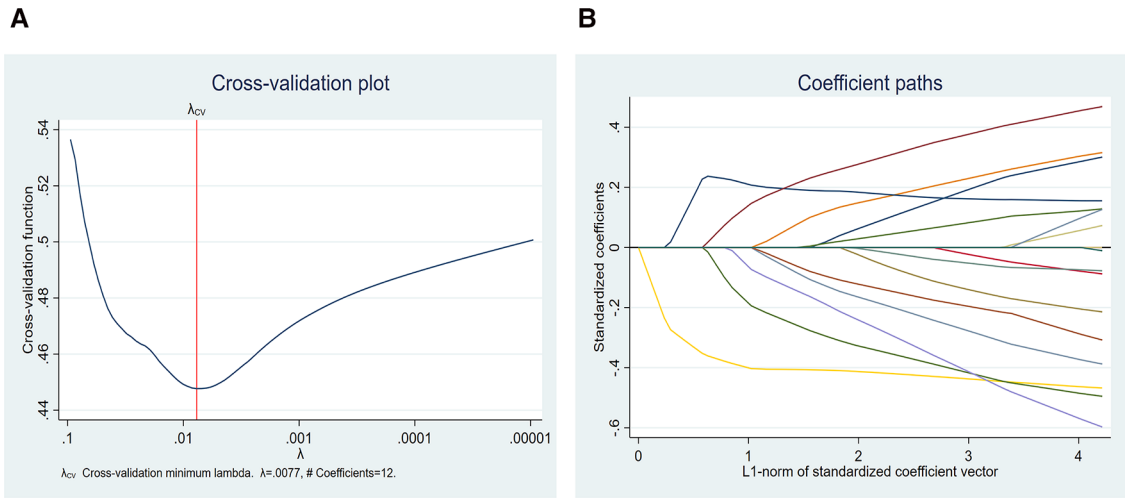


FIGURE 3
LASSO regression showed log (λ) = 0.0077 when the model error was minimized, and 12 variables were selected for further logistic regression analysis.

TABLE 4 Multivariate stepwise logistic regression analysis of risk factors selected by the LASSO-logistic model.

Variables	Odds ratio	95% CI	*P*-value
Gender/male	0.33	0.11–0.95	0.04
Neutrophil > 9.59	3.07	1.22–7.75	0.02
Creatinine > 92.24	3.06	1.23–7.62	0.02
CABG/with	9.54	3.47–26.20	<0.01
ACC	1.01	1.00–1.02	<0.01

CABG: coronary artery bypass graft; ACC: aortic cross-clamp time.

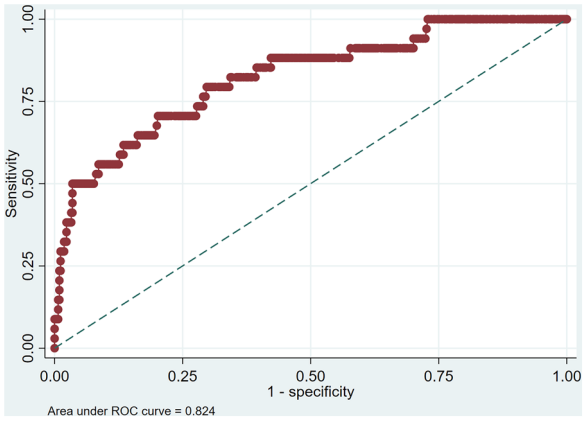


FIGURE 4
Receiver operating characteristic (ROC) curves of the multivariate logistic regression.

level of nomogram is obviously higher than that of "treat all" and "treat none", which indicates that the nomogram has good clinical applicability.

Discussion

Aortic dissection is a very serious health condition, and timely diagnosis is crucial to saving lives. Neutrophil count is a classical

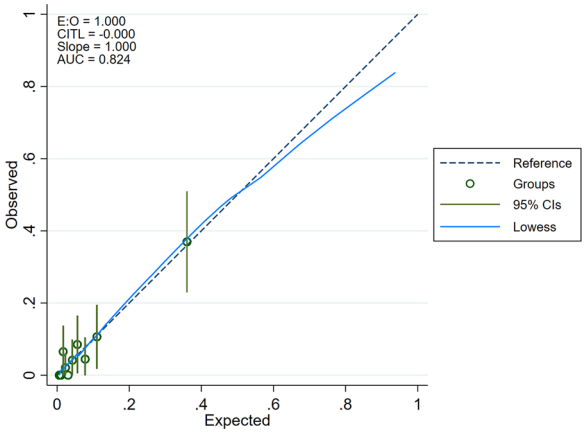


FIGURE 5
Calibration plot of the model that represents perfect prediction that model-predicted probability matches actually observed probability.

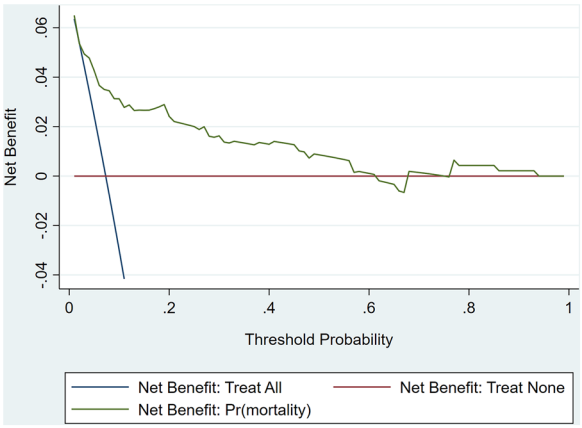


FIGURE 6
Decision curve analysis of the model.

biomarker of inflammation and a central player during acute inflammatory responses. Our findings helped to individualize the patients and may help to reduce in-hospital mortality of patients in the future.

Mechanical injury induced the expression of neutrophil chemoattractant (9). The release of inflammatory factors enhances the inflammatory reaction of the adventitia following the onset of ATAAD, leading to further dissection of the aorta and rupture of the dissection (10, 11). In-hospital mortality was 7.28% in our study. A former study reported that the mortality rates among ATAAD patients who do not receive surgical treatment can reach 30% within 48 h, proving that surgery is still a crucial method to treat patients. When the patient was diagnosed with acute type A aortic dissection, we will arrange surgery for the patient as soon as possible to reduce the impact of the surgery intervention time on the patient. A previous study has proven that elevated levels of inflammatory markers are predictive of cardiovascular events (12). It may be due to injury that a large number of inflammatory factors are released, causing the increase of central granulocytes. The main finding of this study was that elevated neutrophil count was an independent risk factor for in-hospital mortality ($P = 0.02$). Our study also found that gastrointestinal bleeding and low cardiac output syndrome were more common in patients with the elevated neutrophil count. Therefore, neutrophil plays a key role in the development of AD. The increase in the number of neutrophils should be a warning. When the patient's neutrophil number increases fast, the health system will issue an alarm indicating a critical value, which will help to improve clinical vigilance.

WBC count is the most commonly used blood parameter for many medical conditions. Ma et al. found that WBC > 11×10^9/L was an independent risk factor for mortality in ATAAD patients (OR = 3.10, $P < 0.01$) (13). Our research showed that there was no significant difference in the WBC counts of patients between the survivor and non-survivor groups ($P = 0.21$). This study found that neutrophil count was greatly different in the survivor and non-survivor groups. As a cost-effective and convenient

measurement, neutrophil count is a more representative and sensitive inflammation biomarker than NLR and WBC count in this study. NLR and PLR are emerging biomarkers with the combination of hemostatic and inflammatory pathways, which are regarded as potential biomarkers to predict outcomes of cardiac patients. Previous studies have reported the association between NLR, PLR, and in-hospital mortality in patients with cardiovascular diseases, including heart failure, acute coronary syndrome, etc (14–16). A study by Karakoyun et al. showed that elevated NLR may predict in-hospital mortality in patients with ATAAD (15). However, we did not find it to be a risk factor for in-hospital mortality after building a multivariate logistic regression model. Therefore, there was not sufficient evidence to indicate a better predictive value of NLR over neutrophil count in this study. Whether NLR can be a predictive biomarker need further investigation.

RCS found that when neutrophil <9.9 or >16.4 × 10^9/L, it was not the risk factor for in-hospital mortality. The whole curve is approximately C-shaped. Some patients not only suffered from extensive aortic injuries, but also may suffer from stress reactions, which resulted in an abnormal increase of neutrophils that can't predict the patient's physical condition well.

The multivariate regression model showed that ACC time (OR 1.01, 95% CI 1.00–1.02, $P < 0.01$) and CABG surgery (OR 9.54, 95% CI 3.47–26.20, $P < 0.01$) were significantly related to in-hospital mortality. Although CPB was selected in the LASSO analyses, it was not selected in multivariate logistic regression analyses, and it was still of great significance to prognosis. Some previous studies have illustrated that longer CPB time was associated with a greater possibility of suffering from adverse events in ATAAD patients (OR = 1.01) (17). Shorten CPB and ACC time, reduce organ ischemia-reperfusion injury, and improve the prognosis of the aortic disease. CABG surgery is a high-risk procedure, and postoperative complications can result in significant morbidity and mortality (18). Patients with overlapping surgery facing higher risks may have poorer prognoses.

This research had some limitations. First, it's a single-center retrospective study which undermines statistical power. Therefore, external validation of patients from other hospitals is needed. Secondly, detailed information about timing of intervention is not available in our study, which may have an effect on the results. Finally, the changes in neutrophil counts in acute and chronic patients need further study. Further research is needed to apply this prediction model in clinical practice.

Conclusion

Based on our research, neutrophil count is independently associated with in-hospital mortality in patients with ATAAD, which is an effective indicator of inflammation and has the potential to help surgeons make decisions regarding treatment strategy going forward.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving human participants were reviewed and approved by the Ethics Committee of Guangdong Provincial People's hospital. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

WF and HL contributed to the study design. WF, HL and QW participated in the data collection and drafted the manuscript. CL and JY performed data analysis. JW and RF revised the manuscript. All authors contributed to the article and approved the submitted version.

Funding

This work was supported by the National Key Research and Development Program of China (2017YFC1308003).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Gawinecka J, Schonrath F, von Eckardstein A. Acute aortic dissection: pathogenesis, risk factors and diagnosis. *Swiss Med Wkly*. (2017) 147:w14489. doi: 10.4414/smw.2017.14489

2. Mussa FF, Horton JD, Moridzadeh R, Nicholson J, Trimarchi S, Eagle KA. Acute aortic dissection and intramural hematoma: a systematic review. *JAMA*. (2016) 316 (7):754–63. doi: 10.1001/jama.2016.10026

3. Duan XZ, Xu ZY, Lu FL, Han L, Tang YF, Tang H, et al. Inflammation is related to preoperative hypoxemia in patients with acute Stanford type A aortic dissection. *J Thorac Dis*. (2018) 10(3):1628–34. doi: 10.21037/jtd.2018.03.48

4. Silvestre-Roig C, Braster Q, Ortega-Gomez A, Soehnlein O. Neutrophils as regulators of cardiovascular inflammation. *Nat Rev Cardiol*. (2020) 17(6):327–40. doi: 10.1038/s41569-019-0326-7

5. Hermann DM, Gunzer M. Polymorphonuclear neutrophils play a decisive role for brain injury and neurological recovery poststroke. *Stroke*. (2019) 50(3):e40–e1. doi: 10.1161/STROKEAHA.118.021564

6. Anidjar S, Dobrin PB, Eichorst M, Graham GP, Chejfec G. Correlation of inflammatory infiltrate with the enlargement of experimental aortic aneurysms. *J Vasc Surg*. (1992) 16(2):139–47. doi: 10.1016/0741-5214(92)90101-D

7. Bellomo R, Ronco C, Kellum JA, Mehta RL, Palevsky P. Acute renal failure - definition, outcome measures, animal models, fluid therapy and information technology needs: the second international consensus conference of the acute dialysis quality initiative (ADQI) group. *Crit Care*. (2004) 8(4):R204–12. doi: 10.1186/cc2872

8. Kang J, Choi YJ, Kim IK, Lee HS, Kim H, Baik SH, et al. LASSO-Based Machine learning algorithm for prediction of lymph node metastasis in T1 colorectal cancer. *Cancer Res Treat*. (2021) 53(3):773–83. doi: 10.4143/crt.2020.974

9. Anzai A, Shimoda M, Endo J, Kohno T, Katsumata Y, Matsuhashi T, et al. Adventitial CXCL1/G-CSF expression in response to acute aortic dissection triggers local neutrophil recruitment and activation leading to aortic rupture. *Circ Res*. (2015) 116(4):612–23. doi: 10.1161/CIRCRESAHA.116.304918

10. Li M, Xu S, Yan Y, Wang H, Zheng J, Li Y, et al. Association of biomarkers related to preoperative inflammatory and coagulation with postoperative in-hospital deaths in patients with type A acute aortic dissection. *Sci Rep*. (2021) 11(1):18775. doi: 10.1038/s41598-021-98298-w

11. He R, Guo DC, Estrera AL, Safi HJ, Huynh TT, Yin Z, et al. Characterization of the inflammatory and apoptotic cells in the aortas of patients with ascending thoracic aortic aneurysms and dissections. *J Thorac Cardiovasc Surg*. (2006) 131(3):671–8. doi: 10.1016/j.jtcvs.2005.09.018

12. Luo F, Zhou XL, Li JJ, Hui RT. Inflammatory response is associated with aortic dissection. *Ageing Res Rev*. (2009) 8(1):31–5. doi: 10.1016/j.arr.2008.08.001

13. Ma M, Shi J, Feng X, Wang J, Liu L, Wei X. The elevated admission white blood cell count relates to adverse surgical outcome of acute Stanford type a aortic dissection. *J Cardiothorac Surg*. (2020) 15(1):48. doi: 10.1186/s13019-020-1078-5

14. Choi DH, Kobayashi Y, Nishi T, Kim HK, Ki YJ, Kim SS, et al. Combination of mean platelet volume and neutrophil to lymphocyte ratio predicts long-term Major adverse cardiovascular events after percutaneous coronary intervention. *Angiology*. (2019) 70(4):345–51. doi: 10.1177/0003319718768658

15. Karakoyun S, Gursoy MO, Akgun T, Ocal L, Kalcik M, Yesin M, et al. Neutrophil-lymphocyte ratio may predict in-hospital mortality in patients with acute type A aortic dissection. *Herz*. (2015) 40(4):716–21. doi: 10.1007/s00059-014-4121-2

16. Coelho NH, Coelho A, Augusto R, Semiao C, Peixoto J, Fernandes L, et al. Pre-operative neutrophil to lymphocyte ratio is associated with 30 day death or amputation after revascularisation for acute limb ischaemia. *Eur J Vasc Endovasc Surg*. (2021) 62(1):74–80. doi: 10.1016/j.ejvs.2021.03.011

17. Tsai TT, Evangelista A, Nienaber CA, Trimarchi S, Sechtem U, Fattori R, et al. Long-term survival in patients presenting with type A acute aortic dissection: insights from the international registry of acute aortic dissection (IRAD). *Circulation*. (2006) 114(1 Suppl):I350–6. doi: 10.1161/CIRCULATIONAHA.105.000497

18. Montrief T, Koyfman A, Long B. Coronary artery bypass graft surgery complications: a review for emergency clinicians. *Am J Emerg Med*. (2018) 36(12):2289–97. doi: 10.1016/j.ajem.2018.09.014

frontiers | Frontiers in Cardiovascular Medicine

TYPE Original Research
PUBLISHED 04 July 2023
DOI 10.3389/fcvm.2023.1084426

OPEN ACCESS

EDITED BY
Shahzad Raja,
Harefield Hospital, United Kingdom

REVIEWED BY
Michael Ramsay,
Baylor University Medical Center, United States
Antonino S. Rubino,
University of Campania Luigi Vanvitelli, Italy

*CORRESPONDENCE
Alessandro Belletti
belletti.alessandro@hsr.it;
belletti.ale@gmail.com

†PRESENT ADDRESS
Alessandro Belletti,
Department of Anesthesia and Intensive Care, IRCCS San Raffaele Scientific Institute, Milan, Italy

RECEIVED 30 October 2022
ACCEPTED 19 June 2023
PUBLISHED 04 July 2023

CITATION
Belletti A, Lee D-K, Yanase F, Naorungroj T, Eastwood GM, Bellomo R and Weinberg L (2023) Changes in SedLine-derived processed electroencephalographic parameters during hypothermia in patients undergoing cardiac surgery with cardiopulmonary bypass. Front. Cardiovasc. Med. 10:1084426. doi: 10.3389/fcvm.2023.1084426

Changes in SedLine-derived processed electroencephalographic parameters during hypothermia in patients undergoing cardiac surgery with cardiopulmonary bypass

Alessandro Belletti[1,2*†], Dong-Kyu Lee[3], Fumitaka Yanase[1,4], Thummaporn Naorungroj[1,5], Glenn M. Eastwood[1], Rinaldo Bellomo[1,4,6,7] and Laurence Weinberg[6,8]

[1]Department of Intensive Care, Austin Hospital, Heidelberg, VIC, Australia, [2]Department of Anaesthesia and Intensive Care, IRCCS San Raffaele Scientific Institute, Milan, Italy, [3]Department of Anesthesiology and Pain Medicine, Dongguk University Ilsan Hospital, Goyang, Republic of Korea, [4]Australian and New Zealand Intensive Care Research Centre, Monash University, Melbourne, VIC, Australia, [5]Department of Intensive Care, Siriraj Hospital, Mahidol University, Bangkok, Thailand, [6]Department of Critical Care, The University of Melbourne, Melbourne, VIC, Australia, [7]Data Analytics Research and Evaluation Centre, The University of Melbourne and The Austin Hospital, Melbourne, VIC, Australia, [8]Department of Anaesthesia, Austin Hospital, Heidelberg, VIC, Australia

Objective: Processed electroencephalography (pEEG) is used to monitor depth-of-anesthesia during cardiopulmonary bypass (CPB). The SedLine device has been recently introduced for pEEG monitoring. However, the effect of hypothermia on its parameters during CPB is unknown. Accordingly, we aimed to investigate temperature-induced changes in SedLine-derived pEEG parameters during CPB.
Design: Prospective observational study.
Setting: Cardiac surgery operating theatre.
Participants: 28 patients undergoing elective cardiac surgery with CPB.
Interventions: We continuously measured patient state index (PSI), suppression ratio (SR), bilateral spectral edge frequency (SEF) and temperature. We used linear mixed modelling with fixed and random effects to study the interactions between pEEG parameters and core temperature.
Measurements and main results: During CPB maintenance, the median temperature was 32.1°C [interquartile range (IQR): 29.8–33.6] at the end of cooling and 32.8°C (IQR: 30.1–34.0) at rewarming initiation. For each degree Celsius change in temperature during cooling and rewarming the PSI either decreased by 0.8 points [95% confidence interval (CI): 0.7–1.0; $p < 0.001$] or increased by 0.7 points (95% CI: 0.6–0.8; $p < 0.001$). The SR increased by 2.9 (95% CI: 2.3–3.4); $p < 0.001$) during cooling and decreased by 2.2 (95% CI: 1.7–2.7; $p < 0.001$) during rewarming. Changes in the SEF were not related to changes in temperature.
Conclusions: During hypothermic CPB, temperature changes led to concordant changes in the PSI. The SR increased during cooling and decreased during rewarming. Clinicians using SedLine for depth-of-anesthesia monitoring should be aware of these effects when interpreting the PSI and SR values.

KEYWORDS

anesthesia, electroencephalography, cardiac surgery, neuromonitoring, neuroprotection, propofol, delirium, cardiopulmonary bypass

Introduction

Both excessive as well as insufficient anesthesia depth may be associated with worse perioperative outcomes. Excessive anesthesia depth may be associated with the development of delirium, hemodynamic instability, prolonged intensive care unit (ICU) stay and long-term cognitive dysfunction. Conversely, insufficient anesthesia may be associated with the development of delirium, post-traumatic stress disorders, excessive circulating catecholamines and, in worst-case scenarios, intraoperative awareness (1).

Accordingly, monitoring for adequate depth of anesthesia during procedures requiring neuromuscular blockade is recommended by current guidelines, especially when total intravenous anesthesia is used (2). In patients undergoing cardiac surgery, the risk of awareness is considered particularly possible high during cardiopulmonary bypass (CPB) because conventional clinical parameters used to assess depth of anesthesia (i.e., heart rate and blood pressure) are profoundly altered by CPB, aortic cross-clamp and induced cardiac arrest (3–5). This has made the use of depth-of-anesthesia monitoring desirable.

Several depth-of-anesthesia monitoring devices are commercially available (6, 7). These devices obtain continuous, non-invasive processed electroencephalogram (pEEG) signals using adhesive gel electrodes placed on the forehead. The signal is subsequently amplified, filtered and processed through proprietary algorithms to provide a final index of anesthesia depth, as well as other indices such as burst suppression ratio (SR), electromyographic activity, and indices of signal quality (6, 8). Among such devices, the SedLine device (Masimo, Irvine, CA, USA) is one of the most recently introduced (7, 9). However, although some studies have shown that, in patients undergoing cardiac surgery, changes in temperature during CPB can independently affect the bispectral index (BIS) (10–13), there are no available data on the effect of temperature on SedLine-derived parameters.

Accordingly, we conducted a prospective observational study in patients undergoing cardiac surgery to investigate whether and to what extent changes in temperature during CPB affect SedLine-derived parameters.

Methods

This was a single-centre, prospective, observational study performed on adult patients undergoing cardiac surgery requiring CPB in a teaching hospital in Australia. The study was approved by the institutional ethics committee (Austin Health Ethics Committee, Project No.: Audit/19/Austin/59). As the use of SedLine to monitor the depth of anesthesia was the standard of care in our institution, informed consent was waived because of the observational nature of the study.

Inclusion and exclusion criteria

We included adult patients (i.e., over 18 years of age) who underwent elective cardiac surgery with planned use of CPB. We excluded patients if their procedure was planned to be off-pump, or if they had known baseline electroencephalography (EEG) alterations, pre-operative cognitive impairment or intellectual disability, a history of epilepsy, or a suspected or confirmed pregnancy.

Anesthesia and cardiopulmonary bypass management protocol

All procedures were performed under general anesthesia. In all patients, monitoring included 5-lead electrocardiogram, pulse oximetry, invasive arterial blood pressure, central venous pressure, pulmonary artery pressure and cardiac index (obtained with a pulmonary artery catheter), body temperature (bladder temperature), diuresis and transesophageal echocardiography. Depth of anesthesia was monitored with SedLine in all patients. Regional cerebral oximetry was monitored at the discretion of the attending anesthesiologist. The anesthetic regimen was at the discretion of the attending anesthesiologist. However, in all cases, anesthesia was maintained with an intravenous continuous propofol infusion during CPB.

Technology

Processed EEG was recorded using the Next Generation SedLine® Brain Function Monitoring device (Masimo, Irvine, CA, USA). The depth-of-anesthesia parameter displayed by SedLine is the Next Generation Patient State Index (PSI), a dimensionless index of sedation depth ranging from 0 to 100 (with 0 indicating a deeply sedated patient with an isoelectric EEG and 100 a fully awake patient), elaborated through a proprietary algorithm (7, 9). Additionally, the SedLine monitor displays two bilateral raw EEG traces, bilateral density spectral array and spectral edge frequency (SEF) (14), the burst suppression ratio (SR), the percentage of artefacts detected by the device as a measure of signal quality, and electromyographic (EMG) activity.

Data collection and data cleaning

In all patients, SedLine monitoring was started on arrival in the operating theatre before the induction of anesthesia and then discontinued at skin closure before transferring the patient to the ICU. The SedLine device recorded all parameters at 2 s intervals. All SedLine-derived data were downloaded from the device immediately after surgery.

Temperature data obtained through the nasopharyngeal or bladder probe were automatically recorded every 20 s on the CPB machine software and downloaded at the end of the procedure. The CPB machine also automatically measured and recorded data on PaO_2, $PaCO_2$ and hemoglobin every 20 s. Additionally, one of the investigators manually recorded SedLine and temperature data on a case report form every 10 min to allow for a check of data accuracy.

In addition to SedLine and temperature data, baseline characteristics, the type of procedure, the duration of CPB and aortic cross-clamp, and outcome data were also recorded.

These data were subsequently merged into one electronic data sheet for each patient using Microsoft Excel, version 1911 (Microsoft, 2019, USA). According to the recorded event marks, the data on hypothermia induction, maintenance and recovery phases were separated and merged into another database. Before data analysis, all recordings were reviewed by two investigators to check for data accuracy and possible outliers. Using the scatter plots of each pEEG value against time or core temperature, a visual check was performed for artefact values with recorded artefact levels as reference. After manual trimming, all starting time points were set to zero. For the SR, all recorded values of zero were removed.

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics for Windows, version 23 (IBM, 2015, Armonk, NY, USA) and R, version 3.6.1 (R Development Core Team, 2018, Vienna, Austria). A normality test was performed for continuous variables, and, when the normality assumption was violated, a non-parametric statistical method was applied. Descriptive statistics were used for demographic data presentation.

Linear mixed modelling with fixed and random effects

Considering general concepts concerning EEG and hypothermia, we presumed that, at a certain point, all pEEG values would converge into one point as the core temperature decreased (15). At the start of CPB, each patient had a various core temperature and their own pEEG value. The target temperature of hypothermia was also varied individually, as was the duration of hypothermia according to the type of surgery. Additionally, the patient's response to lowering their core temperature could vary.

Therefore, to evaluate the effect of hypothermia on the pEEG at the different hypothermia targets, we performed a visual check of each patient's pEEG response over the measured time points. We hypothesized that the response of the pEEG to the lowering, maintaining, or increasing of the patient's core temperature would follow random slopes (i.e., each patient would have their own response) and converge into one point (i.e., we presumed a fixed intercept where the core temperature was 0°C). With this assumption, and the results of a visual check for the linear relationship between the pEEG and the core temperature of each patient, we evaluated the linear mixed model with random slope–effects between the pEEG and the core temperature during hypothermia induction and recovery periods.

For hypothermia induction and recovery periods, the major variation was the core temperature, which was measured every 20 s. We prespecified a linear mixed model with random effects for the core temperature for both periods. During the maintenance period, the core temperature was maintained within a narrow range, and the main variation was the duration of hypothermia. To evaluate the effect of hypothermia duration on the pEEG for this period, we used a linear mixed model with random effects by time instead of core temperature.

Given the differences in the responses of individual patients and in the values of pEEGs at the beginning of hypothermia maintenance, the random effects were considered for both the slope and intercept for the linear mixed-effects model during the hypothermia maintenance period. Unstructured covariance was used for random effects. The whole model was evaluated for fitness with −2 restricted log-likelihood and Schwarz's Bayesian criterion. A Type III sum of squares was used for fixed-effects evaluation. The variances of covariance parameter estimates were also tested with the Wald *Z*-test to evaluate the random effect between subjects.

Association between PSI values and postoperative complications

PSI values were divided into three different categories: <25 (deep anaesthesia), 25–50 (optimal anaesthesia depth), and >50 (light anaesthesia), as recommended by manufacturer (7, 9). During CPB (from pump-on to pump-off), the relative duration of each PSI level (>50, 25–50, <25) was identified and expressed as percentage of time over the total CPB duration.

Spearman/Pearson correlation analysis was performed according to the characteristics of the outcome variables.

Results

Between 11 June and 29 July 2019, a total of 54 patients underwent cardiac surgery with CPB. Of these, 21 were excluded due to the unavailability of research personnel to collect complete intraoperative data, one was excluded as an emergency case, two were excluded due to exclusion criteria, and two were excluded due to technical failure in data recording by the CPB machine. Therefore, the remaining 28 patients were included in the analysis.

Characteristics of the participants

The median duration of analyses was 2,380 s (IQR: 1,645 s–3,365 s), 4,160 s (IQR: 1,790 s–6,155 s) and 1,520 s (IQR: 1,260 s–2,085 s) for the hypothermia induction, maintenance and recovery periods, respectively. The total included time was 74,000, 125,760 and 48,460 s for the induction, maintenance and recovery periods, respectively. Patients' baseline and procedural characteristics are summarized in **Table 1**.

The median core temperatures measured during CPB were 35.2°C (IQR: 34.7°C–35.6°C) and 32.7°C (IQ: 30.4°C–34.0°C) at the start and end of the hypothermia induction period, respectively (Wilcoxon signed-rank test, $p < 0.001$). During the maintenance period, the core temperatures were 32.1°C (IQR: 29.8°C–33.6°C) and 32.8°C (IQR: 30.1°C–34.0°C) at the start and end of the period, respectively (Wilcoxon signed-rank test,

TABLE 1 Patients' characteristics.

Characteristic	Value
Age (years), Mdn (IQR)	72.3 (60.3–79.6)
Height (cm), Mdn (IQR)	167 (158–173)
Weight (kg), Mdn (IQR)	79 (67–91)
Female	8 (28.6)
Diabetes	12 (37.5)
Hypertension	25 (89.3)
Congestive heart failure	14 (50.0)
Ischemic heart disease	20 (71.4)
Dyslipidemia	17 (60.7)
Cerebrovascular disease	8 (28.6)
Peripheral vascular disease	5 (17.8)
Chronic liver disease	1 (3.5)
Chronic respiratory disease	3 (10.7)
Chronic kidney disease	7 (25.0)
Surgical procedure	
Aortic arch replacement + stenting	1 (3.5)
AVR	2 (7.1)
AVR (mini-invasive)	1 (3.5)
Bentall's procedure	1 (3.5)
CABG	12 (37.5)
CABG and AVR	6 (21.4)
CABG and MV repair	1 (3.5)
David's procedure	1 (3.5)
MV repair	1 (3.5)
MVR	1 (3.5)
MVR (mini-invasive)	1 (3.5)
Ross procedure	2 (7.1)
Cardiopulmonary bypass time (min), Mdn (IQR)	124 (99–201)
Cross-clamp time (min), Mdn (IQR)	99 (74–160)
Deep hypothermic circulatory arrest	1 (3.5)
Selective cerebral perfusion	1 (3.5)

$N = 28$. Values are given as n (%) unless otherwise specified. IQR, interquartile range; AVR, aortic valve replacement; CABG, coronary artery bypass grafting; MVR, mitral valve replacement.

$p < 0.001$). Statistically, the core temperatures were significantly different between at the start and end of the maintenance period despite core temperatures being kept within 2.0°C during this period. For the recovery period, the core temperatures were 32.7°C (IQR: 30.4°C–33.9°C) and 36.5°C (IQR: 36.5°C–36.7°C) at the start and end of the period, respectively (Wilcoxon signed-rank test, $p < 0.001$). Data on PaO_2, $PaCO_2$, hemoglobin and anesthetic agents used during CPB are presented in Table 2.

Analysis of the processed electroencephalography

Hypothermia induction period

Details on changes in pEEG-derived parameters during hypothermia induction period are presented in Table 3. According to the random-slope linear mixed modelling, the PSI decreased 0.84 points with each degree Celsius core temperature drop [95% confidence interval (CI): 0.68–0.99; $p < 0.001$]. The SR increased by 2.9 (95% CI: 2.3–3.4; $p < 0.001$).

TABLE 2 Anaesthesia and arterial blood Gas analysis results during cardiopulmonary bypass.

Variable	Value
PaO_2 (mmHg), M ± SD	
Cooling	351.4 ± 71.5
Maintenance	354.5 ± 53.9
Rewarming	320.1 ± 61.3
$PaCO_2$ (mmHg), M ± SD	
Cooling	39.1 ± 8.7
Maintenance	42.4 ± 4.6
Rewarming	46.1 ± 5.3
Hemoglobin (g/dl), M ± SD	
Cooling	9.4 ± 2.9
Maintenance	9.9 ± 1.8
Rewarming	9.8 ± 2.3
Anesthesia medications during CPB, *n* (%)	
Propofol continuous infusion	28 (100)
Isoflurane in CPB oxygenator	22 (78.6)
Fentanyl bolus	13 (46.4)
Fentanyl continuous infusion	9 (32.1)
Alfentanil continuous infusion	9 (32.1)
Remifentanil continuous infusion	1 (3.5)
Dexmedetomidine continuous infusion	1 (3.5)

CPB, cardiopulmonary bypass.

With lower temperatures, the SEF significantly decreased: by 0.18 Hz (95% CI: 0.12 Hz–0.24 Hz; $p < 0.001$), and by 0.22 Hz (95% CI: 0.16 Hz–0.28 Hz; $p < 0.001$), for the right and left, respectively (see Figure 1). Details on variation between patients are presented in the Supplementary Table S1.

Maintenance period

As previously described, the effects of hypothermia on the pEEG during the maintenance period were evaluated in relation to the duration of hypothermia. Details are presented in Table 4. The PSI had significant random variation at the beginning of hypothermia maintenance ($p < 0.001$); the random effect of

TABLE 3 Changes in processed electroencephalography-derived parameters during cooling and rewarming.

	Change per 1°C change in temperature	95% CI	*F* statistics	*p*-value
Cooling				
PSI	−0.84	−0.99 to −0.68	$F(1, 193.4) = 111.1$	<0.001
SR	2.9	2.3–3.4	$F(1, 315.2) = 104.6$	<0.001
SEF, R (Hz)	−0.18	−0.24 to −0.12	$F(1, 683.7) = 34.7$	<0.001
SEF, L (Hz)	−0.22	−0.28 to −0.16	$F(1, 333.0) = 47.5$	<0.001
Rewarming				
PSI	0.70	0.60–0.81	$F(1, 27.1) = 194.4$	<0.001
SR	−2.2	−2.7 to −1.7	$F(1, 188.5) = 85.4$	<0.001
SEF, R (Hz)	0.18	0.10–0.26	$F(1, 210.2) = 19.3$	<0.001
SEF, L (Hz)	0.28	0.21–0.36	$F(1, 215.7) = 50.3$	<0.001

CI, confidence interval; PSI, patient state index; SEF, R, spectral edge frequency, right cerebral hemisphere; SEF, L, spectral edge frequency, left cerebral hemisphere; SR, suppression ratio.

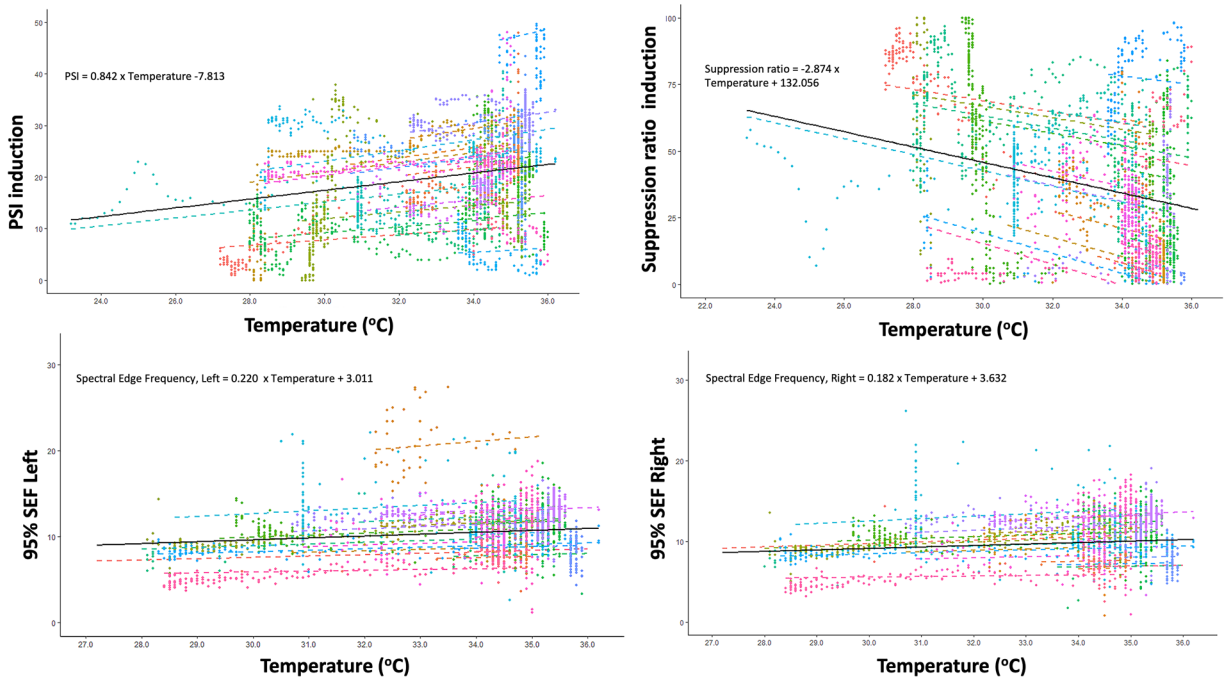


FIGURE 1
Correlation between changes in core temperature and changes in Patient State Index (PSI), Suppression ratio, Spectral Edge Frequency (SEF) of left brain hemisphere and SEF of right brain hemisphere during induction of hypothermia.

hypothermia maintenance duration was not significant ($p = 0.5$). Considering this variability, the estimated linear relationship was not significant ($p = 0.224$). The estimated intercept was 21.3 (95% CI: 17.3–25.2; $p < 0.001$).

The SR showed a significant random effect at the beginning of hypothermia maintenance ($p = 0.001$), and the duration of hypothermia maintenance was not a significant random effect ($p = 0.891$). Accepting that the intercepts were variable between patients, there was no significant linear relationship between the SR and the duration of hypothermia maintenance ($p = 0.978$).

The SEFs also had variability in the intercepts ($p = 0.001$ for both right and left), and the duration of hypothermia maintenance was not a significant random effect (right, $p = 0.996$; left, $p = 0.072$). During this period, there was no relationship between the right or left SEF and the duration of hypothermia maintenance (right, $p = 0.077$; left, $p = 0.346$). The estimated intercepts were 10.7 (95% CI: 9.1–12.3; $p < 0.001$), and 11.3 (95% CI: 9.4–13.2; $p < 0.001$) for the right and left, respectively.

Rewarming period

Details on changes in pEEG-derived parameters during rewarming period are presented in **Table 3**. During the rewarming period, every degree Celsius increase in core temperature was associated with an increase in the PSI by 0.70 (95% CI: 0.60–0.81, $p < 0.001$).

The SR decreased by 2.2 for each degree Celsius increase of the core temperature (95% CI: 1.7–2.7; $p < 0.001$). The SEF of both sides decreased according to increased core temperature. The right and left SEF were predicted to be, in response to increased core temperature of one unit, 0.18 Hz (95% CI: 0.10 Hz–0.26 Hz, $p < 0.001$) and 0.28 Hz (95% CI: 0.21 Hz–0.36 Hz, $p < 0.001$) for right and left, respectively (**Figure 2**). Details on variation between patients are presented in the **Supplementary Table S1**.

TABLE 4 Effect of hypothermia duration on processed electroencephalography-derived parameters during maintenance period.

EEG variable	Coefficient			Estimated variance of intercept		Estimated variance of duration	
	Coefficient	95% CI	*p*-value	Variance	*p*-value	Variance	*p*-value
PSI	5×10^{-4}	-3×10^{-4} to 1.2×10^{-3}	0.224	103.7	<0.001	2.2×10^{-3}	0.500
SR	-4.1×10^{-5}	-3×10^{-3} to 2.9×10^{-3}	0.980	908.7	0.001	-6.3×10^{-3}	0.890
SEF, R (Hz)	2.1×10^{-4}	-3×10^{-5} to 4.4×10^{-4}	0.077	15.2	0.001	2.0×10^{-6}	0.996
SEF, L (Hz)	1.9×10^{-4}	-2.3×10^{-4} to 5.9×10^{-4}	0.346	21.4	0.001	-2.0×10^{-3}	0.072

CI, confidence interval; PSI, patient state index; SEF, R, spectral edge frequency, right cerebral hemisphere; SEF, L, spectral edge frequency, left cerebral hemisphere; SR, suppression ratio.

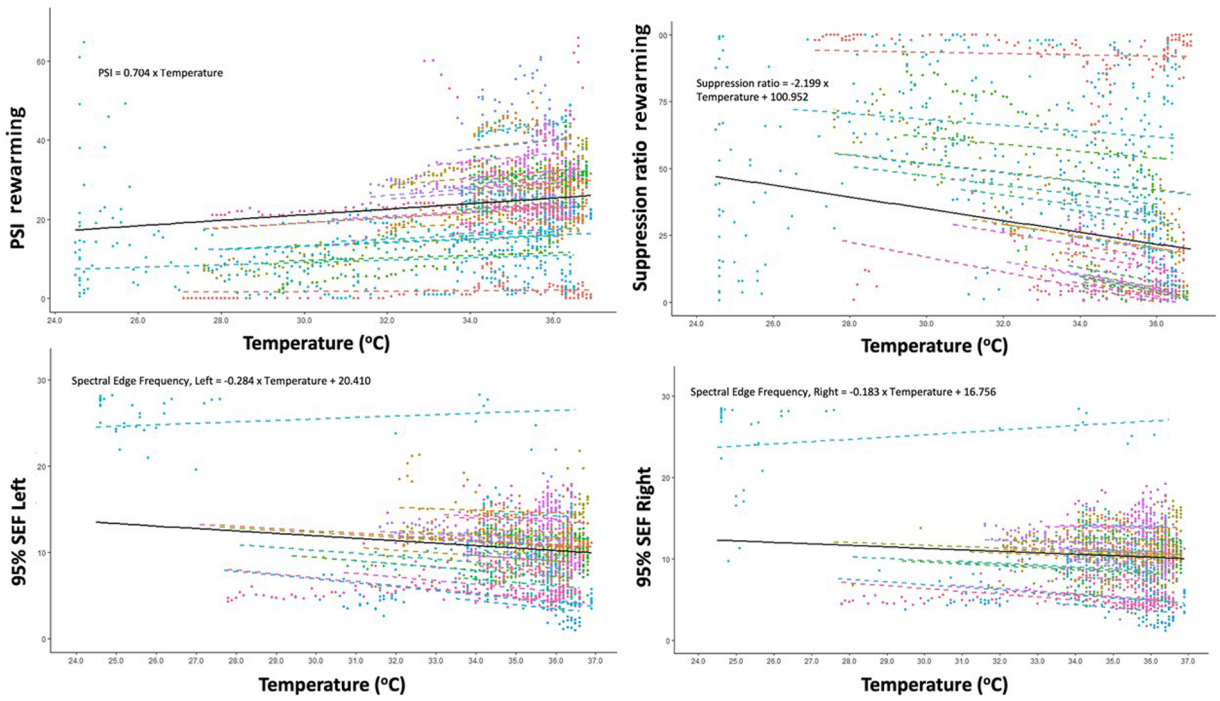


FIGURE 2
Correlation between changes in core temperature and changes in Patient State Index (PSI), Suppression ratio, Spectral Edge Frequency (SEF) of left brain hemisphere and SEF of right brain hemisphere during rewarming.

Outcomes

Major complications are presented in **Supplementary Table S2**. A total of 13 patients (46%) required inotropic support in ICU, with three (10.7%) requiring inotropic support for >48 h. Acute kidney injury occurred in 14 patients (50%), with two patients requiring renal replacement therapy (7.1%). No patient had postoperative myocardial infarction. Delirium occurred in nine (32.1%) patients. Three patients (10.7%) required re-exploration for bleeding. One of them had a massive hemorrhage requiring immediate re-sternotomy at the bedside in ICU. The subsequent course was complicated by cardiogenic shock requiring extracorporeal membrane oxygenation, renal failure requiring renal replacement therapy and ischemic stroke. The patient died in ICU. There were no other in-hospital deaths.

Association between PSI levels and complications is presented in **Supplementary Table S3**. We found that exposure to a PSI level >50 during CPB was moderately associated with development of postoperative delirium, while no association with other complications was found.

Discussion

Key findings

In this single-center observational study, we quantified changes in the SedLine parameters in relation to changes in body temperature. We found that the PSI decreased by 0.84 points for each degree Celsius decrease in core temperature during the induction of hypothermia and increased by 0.70 points for each degree during the recovery phase. Similarly, SR increased during induction and decreased during the recovery period, while changes in the SEF were not related to changes in body temperature.

Relationship to previous studies

Changes in EEG during hypothermia have been previously described in several studies. Early studies performed in the 50 s and 60 s showed that hypothermia has a suppressive effect on EEG (16, 17). More recently, since the introduction of pEEG monitoring in anesthesia, several studies have investigated the effect of hypothermia on the BIS (4), the most widely described pEEG parameter (7).

Overall, these studies found that hypothermia induced a decrease in the BIS of approximately 1 point per degree Celsius (11, 12, 18). A strong correlation between the BIS and temperature was also found in studies investigating cardiac surgery under deep hypothermic circulatory arrest (19, 20). A study comparing the BIS with entropy showed that the two indices have good correlation under normothermic conditions but poor agreement during hypothermia, suggesting that different depth-of-anesthesia indices may not be equivalent under hypothermic conditions (21).

In our study, we investigated, for the first time, the effect of hypothermia on the PSI, a novel depth-of-anesthesia index displayed by the SedLine monitor. Our results showed that changes in the PSI are comparable to those reported for the BIS (i.e., approximately 1 PSI point for each degree Celsius decrease).

Interestingly, changes in the PSI were more pronounced during the induction of hypothermia than during rewarming. To the best of our knowledge, there have been no studies comparing changes in the PSI and the BIS during hypothermia. Thus, comparison between these two indices in this specific condition remains speculative.

In addition, we investigated changes in the SEF. Some studies have reported that the SEF remains relatively stable during hypothermia (22, 23), although such findings have not been consistent (3, 18, 24). Our results demonstrate the possible independence of the SEF from core temperature during hypothermia induction, maintenance and recovery periods. These findings imply that SEF variations during hypothermia may differ from PSI or SR responses to core temperature changes. These findings are consistent with previous reports that changes in the SEF are not consistent with BIS changes in the late phase of CPB (3) and during rewarming (24). Further, our findings confirm those from previous studies that have shown that EEG changes during the cooling phase of hypothermia differ from changes during rewarming (25, 26). However, it should be noted that absolute changes in the SEF in our study are unlikely to be clinically relevant (approximately 0.2 Hz for each degree Celsius; i.e., less than 1 Hz from 36 to 32°C) considering the wide range of SEF values (approximately 10–23 Hz) even in healthy volunteers (27).

Study implications

The results of our study imply that SedLine-derived indices during CPB are influenced by changes in body temperature. Findings from our study further suggest that changes in body temperature have different effects on different SedLine-derived parameters. Our data suggest that the PSI correctly reflects EEG alterations during temperature changes and may be used to monitor the depth of anesthesia during hypothermic CPB. Furthermore, for the first time, we were able to provide an approximate estimation of the relative contribution of mild hypothermia to PSI changes: approximately 1 PSI point for each degree Celsius. Clinicians using pEEG monitoring during anesthesia or in the ICU should be aware of these changes when interpreting the values of pEEG indices and making clinical decisions.

Interestingly, we found that inadequately deep anesthesia was associated with development of postoperative delirium. The association between inadequate anesthesia depth (either too deep or to light) and delirium has already been described, and our findings are in line with this concept (1, 28), although our study was underpowered to investigate association between PSI levels and complications.

Strengths and limitations

The strengths of our study include the continuous recording and analysis of temperature and pEEG data, unlike previous studies that had limited data collection and fixed time points (3, 21, 23). Furthermore, our data collection was not limited to the PSI but also included additional SedLine-derived parameters, such as the SEF and SR, and analysis their correlations.

Our study has several limitations. The anesthesia protocol was not mandatory; therefore, we cannot exclude the possibility of different effects of different anesthesia techniques on the pEEG-derived indices. However, our study reflects current clinical practice. Additionally, the study has all the limitations of a single-center trial. Accordingly, our findings may not be generalizable to other hospitals or to other non-cardiac surgery adult patients. We used only SedLine as depth-of-anaesthesia monitor. Accordingly, we were unable to make a direct comparison with other devices. Future studies should address whether different anesthetic regimens have different effects on SedLine-derived parameters during hypothermia induction, maintenance, and recovery. Furthermore, future studies should investigate whether different PSI-guided anesthesia regimens during hypothermia influence neurological outcomes after cardiac surgery with CPB. Our study provides baseline data that allow for the planning and design of such future studies.

Conclusions

During hypothermic CPB using the SedLine device, temperature changes led to a decrease in the PSI with cooling and an increase with warming with changes of approximately 1 PSI point for each degree Celsius. The SR increased during cooling as the PSI decreased and decreased as the PSI increased during rewarming. In contrast, changes in the SEF were not related to changes in body temperature. Clinicians using SedLine for depth-of-anesthesia monitoring during CPB with induced hypothermia should be aware of these effects when interpreting the PSI and SR values.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The study was approved by Austin Health Ethics Committee, Project No. Audit/19/Austin/59. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

AB: study design; data acquisition, analysis, and interpretation; manuscript drafting. D-KL: data acquisition, analysis, and interpretation; manuscript drafting. FY: data

acquisition, analysis, and interpretation; manuscript review. TN: data acquisition, analysis, and interpretation; manuscript review. GE: data acquisition, analysis, and interpretation; manuscript review. RB: study design; data acquisition, analysis, and interpretation; manuscript review. LW: study design; data acquisition, analysis, and interpretation; manuscript drafting. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2023.1084426/full#supplementary-material

References

1. Escallier KE, Nadelson MR, Zhou D, Avidan MS. Monitoring the brain: processed electroencephalogram and peri-operative outcomes. *Anaesthesia*. (2014) 69 (8):899–910. doi: 10.1111/ANAE.12711

2. Klein AA, Meek T, Allcock E, Cook TM, Mincher N, Morris C, et al. Recommendations for standards of monitoring during anaesthesia and recovery 2021. *Anaesthesia*. (2021) 76(9):1212–23. doi: 10.1111/ANAE.15501

3. Hayashi K, Mita K, Sawa T. Electroencephalographic changes in the late cardiopulmonary bypass period are not reflected in the bispectral index. *Clin Neurophysiol*. (2010) 121(8):1198–204. doi: 10.1016/J.CLINPH.2010.03.018

4. Kertai MD, Whitlock EL, Avidan MS. Brain monitoring with electroencephalography and the electroencephalogram-derived bispectral index during cardiac surgery. *Anesth Analg*. (2012) 114(3):533–43. doi: 10.1213/ANE.0B013E31823EE030

5. Barry AE, Chaney MA, London MJ. Anesthetic management during cardiopulmonary bypass: a systematic review. *Anesth Analg*. (2015) 120(4):749–69. doi: 10.1213/ANE.0000000000000612

6. Fahy BG, Chau DF. The technology of processed electroencephalogram monitoring devices for assessment of depth of anesthesia. *Anesth Analg*. (2018) 126 (1):111–7. doi: 10.1213/ANE.0000000000002331

7. Heyse B, Van Ooteghem B, Wyler B, Struys MMRF, Herregods L, Vereecke H. Comparison of contemporary EEG derived depth of anesthesia monitors with a 5 step validation process. *Acta Anaesthesiol Belg*. (2009) 60(1):19–33.

8. Hajat Z, Ahmad N, Andrzejowski J. The role and limitations of EEG-based depth of anaesthesia monitoring in theatres and intensive care. *Anaesthesia*. (2017) 72(Suppl 1):38–47. doi: 10.1111/ANAE.13739

9. Drover D, Ortega HR. Patient state index. *Best Pract Res Clin Anaesthesiol*. (2006) 20(1):121–8. doi: 10.1016/J.BPA.2005.07.008

10. Mathew PJ, Puri GD, Dhaliwal RS. Propofol requirement titrated to bispectral index: a comparison between hypothermic and normothermic cardiopulmonary bypass. *Perfusion*. (2009) 24(1):27–32. doi: 10.1177/0267659109106071

11. Mathew JP, Weatherwax KJ, East CJ, White WD, Reves JG. Bispectral analysis during cardiopulmonary bypass: the effect of hypothermia on the hypnotic state. *J Clin Anesth*. (2001) 13(4):301–5. doi: 10.1016/S0952-8180(01)00275-6

12. Schmidlin D, Hager P, Schmid ER. Monitoring level of sedation with bispectral EEG analysis: comparison between hypothermic and normothermic cardiopulmonary bypass. *Br J Anaesth*. (2001) 86(6):769–76. doi: 10.1093/BJA/86.6.769

13. Dewandre PY, Hans P, Bonhomme V, Brichant JF, Lamy M. Effects of mild hypothermic cardiopulmonary bypass on EEG bispectral index. *Acta Anaesthesiol Belg*. (2000) 51(3):185–90.

14. Purdon PL, Sampson A, Pavone KJ, Brown EN. Clinical electroencephalography for anesthesiologists part I: background and basic signatures. *Anesthesiology*. (2015) 123(4):937–60. doi: 10.1097/ALN.0000000000000841

15. Crippen D. Role of bedside electroencephalography in the adult intensive care unit during therapeutic neuromuscular blockade. *Crit Care*. (1997) 1(1):15–24. doi: 10.1186/CC3

16. Woodhall B, Sealy WC, Hall KD, Floyd WL. Craniotomy under conditions of quinidine-protected cardioplegia and profound hypothermia. *Ann Surg*. (1960) 152 (1):37–44. doi: 10.1097/00000658-196007000-00006

17. Woodhall B, Reynolds DH, Mahaley Jr S, Sanders AP. The physiologic and pathologic effects of localized cerebral hypothermia. *Ann Surg*. (1958) 147 (5):673–83. doi: 10.1097/00000658-195805000-00011

18. Doi M, Gajraj RJ, Mantzaridis H, Kenny GN. Effects of cardiopulmonary bypass and hypothermia on electroencephalographic variables. *Anaesthesia*. (1997) 52 (11):1048–55. doi: 10.1111/J.1365-2044.1997.229-AZ0364.X

19. Hayashida M, Sekiyama H, Orii R, Chinzei M, Ogawa M, Arita H, et al. Effects of deep hypothermic circulatory arrest with retrograde cerebral perfusion on electroencephalographic bispectral index and suppression ratio. *J Cardiothorac Vasc Anesth*. (2007) 21(1):61–7. doi: 10.1053/J.JVCA.2006.02.029

20. Ziegeler S, Buchinger H, Wilhelm W, Larsen R, Kreuer S. Impact of deep hypothermic circulatory arrest on the BIS index. *J Clin Anesth*. (2010) 22(5):340–5. doi: 10.1016/J.JCLINANE.2009.09.007

21. Meybohm P, Gruenewald M, Höcker J, Renner J, Graesner J-T, Ilies C, et al. Correlation and agreement between the bispectral index vs. state entropy during hypothermic cardio-pulmonary bypass. *Acta Anaesthesiol Scand*. (2010) 54 (2):169–75. doi: 10.1111/J.1399-6576.2009.02138.X

22. Karadeniz Ü, Erdemli O, Yamak B, Genel N, Tutun U, Aksoyek A, et al. On-pump beating heart versus hypothermic arrested heart valve replacement surgery. *J Card Surg*. (2008) 23(2):107–13. doi: 10.1111/J.1540-8191.2007.00536.X

23. Russ W, Kling D, Sauerwein G, Hempelmann G. Spectral analysis of the EEG during hypothermic cardiopulmonary bypass. *Acta Anaesthesiol Scand*. (1987) 31 (2):111–6. doi: 10.1111/J.1399-6576.1987.TB02531.X

24. Levy WJ. Quantitative analysis of EEG changes during hypothermia. *Anesthesiology*. (1984) 60(4):291–7. doi: 10.1097/00000542-198404000-00004

25. Stecker MM, Cheung AT, Pochettino A, Kent GP, Patterson T, Weiss SJ, et al. Deep hypothermic circulatory arrest: I. Effects of cooling on electroencephalogram and evoked potentials. *Ann Thorac Surg*. (2001) 71(1):14–21. doi: 10.1016/S0003-4975(00)01592-7

26. Stecker MM, Cheung AT, Pochettino A, Kent GP, Patterson T, Weiss SJ, et al. Deep hypothermic circulatory arrest: II. Changes in electroencephalogram and evoked potentials during rewarming. *Ann Thorac Surg*. (2001) 71(1):22–8. doi: 10.1016/S0003-4975(00)02021-X

27. Belletti A, Naorungroj T, Yanase F, Eastwood GM, Weinberg L, Bellomo R. Normative values for SedLine-based processed electroencephalography parameters in awake volunteers: a prospective observational study. *J Clin Monit Comput*. (2021) 35(6):1411–9. doi: 10.1007/s10877-020-00618-4

28. Long Y, Feng X, Liu H, Shan X, Ji F, Peng K. Effects of anesthetic depth on postoperative pain and delirium: a meta-analysis of randomized controlled trials with trial sequential analysis. *Chin Med J (Engl)*. (2022) 135(23):2805–14. doi: 10.1097/CM9.0000000000002449

frontiers | Frontiers in Cardiovascular Medicine

TYPE Original Research
PUBLISHED 11 August 2023
DOI 10.3389/fcvm.2023.1093616

OPEN ACCESS

EDITED BY
Massimo Bonacchi,
University of Florence, Italy

REVIEWED BY
Xiao-Qiao Dong,
Zhejiang Chinese Medical University, China
Shahzad Raja,
Harefield Hospital, United Kingdom

*CORRESPONDENCE
Huili Li
lhl100761@163.com
Jinlin Wu
wujinlin@gdph.org.cn

†These authors share co-first authorship

RECEIVED 09 November 2022
ACCEPTED 24 July 2023
PUBLISHED 11 August 2023

CITATION
Li H, Feng W, Wang Q, Li C, Zhu J, Sun T and Wu J (2023) Inclusion of interleukin-6 improved the performance of postoperative acute lung injury prediction for patients undergoing surgery for thoracic aortic disease. Front. Cardiovasc. Med. 10:1093616. doi: 10.3389/fcvm.2023.1093616

Inclusion of interleukin-6 improved the performance of postoperative acute lung injury prediction for patients undergoing surgery for thoracic aortic disease

Huili Li*†, Weiqi Feng†, Qiuji Wang†, Chenxi Li, Jiade Zhu, Tucheng Sun and Jinlin Wu*

Department of Cardiac Surgery, Guangdong Cardiovascular Institute, Guangdong Provincial People's Hospital, Guangdong Academy of Medical Sciences, Guangzhou, China

Background: We studied acute lung injury (ALI) in thoracic aortic disease (TAD) patients and investigated the predictive effect of interleukin-6 (IL-6) in acute lung injury after thoracic aortic disease.
Methods: Data on 188 TAD patients, who underwent surgery between January 2016 to December 2021 at our hospital, were enrolled in. We analyzed acute lung injury using two patient groups. Patients with No-ALI were 65 and those with ALI were 123. Univariate logistic, LASSO binary logistic regression model and multivariable logistic regression analysis were performed for acute lung injury.
Results: Preoperative IL-6 level was lower (15.80[3.10,43.30] vs. 47.70[21.40,91.60] pg/ml, $p < 0.001$) in No-ALI group than in ALI group. The cut-off points, determined by the ROC curve, were preoperative IL-6 > 18 pg/ml (area under the curve: AUC = 0.727). Univariate logistic regression analysis showed 19 features for TAD appeared to be early postoperative risk factors of acute lung injury. Using LASSO binary logistic regression, 19 features were reduced to 9 potential predictors (i.e., Scrpost + PLTpost + CPB > 182 min + D-dimerpost + D-dimerpre + Hypertension + Age > 58 years + IL6 > 18 pg/ml + IL6). Multivariable logistic regression analysis showed that Postoperative creatinine, CPB > 182 min and IL-6 > 18 pg/ml were early postoperative risk factors for ALI after TAD, and the odds ratios (ORs) of postoperative creatinine, CPB > 182 min and IL-6 > 18 pg/ml were 1.006 (1.002–1.01), 4.717 (1.306–19.294) and 2.96 (1.184–7.497), respectively. When postoperative creatinine, CPB > 182 min and IL-6 > 18 pg/ml (AUC = 0.819), the 95% confidence interval [CI] was 0.741 to 0.898. Correction curves were nearly diagonal, suggesting that the nomogram fit well. The DCA curve was then drawn to demonstrate clinical applicability. The DCA curve showed that the threshold probability of a patient is in the range of 30% to 90%.
Conclusions: The inclusion of interleukin-6 demonstrated good performance in predicting ALI after TAD surgery.

KEYWORDS
interleukin-6, acute lung injury, thoracic aortic disease, postoperative, predict

Introduction

Thoracic aortic disease (TAD) contains aneurysms and acute aortic syndromes (intramural hematomas, dissections, penetrating atherosclerotic ulcers). Thoracic aortic disease (TAD) is a type of vascular disease, which if left untreated, could cause fatal complications. Surgery is an important treatment modality for TAD. Aortic dissection is

the worst complication of thoracic aortic disease (1). Acute aortic dissection is commonly reported to be accompanied by acute lung injury (ALI), where oxygenation of the lungs has been impaired severely (2). Acute lung injury (ALI) is a devastating and potentially life-threatening postoperative complication that can also prolong the duration of ventilator support and hospital stay. In large series of patients undergoing open surgery for thoracoabdominal aortic aneurysm, serious lung damage was reported at 60% (3, 4), and in recent publications, it was reported that it was between 40% and 50% (5). Acute hypoxic respiratory insufficiency caused by ALI is caused by a variety of factors that damage alveolar epithelial cells and capillary endothelial cells. Due to these pathological changes, ventilation is reduced, gas exchange is impaired, the ventilation-perfusion imbalance is severe, hypoxia is experienced, and pulmonary compliance is poor (6).

A significant role is played by inflammation in TAD development. A significant association between TAD and elevated plasma inflammatory markers has been reported (7, 8). On the one hand, the lung contains a considerable number of monocytes and macrophages in the cytoplasm to perform a protective function since the trachea is in direct touch with the outside environment; On the other hand, large and slow amount of circulating blood is needed to exchange gas between erythrocytes and alveoli. Lungs are therefore susceptible to inflammatory damage due to this feature (9). The role of inflammatory biomarkers as prognostic indicators after cardiac surgery has gained increasing attention. The impact of perioperative inflammatory biomarkers on clinical outcomes has been understudied in patients undergoing surgery for TAD.

There is a close correlation between cardiovascular disease and intercellular IL-6, which is an inflammatory cytokine that plays an important role in inflammation and immune response (10). The association between interleukin-6 (IL-6) and the pathology of aortic dissection has been demonstrated in numerous basic studies. As well, clinical studies have shown that elevated IL-6 can lead to complications after cardiovascular surgery (11–13). Therefore, to explore if IL-6 plays a predictive role in the occurrence of postoperative ALI in TAD patients undergoing surgery, we collected data on TAD patients from January 2016 to December 2021 who had IL-6 measurement.

Methods

Patient population

The cohort of this retrospective study consisted of 188 patients with surgical treatment for TAD who had preoperative levels of IL-6 measured from Guangdong Provincial People's Hospital (Guangzhou, China) from January 2016 to December 2021. **Figure 1** shows the patient screening process. ALI and Non-ALI are classified according to the presence or absence of acute lung injury after surgery. In our study, we included patients whose CT angiography or

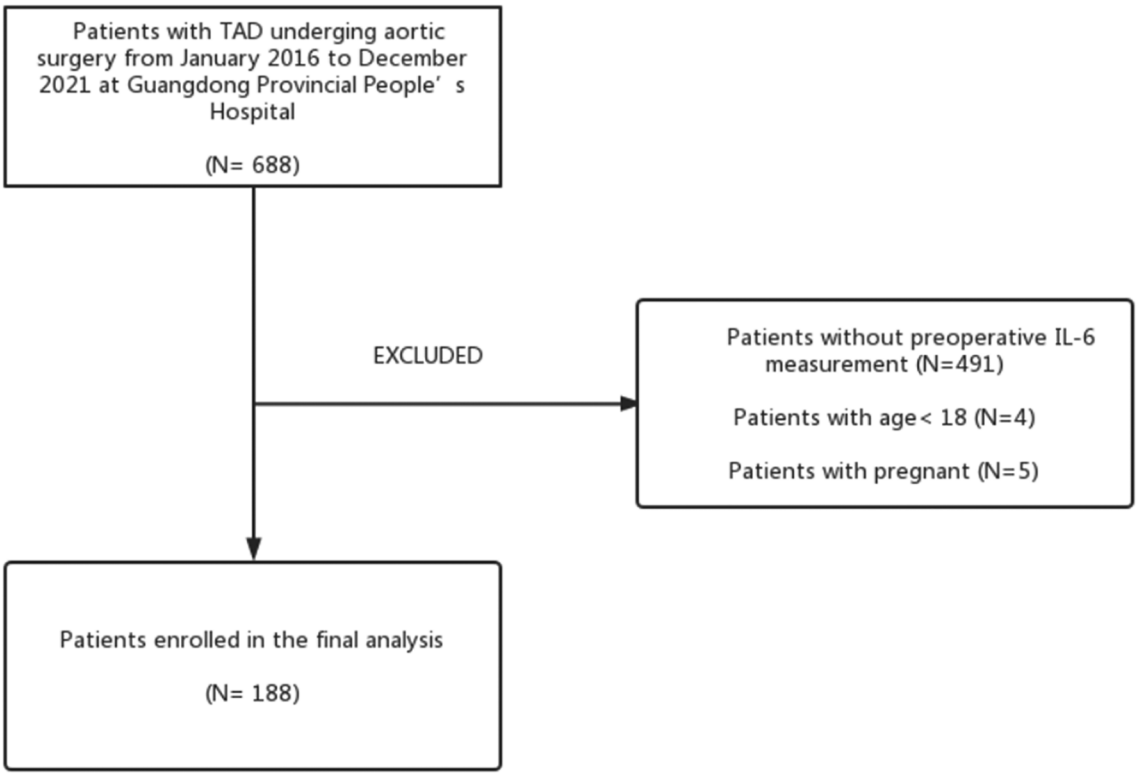


FIGURE 1
Patient screening chart.

magnetic resonance angiography (MRA) confirmed the presence of TAD. The exclusion criteria were as follows: (1) No preoperative IL-6 measurement; (2) Age less than 18 years; (3) Death before surgery; (4) Malignant tumors. An overview of the clinical characteristics of the TAD patients included in the study can be found in Table 1. This study protocol was approved by the Institutional Ethics Committee of Guangdong Provincial People's Hospital (KY-Q-2021-183-02) and conformed to the Declaration of Helsinki.

Definitions

ALI severity was put into the following categories based on the Berlin definition: mild ALI [200 mmHg < oxygen index (OI) ≤ 300 mmHg], moderate ALI (100 mmHg < OI ≤ 200 mmHg), and severe ALI (OI ≤ 100 mmHg) (14). Early mortality was defined as all-cause mortality in-hospital. Stroke was defined as a permanent neurologic injury with clinical or radiographic evidence, such as CT or MRI. The term MODS refers to the acute and potentially reversible dysfunction of two or more organ systems resulting from a variety of clinical factors (15).

Data collection

Before surgery, venous blood was extracted from all patients (within 48 h of the onset of symptoms). The preoperative indices of all patients were observed and summarized in Table 1. Preoperative indicators included age, gender, body mass index (BMI), IL-6, D-dimer, serum creatinine (SCR), triglyceride, low density lipoprotein (LDL), alanine aminotransferase (ALT), aspartic transaminase (AST), glucose, white blood cells (WBC), platelets (PLT), cardiac effusion, hypertension, and other previous history. Intraoperative and postoperative indicators were summarized in Table 2. Intraoperative and postoperative indicators included cardiopulmonary bypass (CPB), aortic cross-clamp time (ACC), deep hypothermic circulation arrest (DHCA), WBC, PLT, D-dimer, glucose, SCR, AST, ALT, mediastinitis, and other postoperative complications.

Statistical analysis

Continuous variables were reported with mean ± standard deviation (SD) and were compared using Student's independent

TABLE 1 Preoperative data.

Variables	All ($n = 188$)	No-ALI ($n = 65$)	ALI ($n = 123$)	p
Age, mean (±SD), years	54.31 ± 12.02	50.97 ± 11.49	56.07 ± 11.92	0.005
Male, *n* (%)	142 (75.53)	54 (83.08)	88 (71.54)	0.080
BMI, mean (±SD), kg/m^2	24.34 ± 3.79	23.62 ± 3.33	24.72 ± 3.96	0.069
Atria fibrillation, *n* (%)	10 (5.75)	4 (6.25)	6 (5.45)	0.828
Hypertension, *n* (%)	131 (69.68)	38 (58.46)	93 (75.61)	0.015
Diabetes mellitus, *n* (%)	7 (3.72)	3 (4.62)	4 (3.25)	0.639
Hyperlipidemia, *n* (%)	63 (34.62)	19 (29.23)	44 (37.61)	0.255
Chronic kidney disease, *n* (%)	20 (10.64)	4 (6.15)	16 (13.01)	0.147
Smoking, *n* (%)	36 (19.25)	9 (14.06)	27 (21.95)	0.194
Cardiac aortic surgery, *n* (%)	11 (5.85)	4 (6.15)	7 (5.69)	0.898
COPD, *n* (%)	32 (17.20)	14 (21.88)	18 (14.75)	0.222
BAV, *n* (%)	7 (3.74)	4 (6.15)	3 (2.46)	0.205
MFS, *n* (%)	8 (4.28)	5 (7.69)	3 (2.46)	0.092
Shock hypotension, *n* (%)	2 (1.06)	0 (0.00)	2 (1.63)	0.301
AI greater than 2, *n* (%)	28 (46.67)	10 (43.48)	18 (48.65)	0.696
Pleural Effusion, *n* (%)	27 (14.36)	6 (9.23)	21 (17.07)	0.145
Cardiac Effusion, *n* (%)	50 (26.60)	9 (13.85)	41 (33.33)	0.004
Renal Cyst, *n* (%)	65 (35.14)	19 (30.16)	46 (37.70)	0.308
Liver cyst, *n* (%)	55 (29.73)	19 (30.16)	36 (29.51)	0.927
Preoperative condition				
IL6, median [IQR], pg/ml	39.30[10.60,74.20]	15.80[3.10,43.30]	47.70[21.40,91.60]	<0.001
WBC, mean (±SD), 10^9/L	10.74 ± 3.92	9.61 ± 4.10	11.31 ± 3.69	0.005
PLT, mean (±SD), 10^9/L	212.32 ± 104.96	220.98 ± 105.36	207.89 ± 104.48	0.424
D-dimer, mean (±SD), ng/ml	8,121.19 ± 7,824.67	4,848.88 ± 6,284.89	9,730.52 ± 8,003.38	<0.001
glucose, mean (±SD), mg/dl	7.07 ± 2.10	6.58 ± 2.21	7.32 ± 2.00	0.023
AST, mean (±SD), U/L	78.55 ± 376.31	24.34 ± 13.61	105.44 ± 457.79	0.171
ALT, mean (±SD), U/L	61.68 ± 245.19	21.88 ± 16.22	81.08 ± 296.89	0.133
LDL, mean (±SD), mmol/L	2.93 ± 0.77	2.85 ± 0.76	2.97 ± 0.77	0.339
Tirglyceride, mean (±SD), mh/dl	1.33 ± 0.58	1.28 ± 0.56	1.36 ± 0.59	0.426
Scr, mean (±SD), umoI/L	103.45 ± 68.32	86.50 ± 25.61	112.13 ± 80.63	0.002
Maximum Diameter, median [IQR], mm	48.00[42.00,57.00]	50.00[41.00,59.00]	48.00[43.00,57.00]	0.877

TABLE 2 Surgical data.

Variables	All ($n = 188$)	No-ALI ($n = 65$)	ALI ($n = 123$)	p
Elective surgery, n (%)	98 (52.128)	40 (62.500)	58 (46.774)	0.041
Concomitant procedures, n%				
Bentall, n (%)	55 (29.255)	25 (39.063)	30 (24.194)	0.034
Cabrol, n (%)	6 (3.191)	2 (3.125)	4 (3.226)	0.970
Wheats, n (%)	8 (4.255)	6 (9.375)	2 (1.613)	0.012
FET, n (%)	160 (85.106)	47 (73.438)	113 (91.129)	0.001
Operation details				
CPB time, median [IQR], min	232.00[198.00,263.00]	227.00[182.00,245.00]	233.00[202.00,266.00]	0.045
ACC time, median [IQR], min	119.00[91.00,146.00]	117.00[87.00,146.00]	119.00[94.00,146.00]	0.520
DHCA time, mean (±SD) min,	16.84 ± 8.86	15.84 ± 9.48	17.38 ± 8.46	0.260
DHCA temp nasal, median [IQR], °C	24.00[22.20,25.00]	24.00[22.50,25.30]	23.80[22.10,25.00]	0.221
CPB > 187.5 min, n (%)	160 (85.11)	47 (72.31)	113 (91.87)	<0.001
ACC > 172.5 min, n (%)	25 (13.30)	4 (6.15)	21 (17.07)	0.036
DHCA, n (%)	164 (87.23)	52 (80.00)	113 (91.87)	0.018
RBC transfusionU, median [IQR]	5.000[1.500,8.000]	4.000[1.000,6.000]	5.500[3.500,10.000]	0.008

t-test. If normality was not assumed (Kolmogorov-Smirnov test), median (interquartile range, IQR) and Mann-Whitney U test would be used. Categorical variables were presented as numbers and percentages and were compared using the Chi-square test or Fisher's exact test (if expected value ≤5 was found). Univariate and multivariate logistic regression models and estimated odds ratio (OR) were used to investigate the association between independent variables and in-hospital mortality. Partial nonbinary variables were dichotomized via univariable logistic regression and optimal cut-off points were estimated via receiver operating characteristic (ROC) curve analysis and determined based on the maximum Youden index.

We selected the most useful predictive features from the primary data set by using the least absolute shrinkage and selection operator (LASSO) method. This method is suitable for reducing high-dimensional data. With the LASSO binary logistic regression model, all the clinicopathologic variables in the cohort were reduced to a limited set of potential predictors. If the penalization coefficient lambda (λ) is high, the predicted regression parameters are unaffected; but, as the coefficients decrease, certain

TABLE 3 Postoperative data.

Variables	All ($n = 188$)	No-ALI ($n = 65$)	ALI ($n = 123$)	p
Postoperative condition				
SCR > 200.10 umoI/L, n (%)	64 (34.0)	9 (13.80)	55 (44.72)	<0.001
Early Mortality, n (%)	18 (9.57)	2 (3.08)	16 (13.01)	0.028
Stroke, n (%)	17 (9.04)	3 (4.62)	14 (11.38)	0.124
Delirium, n (%)	35 (18.62)	5 (7.69)	30 (24.39)	0.005
Paresis, n (%)	5 (2.66)	0 (0.00)	5 (4.07)	0.099
CRRT, n (%)	37 (19.68)	0 (0.00)	37 (30.08)	<0.001
Re-exploration for bleeding delayed chest closure, n (%)	10 (5.32)	1 (1.54)	9 (7.32)	0.093
Tracheotomy, n (%)	9 (4.79)	0 (0.00)	9 (7.32)	0.029
MODS, n (%)	25 (13.30)	1 (1.54)	24 (19.51)	<0.001
ECMO, n (%)	8 (4.26)	0 (0.00)	8 (6.50)	0.052
Mediastinitis, n (%)	3 (1.60)	1 (1.54)	2 (1.63)	0.964
Acute Liver Injury, n (%)	74 (39.36)	18 (27.69)	56 (45.53)	0.017
Post-op Hospital stay, median [IQR], day	21.00[16.00,31.00]	18.00[14.00,27.00]	23.00[18.00,39.00]	<0.001
ICU stay, median [IQR], day	89.00[14.00,186.00]	43.00[7.00,80.00]	148.00[21.00,257.00]	<0.001
Ventilation time, mean (±SD), h	159.55 ± 236.03	48.22 ± 44.96	217.48 ± 271.74	<0.001
WBC, mean (±SD), 10^9/L	19.14[15.97,22.57]	18.03[15.67,20.44]	19.54[16.37,23.17]	0.089
PLT, mean (±SD), 10^9/L	103.00[56.00,128.00]	116.00[79.00,147.00]	94.00[50.00,120.00]	0.003
D-dimer, mean (±SD), ng/ml	9,635.20 ± 6,717.40	7,694.63 ± 6,691.10	10,628.05 ± 6,510.87	0.018
Glucose, mean (±SD), mg/dl	12.04[9.38,14.27]	11.95[9.63,14.13]	12.04[9.25,14.33]	0.899
AST, mean (±SD), U/L	403.12 ± 1,911.01	106.60 ± 190.18	562.41 ± 2,349.84	0.122
Scr, mean (±SD), umoI/L	160.96[109.60,259.50]	124.10[95.90,172.36]	179.60[121.60,360.20]	<0.001
ALT, mean (±SD),U/L	62.00[29.00,117.00]	40.00[26.00,88.00]	70.00[30.00,152.00]	0.023

coefficients may be reduced to zero. We then selected the optimal λ in the LASSO model by using 10-fold cross-validation via minimum criteria and one standard error of the minimum criteria (the 1-SE criterion). Lastly, the Lasso method was used to select all coefficients that were not zero (**Figure 1**) to re-fit the model. The variables that were also significant in multivariate results would be recognized as associated factors in patients' ALI. A $p < 0.05$ would be recognized as reaching significance for each test, two-tailed. All analyses were performed using IBM SPSS Version 25 (SPSS Statistics V25, IBM Corporation, Somers, New York) and R software (version 4.2.1).

The model was assessed using the area under the ROC curve. The AUC (area under the receiver operating characteristic curve) was measured to quantify the discrimination performance of the model. Based on the cohort, we conducted decision curve analyses to determine the clinical usefulness of the model. This model's calibration was assessed using calibration plots.

Results

Clinical characteristics

In this study, 188 patients undergoing TAD surgery were included after inclusion and exclusion criteria were met. The mean age of patients was 54.31 ± 12.02 years. In addition, 75.53% of patients were male. According to the postoperative OI, ALI was identified in 123 patients and the incidence of preoperative ALI was 65.4%. As shown in **Table 1**, patients who have and do not have postoperative ALI are compared in terms of their basic characteristics. The ALI patients were older (56.07 ± 11.92 vs. 50.97 ± 11.49; $p = 0.005$), had a higher IL6 (15.8 VS. 47.7; $p < 0.001$), a higher WBC (9.61 ± 4.10 VS. 11.31 ± 3.69; $p = 0.005$), a higher D-dimer (4,848.88 ± 6,284.89 vs. 9,730.52 ± 8,003.38; $p < 0.001$), a higher glucose (6.58 ± 2.21 vs. 7.32 ± 2.00; $p = 0.023$) and SCR (86.50 ± 25.61 vs. 112.13 ± 80.63; $p = 0.002$). Compared

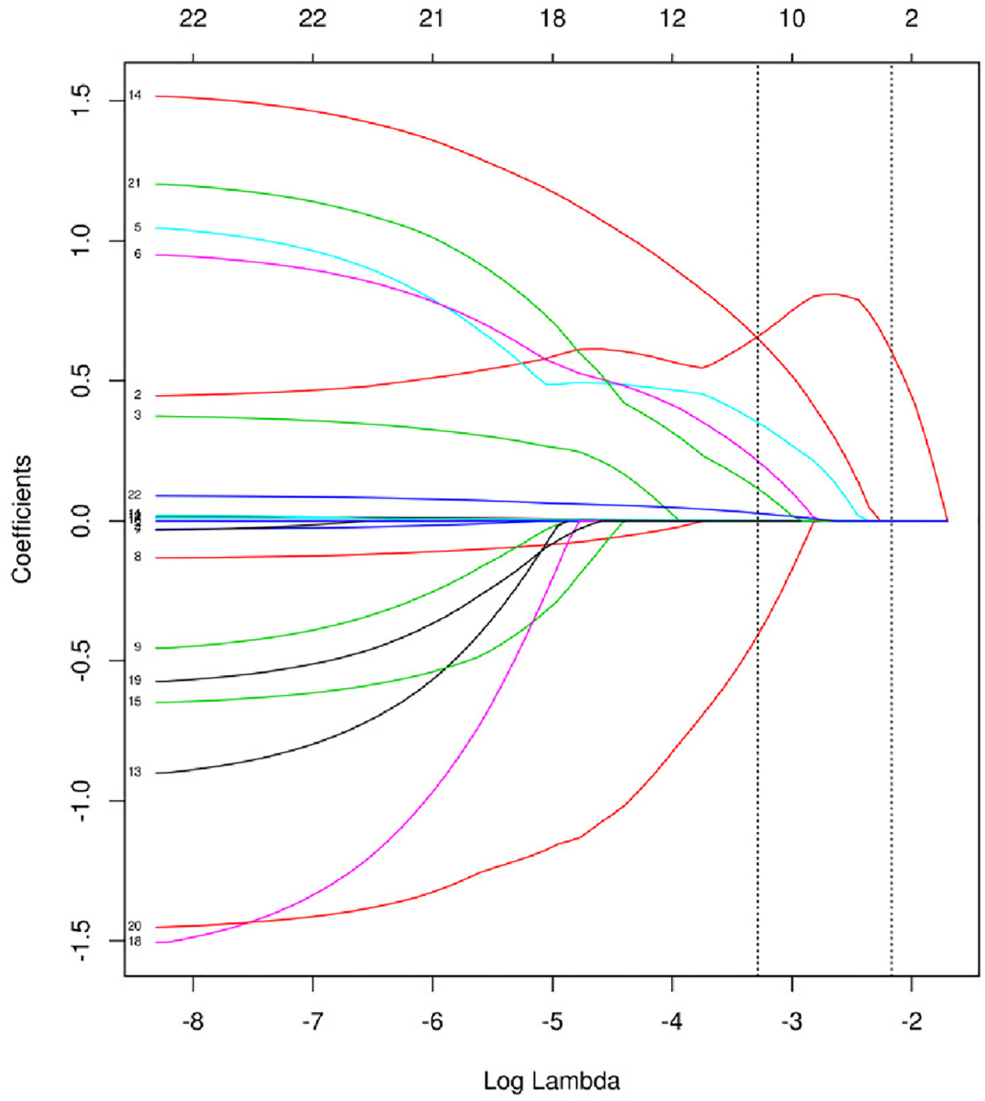


FIGURE 2
Texture feature selection using the least absolute shrinkage and selection operator (LASSO) binary logistic regression model.

to no-ALI, a greater percentage of ALI were hypertensive (75.61% vs. 58.46%; $p = 0.015$). There was no statistically significant difference between the ALI and no-ALI groups in other factors. Patients with lung injury have a relatively worse postoperative situation (Table 3).

Feature selection

The model was built using LASSO binary logistic regression because the sample size in this study was insufficient to satisfy the advised number of events per variable. The λ value was 0.034. Of all the relevant variables, 24 features were reduced to 10 potential predictors on the basis of the cohort. The 10 variables with non-zero coefficients in the LASSO logistic regression model (i.e., Scrpost + RBC transfusionU + CPB > 182mi + D-dimerpre + Hypertension + Age > 58 years + Wheats + FET + IL6 > 18 pg/ml + IL6) were used in the final model (in Figures 2, 3). Next, the above mentioned 10 variables were included in the multivariable logistic regression analysis to determine the risk factors associated with postoperative ALI. Finally, postoperative creatinine, age > 58 and IL-6 > 18 pg/ml were as ALI risk factors (Tables 4, 5 and Figure 4). And ROC curve was shown in Figure 5.

Nomogram construction

The above-mentioned variables were screened using logistic regression backwards selection. Postoperative creatinine, age > 58 and IL-6 > 18 pg/ml were identified as ALI risk factors. The ALI risk was 2.78-fold higher in IL > 18 than in IL < 18

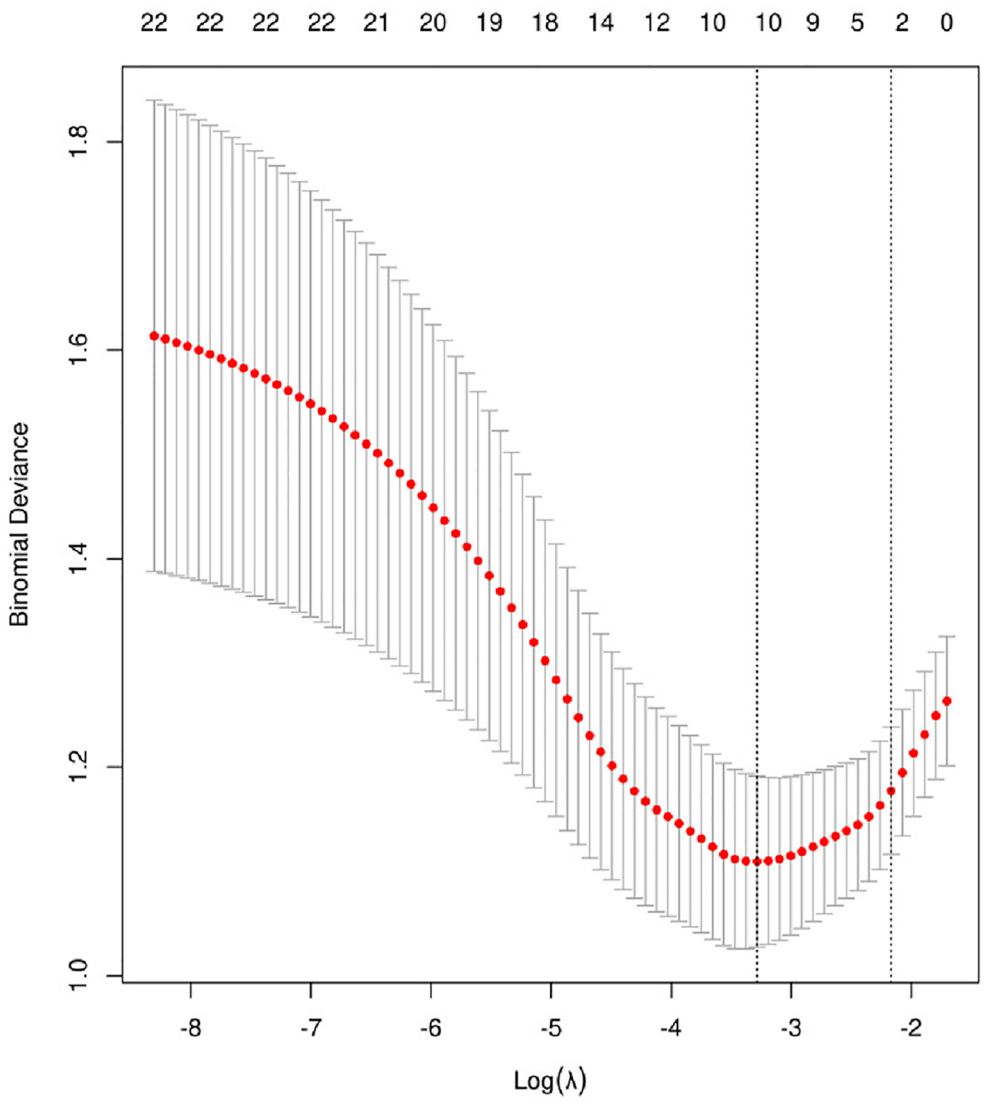


FIGURE 3
Cross-validation of the LASSO regression model. Lasso coefficient profiles of the 24 features. A coefficient profile plot was produced against the log (λ) sequence. The vertical line was drawn at the value selected using 10-fold cross-validation, where optimal resulted in 10 features with non-zero coefficients.

TABLE 4 Univariate analyses for postoperative acute lung injury.

Variables	OR	95%CI	*p*-value
Preoperative condition			
IL6, pg/ml	1.02	[1.010,1.031]	0.000
Age, years	1.04	[1.010,1.064]	0.007
BMI, kg/m^2	1.08	[0.993,1.179]	0.071
Age > 58 years	3.57	[1.738,7.342]	0.001
Male	0.51	[0.240,1.092]	0.083
Atria_fibrillation	0.87	[0.235,3.189]	0.828
Hypertension	2.20	[1.159,4.187]	0.016
Diabetes mellitus	0.70	[0.151,3.202]	0.640
Hyperlipidemia	1.46	[0.760,2.802]	0.256
Chronic kidney disease	2.28	[0.729,7.129]	0.156
Smoking	1.72	[0.754,3.918]	0.198
Hx of cardiac aortic surgery	0.92	[0.259,3.267]	0.898
COPD	0.62	[0.285,1.343]	0.224
Pleural Effusion	2.03	[0.773,5.299]	0.151
Cardiac Effusion	3.111	[1.401,6.906]	0.005
Renal Cyst	1.402	[0.731,2.687]	0.309
Liver cyst	0.969	[0.499,1.883]	0.927
WBC, 10^9/L	1.127	[1.035,1.227]	0.006
PLT, 10^9/L	0.999	[0.996,1.002]	0.423
D-dimer, ng/ml	1.000	[1.000,1.000]	0.000
Glucose, mg/dl	1.205	[1.023,1.420]	0.025
AST, U/L	1.018	[0.997,1.039]	0.089
ALT, U/L	1.008	[0.994,1.022]	0.258
LDL, mmol/L	1.235	[0.802,1.901]	0.337
SCR, umoI/L	1.010	[1.002,1.019]	0.017
WBC > 9.27 10^9/L	2.651	[1.375,5.110]	0.004
SCR > 95.28 umoI/L	3.065	[1.331,7.060]	0.008
IL > 18 pg/ml	5.517	[2.850,10.679]	0.000
Elective surgery, *n* (%)	0.527	[0.285,0.977]	0.042
Concomitant procedures, *n*%			
Bentall, *n* (%)	0.498	[0.260,0.953]	0.035
Wheats, *n* (%)	0.158	[0.031,0.809]	0.027
FET, *n* (%)	3.716	[1.618,8.532]	0.002
Operation details			
CPB time, min	1.005	[1.000,1.011]	0.052
ACC time, min	1.003	[0.995,1.010]	0.472
DHCA time, min	1.020	[0.986,1.055]	0.259
DHCA temp nasal, °C	0.938	[0.851,1.034]	0.201
Ventilation time, min	1.019	[1.011,1.027]	0.000
CPB > 182 min	4.328	[1.860,10.070]	0.001
Acc > 172.5 min	3.140	[1.029,9.578]	0.044
DHCA	2.880	[1.185,7.002]	0.020
RBC transfusionU, median [IQR]	1.124	[1.041,1.213]	0.003
Postoperative condition			
RBC, 10^9/L	0.194	[0.064,0.587]	0.004
WB, 10^9/L	0.994	[0.975,1.013]	0.510
PLT, 10^9/L	0.993	[0.988,0.998]	0.007
D-dimer, ng/ml	1.000	[1.000,1.000]	0.020
Glucose, mg/dl	0.975	[0.912,1.042]	0.457
AST, U/L	1.001	[1.000,1.003]	0.139
Scr,umoI/L	1.007	[1.004,1.011]	0.000
ALT, U/L	1.002	[0.999,1.005]	0.117
Scr > 200 umoI/L	5.033	[2.287,11.073]	0.000

patients (95% CI = 1.0–7.54; $p = 0.048$), postoperative creatinine (OR for ALI: 1.006, 95% CI: 1.002–1.01, $p = 0.005$) and Age > 58 years (OR for ALI: 2.654, 95% CI: 1.171–6.379, $p = 0.023$) were independent risk factors for postoperative ALI (Table 4). These results were used to construct a nomogram for estimating the postoperative ALI risk in TAD patients during hospitalization (Figure 4). Three independent predictors of postoperative ALI were used. The three factors used to estimate the postoperative risk of ALI each received a score based on their value, and the total score was calculated by summing the scores.

TABLE 5 Multivariate analyses for postoperative acute lung injury.

Variables	OR (95% CI)	*p*
Postoperative serum creatinine	1.006 (1.002–1.01)	0.003
CPB > 182 min	2.141 (0.762–6.26)	0.152
Age > 58 years	2.654 (1.171–6.379)	0.023
IL6 > 18 pg/ml	2.781 (0.996–7.54)	0.048
IL	1.009 (0.999–1.025)	0.177

Nomogram validation

Figure 4 indicated that the AUC values of the nomogram were 0.811(95% CI = 0.747–0.875) for the cohort. Figure 6 displayed the calibration curves of the nomogram. The Hosmer–Lemeshowchi-square statistic was 4.24 and the *p* value was 0.8348, demonstrating good calibration. In the cohort, correction curves were nearly diagonal, suggesting that the nomogram fit well. The DCA curve was then drawn to demonstrate clinical applicability (Figure 7). The DCA curve showed that the threshold probability of a patient is in the range of 30% to 90%, the use of a nomogram to predict postoperative ALI in patients with TAD is more beneficial than either the treat-all-patients or treat-none scheme.

Discussion

We looked into whether adding IL-6 could predict occurrence of ALI after TAD surgery. Association of interleukin-6 with postoperative complications is an area of ongoing research. This study explores the predictive role of interleukin-6 in the occurrence of postoperative ALI after surgery for TAD and demonstrates that the inclusion of interleukin-6 improves the performance of predictive model for ALI after TAD surgery. We developed a diagnostic nomogram for the postoperative individualized prediction of ALI in patients with TAD during hospitalization. The nomogram incorporated three items: IL-6 > 18 pg/ml, ventilation time and postoperative serum creatinine. We selected an optimum IL-6 cutoff value based on the Youden index (Youden index = sensitivity + specificity-100%) for the prediction of patients with postoperative ALI, and also considered the sensitivity and specificity. In practice, the choice of cutoff value might be dependent on the risk of misdiagnosis and missed diagnosis. Our study indicated that IL-6 > 18 pg/ml, ventilation time and postoperative serum creatinine are independent risk factors for postoperative ALI in TAD patients. The validity of our nomogram model was determined using multiple indicators, including AUC, correction curve, Hosmer-

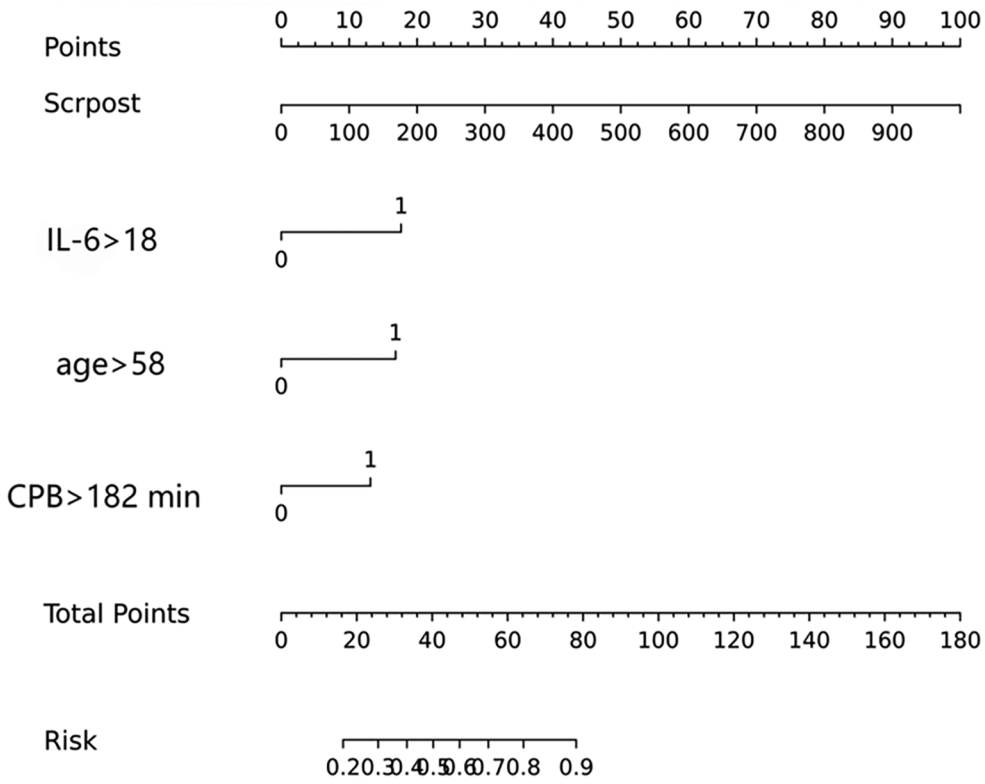


FIGURE 4
Nomogram to predict ALI in patients with TAD.

Lemeshow test and DCA. Through shrinking the regression coefficients that represent the correlation between predictor and outcome, 24 candidate features were reduced to 10 potential predictors for the construction of the model. This strategy outperforms the method of selecting predictors based on the strength of their univariable correlation with the result.

A number of studies found that blood or lung tissues from aortic dissection and thoracic aortic aneurysm patients with postoperative ALI showed significant increases in C-reactive protein (CRP), interleukin-6 (IL-6), and monocyte chemoattractant protein-1 (MCP-1). As a result of these reactions, pulmonary microvascular endothelial cells (PMVECs) are subject to apoptosis and barrier dysfunction, which lead to the elevation of endothelial permeability and the development of ALI (5, 16). Patients with ALI had higher levels of blood IL-6. Experimental evidence suggested aortic aneurysms can secrete IL-6 (17–19). In basic experimental research, Paige et al. found aortic rupture and death can be decreased in AAA mouse models by selectively inhibiting the IL-6 trans-signaling pathway, which suggested a possible therapeutic target (20). The expression of IL-6 was increased in AD rat models. MMP-2 expression may be enhanced by IL-6, which may promote extracellular matrix degradation, which promoted AD development (19). In clinical studies, the IL-6 level was found to be elevated in postoperative delirium (POD) patients following aortic dissection surgery by Lv Xiaochai et al. In this way, plasma IL-6 levels could be used to evaluate the outcomes of POD in AAD patients (21). Furthermore, high levels of IL-6 and D-dimer have predictive significance in predicting poor prognosis following acute Stanford type A aortic dissection surgery (13). Researchers have looked into the link between IL-6 (22), but it is not clear if IL-6 also affects ALI after TAD surgery.

In our study, patients with postoperative ALI had higher levels of IL-6 than those who did not have ALI, and the difference was statistically significant. After using logistic regression, it was found that IL-6 > 18 was a risk factor for postoperative ALI. Moreover, the AUC in the development cohort was 0.811 and correction curves were nearly diagonal. Compared with other studies, we selected the cutoff value, but whether this cutoff value can be applied to the subsequent validation still needs to be explored by expanding the sample size.

In cardiac surgery, a serious problem associated with cardiopulmonary bypass (CPB) was that it triggers an inflammatory response that contributed to the dysfunction of various organs, including the lungs (23, 24). The complement system in the blood and many different body cells were broadly stimulated in the particular cardiopulmonary bypass environment, and their functions were progressively damaged. During the postoperative period, their own inflammatory response was amplified, leading to SIRS and further causing organ dysfunction, including ALI. Lung immunological and

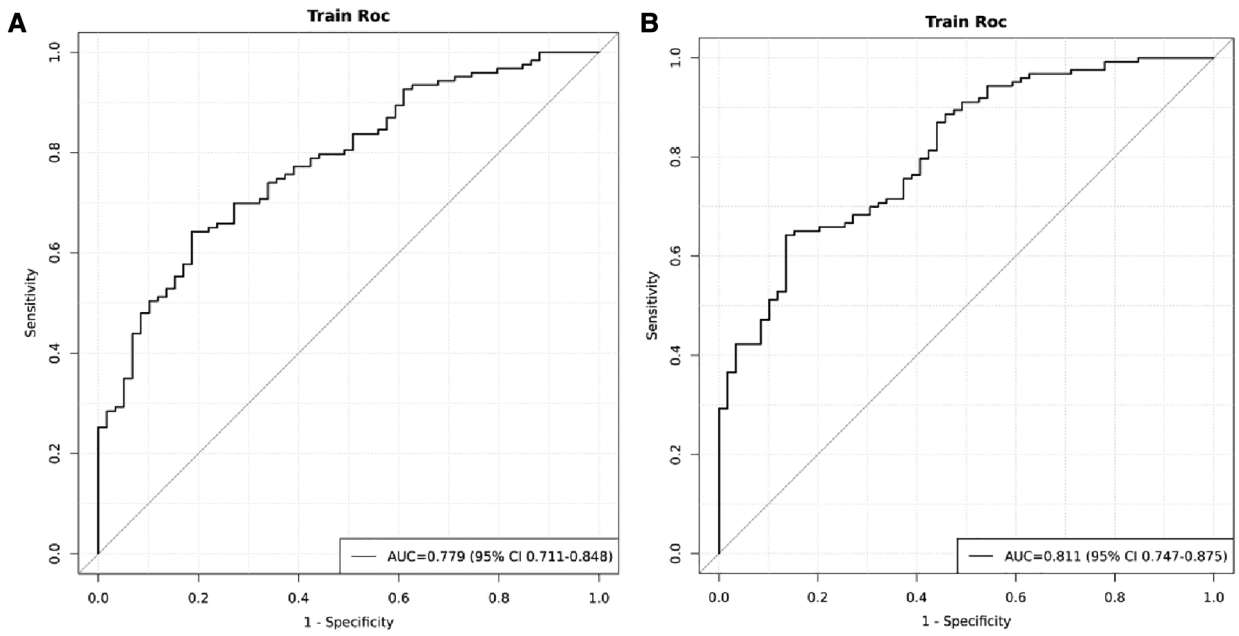


FIGURE 5
ROC of the cohort. (A) ROC curves when IL > 18 was excluded. (B) ROC curves at inclusion of postoperative creatinine, age > 58 and IL-6 > 18 pg/ml.

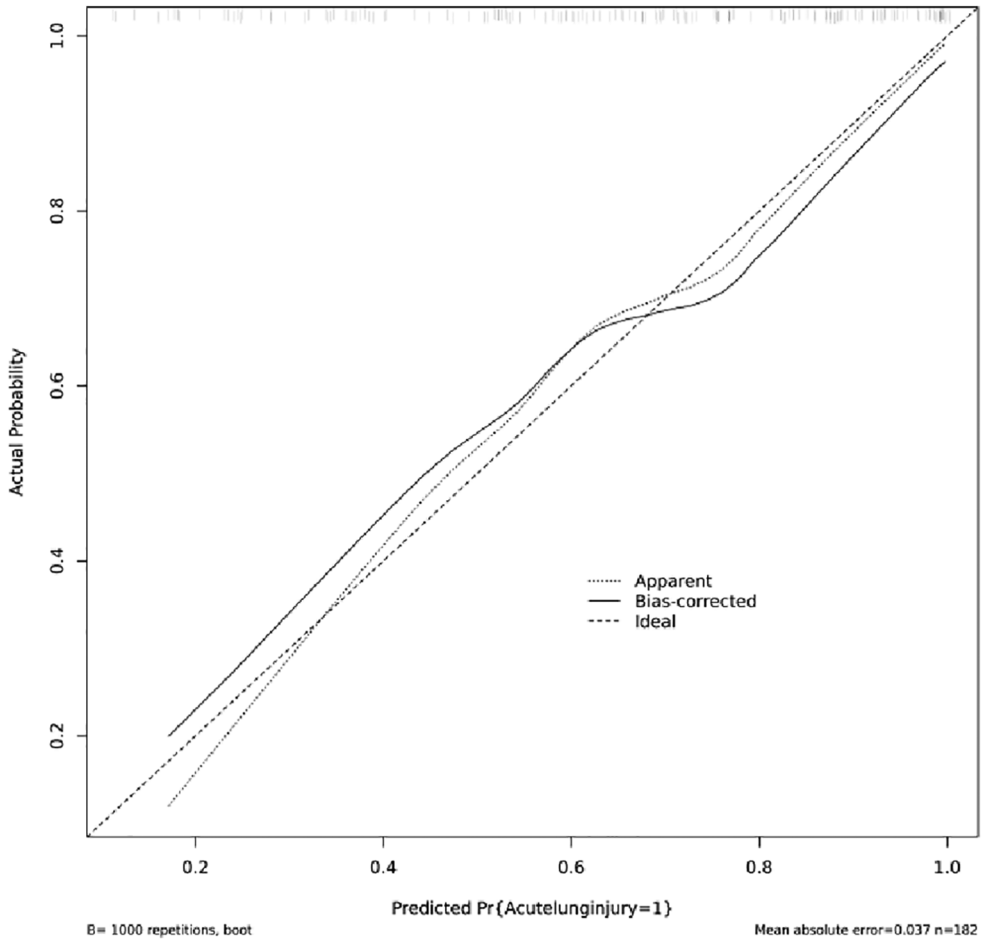


FIGURE 6
A calibration plot based on the nomogram.

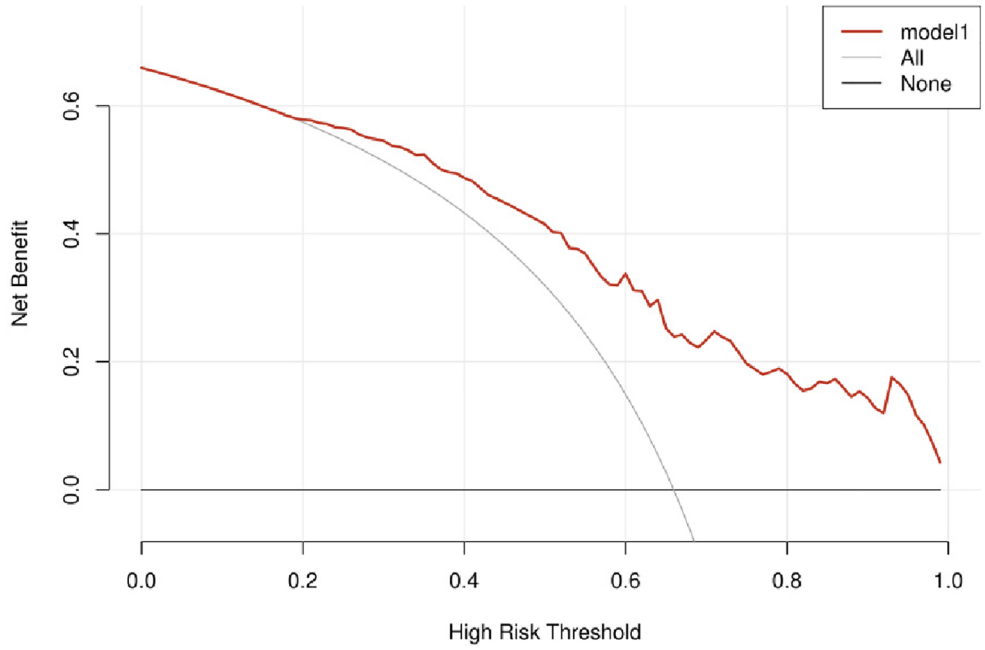


FIGURE 7
Decision curve.

anatomical characteristics contribute directly to the poor prognosis of ALI, which makes it one of the main targets of inflammation (25). A prolonged cardiopulmonary bypass will, therefore, result in postoperative ALI in ATAAD surgery.

The kidneys were responsible for eliminating creatinine from the body. Increased creatinine levels suggest renal disease. However, a statistically significant difference was found in postoperative serum creatinine levels higher in the ALI group in our study. Despite being different organs with their own location, structure, and function, lung and kidney tissue could be damaged simultaneously in the course of systemic diseases since they were not completely independent from one another (26). It has been proposed that renal ischemia/reperfusion could cause the systemic release of injurious factors that activated pulmonary capillary endothelial cells, resulting in increasing permeability and expression of adhesion molecules in the cells (27). Previous studies have found in the univariate analysis, increased creatinine levels were a significant risk factor for hypoxemia at 1 and 12 h after surgery (28). A possible explanation was that this group of patients was more susceptible to fluid overload during surgery due to their increased sensitivity to fluids. Some investigators have identified excessive hemodilution during CPB as an important factor in the development of lung injury (28). We were unable to determine hemodilution and fluid load because fluid intake and output were not counted in our data.

The shortcoming of our study is that relatively few previous tests for IL-6 have been performed, so the number of patients included in the study is relatively insufficient, which may make the results less perfect. Therefore, for follow-up studies, preoperative testing of IL-6 in patients should be increased. IL-6 testing is not included as a mandatory preoperative test, but since studies have found that IL-6 has a predictive effect on lung injury, it should be recommended that patients be tested for IL-6 prior to surgery in order to better prevent postoperative lung injury. We also hope to explore the cut-off value of IL-6 in a follow-up study.

Data availability statement

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding authors.

Ethics statement

The study protocol was approved by the institutional Ethics Committee of Guangdong Provincial People's Hospital (KY-Q-2021-183-02) and written informed consent was not required in accordance with the national legislation.

Author contributions

JW and HL: contributed to the study design. HL and WF: participated in the data measurement and analysis. QW and CL: conducted the statistical analysis. HL, WF, and JZ: drafted the manuscript. TS and JW: revised the manuscript. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2023.1093616/full#supplementary-material

References

1. Aldridge A, Desai A, Owston H, Jennings LM, Fisher J, Rooney P, et al. Development and characterisation of a large diameter decellularised vascular allograft. *Cell Tissue Bank*. (2018) 19:287–300. doi: 10.1007/s10561-017-9673-y

2. Kurabayashi M, Okishige K, Azegami K, Ueshima D, Sugiyama K, Shimura T, et al. Reduction of the PaO2/FiO2 ratio in acute aortic dissection—relationship between the extent of dissection and inflammation. *Circ J*. (2010) 74:2066–73. doi: 10.1253/circj.CJ-10-0336

3. Conrad MF, Crawford RS, Davison JK, Cambria RP. Thoracoabdominal aneurysm repair: a 20-year perspective. *Ann Thorac Surg*. (2007) 83:S856–61; discussion S890–2. doi: 10.1016/j.athoracsur.2006.10.096

4. Rigberg DA, McGory ML, Zingmond DS, Maggard MA, Agustin M, Lawrence PF, et al. Thirty-day mortality statistics underestimate the risk of repair of thoracoabdominal aortic aneurysms: a statewide experience. *J Vasc Surg*. (2006) 43:217–22; discussion 223. doi: 10.1016/j.jvs.2005.10.070

5. Estrera AL, Sandhu HK, Charlton-Ouw KM, Afifi RO, Azizzadeh A, Miller CC 3rd, et al.. A quarter century of organ protection in open thoracoabdominal repair. *Ann Surg*. (2015) 262:660–8. doi: 10.1097/SLA.0000000000001432

6. Liu K, Liu J, Wu S. Association of dynamic changes in serum cytokine levels with the severity of injury in patients suffering from closed chest traumas complicated with pulmonary contusions. *Exp Ther Med*. (2011) 2:563–7. doi: 10.3892/etm.2011.241

7. Zhang J, Liu J, Zhao M, Ye J, Xu Y, Wang Z, et al. The expression of interleukin 20 increases in plasma and aortic tissues from patients with acute aortic dissection. *Clin Chim Acta*. (2020) 510:373–80. doi: 10.1016/j.cca.2020.07.049

8. Ramprasath T, Han YM, Zhang D, Yu CJ, Zou MH. Tryptophan catabolism and inflammation: a novel therapeutic target for aortic diseases. *Front Immunol*. (2021) 12:731701. doi: 10.3389/fimmu.2021.731701

9. Duan XZ, Xu ZY, Lu FL, Han L, Tang YF, Tang H, et al. Inflammation is related to preoperative hypoxemia in patients with acute Stanford type A aortic dissection. *J Thorac Dis*. (2018) 10:1628–34. doi: 10.21037/jtd.2018.03.48

10. Nagareddy P, Smyth SS. Inflammation and thrombosis in cardiovascular disease. *Curr Opin Hematol*. (2013) 20:457–63. doi: 10.1097/MOH.0b013e328364219d

11. Ju X, Ijaz T, Sun H, Ray S, Lejeune W, Lee C, et al. Interleukin-6-signal transducer and activator of transcription-3 signaling mediates aortic dissections induced by angiotensin II via the T-helper lymphocyte 17-interleukin 17 axis in C57BL/6 mice. *Arterioscler Thromb Vasc Biol*. (2013) 33:1612–21. doi: 10.1161/ATVBAHA.112.301049

12. Brocca A, Virzi GM, de Cal M, Giavarina D, Carta M, Ronco C. Elevated levels of procalcitonin and interleukin-6 are linked with postoperative complications in cardiac surgery. *Scand J Surg*. (2017) 106:318–24. doi: 10.1177/1457496916683096

13. Wu Q, Li J, Chen L, Yan LL, Qiu Z, Shen Y, et al. Efficacy of interleukin-6 in combination with D-dimer in predicting early poor postoperative prognosis after acute Stanford type A aortic dissection. *J Cardiothorac Surg*. (2020) 15:172. doi: 10.1186/s13019-020-01206-y

14. Force ADT, Ranieri VM, Rubenfeld GD, Thompson BT, Ferguson ND, Caldwell E, et al. Acute respiratory distress syndrome: the Berlin definition. *JAMA*. (2012) 307:2526–33. doi: 10.1001/jama.2012.5669

15. Gourd NM, Nikitas N. Multiple organ dysfunction syndrome. *J Intensive Care Med*. (2020) 35:1564–75. doi: 10.1177/0885066619871452

16. Wu Z, Wang Z, Dai F, Liu H, Ren W, Chang J, et al. Dephosphorylation of Y685-VE-cadherin involved in pulmonary microvascular endothelial barrier injury induced by angiotensin II. *Mediat Inflamm*. (2016) 2016:8696481. doi: 10.1155/2016/8696481

17. Dawson J, Cockerill GW, Choke E, Belli A-M, Loftus I, Thompson MM. Aortic aneurysms secrete interleukin-6 into the circulation. *J Vasc Surg*. (2007) 45:350–6. doi: 10.1016/j.jvs.2006.09.049

18. Dawson J, Cockerill G, Choke E, Loftus I, Thompson MM. Aortic aneurysms as a source of circulating interleukin-6. *Ann N Y Acad Sci*. (2006) 1085:320–3. doi: 10.1196/annals.1383.009

19. Cai YL, Wang ZW. The expression and significance of IL-6, IFN-γ, SM22α, and MMP-2 in rat model of aortic dissection. *Eur Rev Med Pharmacol Sci*. (2017) 21:560–8.

20. Paige E, Clement M, Lareyre F, Sweeting M, Raffort J, Grenier C, et al. Interleukin-6 receptor signaling and abdominal aortic aneurysm growth rates. *Circ Genom Precis Med*. (2019) 12:e002413. doi: 10.1161/CIRCGEN.118.002413

21. Lv XC, Lin Y, Wu QS, Wang L, Hou YT, Dong Y, et al. Plasma interleukin-6 is a potential predictive biomarker for postoperative delirium among acute type a aortic dissection patients treated with open surgical repair. *J Cardiothorac Surg*. (2021) 16:146. doi: 10.1186/s13019-021-01529-4

22. Butt Y, Kurdowska A, Allen TC. Acute lung injury: a clinical and molecular review. *Arch Pathol Lab Med*. (2016) 140:345–50. doi: 10.5858/arpa.2015-0519-RA

23. Deng Y, Hou L, Xu Q, Liu Q, Pan S, Gao Y, et al. Cardiopulmonary bypass induces acute lung injury via the high-mobility group box 1/toll-like receptor 4 pathway. *Dis Markers*. (2020) 2020:8854700. doi: 10.1155/2020/8854700

24. Ge J, Tian J, Yang H, Hou L, Wang Z, He Z, et al. Alpha7 nicotine acetylcholine receptor agonist PNU-282987 attenuates acute lung injury in a cardiopulmonary bypass model in rats. *Shock*. (2017) 47:474–9. doi: 10.1097/SHK.0000000000000744

25. Sreeramkumar V, Adrover JM, Ballesteros I, Cuartero MI, Rossaint J, Bilbao I, et al. Neutrophils scan for activated platelets to initiate inflammation. *Science*. (2014) 346:1234–8. doi: 10.1126/science.1256478

26. Visconti L, Santoro D, Cernaro V, Buemi M, Lacquaniti A. Kidney-lung connections in acute and chronic diseases: current perspectives. *J Nephrol*. (2016) 29:341–8. doi: 10.1007/s40620-016-0276-7

27. Karimi Z, Ketabchi F, Alebrahimdehkordi N, Fatemikia H, Owji SM, Moosavi SM. Renal ischemia/reperfusion against nephrectomy for induction of acute lung injury in rats. *Ren Fail*. (2016) 38:1503–15. doi: 10.1080/0886022X.2016.1214149

28. Weiss YG, Merin G, Koganov E, Ribo A, Oppenheim-Eden A, Medalion B, et al. Postcardiopulmonary bypass hypoxemia: a prospective study on incidence, risk factors, and clinical significance. *J Cardiothorac Vasc Anesth*. (2000) 14:506–13. doi: 10.1053/jcan.2000.9488

frontiers | Frontiers in Cardiovascular Medicine

TYPE Original Research
PUBLISHED 30 August 2023
DOI 10.3389/fcvm.2023.1166093

OPEN ACCESS

EDITED BY
Shahzad Raja,
Harefield Hospital, United Kingdom

REVIEWED BY
Gianluca Paternoster,
San Carlo Hospital, Italy
David Santer,
University Hospital of Basel, Switzerland

*CORRESPONDENCE
Daniela Geisler
daniela.geisler@gesundheitsverbund.at

RECEIVED 14 February 2023
ACCEPTED 14 August 2023
PUBLISHED 30 August 2023

CITATION
Geisler D, Arleth N, Grabenwöger J, Arnold Z, Aschacher T, Winkler B, Mach M and Grabenwöger M (2023) Impact of CytoSorb® on interleukin-6 in cardiac surgery.
Front. Cardiovasc. Med. 10:1166093.
doi: 10.3389/fcvm.2023.1166093

Impact of CytoSorb® on interleukin-6 in cardiac surgery

Daniela Geisler[1,2]*, Noemi Arleth[3], Johannes Grabenwöger[3], Zsuzsanna Arnold[1,2], Thomas Aschacher[1,2], Bernhard Winkler[1,2,3], Markus Mach[4] and Martin Grabenwöger[1,2,3]

[1]Department of Cardiovascular Surgery, Clinic Floridsdorf, Vienna, Austria, [2]Institute of Cardiovascular Research, Karl Landsteiner Society, Vienna, Austria, [3]Medical Faculty, Sigmund Freud Private University, Vienna, Austria, [4]Department of Cardiac Surgery, Medical University of Vienna, Vienna, Austria

Objective: Cardiac surgery is known to activate a cascade of inflammatory mediators leading to a systemic inflammatory response. Hemadsorption (HA) devices such as CytoSorb® have been postulated to mitigate an overshooting immune response, which is associated with increased morbidity and mortality, and thus improve outcome. We aimed to investigate the effect of CytoSorb® on interleukin (IL)-6 levels in patients undergoing complex cardiac surgery in comparison to a control group.

Methods: A total of 56 patients (28 CytoSorb®, 28 control) undergoing acute and elective cardiac surgery between January 2020 and February 2021 at the Department of Cardiac and Vascular Surgery, Clinic Floridsdorf, Vienna, were retrospectively analyzed. The primary endpoint was the difference in IL-6 levels between the CytoSorb® and control group. Secondary endpoint was periprocedural mortality.

Results: CytoSorb®, installed in the bypass circuit, had no significant effect on IL-6 levels. IL-6 peaked on the first postoperative day (HA: 775.3 ± 838.4 vs. control: 855.5 ± 1,052.9 pg/ml, $p = 0.856$). In total, three patients died in the HA group, none in the control (logistic regression model, $p = 0.996$). Patients with an increased Euroscore II of 7 or more showed a reduced IL-6 response compared to patients with an Euroscore II below 7 (178.3 ± 63.1 pg/ml vs. 908.6 ± 972.6 pg/ml, p-value = 0.00306).

Conclusions: No significant reduction of IL-6 levels or periprocedural mortality through intraoperative HA with CytoSorb® in patients undergoing cardiac surgery was observed. However, this study was able to show a reduced immunologic response in patients with a high Euroscore II. The routine application of CytoSorb® in cardiac surgery to reduce inflammatory mediators has to be scrutinized in future prospective randomized studies.

KEYWORDS

IL-6, cytokine storm, CytoSorb®, hemadsorption, cardiac surgery

Introduction

Cardiac surgery evokes an unpredictable activation of the complement cascade and stimulation of the immune system induced by surgical trauma, cardiopulmonary bypass (CPB) through sheer stress, artificial surfaces and reperfusion injury. A normal immune response results in a controlled inflammation process involving pro- and anti-

Abbreviations
ACC, aortic cross clamp; AUC, area under curve; CPB, cardiopulmonary bypass; CRP, c-reactive protein; CARS, compensatory anti-inflammatory response syndrome; ES II, Euroscore II; HA, hemadsorption; ICU, intensive care unit; IL, interleukin; POD, postoperative day; RCT, randomized controlled trials; ROC, receiver operating characteristic; SIRS, systemic inflammatory response syndrome.

inflammatory cytokines. In case of a dysregulation, inflammatory mediators are excessively released, which is referred to as "cytokine storm" (1, 2). This hyperactivation may result in a systemic inflammatory response syndrome (SIRS) and consequently septic shock. Cytokine-induced vasodilatation and increased capillary permeability cause hemodynamic depression and organ dysfunction, linked to increased morbidity and mortality (3–5). Interleukin (IL-) 6 plays a crucial role as early indicator of inflammation prior to C-reactive protein (CRP) and is therefore routinely used in the intensive care setting (6). IL-6 is induced by tumor necrosis factor in response to severe injury and infection and stimulates the synthesis of acute-phase-proteins such as CRP in the liver. Elevated IL-6 levels were not only shown to correlate with the severity of sepsis but also to be highly predictive of adverse outcome following cardiac surgery (1, 7, 8). Bauer et al. also found IL-6 to be predictive for prolonged mechanical ventilation and thus longer stay at the ICU (7).

Hemadsorption (HA) devices have been postulated to reduce excess cytokine levels—as produced in a cytokine storm—and thus attenuate an overshooting immune response and ultimately prevent multi-organ failure (9). The CytoSorb® adsorber (CytoSorbents Europe GmbH, Berlin, Germany) is the most widely-used cytokine filter and consists of a crosslinked divinyl benzol-polymer filtering small and mid-size hydrophobic molecules up to a size of 60 kDa. It was designed for the removal of inflammatory mediators in SIRS, sepsis and septic shock and is now increasingly adopted in cardiac surgery to mitigate the inflammatory response induced by CPB (10, 11). Reported results are inconsistent and in the scarce literature of HA in cardiac surgery the effect of CytoSorb® on inflammatory cytokines remains questionable (10–13). The aim of this retrospective study was to investigate whether the use of CytoSorb® during CPB in patients undergoing cardiac surgery has an effect on IL-6 levels and secondarily on periprocedural mortality. Furthermore, possible factors leading to increased IL-6 were analyzed and reported out of concurrence.

Materials and methods

Study design and patients

The study protocol was reviewed and approved by the ethics committee of Vienna, reference number EK 21-039-VK. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Study cohort

A total of 56 patients who underwent elective or acute cardiac surgery with ($n = 28$) and without (=28) CytoSorb® between January 2020 and February 2021 at the Department of Cardio-Vascular Surgery Vienna, Clinic Floridsdorf, Vienna, Austria, were retrospectively analyzed. CytoSorb® was employed non-randomly at the surgeon's discretion, predominantly in endocarditis, redo- and high-risk surgeries. A control group was chosen within the same time period.

Inclusion criteria

Criteria for inclusion for both groups were: coronary artery bypass surgery, aortic-, mitral- and/or tricuspid valve repair/replacement, surgery of the ascending aorta and aortic arch including surgeries with circulatory arrest or combined procedures and serum levels of IL-6 available at baseline and in the postoperative period.

Hemadsorption protocol

According to the manufacturer's recommendation the CytoSorb® 300 ml adsorber had been installed in the CPB circuit (Stöckert S5 LivaNova, USA, Inc., Arvada, CO.) with a side arm coming from the venous outflow tube and given back to the venous reservoir prior to the oxygenator. Blood was pumped actively through the CytoSorb® cartridge with a standardized rate of 200 ml/min by a roller pump. The CPB circuit was primed according to institutional standards (1,700 ml Elomel saline solution + 10.000 IE heparin). In this study, CytoSorb® filtering was active only during CPB time.

IL-6 is routinely assessed in laboratory measurements at the intensive care unit (ICU) at our department to monitor the postoperative course of inflammation, as elevated IL-6 levels were shown to be predictive of the course in the ICU following cardiac surgery. In general, the first measurement postoperatively is about 6 h after the operation, then routine laboratory measurements are around 6 a.m. Thus, time points can be described as follows: before surgery, 6 h after end of surgery, first postoperative (POD 1) and second postoperative day (POD 2). For the quantification of IL-6 electrochemiluminescence sandwich immunoassay ECLIA (Roche Diagnostics) was used.

Endpoints

Primary endpoint was difference in IL-6 levels at its peak, which was on the first POD, between the HA and control group. Secondary endpoint was periprocedural mortality, defined as death occurring ≤30 days after the index procedure, >30 days but during the index hospitalization. Additionally, clinical parameters, duration of surgery, aortic cross-clamp and CPB time, catecholamine use, ICU and overall hospital stay as well as relevant laboratory parameters such as leucocytes count, c-reactive protein (CRP) were assessed.

Sample size calculation

Sample size calculations were performed for the primary outcome. A strong effect of CytoSorb® on IL-6 levels was assumed (effect size $d = 0.8$), at a level of significance of $\alpha = 5\%$ and a power of 80%. The number of patients per group was calculated to be 21. We estimated a drop-out quote of one third due to missing data, therefore an additional of 7 patients were included.

Statistical analysis

Patient and perioperative data was collected from the electronic hospital records. Patient records were pseudoanonymized for further processing. Statistical analysis was performed using the open-source statistical software package R [version 4.1.0, 2021-05-18, R Core Team (2021). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org].

The *primary endpoint* IL-6 serum levels [in pg/ml] on the first POD (IL-6 POD 1) were compared in a Wilcoxon sum rank test, as IL-6 was not normally distributed. Comparison of IL-6 levels at all timepoints were additionally reported using Wilcoxon signed rank tests and *p*-values were Bonferroni corrected. Nevertheless, the primary endpoint remained IL-6 POD 1 levels and these outcomes were reported out of concurrence.

For the *secondary endpoint* periproceural mortality, a logistic regression model was used.

As patients who had Cytosorb installed in the CPB are generally sicker, this patient cohort represents a real-world setting. In *confounder analyses* the influence of significantly different baseline parameters (ES II, number of redo surgeries, ascending aortic replacements, aortic arch replacements, surgery time, and cardiopulmonary bypass time) on peak IL-6 levels were assessed. In case of ratio scaled parameters linear regression models were used to estimate the logarithm of IL-6. The logarithm was used to reduce the right-skewness of IL-6. In case of dichotomous baseline parameters, a Wilcoxon sum rank test was used. Additionally, a propensity score matching was performed matching age, Euroscore II, CPB time, surgical duration, see **Supplementary Tables S1–S4**, which contain all statistics of **Tables 1–4** based on the propensity score matched data set.

In *exploratory analyses* we investigated possible factors (impact of oxygenators, steroid application) affecting IL-6 levels, since those were high in comparison to other studies. Three different oxygenators were used: Terumo Capiox® FX25 Advance Oxygenator, Eurosets Horizon and Getinge Quadrox-i®. Additionally, the effect of HA on CRP serum levels, leukocyte count, norepinephrine levels was tested in Wilcoxon rank sum tests. Postoperative outcome parameters including ICU stay, atrial fibrillation *de novo*, stroke rate, ventilation time and reintubation were evaluated. The length of the ICU stay was tested in a Poisson regression model. To evaluate the validity of the study sample, the prognostic effect on 30-day mortality of known risk factors such as ES II, troponin I, and lactate levels on POD 1 were analyzed in a ROC analysis and opposed to peak IL-6. The area under curve (AUC), the sensitivity, and the specificity were reported.

Results

In this retrospective study, 56 patients who underwent cardiac surgery for different indications were included: 28 patients with CytoSorb® installed in the bypass circuit and 28 patients in the control group, with no HA device. The groups were comparable in terms of age and sex (see **Tables 1**, **2**). Following baseline parameters showed to be significantly different between both groups: ES II, number of redo surgeries, ascending aortic replacements, aortic arch replacements, and cardiopulmonary bypass time (CPB, see **Tables 1**, **2**). Baseline parameters are depicted in **Table 1** (containing the normal distributed parameters) and **Table 2** (containing the dichotomous parameters).

Primary endpoint: impact of CytoSorb® on IL-6 levels

Primary endpoint results are depicted in **Table 3** and **Figure 1**. Preoperative IL-6 levels were 16.6 ± 39.5 pg/ml in the CytoSorb®

TABLE 1 Interval and ratio scaled baseline parameters of CytoSorb® and control group.

Time	Parameter	HA ($n = 28$)				Control ($n = 28$)				*t*-Test		
		Mean	SD	Min	Max	Mean	SD	Min	Max	*t*	Dof	*p*
Preop	Age	62.8	14.7	32.0	82.0	67.3	14.5	24.0	81.0	−1.160	53.98738	0.251
	BMI	29.4	14.7	18.7	101.0	26.8	4.1	20.5	35.2	0.904	31.27468	0.373
	Euroscore II	5.4	6.0	0.7	23.7	2.5	2.2	0.5	9.8	2.395	34.07519	**0.022**
	Heart rate in bpm	73.5	10.1	54.0	90.0	74.0	11.8	53.0	95.0	−0.183	52.75003	0.856
	Respiratory rate in bpm	11.8	1.0	9.0	14.0	11.9	0.6	10.0	13.0	−0.806	44.27324	0.425
	Body temperature in °C	37.1	0.4	36.0	37.7	37.0	0.4	36.0	37.5	0.710	53.74381	0.481
Baseline	Hemoglobin (g/dl)	13.3	1.7	11.2	18.0	13.6	1.5	10.5	16.8	−0.713	52.97789	0.479
	Leukocyte count (G/L)	7.3	2.2	3.0	12.4	7.6	2.4	3.9	13.3	−0.449	53.77825	0.655
	Albumin (g/dl)	42.5	2.2	37.0	48.0	41.6	5.0	29.0	49.0	0.799	37.36149	0.429
	Bilirubin (mg/dl)	0.6	0.3	0.2	1.4	0.6	0.4	0.2	2.4	−0.529	50.49803	0.599
	CRP (mg/L)	9.0	25.3	0.3	135.8	6.0	15.8	0.3	83.2	0.522	45.14523	0.604
	IL6 (pg/ml)	16.6	39.5	1.5	206.0	7.8	12.5	1.5	67.7	1.124	32.33027	0.269
	Procalcitonin (ng/ml)	0.0	0.0	0.0	0.1	0.0	0.1	0.0	0.3	−1.311	30.04276	0.200
Intraop	Surgery duration (min.)	300.7	72.0	168.0	454.0	260.1	63.7	183.0	422.0	2.231	53.21981	**0.030**
	CPB (min.)	155.7	50.2	81.0	253.0	125.2	41.5	56.0	239.0	2.472	52.18499	**0.017**
	ACC (min.)	93.3	32.7	0.0	155.0	86.1	38.1	27.0	202.0	0.764	52.80028	0.448

Bold parameters indicate a significant difference between both groups.
BMI, body mass index; Dof, degrees of freedom; Max, maximum; Min, minimum; SD, standard deviation, *p*, *p*-value. ACC, aortic cross clamp, BMI, body mass index; CPB, cardiopulmonary bypass; CRP, c-reactive protein; HA, hemadsorption; IL6, interleukin 6.

TABLE 2 Dichotomous distributed baseline parameters of CytoSorb® and control group.

Time	Parameters	HA ($n = 28$)	Control ($n = 28$)	χ^2 test	
				χ^2	p-value
Preop	Sex (male)	15 (53.6)	21 (75.0)	1.944	0.163
	Art. Hypertension	21 (75.0)	21 (75.0)	0.000	1.000
	Pulmonary hypertension	2 (7.1)	4 (14.3)	0.187	0.666
	Hyperlipidemia	20 (71.4)	19 (67.9)	0.000	1.000
	COPD	5 (17.9)	5 (17.9)	0.000	1.000
	Diabetes mellitus	7 (25.0)	9 (32.1)	0.350	0.774
	Peripheral artery disease	0 (0)	0 (0)	NA	NA
	Cerebrovascular disease	7 (25.0)	5 (17.9)	0.106	0.745
	Marfan syndrome	3 (10.7)	0 (0)	1.409	0.234
Indication	Aortic stenosis	7 (25.0)	9 (32.1)	0.088	0.767
	Aortic regurgitation	5 (17.9)	2 (7.1)	0.653	0.419
	Combined aortic vitium	0 (0)	1 (3.6)	0.000	1.000
	Mitral regurgitation	5 (17.9)	8 (28.6)	0.401	0.527
	Mitral stenosis	1 (3.6)	1 (3.6)	0.000	1.000
	Tricuspid regurgitation	2 (7.1)	4 (21.4)	0.187	0.666
	Aneurysm	12 (42.9)	3 (10.7)	5.828	**0.016**
	Dissection	5 (17.9)	0 (0)	3.514	0.060
	Coronary artery disease	7 (25.0)	15 (53.6)	3.668	0.055
	Myocardial infarction	0 (0)	2 (7.1)	0.519	0.471
Surgery	Re-do surgery	12 (42.9)	0 (0)	12.833	**0.000**
	Combined surgery	19 (67.9)	13 (46.4)	1.823	0.177
	AVR mechanical	2 (7.1)	1 (3.6)	0.000	1.000
	AVR biological	8 (28.6)	10 (35.7)	0.082	0.775
	Mitral valve replacement	5 (17.9)	4 (14.3)	0.000	1.000
	Mitral valve repair	1 (3.6)	5 (17.9)	1.680	0.195
	Tricuspid valve repair	2 (7.1)	4 (14.3)	0.187	0.666
	CABG	8 (28.6)	15 (53.6)	2.656	0.103
	Ascending aorta replacement	11 (39.9)	3 (10.7)	7.547	**0.023**
	Aortic arch replacement (partial/full)	10 (35.7)	1 (3.6)	7.240	**0.007**
	Bentall procedure	1 (3.6)	1 (3.6)	0.000	1.000
	ECMO	1 (3.6)	2 (7.1)	0.000	1.000

Bold parameters indicate a significant difference between both groups. Columns 3 and 4 show absolute numbers (n=) and percentages in brackets (%).
AVR, aortic valve replacement; COPD, chronic obstructive pulmonary disease; CABG, coronary artery bypass grafting; ECMO, extracorporeal membrane oxygenation; HA, Hemadsorption.

vs. 7.8 ± 12.5 pg/ml in the control group. Six hours after surgery the IL-6 levels showed a strong increase (HA: 512.6 ± 391.2 vs. control: 554.1 ± 340.8 pg/ml) and peaked on the first POD (HA: 775.3 ± 838.4 vs. control: 855.5 ± 1,052.9 pg/ml, not significant). The IL-6 POD 1 levels showed no significant difference in a Wilcoxon sum rank test ($p = 0.856$, see Table 3). Two days after surgery the IL-6 levels halved (IL-6 POD 2, HA: 347.6 ± 310.8 vs. control: 283.5 ± 225.9 pg/ml). *At all times CytoSorb® did not exhibit a significant effect on IL-6 levels when compared to the control group*. All significantly different baseline parameters were

TABLE 3 Inflammatory blood parameters of both groups specified by median (1st quartile, 3rd quartile).

		HA ($n = 28$)	Control ($n = 28$)	p-value
IL6 [pg/ml]	Baseline	3.9 (2.4, 9.8)	4.0 (2.8, 7.3)	0.974
	6 h postop	357.5 (261.0, 667.8)	479.5 (238.0, 883.8)	0.533
	POD1	398.5 (215.8, 918.8)	392 (245.3, 1,073.3)	0.856
	POD2	203.0 (133.5, 458.5)	253.0 (91.0, 323.3)	0.720
CRP [mg/L]	Baseline	2.1 (0.9, 6. 5)	1.0 (0.5, 4.9)	0.359
	6 h postop	14.9 (8.2, 18.7)	11.41 (8.9, 14.8)	0.599
	POD1	67.3 (48.9, 90.8)	73.1 (56.1, 89.5)	0.890
	POD2	232.5 (157.7, 275.4)	214.0 (186.4, 273.7)	0.955
Leukocyte count (G/L)	Baseline	7.2 (5.8, 7.9)	7 (5.9, 9.2)	0.831
	POD1	11.0 (7.7, 15.1)	12.2 (9.9, 15.2)	0.298
	POD2	12.2 (8.8, 17.2)	11.2 (9.3, 15.1)	0.699

The p-value refers to the Wilcoxon rank sum test.
CRP, c-reactive protein; HA, hemadsorption; IL6, interleukin 6; POD, postoperative.

TABLE 4 Postoperative outcome parameters.

	HA ($n = 28$)	Control ($n = 28$)	χ^{2a}/W^b	p-value
Periprocedural mortality, n (%)	3 (10.7)	0 (0)	0.011[a]	0.996
ICU stay, in days median ± mad	5.0 ± 2.2	5.0 ± 2.2	374[b]	0.771
Atrial fibrillation *de novo*, n (%)	4 (14.3)	6 (21.4)	0.0004[a]	0.984
Stroke rate, n (%)	1 (3.6)	1 (3.6)	0[a]	1.000
Ventilation time median ± mad	17.5 ± 17.0	9.0 ± 7.4	344[b]	0.431
Reintubation, n (%)	4 (14.3)	0 (0)	0.043[a]	0.836
Acute kidney injury, n (%)	4[c] (14.3)	7[c] (25)	0.452[a]	0.501
Renal replacement therapy, n (%)	0 (0)	0 (0)	0.000[a]	1.000

[a]Pearson's χ^2 test.
[b]Wilcoxon rank sum test with continuity correction, mad ... median deviation of the median.
[c]Patients with acute kidney injury (AKI) stage 1: increase in serum creatinine by 0.3 mg/dl or more within 48 h or increase in serum creatinine to 1.5 times baseline according to KDIGO-criteria.

investigated in confounder analyses (see "**Confounder analyses**" at the end of the "Results" section). In the propensity score matched data set, IL-6 did not show to be significantly different between groups (**Supplementary Table S3**). Previously significant baseline parameters such as Euroscore II, surgical duration, and CPB time were not significantly different following propensity score matching (**Supplementary Table S1**).

Secondary endpoint: impact of CytoSorb® on periprocedural mortality

Although all of three deceased patients were in the HA group, no significant effect was shown in the logistic regression model (OR = $e^{-18.4}$, 95%- confidence interval = 0.0–437.5, $p = 0.996$). The three deceased patients were female with ages of 62, 76, and 77 showing high ES II values of 11.3, 14.2, and 19.6, respectively. Neither IL-6 levels of the first POD were indicative (206, 154, 219 pg/ml), nor body temperature (37.4, 37.1, 36.9°C). Serum levels of troponin I on the first POD were 11,563, 1,149, 3,611 µg/L, lactate levels were 3.3, 5.71, 3.22 mmol/L. Indications for surgery were acute aortic dissection, re-aortic valve stenosis and aortic aneurysm, respectively. Surgeries were replacement of the ascending aorta and hemiarch in mild hypothermic circulatory arrest, redo aortic valve replacement and coronary artery bypass grafting, and reoperation with replacement of the ascending aorta and hemiarch in mild hypothermic circulatory arrest. The 62-year old patient died due to liver failure resulting in multiorgan failure on the 4th postoperative day, the 76-year old patient died on the first postoperative day due to right ventricular failure after revision due to hemothorax, and the 77-year old patient died on the 20nd postoperative day due to multiple strokes, bilateral pneumonia requiring veno-venous extracorporeal membrane oxygenation and consequently followed by SIRS.

Confounder analysis

The impact of the significantly different baseline parameters on IL-6 levels on the first POD (i.e., IL-6 POD 1 = peak of IL-6) were further analyzed, i.e., ES II, number of redo surgeries, ascending aortic replacements, aortic arch replacements, surgery time, and CPB time.

ES II showed a significant effect on IL-6 POD 1 levels [linear regression model: log(IL-6 POD 1) = 6.4–0.069 × ES II, Wald's test p (ES II) = 0.023]. Moreover, patient with an increased ES II

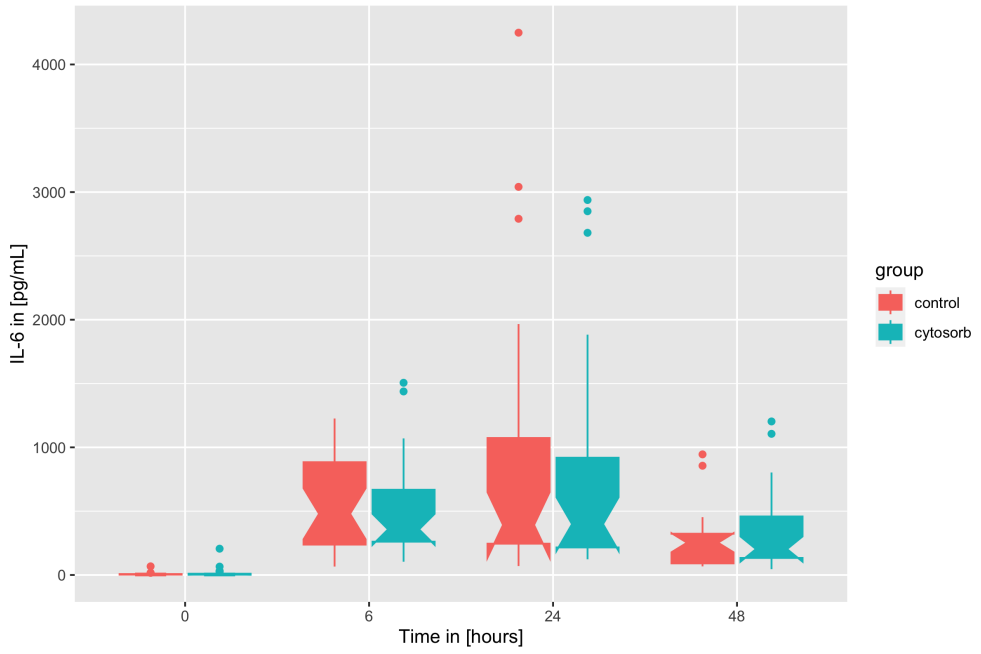


FIGURE 1
Timely course of IL-6 as boxplots over time at baseline, 6 h after surgery, on the first postoperative day (POD 1), on the second postoperative day (POD 2).

of 7 or more showed significantly reduced IL-6 POD 1 response (ES II > 7: 178.3 ± 63.1 pg/ml vs. IL-6 levels in ES II < 7: 908.6 ± 972.6 pg/ml, Wilcoxon rank sum test: $W = 280$, p-value = 0.003, see Figure 2). Among the significantly different intraoperative parameters, the surgery duration did not show a significant effect on IL-6 POD 1 levels [linear regression model: log(IL-6) = 6.15–0.00002 × surgery duration, p(surgery duration) = 0.993], similarly to CPB time [linear regression model: log(IL-6) = 6.19–0.0003 × CPB, p(CPB) = 0.923].

IL-6 POD 1 levels were equally high for redo surgery patients vs. in all other patients (439.8 ± 390 pg/dl vs. 559.1 ± 357.9 pg/dl, Wilcoxon rank sum test with continuity correction, $W = 324.5$, p-value = 0.1341).

Neither ascending aorta replacement (replacement group: 396.4 ± 319.6 vs. 585.0 ± 371.5 pg/dl in the others, Wilcoxon rank sum test with continuity correction, $W = 358.5$, p-value = 0.2059), nor aortic arch replacement did affect the IL-6 POD 1 levels, in case of IL-6 POD 1 was 402.5 ± 376.6 in the group with arch replacement vs. 565.9 ± 358.8 in all others (Wilcoxon rank sum test with continuity correction, $W = 318.5$, p-value = 0.08003).

Exploratory analysis: laboratory parameters and clinical course

Three different *oxygenators* were used in the CPB circuit: horizon, fx25 m, and quadrox-i. There was no significant effect of the different oxygenators on IL-6 POD 1 [horizon: 659.5 ± 755.9 pg/dl, fx25: 825.4 ± 1,048.5 pg/dl, quadrox-i: 938.8 ± 941.1 pg/dl, linear regression model estimating log(IL-6 POD 1) = 6.0 + 0.13 × fx25 (yes or no) + 0.37 × quadrox (yes or no) with horizon as reference, p(fx25) = 0.721, p(quadrox) = 0.356]. Intraoperative single shot administration of *steroids* did not decrease IL-6 POD 1 [linear regression model: log(IL-6 POD 1) = 6.1 + 0.09 × steroids (yes or no), p(steroids) = 0.786]. At the end of the surgery *norepinephrine* dosage was comparable in both groups, i.e., 5.2 ± 4.0 ml/h in the CytoSorb® group and 4.8 ± 6.6 in the control group (Wilcoxon rank sum test with continuity correction, $W = 336$, p-value = 0.624).

Similarly to IL-6, *CRP* serum levels are driven by the surgery but in contrast to IL-6 with a known temporal lag of 24 h (see Figure 3). Again, no effect of CytoSorb® on CRP and leukocyte count was found (see Table 3). The peak CRP levels on the second POD are correlated to the peaking logarithm of IL-6 levels of the first POD [linear regression model: CRP POD 2 = −63.2 + log(IL-6 POD 1), adjusted $R^2 = 0.37$, Wald's test $p < 0.001$, see Figure 4].

The ICU stay was 6.6 ± 5.6 days in the CytoSorb® group and 5.3 ± 2.6 in the control group. A Poisson regression model of ICU stay did not significantly differ between both groups (Poisson regression model: $e^{1.7 + 0.2 \times \text{Cytosorb}}$, Wald's test $p = 0.053$).

A powerful prognostic effect of ES II on periprocedural mortality was shown (ROC analysis: AUC = 0.97, Threshold = 10.54, Sensitivity = 100%, Specificity = 96.2%) of troponin I POD 1 (ROC analysis: AUC = 0.93, threshold = 1,137, Sensitivity = 100%, Specificity = 82.7%) and of lactate levels POD 1 (ROC analysis: AUC = 0.96, threshold = 3.105, Sensitivity = 100%, Specificity = 92.5%) but not of IL-6 POD1 (ROC analysis: AUC = 0.79, Threshold = 230.5, Sensitivity = 100%, Specificity = 78.4%).

Postoperative outcome parameters were compared using Pearson's χ^2 test in case of count data and Wilcoxon rank sum

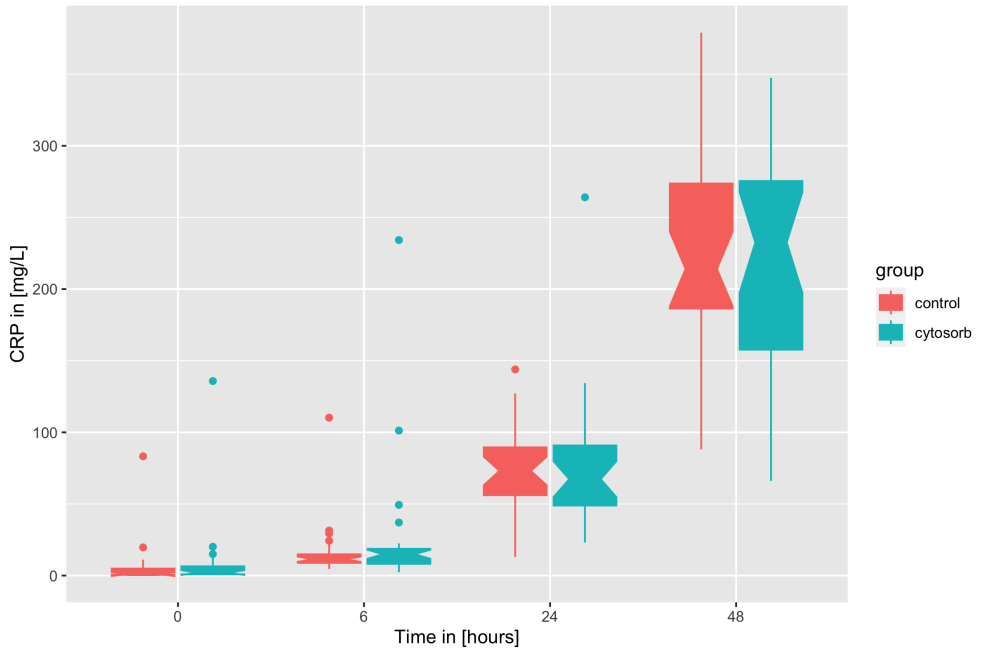


FIGURE 2
Timely course of CRP as boxplots over time at baseline, six hours after surgery, on the first postoperative day (POD 1), and on the second postoperative day (POD 2).

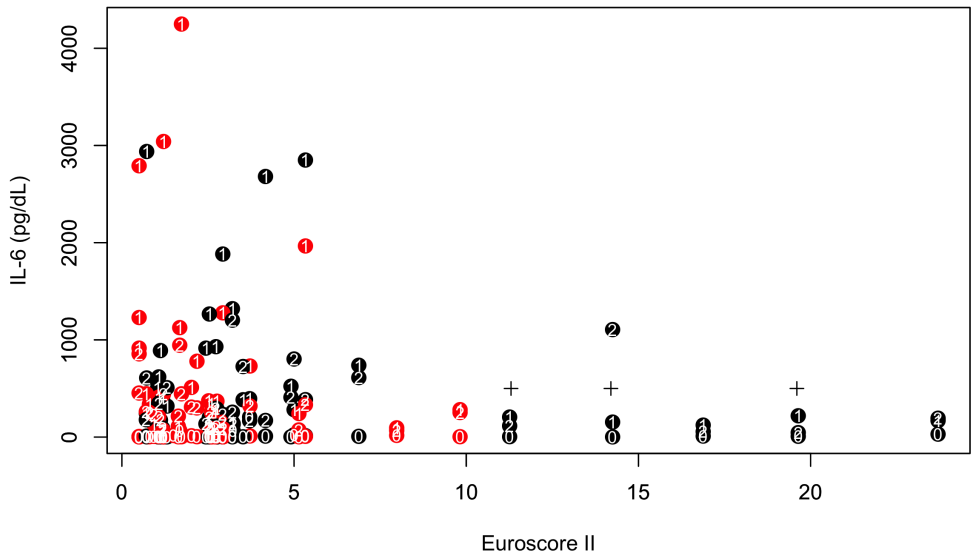


FIGURE 3
IL-6 at three different timepoints (indicated as white letters: 0 ... baseline, 1 ... first postoperative day, 2 ... second postoperative day) vs. Euroscore II. Red refers to HA, black to control. The cross symbols mark the three deceased patients.

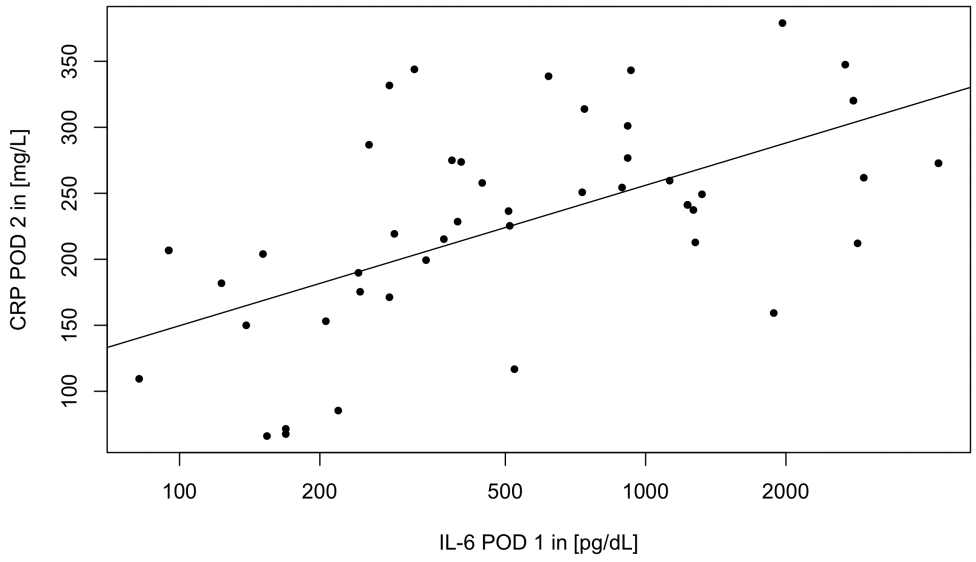


FIGURE 4
Scatterplot of CRP POD 2 vs. IL-6 POD 1. A regression line was plotted.

test for metric data, respectively, and were not significantly different between groups (see Table 4).

Discussion

The effect of intraoperative application of CytoSorb® on IL-6

CytoSorb® applied during CPB in patients undergoing cardiac surgery showed to have no effect on postoperative IL-6 levels in this retrospective single center cohort. Furthermore, no difference in mortality between the CytoSorb® and the control group was observed, i.e., the most relevant clinical endpoint.

CytoSorb® therapy is approved for the non-selective removal of excessive levels of cytokines. To date, there are seven randomized controlled trials (RCT) investigating this role of CytoSorb® applied in cardiac surgery (10–16). Comparing these RCTs, IL-6 levels showed a heterogenous course with peak values ranging from immediately after surgery (12, 16), on admission at ICU (14), 2 h after surgery (11), and 6 h after surgery (10, 13). The fact that the half-life of IL-6 lies within minutes, suggests that once the causative trigger is eliminated, IL-6 levels should decrease rapidly, i.e., after surgery, remaining elevated only in

cases of prolonged immune answer e.g., sepsis (17). Albeit the IL-6 course, with an elevation about 6 h after surgery, corresponds to our data, we found in contrast prolonged elevated IL-6 levels postoperatively with a peak on the first POD, in line with Puchinger et al. (18) However, HA consistently had no significant effect on systemic inflammatory response or clinical outcome, respectively, supporting our results.

The effect of confounding variables on IL-6 levels

Although present differences between groups, CytoSorb® and control group, including increased ES II, longer surgery duration and CBP times, re-do surgeries, surgeries of the ascending aorta and arch, respectively, a significant impact of these factors on IL-6 levels was only observed for ES II in the confounder analysis. Despite the assumption that patients with an increased ES II will show increased IL-6 levels due to higher morbidity, this was not the case. Interestingly, in those patients with a high ES II of 7 or more and thus suspected high proinflammatory activity, IL-6 levels did not raise above 500 pg/ml. This is explained by the immunologic phenomenon, that critically ill patients are often anergic, characterized by a decrease in cytokine response, described in literature as *compensatory anti-inflammatory response syndrome* (CARS) (18, 19). Putting this into practice, a high ES II renders hemadsorption, with the aim to reduce cytokine levels following CPB, questionable.

Comparison of IL-6 levels to literature

Moreover, we observed relatively high IL-6 levels with regard to existing literature, with maximum serum concentrations greater than 500 pg/ml, comparable to sepsis patients (20–24). We hypothesized that the significantly elevated IL-6 levels, might be due to the fact that we included not only elective but also acute cardiac surgeries involving complex aortic arch surgeries and circulatory arrest. We did not confirm this hypothesis due to the heterogenous patient population and therefore small number of patients receiving certain operations and due to missing randomization, which is the main limitation of this study. But on another note, as per Schadler et al., the removal of cytokines is described to be concentration-dependent, while low cytokine plasma concentrations show to be not affected, high cytokine plasma levels are ought to be reduced effectively (21). This, although the patients in our cohort exhibited considerably elevated IL-6 levels, was not the case.

Exploratory analyses

Factors believed to contribute to the inflammatory response following cardiac surgery were included in an exploratory analysis. Literature on differences between oxygenator used in cardiac surgery and postoperative cytokine levels is lacking. We were not able to detect a difference between the three oxygenators used on IL-6 on POD 1. On the contrary, the anti-inflammatory effects of steroids on clinical outcome in cardiac surgery have been investigated in several trials (25–27). The meta-analysis by Dvirnik et al. showed that steroid administration at the time of cardiac surgery had no impact on mortality in over 16,000 patients (27). We analyzed the effect of intraoperative single shot steroid administration [100 mg SOLU-CORTEF® (hydrocortisone sodium succinate)] on IL-6 POD 1 between groups, with no significant difference. CytoSorb® was described to be associated with reduced catecholamine use, which we did not confirm as norepinephrine dosage was comparable in both groups (5.2 ± 4.0 ml/h in the CytoSorb® group vs. 4.8 ± 6.6 in the control group in our study sample) (28, 29). Contrarywise, Santer et al. reported a significantly higher demand for norepinephrine in the HA group in patients undergoing valve surgery for infectious endocarditis. He also found higher reoperation rates due to bleeding going along with a higher need for red blood cell concentrates and platelets with an overall longer hospital stay (30).

We further analyzed other important markers of inflammation including CRP and leucocyte count, which were not influenced by intraoperative HA with CytoSorb®. Also, the ICU stay and overall hospital stay in our cohort was comparable between groups.

Additionally, we were able to confirm the known prognostic effect of ES II, troponin I POD 1 and lactate levels POD 1 on periprocedural mortality in a receiver operating characteristic (ROC) analysis. IL-6 on POD 1 did not show to be of prognostic relevance in our cohort.

Other available data on CytoSorb® in cardiac surgery are smaller retrospective studies and case reports (28, 30–33). In sepsis studies, however, HA already finds an ample field of application (23). In a retrospective septic shock study cohort, the duration of application of CytoSorb® and thus the amount of blood purified seemed to be of clinical importance (23). Asch et al. applied CytoSorb® during CPB, and then continuously for 24 h following cardiac surgery, changing the cartridge every 8 h. However, this had no effect on postoperative inflammatory mediators (12). Gleason et al. combined two Cytosorb cartridges placed in a parallel configuration to reach a total blood flow of about 600 ml/min during CPB (mean duration of CytoSorb® treatment 2.5 ± 1.2 h, range 0.8–5.0 h) to enhance the effect of HA therapy. They reported an initial reduction of the complement factors C3a and C5a, also in the HA group, but this also did not affect outcome (15).

Limitations

First, one limitation of this study was that the Cytosorb group represented a frailer patient cohort showing significantly higher ES II values. This is partly due to the fact that only the CytoSorb® group included redo surgeries (12 out of 28), a 4.3-fold (11 vs. 3) of ascending aorta replacements, and a 10-fold (10 vs. 1) of aortic arch replacements in comparison to the control group.

Hence, significantly longer surgeries and CPB times were found in the CytoSorb® group.

Second this study was not randomized through its retrospective character and a significant negative selection bias might have been introduced by choosing sicker patients for the use of CytoSorb®. On the other side, reduced IL-6 levels were found in patients with an ES II above 7. A reduced general condition is linked to a limited immunologic response in such patients.

Third, in our study we did not find a significant influence of CytoSorb® on mortality. With a mortality of 5.4% (3 out of 56), a sample size of 1,414 patients is required for a logistic regression model with an alpha of 5% (and not 1% as in our study), a power of 95%, a supposed odds ratio of 2.0 and an allocation strategy of 50% for each group. Nevertheless, mortality as endpoint is of use in a meta-analysis and thus just has to be reported also in smaller scale studies.

In future studies we recommend to use ES II for stratification to exclude its effect on immunologic response.

Conclusion

In conclusion, literature on CytoSorb® in cardiac surgery is diverging and although no clear benefit on the inflammatory response was demonstrated, CytoSorb® is routinely installed in the CPB circuit for the removal of cytokines in complex cardiac surgeries. No significant reduction of postoperative IL-6 levels nor periprocedural mortality through intraoperative HA with CytoSorb®, installed in the CPB in patients undergoing cardiac surgery, was observed. The immunologic response, i.e., IL-6 levels, seems to be reduced in those patients with a high ES II—a poor clinical prognosis, therefore a reduction of IL-6 through HA is not of relevance in these patients. The routine application of CytoSorb® in cardiac surgery to reduce IL-6 needs to be reconsidered. A large multi-institutional trial with stringent entry criteria is required to verify the beneficial impact of hemadsorption in cardiac surgery.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by the ethical committee Vienna (EK-21-039 VK). The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and institutional requirements.

Author contributions

DG, MG conceptualization. DG writing–original draft. ZA, TA, MM writing–review & editing. NA, JG data curation. BW and MG project administration & supervision. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2023.1166093/full#supplementary-material

References

1. Chousterman BG, Swirski FK, Weber GF. Cytokine storm and sepsis disease pathogenesis. *Semin Immunopathol.* (2017) 39(5):517–28. doi: 10.1007/s00281-017-0639-8

2. Ferrara JL, Abhyankar S, Gilliland DG. Cytokine storm of graft-versus-host disease: a critical effector role for interleukin-1. *Transplant Proc.* (1993) 25(1 Pt 2):1216–7.

3. Chenoweth DE, Cooper SW, Hugli TE, Stewart RW, Blackstone EH, Kirklin JW. Complement activation during cardiopulmonary bypass: evidence for generation of C3a and C5a anaphylatoxins. *N Engl J Med.* (1981) 304(9):497–503. doi: 10.1056/NEJM198102263040901

4. Kohler T, Schwier E, Praxenthaler J, Kirchner C, Henzler D, Eickmeyer C. Therapeutic modulation of the host defense by hemoadsorption with CytoSorb®—basics, indications and perspectives—a scoping review. *Int J Mol Sci.* (2021) 22(23). doi: 10.3390/ijms222312786

5. Papazisi O, Bruggemans EF, Berendsen RR, Hugo JDV, Lindeman JHN, Beeres S, et al. Prevention of vasoplegia with CytoSorb in heart failure patients undergoing cardiac surgery (CytoSorb-HF trial): protocol for a randomised controlled trial. *BMJ Open.* (2022) 12(9):e061337. doi: 10.1136/bmjopen-2022-061337

6. Tanaka T, Narazaki M, Kishimoto T. IL-6 in inflammation, immunity, and disease. *Cold Spring Harb Perspect Biol.* (2014) 6(10):a016295. doi: 10.1101/cshperspect.a016295

7. Bauer A, Korten I, Juchem G, Kiesewetter I, Kilger E, Heyn J. Euroscore and IL-6 predict the course in ICU after cardiac surgery. *Eur J Med Res*. (2021) 26(1):29. doi: 10.1186/s40001-021-00501-1

8. Wu HP, Chen CK, Chung K, Tseng JC, Hua CC, Liu YC, et al. Serial cytokine levels in patients with severe sepsis. *Inflamm Res*. (2009) 58(7):385–93. doi: 10.1007/s00011-009-0003-0

9. Trager K, Fritzler D, Fischer G, Schroder J, Skrabal C, Liebold A, et al. Treatment of post-cardiopulmonary bypass SIRS by hemoadsorption: a case series. *Int J Artif Organs*. (2016) 39(3):141–6. doi: 10.5301/ijao.5000492

10. Poli EC, Alberio L, Bauer-Doerries A, Marcucci C, Roumy A, Kirsch M, et al. Cytokine clearance with CytoSorb(R) during cardiac surgery: a pilot randomized controlled trial. *Crit Care*. (2019) 23(1):108. doi: 10.1186/s13054-019-2399-4

11. Bernardi MH, Rinoesl H, Dragosits K, Ristl R, Hoffelner F, Opfermann P, et al. Effect of hemoadsorption during cardiopulmonary bypass surgery—a blinded, randomized, controlled pilot study using a novel adsorbent. *Crit Care*. (2016) 20:96. doi: 10.1186/s13054-016-1270-0

12. Asch S, Kaufmann TP, Walter M, Leistner M, Danner BC, Perl T, et al. The effect of perioperative hemadsorption in patients operated for acute infective endocarditis—a randomized controlled study. *Artif Organs*. (2021) 45(11):1328–37. doi: 10.1111/aor.14019

13. Garau I, Marz A, Sehner S, Reuter DA, Reichenspurner H, Zollner C, et al. Hemadsorption during cardiopulmonary bypass reduces interleukin 8 and tumor necrosis factor alpha serum levels in cardiac surgery: a randomized controlled trial. *Minerva Anestesiol*. (2019) 85(7):715–23. doi: 10.23736/S0375-9393.18.12898-7

14. Stupica GT, Sostaric M, Bozhinovska M, Rupert L, Bosnic Z, Jerin A, et al. Extracorporeal hemadsorption versus glucocorticoids during cardiopulmonary bypass: a prospective, randomized, controlled trial. *Cardiovasc Ther*. (2020) 2020:7834173. doi: 10.1155/2020/7834173

15. Gleason TG, Argenziano M, Bavaria JE, Kane LC, Coselli JS, Engelman RM, et al. Hemoadsorption to reduce plasma-free hemoglobin during cardiac surgery: results of REFRESH I pilot study. *Semin Thorac Cardiovasc Surg*. (2019) 31(4):783–93. doi: 10.1053/j.semtcvs.2019.05.006

16. Diab M, Lehmann T, Bothe W, Akhyari P, Platzer S, Wendt D, et al. Cytokine hemoadsorption during cardiac surgery versus standard surgical care for infective endocarditis (REMOVE): results from a multicenter randomized controlled trial. *Circulation*. (2022) 145(13):959–68. doi: 10.1161/CIRCULATIONAHA.121.056940

17. Jawa RS, Anillo S, Huntoon K, Baumann H, Kulaylat M. Analytic review: interleukin-6 in surgery, trauma, and critical care: part I: basic science. *J Intensive Care Med*. (2011) 26(1):3–12. doi: 10.1177/0885066610395678

18. Puchinger J, Ryz S, Nixdorf L, Edlinger-Stanger M, Lassnigg A, Wiedemann D, et al. Characteristics of interleukin-6 signaling in elective cardiac surgery—a prospective cohort study. *J Clin Med*. (2022) 11(3). doi: 10.3390/jcm11030590

19. Ward NS, Casserly B, Ayala A. The compensatory anti-inflammatory response syndrome (CARS) in critically ill patients. *Clin Chest Med*. (2008) 29(4):617–25, viii. doi: 10.1016/j.ccm.2008.06.010

20. Paul R, Sathe P, Kumar S, Prasad S, Aleem M, Sakhalvalkar P. Multicentered prospective investigator initiated study to evaluate the clinical outcomes with extracorporeal cytokine adsorption device (CytoSorb®) in patients with sepsis and septic shock. *World J Crit Care Med*. (2021) 10(1):22–34. doi: 10.5492/wjccm.v10.i1.22

21. Schadler D, Pausch C, Heise D, Meier-Hellmann A, Brederlau J, Weiler N, et al. The effect of a novel extracorporeal cytokine hemoadsorption device on IL-6 elimination in septic patients: a randomized controlled trial. *PLoS One*. (2017) 12(10):e0187015. doi: 10.1371/journal.pone.0187015

22. Scharf C, Schroeder I, Paal M, Winkels M, Irlbeck M, Zoller M, et al. Can the cytokine adsorber CytoSorb® help to mitigate cytokine storm and reduce mortality in critically ill patients? A propensity score matching analysis. *Ann Intensive Care*. (2021) 11(1):115. doi: 10.1186/s13613-021-00905-6

23. Schultz P, Schwier E, Eickmeyer C, Henzler D, Kohler T. High-dose CytoSorb hemoadsorption is associated with improved survival in patients with septic shock: a retrospective cohort study. *J Crit Care*. (2021) 64:184–92. doi: 10.1016/j.jcrc.2021.04.011

24. Mat-Nor MB, Md Ralib A, Abdulah NZ, Pickering JW. The diagnostic ability of procalcitonin and interleukin-6 to differentiate infectious from noninfectious systemic inflammatory response syndrome and to predict mortality. *J Crit Care*. (2016) 33:245–51. doi: 10.1016/j.jcrc.2016.01.002

25. Cappabianca G, Rotunno C, de Luca Tupputi Schinosa L, Ranieri VM, Paparella D. Protective effects of steroids in cardiac surgery: a meta-analysis of randomized double-blind trials. *J Cardiothorac Vasc Anesth*. (2011) 25(1):156–65. doi: 10.1053/j.jvca.2010.03.015

26. Ho KM, Tan JA. Benefits and risks of corticosteroid prophylaxis in adult cardiac surgery: a dose-response meta-analysis. *Circulation*. (2009) 119(14):1853–66. doi: 10.1161/CIRCULATIONAHA.108.848218

27. Dvirnik N, Belley-Cote EP, Hanif H, Devereaux PJ, Lamy A, Dieleman JM, et al. Steroids in cardiac surgery: a systematic review and meta-analysis. *Br J Anaesth*. (2018) 120(4):657–67. doi: 10.1016/j.bja.2017.10.025

28. Saller T, Hagl C, Woitsch S, Li Y, Niedermayer S, Born F, et al. Haemadsorption improves intraoperative haemodynamics and metabolic changes during aortic surgery with hypothermic circulatory arrest. *Eur J Cardiothorac Surg*. (2019) 56(4):731–7. doi: 10.1093/ejcts/ezz074

29. Nemeth E, Kovacs E, Racz K, Soltesz A, Szigeti S, Kiss N, et al. Impact of intraoperative cytokine adsorption on outcome of patients undergoing orthotopic heart transplantation-an observational study. *Clin Transplant*. (2018) 32(4):e13211. doi: 10.1111/ctr.13211

30. Santer D, Miazza J, Koechlin L, Gahl B, Rrahmani B, Hollinger A, et al. Hemoadsorption during cardiopulmonary bypass in patients with endocarditis undergoing valve surgery: a retrospective single-center study. *J Clin Med*. (2021) 10(4). doi: 10.3390/jcm10040564

31. Trager K, Skrabal C, Fischer G, Datzmann T, Schroeder J, Fritzler D, et al. Hemoadsorption treatment of patients with acute infective endocarditis during surgery with cardiopulmonary bypass—a case series. *Int J Artif Organs*. (2017) 40(5):240–9. doi: 10.5301/ijao.5000583

32. Trager K, Skrabal C, Fischer G, Schroeder J, Marenski L, Liebold A, et al. Hemoadsorption treatment with CytoSorb® in patients with extracorporeal life support therapy: a case series. *Int J Artif Organs*. (2020) 43(6):422–9. doi: 10.1177/0391398819895287

33. Haidari Z, Wendt D, Thielmann M, Mackowiak M, Neuhauser M, Jakob H, et al. Intraoperative hemoadsorption in patients with native mitral valve infective endocarditis. *Ann Thorac Surg*. (2020) 110(3):890–6. doi: 10.1016/j.athoracsur.2019.12.067

frontiers | Frontiers in Surgery

TYPE Review
PUBLISHED 03 July 2024
DOI 10.3389/fsurg.2024.1224068

OPEN ACCESS

EDITED BY
Shahzad Raja,
Harefield Hospital, United Kingdom

REVIEWED BY
Nobuyuki Ishibashi,
Children's National Hospital, United States
Gianluca Paternoster,
ICU San Carlo Hospital Potenza Italy, Italy

*CORRESPONDENCE
Frank W. Sellke
frank_sellke@brown.edu

RECEIVED 17 May 2023
ACCEPTED 07 June 2024
PUBLISHED 03 July 2024

CITATION
Banerjee D, Feng J and Sellke FW (2024) Strategies to attenuate maladaptive inflammatory response associated with cardiopulmonary bypass.
Front. Surg. 11:1224068.
doi: 10.3389/fsurg.2024.1224068

Strategies to attenuate maladaptive inflammatory response associated with cardiopulmonary bypass

Debolina Banerjee, Jun Feng and Frank W. Sellke*

Division of Cardiothoracic Surgery, Department of Surgery, Brown University/Rhode Island Hospital, Providence, RI, United States

Cardiopulmonary bypass (CPB) initiates an intense inflammatory response due to various factors: conversion from pulsatile to laminar flow, cold cardioplegia, surgical trauma, endotoxemia, ischemia-reperfusion injury, oxidative stress, hypothermia, and contact activation of cells by the extracorporeal circuit. Redundant and overlapping inflammatory cascades amplify the initial response to produce a systemic inflammatory response, heightened by coincident activation of coagulation and fibrinolytic pathways. When unchecked, this inflammatory response can become maladaptive and lead to serious postoperative complications. Concerted research efforts have been made to identify technical refinements and pharmacologic interventions that appropriately attenuate the inflammatory response and ultimately translate to improved clinical outcomes. Surface modification of the extracorporeal circuit to increase biocompatibility, miniaturized circuits with sheer resistance, filtration techniques, and minimally invasive approaches have improved clinical outcomes in specific populations. Pharmacologic adjuncts, including aprotinin, steroids, monoclonal antibodies, and free radical scavengers, show real promise. A multimodal approach incorporating technical, circuit-specific, and pharmacologic strategies will likely yield maximal clinical benefit.

KEYWORDS

cardiac surgery, cardiopulmonary bypass, inflammation, ischemiareperfusion injury, organ damage

1 Introduction

The advent of cardiopulmonary bypass (CPB) revolutionized cardiac surgery and dramatically improved patient outcomes. CPB activates various inflammation, coagulation, fibrinolysis, apoptosis, and oxidation pathways. Exposure to nonphysiologic conditions during CPB leads to an intense immunologic response that can affect the function and recovery of multiple organ systems (1).

The body's immunologic response is designed to sequester and destroy what it recognizes as foreign. The initial stimulus or signal undergoes amplification due to redundant and synergistic inflammatory cascades. Frequently, this results in activation of both humoral and cellular components of the immune system, and the initial inflammatory response to CPB is no different. The activation of these pathways leads to release of multiple humoral mediators. Leukocytes, chiefly neutrophils, are then drawn to the site of production of these mediators, become activated, and subsequently adhere to endothelial cells by way of receptor interactions with adhesion molecules. Endothelial

cells in turn become activated, rendering regional and remote capillary endothelium permeable to further migration of neutrophils and other intravascular molecules. Extravasation of neutrophils en masse results in release of large amounts of cytokines, chemokines, vasoactive substances, proteases of the coagulation and fibrinolysis systems, cytotoxins, and reactive oxygen species (ROS). Elimination of foreign antigen is the final step of this cascade of events.

Immunologic activation leading to inflammation is usually physiologic and protective, often leading to self-limited or subclinical organ dysfunction. However, on occasion, nonimmunologic activation that persists following CPB can be maladaptive and may progress beyond restoring homeostasis to marked fluid shifts and formation of microemboli, particularly in high-risk patients with limited functional reserve. This exuberant systemic inflammatory response to CPB, often characterized as systemic inflammatory response syndrome (SIRS), may manifest as clinically significant increase in capillary permeability, interstitial edema, and organ dysfunction. The link between CPB-induced inflammatory response and adverse clinical outcomes is still not well delineated. Several hypotheses have been proposed. One hypothesis suggests the balance between pro-inflammatory and anti-inflammatory cytokine release correlates with the magnitude of multiorgan injury (2). Temporal and magnitude changes in cytokine production patterns may further influence the clinical presentation and course of SIRS postoperatively (3, 4). A second hypothesis suggests SIRS is the result of a multifaceted response with overall cytokine upregulation leading to both a proinflammatory state (SIRS) as well as homeostatic, compensatory anti-inflammatory response syndrome (CARS) leading to systemic deactivation of the immune response, predisposing the patient to immunosuppression and infectious complications (5). The multi-hit hypothesis suggests CPB primes polymorphonuclear leukocytes such that subsequent exposure to stimuli that otherwise may be self-limiting (i.e., postoperative infection or ongoing ischemia) results in enhanced cytotoxin release and downstream organ dysfunction (5). This priming is thought to occur through various processes, including the secretion of cytokines, leading to pulmonary leucosequestration (5).

These harmful inflammatory effects are due to the interactions of a wide spectrum of compounds, including inflammatory triggers (complement-derived factors), mediators (cytokines and adhesion molecules), or effectors (proteolytic enzymes, oxygen free radicals, arachidonic acid metabolites, and immune cells). Hallmarks of ensuing multiorgan dysfunction include coagulopathy, myocardial dysfunction, pulmonary and renal insufficiency, neurocognitive deficits, hepatic injury, splanchnic bed hypoperfusion and bacterial translocation. Collectively, these multiorgan effects have been linked to post-perfusion/post-pump syndrome and ischemia-reperfusion injury.

Despite drawbacks attributed to the attendant inflammatory response, CPB remains a mainstay technique in cardiothoracic surgery, as it allows for adequate exposure of the lateral and posterior coronary arteries and facilitates a bloodless field in which to operate. While more drastic strategies have been employed to blunt excessive inflammatory response and its sequelae (including off-pump coronary artery bypass and minimally invasive techniques), an integrated approach to modulate the stress response incorporating pharmacologic measures and technical refinements has shown promise. Herein, we characterize the pathophysiology of the inflammatory response, and discuss potential strategies to intercept and attenuate this response.

2 Pathophysiology of inflammatory response following cardiopulmonary bypass

Compared with off-pump approaches, CPB may trigger an intense physiologic response due to several stimuli (Figure 1) (6, 7):

- Blood contact with synthetic surfaces within the perfusion circuit and multiple tissues within the wound
- Abnormal blood-gas interface
- Pulsatile flow converted to laminar flow
- Hypothermia
- Surgical trauma
- Global myocardial ischemia during cardioplegic protection
- Ischemia-reperfusion injury to end-organs
- Endotoxemia proceeding from splanchnic hypoperfusion and bacterial translocation

The inflammatory response following CPB is multifactorial, and may become generalized and uncontrolled, leading to SIRS. The "early" phase is initiated by blood contact with nonendothelial surfaces of the extracorporeal circuit and ultimately involves both humoral and cellular constituents of the immune system. The "late" phase that perpetuates inflammatory cascades is characterized by ischemia-reperfusion injury, endotoxemia, coagulopathy, and heparin-protamine complex reactions (Table 1). The link between inflammatory, coagulation, and fibrinolytic cascades is complex and may partially be explained by acute phase reactions during CPB similar to those seen in sepsis (8). Another link may be nuclear factor kappa B (NFκB), a ubiquitous and inducible transcription factor that is implicated during all phases of the response but plays a central role in regulating pro-inflammatory genes during the acute phase reaction (9).

2.1 Contact activation

The exposure of blood to air and nonphysiologic surfaces of the extracorporeal circuit leads to simultaneous activation of coagulation and fibrinolysis cascades as well as the complement pathways of innate immunity (Figure 2).

Four proteins are involved in the contact activation pathway: factor XII (Hageman factor), factor XI, prekallikrein, and high-molecular-weight kininogen (HMWK). Upon exposure of blood to foreign material of the CPB circuit, factor XII is converted to its active form in the presence of prekallikrein and HMWK. Activated factor XII activates factor XI of the intrinsic pathway, ultimately leading to thrombin formation. Activated factor XII

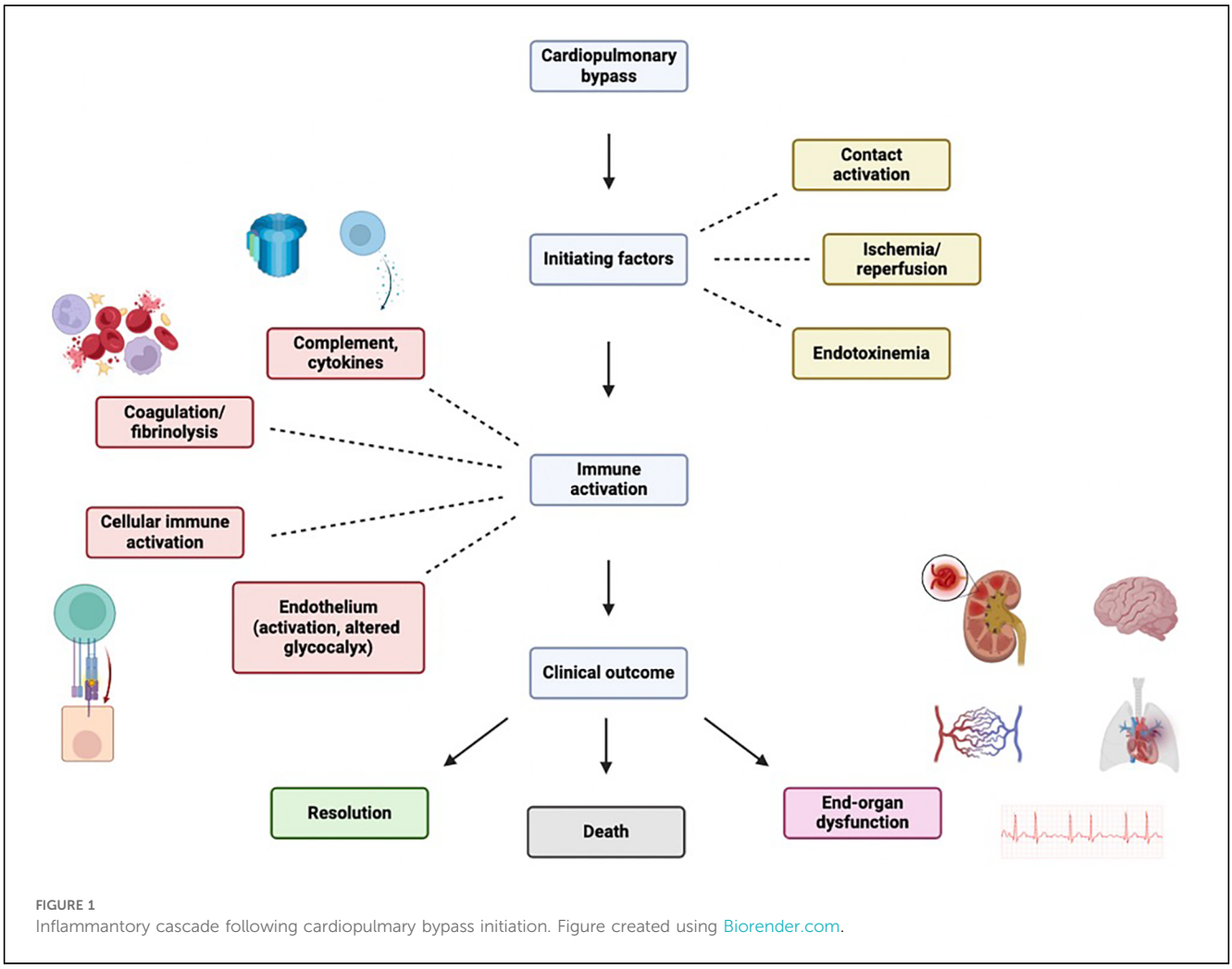


FIGURE 1
Inflammantory cascade following cardiopulmary bypass initiation. Figure created using Biorender.com.

TABLE 1 Humoral and cellular factors influencing systemic inflammatory response associated with CPB.

Humoral	Cellular
Contact activation	Endothelial cell (EC) activation
Intrinsic coagulation	Adhesion molecules
Extrinsic coagulation	Leukocyte activation
Complement activation	○ Neutrophils
Fibrinolysis activation	○ Lymphocytes
Cytokines	○ Platelets
Endotoxin	○ Monocytes

also converts prekallikrein to kallikrein. Kallikrein then: (1) feeds back to facilitate continued activation of factor XII, (2) induces cleavage of HMWK to form bradykinin, (3) potentiates mediators of the alternative complement pathway, and (4) promotes conversion of plasminogen to plasmin. Plasmin is an important link to the fibrinolytic cascade. Plasminogen is activated to plasmin by activated factor XII in the intrinsic pathway during contact activation, and by tPA as part of the extrinsic pathway later during CPB. In addition to thrombin, inflammatory mediators (cytokines and endotoxin) can activate plasminogen. Activated factor XII and plasmin both play a role in stimulating the classical complement pathway (10).

Activation of prothrombin to thrombin by way of the extrinsic pathway contributes significantly to systemic thrombin generation and thrombus formation. Circulating levels of tissue factor and activated factor VII increase following CPB and surgical trauma, correlating with stimulation of pro-inflammatory mediators interleukin-1 (IL-1), tumor necrosis factor-α (TNF-α), and endotoxin (11–13). CPB necessitates anticoagulation in the form of high-dose heparin to prevent immediate clotting within the circuit. Heparin potentiates antithrombin III to inhibit thrombin and other coagulation factors (activated X, IX, XI, XII, and kallikrein). During CPB, fibrinogen and fibrin are readily deposited onto circuitry, thus creating a surface to which thrombin avidly adheres. Upon binding to deposited fibrinogen/fibrin, thrombin undergoes a conformational change that renders it resistant to inhibition by heparin-activated antithrombin III. Thus, while heparin can inhibit systemic thrombin, it is unable to inhibit surface-bound thrombin. The surface-bound thrombin continues to generate more circulating thrombin, that can in turn activate a number of constituent blood elements, including platelets. Activated platelets can bind fibrin- and fibrinogen-coated surface of CPB circuitry, and also can individually provide a scaffold for prothrombinase complexes to form and convert prothrombin to thrombin. While high-dose heparin can limit

10.3389/fsurg.2024.1224068

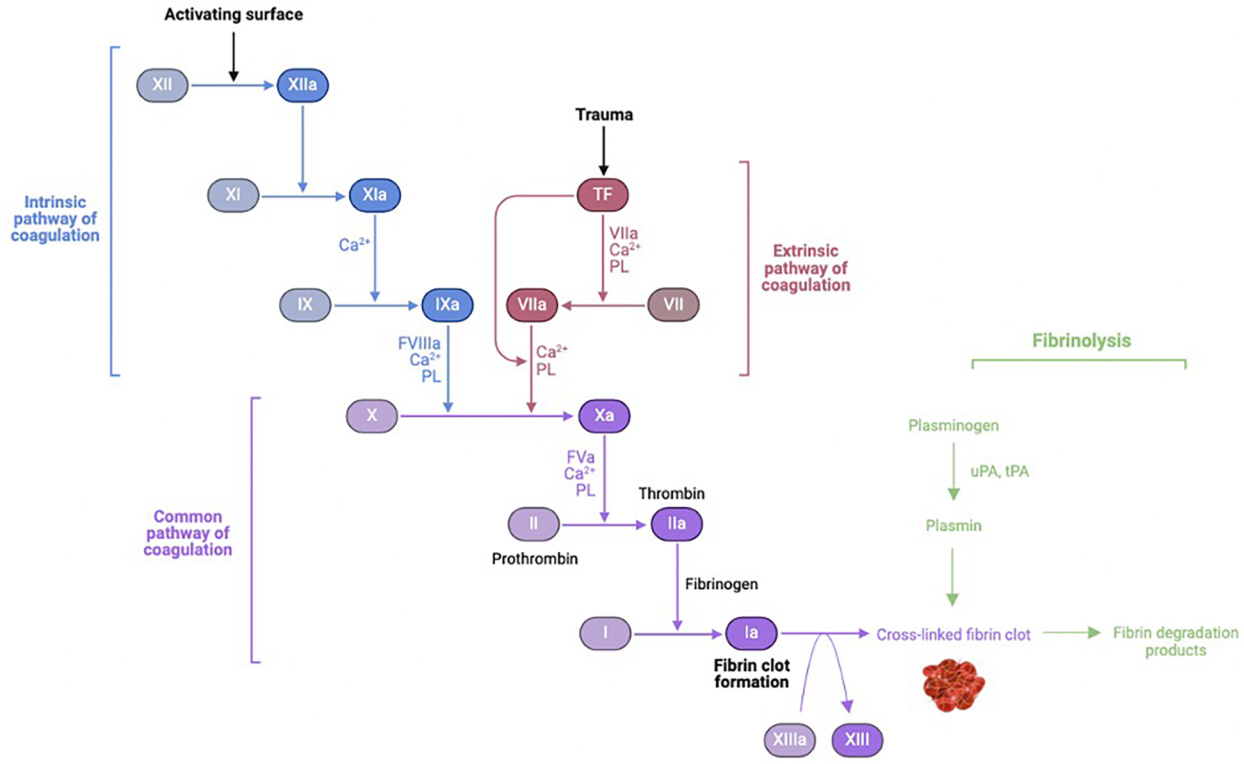


FIGURE 2
Coagulation-fibrinolysis pathway activation. uPA, urokinase; tPa, tissue plasminogen activator. Figure created using Biorender.com.

fibrin-rich thrombus during CPB, it cannot prevent thrombin generation *per se*. Thrombin levels, measured as thrombin-antithrombin complex and prothrombin fragment, increase within minutes of initiating CPB, further increase following discontinuation of CPB, and persist for up to 60 days after surgery (14–17). Heparin concentrations, antithrombin III levels, and activated coagulation time are not associated with thrombin generation (10, 18).

Activated factor XII, thrombin, kallikrein, and products of fibrinolysis potentiate the inflammatory response. Activated factor XII and kallikrein stimulate neutrophil aggregation and degranulation. Thrombin induces endothelial cells to express receptors to facilitate neutrophil binding. Fibrinogen fragment D (D-dimer) disrupts the integrity of endothelial cells and stimulates continued complement activation.

2.2 Complement activation

Contact activation by biomaterials is a critical inciting event to downstream generation of humoral mediators through its activation of the alternative complement pathway. The CPB circuit lacks inhibitors normally found on endothelial cells that limit cofactor C3 binding and activation. This contact activation, potentiated by kallikrein, leads to activation of C3 and C5 (19). Their active split products, C3a and C5a, are anaphylatoxins that are potent chemoattractants (20). Their activity is mediated by complement receptor type 1 (CR1), a transmembrane glycoprotein expressed on leukocytes that regulates complement pro-inflammatory activity while inhibiting other complement pathways. C3a is a potent stimulator of platelet aggregation. There is a significant rise in plasma C3a levels with CPB associated with its duration (2), which is not observed when CPB is avoided (19, 21–26). C5a avidly binds to neutrophil receptors (19, 24, 27, 28) thereby stimulating them to be chemotactically drawn to sites of C5a production (29, 30), aggregate and adhere to endothelial cells (31–33), degranulate to release proteases (29, 34), and produce ROS (35). C5a levels are more difficult to directly measure as C5a is internalized rapidly upon binding to neutrophil receptors, but several studies have shown decreased available C5a receptors and increased levels of terminal complement complex C5b-C9, which provide evidence for C5a generation during CPB (28, 36).

Endotoxin is a powerful activator of the alternative complement pathway, with a smaller role in activating the classical complement pathway (25). Endotoxin, or lipopolysaccharide Lipid A, arises from cell walls of gram-negative bacteria and is released upon disruption of their cell walls. They may appear in circulation during CPB due to contamination of CPB circuitry, pulmonary arterial catheters, intravenous fluids, or banked blood production. The primary mechanism for endotoxemia, however, is due to splanchnic hypoperfusion and vasoconstriction during aortic cross-clamping and resultant transient gastrointestinal bacterial translocation (37–40). Transient ischemia and laminar flow in the gut increases intestinal permeability (37, 41), facilitating endotoxin

release into circulation (42). Here, endotoxin can activate complement and stimulate production of inflammatory cytokines (43, 44). Endotoxin circulates in plasma by binding to LPS-binding protein. This complex binds to CD14 receptors on macrophages, enhancing TNF-α production (25, 45, 46). Endotoxin also stimulates endothelial cells to produce IL-6 (43). Levels of circulating endotoxin rise during and after CPB (25, 40, 42, 47–50), and are correlated with duration of aortic cross-clamping during CPB (48). Elevated LPS levels lead to myocardial dysfunction (46). Increased endotoxin levels in children with congenital heart defects undergoing CPB are associated with increased mortality after CPB (51).

CPB induces the classical complement pathway through contact activation (activated factor XII and plasmin) and heparin reversal with protamine, as evidenced by increased levels of the terminal complex C5b-C9, C3a, and C4a (27, 28, 52, 53). This further augments levels of C3a and C5a. The extent of complement activation has been correlated with duration of CPB (19). It remains unclear whether complement activation portends worse outcomes. While higher levels of C3a have been reported in those requiring prolonged mechanical ventilation, other groups have failed to identify a correlation between complement activation and acute lung injury or adverse hemodynamic responses (54–56). Equivocal findings may be due to the difficulty of parsing out the role of the complement system in the context of the complex inflammatory response associated with CPB (57).

2.3 Conversion to laminar flow

Following initiation of CPB, physiologic pulsatile aortic flow is converted to continuous laminar flow. Endothelial cells sense mechanical stresses through their attachments to the basement membrane and through membrane proteins on their luminal surface. Changes in mechanical stresses result in changes in downstream transcription of genes regulated by promoter regions containing shear stress-responsive elements. Conversion from pulsatile to laminar flow may induce expression of genes related to a pro-inflammatory phenotype. One group showed differential gene expression was responsible for a quiescent endothelial phenotype (lung Kruppel-like factor) after endothelial cells were exposed to pulsatile or laminar flow (58). Antioxidant proteins, including thioredoxin reductase and ferritin, were among shear-regulated gene products. Other studies have shown Mn^{2+}- and Cu^{2+}/Zn^{2+}-superoxide dismutase to be shear-regulated as well (59). Reduced activation of transcription factor NFκB and expression of pro-inflammatory cytokines, including IL-6, TNF-α, and IL-1 (60, 61) as well as decreased endothelial activation (62, 63) in the group undergoing pulsatile perfusion compared to the group undergoing non-pulsatile perfusion has been described.

2.4 Oxidative stress

Free radicals are molecules with unpaired electrons that render them highly reactive. ROS are free radicals derived from oxygen, and can be formed by activated neutrophils during their cytotoxic oxidative burst as a response to C5a stimulation (64–66). Exposure of blood to material of the CPB circuit as well as ischemia-reperfusion both generate various ROS implicated in oxidative damage, including superoxide anion, hydrogen peroxide, hydroxyl radical, peroxynitrite, hypochlorous acid, and singlet oxygen. Natural defense mechanisms to neutralize ROS and restore balance (redox state) include enzymes and free radical scavengers. Antioxidant enzymes include superoxide dismutase, glutathione peroxidase, and catalases. Mitochondrial scavenger complexes integrate thioredoxin and peroxiredoxin proteins (67–70). Should the body's detoxifying capacity be outstripped, ROS can cause direct damage to endothelial cell and fibroblast membrane lipids compromising membrane integrity, render proteins dysfunctional, induce nucleic acid damage that results in downstream changes in transcriptional programs via NFκB modulation, and provide positive feedback to inflammatory cascades.

Direct measurement of ROS is difficult given its short half-life and highly reactive properties, but indirect methods via measurement of more stable intermediates have shown increased ROS activity during and after CPB (24, 71–74). The onset of CPB and aortic cross-clamping creates transient ischemia, subjecting the myocardium and other organs to direct hypoxic cellular damage. CPB itself may induce generation of ROS in the area drained by the inferior vena cava (73, 74) as well as systemic oxidative stress (75). Reperfusion occurs upon removing the aortic cross-clamp, which generates further oxidative stress through recruitment of activated neutrophils to post-ischemic tissue. Following reperfusion, ROS may impair nitric oxide (NO) availability and predispose myocardial vessels to spasm and thrombose (76–78). A correlation has also been observed between timing of lipid peroxidation and degree of complement activation (24). Hypothermia may also influence ROS production by altering neutrophil-endothelium interactions during CPB (79–82).

2.5 Cytokines

Cytokines are another major group of humoral mediators that play a central role in inflammation and cell signaling. The body produces cytokines constitutively whereby subsets of immune cells maintain baseline levels of cytokines under normal conditions. Cytokines must bind cell membrane receptors to exert their effects, and the action of one or more cytokines is necessary to mount an immune response. These molecules form an intricate network in the development of inflammation, as the production of one cytokine influences the synthesis or response of others. The overlapping actions of different cytokines can be explained by both pleiotropy (single cytokine: multiple effects) and redundancy (multiple cytokines: same effect). The ability of certain cytokines to signal via more than one type of receptor complex also contributes to their pleiotropic actions wherein separately activated pathways contribute to distinct downstream effects. Simultaneously, redundant actions of cytokines allow for signal amplification; different cytokine receptors with similar motifs mediate coupling to other processes, ultimately leading to

activation of converging inflammatory pathways in potentiating the immune response.

Broadly, cytokines include tumor necrosis factor, interleukins, interferons, and several growth factors. The production and release of cytokines is induced by complement factors and their degradation products during CPB-related acute phase reaction. A number of other factors may also contribute, including endotoxin, oxidative stress, ischemia-reperfusion, and effects of cytokines themselves. While cytokines are generally considered to be products of mature leukocytes, their secretion may be modulated by other cell types, including platelets and endothelial cells (83–90). The degree of the inflammatory response is strongly influenced by the balance between pro-inflammatory (TNF-α, IL-1, IL-6, and IL-8) and anti-inflammatory (IL-10) cytokines (3).

2.5.1 Tumor necrosis factor α (TNF-α)

Tumor necrosis factor α (TNF-α, or cachectin) functions within a complex and tightly regulated cytokine network. It has a role in orchestrating the inflammatory response by inducing expression of other pro-inflammatory cytokines (IL-1 and IL-6) as well as increasing its own production. It functions in cell signaling through interactions with p55 and p75 receptors localized to the myocardium (91). It induces nitric oxide synthase and therefore increases concentrations of nitric oxide following CPB. It usually peaks shortly after surgery and then undergoes rapid degradation, but excessive production may lead to organ dysfunction. In the lung, it induces apoptosis and has been implicated in pulmonary complications. Infusion of antibodies to TNF-α prevented pulmonary edema, improved oxygenation, and significantly reduced markers of inflammation (neutrophil count, plasma TNF-α levels, malondialdehyde concentrations) in a rabbit model (92). TNF-α has been implicated in renal dysfunction as it induces fibrin deposition in the kidney glomerulus, promoting cell infiltration and vasoconstriction, thereby reducing glomerular filtration rate (93). TNF-α and IL-1 synergistically suppress myocardial contractility through a mechanism mediated by sphingosine impeding calcium-induced calcium release from the sarcoplasmic reticulum. This may result in low cardiac output, decreased vascular smooth muscle tone, and development of thrombosis, thereby contributing to dysfunction and hemodynamic instability after CPB (46, 94–96). Trends in TNF-α levels following CPB are variable, as some studies have shown increased levels while others have failed to demonstrate this. Additionally, inter-individual differences in TNF-α production may be attributed to genetic variability (97). In contrast to other cytokines (i.e., IL-6), there is no evidence that indicates TNF-α is released in large amounts following CPB (98).

2.5.2 Interleukins

Interleukins (IL) comprise a broad group of cytokines that function as intermediaries between different leukocytes and regulate various stages of the inflammatory response. IL-1 is an endogenous pyrogen. Levels of IL-1β usually increase after CPB, but this cytokine is difficult to detect due to hemodilutional effects of CPB. The IL-1 response pattern is consistent with its role as a key mediator of inflammation, both through its synergistic actions on TNF-α as well as its induction of other pro-inflammatory cytokines, including IL-6. IL-6 is a pleiotropic cytokine role that chiefly coordinates the acute phase reaction. It also induces the expression of adhesion molecules on cardiac myocytes to facilitate neutrophil adhesion, inhibits apoptosis in various cell types, enhances antibody production by activated B lymphocytes, and has negative inotropic effects on cardiac myocytes through induction of local nitric oxide release (99–104). In this way, it may be more a precise marker for progression of inflammation after CPB. A marked increased in IL-6 occurs during CPB, peaking within a few hours following CPB (105, 106), with a gradual decrease toward preoperative levels within 24 h (28). Peak IL-6 concentrations were a function of aortic cross-clamping duration. This characteristic trend in IL-6 levels has been observed in the setting of bubble and membrane oxygenators (105), after hypothermic and normothermic CPB (107), and with and without heparin-coating CPB circuitry (28). The magnitude of increase in IL-6 levels was positively correlated with duration of CPB but not duration of aortic cross-clamp time (108). In the pediatric population, duration of CPB and aortic cross-clamp time were attributed to pronounced postoperative inflammation, with only a modest influence of the degree of hypothermia (109). Rise in IL-6 after CPB has been correlated with increasing age, as those older than seventy years had a greater increase in plasma IL-6 levels during ischemia and reperfusion than their younger counterparts (110). Postoperative IL-6 levels are significantly higher in patients with complications compared to those without (111). Increased levels of IL-6 have been associated with myocardial dysfunction and wall motion abnormalities (112, 113), while effects on hemodynamics are less clear (114). Rise in IL-6 has not been correlated with complement activation.

IL-8 is a potent chemotactic agent involved in the homing of neutrophils and macrophages sites of inflammation (115). It may also play a role in ischemia-reperfusion injury, as postoperative cardiac troponin-I levels correlate strongly with IL-8 levels in patients following coronary artery bypass grafting (CABG) (116, 117). It has been implicated in precipitating vascular damage, particularly in the lungs and kidneys. Increased levels of IL-6 and IL-8 following CPB have been reported (81, 106, 107, 113, 118–123), and correlate with duration of cardiac ischemia during CPB and regional wall motion abnormalities (113, 118).

IL-10 has anti-inflammatory properties via its downregulation of pro-inflammatory cytokine synthesis by type 1 T helper cells, neutrophils, and monocytes (124–126). It has been associated with decreased production of TNF-α, IL-1, IL-2, and interferon-γ, ROS and nitric oxide derivatives (124, 127, 128). While several pro-inflammatory cytokines (TNF-α, IL-6, and IL-8) have been shown to originate from myocardium (120, 121, 129), the liver has been shown to be the primary source of IL-10 in patients undergoing CPB (120, 121, 130, 131). Rapid and transient secretion of IL-10 has been noted following CPB (132, 133). *In vivo* kinetics of IL-10 release are similar to those observed in murine endotoxemia experiments following lipopolysaccharide challenge but contrast with *in vitro* data from human monocytes following lipopolysaccharide stimulation (134, 135). IL-10 produced during CPB may represent an *in vivo* regulatory mechanism for controlling activation of cells that synthesize pro-inflammatory cytokines.

There are several caveats in relating serum cytokine levels associated with CPB to organ dysfunction. Plasma concentrations often do not reflect local effects. Several cytokines may also avidly bind to other plasma proteins, leading to inaccurate detection. Inter-individual genomic differences also contribute to heterogeneity in cytokine levels, and have implications for identifying therapeutic targets and developing preventative strategies that can be broadly applied. Genetic variants of promoter regions encoding IL-6 (−174 G/C polymorphism, −572 G/C polymorphism), TNF-α (308 G/A polymorphism), and antioxidant response elements (NQO1) have been linked to increased cytokine production and postoperative complications (136–142). A prevalent single-nucleotide polymorphism (SNP) of the gene encoding IL-18 was shown to be associated with increased TNF-α levels and decreased IL-10 levels. Apolipoprotein E4 allele has been correlated with increased IL-8 and TNF-α generation and decreased IL-10 levels, with a speculated link to postoperative cerebral injury (143–145). While the A allele of this SNP was associated with 30-day and 50-year mortality in the INFLACOR study, post-hoc analysis revealed the C allele of −572 G/C polymorphism to be significantly associated with reduced benefit of prophylactic administration of dexamethasone on postoperative IL-6 levels compared to the G allele. Its effects on C-reactive protein (CRP), however, did not appear to be genotype-dependent. These genomic differences pose a challenge to randomized controlled trials (RCTs) evaluating clinically relevant responses to prophylactic measures. Second-generation studies, including genome-wide association studies (GWAS), may be able to more completely evaluate genotype-phenotype relationships.

2.6 Cellular immune activation

CPB-induced cellular immune activation plays a key role in the ensuing inflammatory response (146). Recruitment of immune cells is mediated by upregulation of cytokines, chemokines, complement system proteins, and adhesion molecules, including selectins and integrins (147). Primary adhesion occurs when freely moving neutrophils are converted to the "rolling" state following upregulation of P- and E-selectin on endothelium and upregulation of L-selectin on neutrophils. This leads to neutrophils initially traveling in the center of postcapillary venules to tumble along endothelial walls endothelium-neutrophil selectin interactions. C5a, which is released in response to contact activation during CPB, is a potent stimulator of endothelial P-selectin expression (148). E-selectin subsequently replaces P-selectin on endothelium to maintain primary adhesion. Secondary adhesion of neutrophils is mediated by integrins. Integrins CD11a/CD18 and CD11b/CD18 are expressed by neutrophils; IL-8 and C5a are potent stimulators of CD11b/CD18 expression on neutrophils (147, 149, 150). Activated integrins bind adhesion molecules on endothelium (intercellular adhesion molecule-1 and intercellular adhesion molecule-2) and extracellular matrix elements including fibrinogen (147). Both selectins and integrins have been shown to increase following CPB (150). Transmigration of neutrophils follows secondary adhesion (151). CD11b/CD18 binding to endothelium during secondary adhesion and transmigration primes neutrophils to degranulate and undergo respiratory burst for up to 24 h following CPB (49, 147, 151). Elastase, myeloperoxidase, and ROS released from neutrophils result in cytotoxic damage to endothelium and tissues (49, 66, 151). Modulation of adhesion, transmigration, and neutrophil priming is heavily influenced by platelet-activating factor, IL-8, and C5a (147, 152). Normalization of C5a levels limits the extent of CPB-induced inflammatory response (149). Increased secretion of monocyte chemoattractants leads to upregulation of selectins and integrins and further activation of circulating monocytes and macrophages. Naïve monocytes and granulocytes are hyperstimulated following exposure to post-CPB plasma. Impaired oxidative burst and phagocytic activity have been observed 48 h following CPB, suggesting a biphasic course characterized by early tissue cytotoxicity and late neutrophil dysfunction (146, 153, 154).

Clinically, increased cellular immune activation leads to pulmonary leukocyte sequestration that has been associated with severe histologic lung injury (155, 156). Inhibition of CD11/CD18 expression or function improves myocardial function following CPB, and neutrophil adhesion blockade reduces pulmonary injury during CPB (157–159). Reduction of circulating activated leukocytes has been associated with reduced organ injury (160).

2.7 The endothelium and glycocalyx degradation

The endothelium is central to inflammatory pathophysiology following CPB (161). The endothelial glycocalyx protects the endothelial cell monolayer and is composed primarily of transmembrane heparan sulfate and syndecan proteoglycans. The glycocalyx plays important roles in leukocyte and platelet adhesion following immune activation, vascular permeability facilitating leukocyte transmigration, and regulation of the coagulation cascade on the luminal endothelial surface (161–163). Following CPB, activated metalloproteinases and TNF-α induce shedding of syndecan-1 and heparin sulfate from the glycocalyx; increased plasma levels of these glycocalyx breakdown products following CPB initiation have been extensively documented (164–170). Membrane-bound syndecan-1 reduces cytokine production while soluble syndecan-1 results in neutrophil activation and monocyte chemotaxis (171–173). Pro-inflammatory IL-6 and IL-8 levels at two timepoints (preoperatively and 6 horus postoperatively) and anti-inflammatory IL-10 have been correlated with CPB-induced inflammation (174). Syndecan-1 levels have been shown to prognosticate acute kidney injury following CPB in children though levels vary widely between patients and do not correlate intraoperatively (174, 175).

3 Strategies to modulate CPB-associated inflammatory response

Innovations and potential therapeutic targets have been investigated to better modulate the inflammatory response to

CPB. The main challenge remains to balance mitigating the effects of excessive inflammation and immune activation while still preserving host defenses and wound healing (176). The most clinically effective way to curb CPB-associated inflammation involves targeting multiple inflammatory mediators simultaneously using a combination of surgical, pharmacologic, and mechanical pump approaches as no single intervention is supported by strong level A evidence (Figure 3). Interventions with level A evidence include off-pump surgery, miniaturized CPB circuits, coated circuits to improve biocompatibility, leukocyte filtration, complement (C5) inhibition, preoperative aspirin, and corticosteroid prophylaxis. Interventions with level B evidence include, but not limited to, hemofiltration, aprotinin, nitric oxide donors, C1 esterase inhibition, neutrophil elastase inhibition, N-acetylcysteine, and intensive insulin therapy (177). The precise combinations of studied interventions tailored to specific patient populations have yet to be determined.

3.1 Non-pharmacologic strategies

3.1.1 Coated CPB circuits

Heparin-coated circuits improve biocompatibility of the extracorporeal circuit, and translation into clinical benefit has been demonstrated in certain populations (178, 179). Heparin molecules bound to the surface of the CPB circuit resemble heparin sulphate glycosaminoglycans on endothelial cells and reduce direct contact of blood with the otherwise artificial, nonendothelial material lining the CPB circuit. These coated circuits improve biocompatibility through various mechanisms. Heparin-coated circuits reduce cytokine release, complement activation *in vivo* and *in vitro*, kallikrein, and leukocyte activation (180–184). They have been shown to reduce platelet adhesion and improve platelet function, as well as inhibit the release of pro-inflammatory cytokines TNF-α, IL-6, IL-8 as well as soluble TNF receptors (180, 185–187). Significant reduction in mean concentrations of polymorphonuclear (PMN) elastase and soluble C5b-9 ($p < 0.001$ and $p = 0.006$, respectively) at one hour following initiation of CPB have been reported. Heparin coating also reduced postoperative blood loss (188–190). An RCT conducted in the Netherlands showed reduced complement activation that correlated with improved clinical performance scores in patients who underwent CPB with heparin-coated circuits in combination with full systemic heparinization (191). A large, multicenter RCT demonstrated improved clinical outcomes, shorter length of stay (hospital and ICU), and decreased respiratory and renal dysfunction in high-risk patients (192). A meta-analysis of 41 RCTs showed heparin coating was associated with 40% reduction in re-sternotomy rates ($p = 0.002$) and 20% reduction in patients requiring blood transfusion but no significant difference in 24-h blood loss or adverse events. Heparin coating reduced average ventilation time by 80 min, ICU length of stay by 9 h ($p < 0.001$), and average hospital length of stay by 0.5 days ($p = 0.02$) (193). In cardiac reoperations, use of

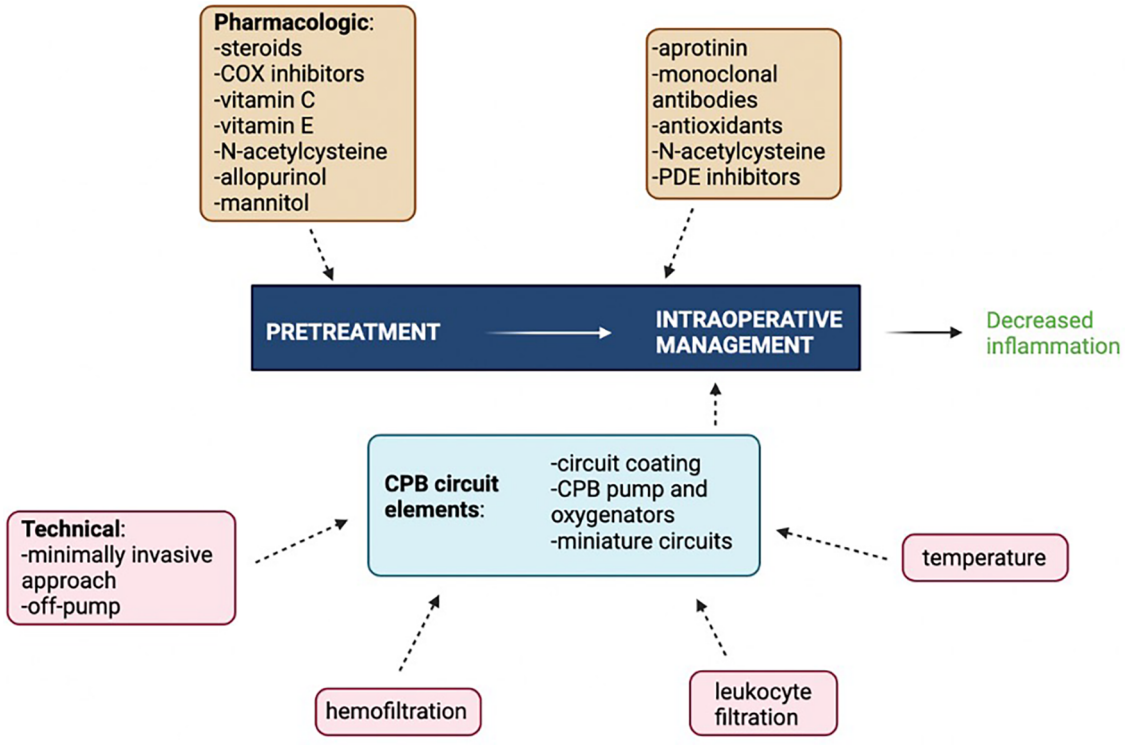


FIGURE 3
Multimodal approach to attenuating CPB-associated inflammation. COX, cyclooxygenase; PDE, phosphodiesterase. Figure created using Biorender.com.

heparin-coated circuits with full systemic heparinization was associated with decreased rates of reoperation for bleeding ($p = 0.058$) and lower blood transfusion requirement ($p = 0.035$) without significant difference in adverse events (189).

A study investigating heparin-coated circuits with administration of reduced systemic heparin dose showed decreased intraoperative platelet counts, maximal intraoperation concentrations of platelet factor 4 and PMN elastase, as well as 12-h blood loss with heparin-coated circuits but no difference adverse events or 30-day mortality (190). A departmental analysis revealed patients undergoing CPB with heparin-coated circuits received lower dose of systemic heparin, showed improvements in clinical parameters, and had a decreased rate of adverse events (10%, $p = 0.035$) compared to their counterparts (194).

Heparin-albumin and polymer-coated circuits as well as phosphorylcholine-coated circuits have been shown to induce fewer inflammatory responses and oxidative stress compared to other circuits (195, 196). However, heparin-coated circuits are associated with better preservation of endothelial glycocalyx compared with phosphorylcholine-coated circuits (197). An RCT investigating albumin-coated circuits in patients undergoing aortic arch replacement with deep hypothermic circulatory arrest showed mitigation in platelet reduction as evidenced by decreased transfusion requirement but no effect on platelet dysfunction (198). A study investigating hyaluronan-based heparin-coated circuits in various risk cohorts showed improved platelet preservation and better perioperative outcomes; ventilation time, hemorrhage, and degree of inflammation were reduced in high-risk groups, which translated to shorter ICU and hospital length of stay ($p = 0.001$ and $p = 0.006$, respectively) (199). New data is still emerging for pediatric populations. A randomized pilot study recently demonstrated application of new ternary polymer, SEC-1 coat^{TM} in pediatric cardiac operations improved biocompatibility with regard to platelet preservation and attenuated coagulation activation and overall inflammatory reaction (200). Another study showed no purported benefit in improving coagulation derangements during pediatric CPB as assessed by primary endpoint of concentration of β-thromboglobulin across all time points, as well as secondary endpoints of other markers of coagulation and platelet function (201).

Multiple studies found reductions in inflammatory markers, marginal improvement in biocompatibility, but minimal or no correlation with improved outcomes (202, 203). One such study showed reduced complement activation and synthesis of pro-inflammatory cytokines but no significant differences in fibrinolysis, platelet activation, time to hemostasis, postoperative blood loss at 12 h, total blood transfusion requirement, or intubation time (202). Another multicenter trial similarly found decreased complement activation but no association with release of specific neutrophil granule enzymes, myeloperoxidase, lactoferrin, or clinical outcome (203). A multimodal approach incorporating heparin-coated circuits, high-dose aprotinin, and pre-CPB hemofiltration reduced inflammatory response and improved clinical outcomes in high-risk patients (179).

Overall, heparin-coated circuits and newer third-generation heparin-polymer-coated circuits induce fewer inflammatory responses and are associated with improved outcomes and, therefore, justify their additional cost. Newer third-generation circuits preserve platelet function and improve perioperative outcomes, including reduced blood loss, reoperation rates, ventilation, time, and length of stay.

3.1.2 Hemofiltration

Hemofiltration, or ultrafiltration, removes excess fluid and low-molecular-weight substances from plasma through a hydrostatic pressure gradient. It has been shown to improve hemodynamic parameters, cardiac and pulmonary function, and reduce inflammation with greatest benefit in pediatric patients. It has been associated with reduced levels of TNF-α, IL-1, IL-6, IL-8, C3a, and myeloperoxidase levels postoperatively in this population (204–208). Clinical benefits include improved hemodynamic stability and early postoperative oxygenation and decreased postoperative blood loss and duration of mechanical ventilation. It has been associated with decreased endothelin-1, potentially explaining improvement in pulmonary hypertension following congenital heart surgery (204, 206, 207, 209–211). Modified ultrafiltration has been associated with improved left ventricular systolic function and diastolic compliance, increased blood pressure, and reduced inotropic drug administration in the early postoperative period in infants (212–214). Hemofiltration has shown less clinical advantage in adults as it is less effective in removing pro-inflammatory cytokines (215). However, modified hemofiltration has been associated with decreased early morbidity, postoperative bleeding, and blood transfusion requirements in adults and its use was not associated with hemodynamic instability in adults (215–217).

3.1.3 Leukocyte filtration

Activated leukocytes form the first line of defense against foreign substances and are critical players in potentiating the inflammatory response. Leukocyte-specific filters that trap activated neutrophils and monocytes implicated in SIRS attenuate inflammation and oxidative stress and have been associated with improved outcomes in the immediate postoperative period (218–222). Filters decrease concentrations of circulating platelets and leukocytes by interfering with endothelial-mediated leukocyte activation and subsequent neutrophil transmigration. This effectively decreases the endothelial-mediated component of the CPB-associated inflammatory response.

Leukocyte filtration may limit postoperative myocardial and pulmonary dysfunction following CPB. Results of a prospective randomized study showed patients undergoing coronary revascularization had decreased total leukocyte counts during and after CPB, and significantly decreased activated leukocyte counts at all timepoints. The rate of alveolar exhaled NO production and alveolar-arterial oxygenation index were significantly increased in the control group, where NO was a marker for lung inflammation. Leukocyte depletion was also associated with lower pro-inflammatory cytokine (IL-6 and IL-8) burst postoperatively in those with normal preoperative oxygenation capacity. There were no differences in intubation time, ICU, or hospital length of stay (223–226). Though, as expected, the rate of alveolar NO

production increased in both groups following CPB, absolute NO production was shown to be lower with leukocyte depletion, suggesting filtration may be lung-protective. Leukocyte depletion at early reperfusion in those with limited preoperative oxygenation capacity (mild lung dysfunction and chronic obstructive pulmonary disease) and with increased duration of CPB time was associated with improved oxygenation, shorter intubation time, and shorter ICU and hospital length of stay (227, 228). Filtration may improve postoperative lung function by mitigating pulmonary reperfusion injury. Leukocyte depletion of residual blood prior to re-transfusion also improved lung function. In patients undergoing urgent CABG for unstable angina, leukocyte depletion of re-transfused blood and during CPB reduced markers of myocardial injury, whereas leukocyte depletion did not confer clinical benefit in low-risk patients (229). In patients with decreased left ventricular function, leukocyte depletion of blood cardioplegia alone improved early myocardial function and attenuated myocardial injury (160, 230). In those with left ventricular hypertrophy undergoing valve surgery, terminal blood cardioplegia reduced myocardial injury and improved heart function (231, 232). A large-scale clinical trial showed reduced overall 60-day mortality with leukocyte depletion of transfused blood, mainly attributed to reduction in noncardiac causes of death including multisystem organ failure. In those who received greater than 3 units of blood, postoperative infection rate was lower with leukocyte depletion (233). More recently, a study showed preoperative neutrophil response to *in vitro* stimuli may predict clinical outcome following CPB, but leukocyte filtration did not offer significant benefit (234).

Leukocyte depletion may provide renal protection. A prospective randomized study of in 40 patients undergoing CABG showed leukocyte filtration decreased indices of glomerular and tubular injury, namely microalbumin/creatinine ratio, urinary excretion of microalbumin, and retinol-binding protein (235). Filtration of neutrophils containing myeloperoxidase decreased apoptosis, caspase-3 activity, and IL-1β activation and effectively improved post-ischemic renal function and structure in a porcine model of isolated kidney perfusion (236).

3.1.4 Cytokine adsorption

Extracorporeal blood purification through hemoadsorption utilizes biocompatible highly porous nonpolar polymer sorbent beads to sequester hydrophobic cytokines based on size exclusion and concentration-dependent surface adsorption throughout the beads. Nonspecific adsorptive characteristics allow for reduction in circulating pro-inflammatory cytokines, sequestration of free hemoglobin and bilirubin, and fortifying the endothelial glycocalyx. Clinical benefits include improved hemodynamic and metabolic stabilization postoperatively. No adverse effects of hemolysis or leukocyte removal have been reported. The first RCT investigating hemoadsorption with Cytosorb in cardiac surgical patients found prolonged anti-inflammatory IL-10 effect in the treatment group (237). A trial at a single center in Switzerland showed neither increased nor decreased cytokine levels (pro- or anti-inflammatory) with use of Cytosorb (238). Results showed no change in relevant clinical outcomes though the procedure was both feasible and safe. The REFRESH 1 pilot RCT showed Cytosorb signgicantly reduced C3a and C5a, as well as plasma-free hemoglobin during valve replacement operations (239). When comparing hemoadsorption to glucocorticoids, methylprednisolone was shown to more effectively reduce inflammatory markers (IL-6, TNF-alpha, and IL-8) though no differences in cardiac index or parameters of clinical outcomes were reported (240, 241). An RCT investigating intraoperative hemoadsorption showed clinical benefit as evidenced by reduced incidence of severe acute respiratory distress syndrome and a trend toward shorter ventilation times (242).

3.1.5 Temperature

Studies comparing the acute phase reaction associated with normothermic vs. hypothermic CPB have conflicting results. This is due in part to inconsistent definitions of hypothermia. Hypothermia has been shown to delay but not completely prevent the expression of inflammatory mediators as increased levels of adhesion molecules and leukocyte proteolytic enzymes were seen at 34°C compared to moderate hypothermia (26°C–28°C) (80–82). Still other studies have shown no difference between patients randomly assigned to undergo CPB at various temperatures: > vs. 27-° in one study; 28°C, 32°C, vs. 37°C in another study (243, 244). Increased levels of NO were seen with CPB at 34°C when compared to CPB at 28°C, resulting in reduced systemic vascular resistance (245). A prospective randomized study in patients undergoing valve operations showed those undergoing normothermic CPB had similar levels of myocardial protection as measured by dynamics of troponin I while those undergoing hypothermic CPB benefitted from significantly lower ventilation times ($p = 0.01$) (246). A study investigating 262 different proteins using high-throughput technology showed deep hypothermic circulatory arrest (DHCA) and rewarming potentially exert a significant effect on the plasma proteome in patients undergoing aortic operations as evidenced by suppression of complement activation during hypothermia. These findings were confirmed by changes in terminal complement complex (C5b-9) levels. Following rewarming, however, these levels of terminal complement complex were more increased with DHCA than with normothermic CPB while 48 other proteins were significantly downregulated (247). In patients with left ventricular dysfunction, normothermia was found to enable decreased requirement for defibrillation after aortic unclamping and postoperative cardiac pacing, translating to improved myocardial protection. Normothermia had no effect on development of postoperative stroke, atrial fibrillation, renal failure, or mortality (248). A large-animal study recently showed hypothermic CPB attenuated platelet degranulation and coagulopathy and better maintained oxygenator performance in swine (249). Several RCTs studying pediatric populations showed normothermic CPB as noninferior to hypothermic CPB with endpoints including inotrope duration, intubation time, hospital stay, and early neurodevelopmental outcomes in low-risk populations (250, 251).

3.1.6 CPB pumps and oxygenators

Studies evaluating the potential benefit of pulsatile pumps have not yielded conclusive results regarding clinical outcomes. Few studies have reported reduced levels of endotoxin and other pro-inflammatory mediators while other studies have not. Routine use of centrifugal pumps for CPB has not shown clear clinical advantages compared to roller pumps, and in fact several studies have showed increased levels of anaphylatoxins, pro-inflammatory cytokines, adhesion molecules, and leukocyte elastase with the use of centrifugal pumps. Radial-flow-patterned oxygenators may limit the extent of inflammation triggered by oxygenators in CPB when compared axial-flow-patterned oxygenators as evidenced by the results of a recent prospective RCT that noted significantly lower levels of humoral inflammatory markers (IL-1, IL-6, and TNF-α) 24 h postoperatively (252). In pediatric patients, controlled reoxygenation during CPB has shown benefit in single-ventricle patients as evidenced by reduced markers of organ damage, inflammation, and oxidative stress when compared to their double-ventricle counterparts (253).

3.1.7 Miniature CPB circuits

Miniature extracorporeal circulation (MECC) systems have been developed to eliminate blood-foreign surface interface, shorten tubing length, reduce priming volume, eliminate venous reservoirs and cardiotomy suction, minimize hemolytic and consumptive effects on blood cells, and maximize blood re-transfusion (254). These systems consist of a centrifugal pump, membrane oxygenator, and arterial filter with all components of the system coated with heparin to optimize biocompatibility. An RCT confirmed a milder induced inflammatory response compared to conventional CPB with reduced levels of IL-6, TNF-α, and elastase release. A more recent RCT also showed reduced migration inhibitory factor levels associated with MECC systems in addition to decreased release of pro-inflammatory cytokines in the immediate postoperative period. This overall reduction correlated with decreased blood transfusion requirement and shorter mechanical ventilation time on bypass (255). The initially reduced levels of inflammatory markers seen with MECC may not be sustained throughout the postoperative period. An RCT investigating type 2 MECC compared to conventional CPB circuit in 50 patients undergoing aortic valve replacement found significantly lower levels of pro-inflammatory markers at 2 h postoperatively ($p = 0.013$) but no difference at 24 h ($p = 0.990$) when adjusting for type of oxygenator and hemoglobin. MECC was still associated with shorter perfusion times and less transfusion requirements (256). A small prospective RCT showed decreased IL-6, decreased hemolysis peaks as evidence by plasma-free hemoglobin levels, higher cardiac index and reduced pulmonary vascular resistance within 30 min postoperatively associated with MECC (257). However, these differences were not significant, and larger prospective RCTs are lacking. Normothermic CPB using MECC systems may be beneficial for perioperative preservation of pulmonary function and hemostasis in low-risk patients (258). These systems offer a promising minimally invasive approach to CPB.

3.1.8 Minimally invasive and off-pump cardiac surgery

Advances in minimally invasive cardiothoracic surgery, including laparoscopic and thoracoscopic operations, allow for comparable outcomes while avoiding a full median sternotomy. The reduced size of surgical incisions significantly decreases the inflammatory response, but these approaches have not infrequently been associated with increased duration of CPB and aortic cross-clamping time. These results have been seen with minimally invasive valve operations as well as minimally invasive pulmonary embolectomy. While ventilator time and ICU length of stay were similar, minimally invasive operations resulted in decreased overall hospital length of stay by almost 5 days (259). In a retrospective analysis comparing video-assisted thoracoscopic surgery (VATS) vs. open operation for mitral valve disease, VATS was associate with longer duration of CPB and aortic cross-clamping but decreased ventilation time and ICU length of stay. Clearance of lactate was increased while levels of pro-inflammatory C-reactive protein, neutrophil-lymphocyte ratio, and cardiac troponin levels were significantly decreased at 24 h postoperatively in those undergoing VATS (260). These results suggest a totally thoracoscopic approach may be superior to conventional median sternotomy with regard to extent of inflammatory reaction, cardiac injury, and postoperative recovery (260).

Miniature aortic valve replacement (mini-AVR) has been evaluated in recent studies. A prospective RCT showed minimal difference in operative time on CPB, cross-clamp time, and overall operating time when evaluating patients undergoing AVR through median sternotomy compared to right anterolateral thoracotomy (261). However, cosmesis and patient satisfaction were significantly higher with reduced length of incision associated with thoracotomy approach. Ministernotomy for AVR has been associated with significant reduction in intraoperative blood loss compared to counterparts undergoing median sternotomy, though transfusion requirements were unchanged (262). This same prospective randomized study also reported no difference in respiratory function between the two groups, which was supported by results of another prospective RCT comparing outcomes in patients undergoing AVR with partial upper sternotomy vs. median sternotomy (263). This study also found the minimally invasive approach did not affect neurological outcomes or myocardial protection. In contrast, a separate randomized trial showed reduced transfusion requirement, shorter ventilation times, greater sternal stability, improved respiratory function, and earlier extubation and hospital discharge with ministernotomy AVR compared to median sternotomy approach (264).

Off-pump cardiac surgery has been attributed to reduced postoperative SIRS, but operative trauma, regional ischemia/reperfusion injury, and endotoxin release induced even in the absence of CPB and aortic cross-clamping may contribute to postoperative biological derangements and clinical morbidity. The endothelium has been increasingly implicated in multiorgan dysfunction following cardiac surgery, particularly in relation to hemostasis and oxidative stress. In a randomized clinical trial, patients undergoing off-pump cardiac surgery experienced

reduction in systemic inflammatory response as measured by decreased plasma TNF-α, IL-10, myeloperoxidase, but avoiding CPB and aortic cross-clamping did not alter circulating levels of endothelial adhesion molecules (265). Differences between on-pump and off-pump cardiac surgery in this context have been limited to the final steps of the operations and early hours thereafter, suggesting that global surgical trauma may play a more important role in activation of systemic inflammatory and coagulation-fibrinolytic pathways (266). Complement activation and release of IL-8 is dependent on extracorporeal CPB circuit, while release of products of endothelial and leukocyte activation are temporally similar but decreased in magnitude in off-pump cardiac surgery. A procoagulant state and no rise in anti-inflammatory IL-10 following off-pump cardiac surgery may offset other benefits. Neurocognitive decline and pulmonary function outcomes following off-pump cardiac surgery are variable (267).

3.2 Pharmacologic strategies

3.2.1 Aprotinin

Serine proteases comprise a large portion of effector proteins downstream of pro-inflammatory cytokines, complement activation, and hemolytic cascades. As these proteins amplify the inflammatory reaction, serine protease inhibitors have been investigated as potential therapies to mitigate excessive inflammation. Aprotinin is one such inhibitor and is perhaps one of the most well-studied. Aprotinin dually functions as an inhibitor of thrombin generation via the intrinsic pathway and has also been shown to preserve cellular junctions and reduce myocardial edema following cardioplegia and regional ischemia (268, 269). Its use has been previously associated with reduced intraoperative blood loss, while higher dosages may suppress the inflammatory response (270–272). These effects include attenuation of platelet activation, maintenance of platelet function, decreased complement and leukocyte activation, inhibition of kallikrein production, inhibition of endogenous cytokine-mediated NOS induction, inhibition of upregulation of adhesion molecules, and reduced release of several pro-inflammatory mediators (TNF-α, IL-6, and IL-8) (9, 150, 273–278). High-dose aprotinin has been associated with reduced post-CPB inflammation, myocyte damage, myocardial ischemia, and hospital length of stay in high-risk patients (7, 279, 280). High-dose aprotinin has also been associated with increased pro-inflammatory 8-isoprostane levels in the lungs relative to plasma levels. This effect disappeared with low-dose aprotinin, suggesting its action varies in a dose-dependent manner. The effect of high-dose aprotinin in decreasing circulating 8-isoprostane as estimated by lung passage (based on blood sampled from pulmonary and radial arteries) may signify a shift toward an anti-inflammatory milieu (281). Aprotinin may also decrease postoperative pulmonary and cerebral injury. Initial concerns of lower graft patency and renal dysfunction with aprotinin appear unfounded (7, 282). A meta-analysis revealed aprotinin reduced surgical blood loss, blood transfusion requirement, and need for redo thoracotomy. It also decreased perioperative mortality almost twofold without an associated increase in risk of myocardial infarction (270). A large, international, multi-institutional prospective study comparatively assessing the safety profile of aprotinin and lysine analogs (aminocaproic acid and tranexamic acid) use in patients presenting for coronary artery bypass surgery found that patients administered aprotinin had doubled risk of renal failure requiring dialysis, 55% increase in risk of myocardial infarction or heart failure, 181% increase in risk of stroke or encephalopathy, and increased risk of mortality. Neither of lysine analogs studied was associated with increased risk of cardiac, renal, or cerebral adverse events (283) and reduction in blood loss during surgery was similar for all three drugs. The BART study, a blinded RCT comparing aprotinin and lysine analogs in patients undergoing high-risk cardiac operations had to be prematurely terminated due to increased mortality associated with aprotinin, leading to suspended use of aprotinin in these patients (284). Subsequent initiatives that revisited limitations of these studies resulted in resumed use of aprotinin in select patients in both Canada and the European Union, while use of aprotinin in USA is still restricted.

3.2.2 Phosphodiesterase inhibitors

Pentoxifylline is a nonspecific phosphodiesterase inhibitor with various anti-inflammatory effects, including attenuation of TNF-α and endotoxin release, cytokine synthesis, pulmonary leukocyte sequestration and vascular resistance, and reduction in indices of endothelial injury and permeability (285–289). Pentoxifylline was associated with decreased levels of pro-inflammatory cytokines (TNF-α and IL-6). Its use was also associated with improved left ventricular ejection fraction, decreased ICU length of stay, ventilation time, requirement of inotropic agents, and transfusion requirement (290).

Other phosphodiesterase inhibitors have been evaluated in the context of maximizing splanchnic perfusion as a strategy to attenuate excessive inflammation associated with CPB. Milrinone has been associated with reduction in venous and hepatic endotoxin levels, decrease in gastric intramucosal pH, and decreased IL-6 levels postoperatively in otherwise healthy patients undergoing cardiac operations (291, 292).

3.2.3 Corticosteroids

Steroids have potent anti-inflammatory effects, and the mechanisms by which they exert their effects are multifactorial. Preoperative administration of glucocorticoids has been shown to attenuate endotoxin release and complement activation in response to CPB (293–295). Methylprednisolone was associated with decreased levels of postoperative pro-inflammatory mediators IL-6, IL-8, and TNF-α along with increased levels of anti-inflammatory IL-10 and IL-1ra (120, 121, 275, 296–300). Corticosteroids blunted the activation of leukocytes, upregulation of neutrophil adhesion molecules, and sequestration of neutrophils in the pulmonary parenchyma and vasculature (275, 294, 301, 302). Combination treatment of patients with methylprednisolone and aprotinin resulted in improved postoperative indices of cardiovascular, pulmonary, hemostatic,

and renal function (303). Low-dose aprotinin had similar effect as methylprednisolone in blunting TNF-α release and neutrophil integrin CD11b upregulation (275). Another study showed methylprednisolone pretreatment was associated with improved cardiac performance and decreased bronchial inflammation post-CPB (275, 300). Short course of methylprednisolone has been shown to reduce incidence of postoperative atrial fibrillation (304). Low-dose methylprednisolone in pump priming solutions attenuated degree of myocardial damage (305). Preoperative plus pre-CPB administration may be superior to pre-CPB administration alone. Methylprednisolone prophylaxis was associated with lower levels of neuron-specific enolase, a biomarker for neuronal damage, suggesting it may be useful in reducing post-CPB cerebral injury (296). Benefits of corticosteroid use for reducing pulmonary inflammation, endotoxemia, and complemented activation are still disputed given the results of more recent RCTs, and differences in dosing regimen, formulation, and timing of administration may partially account for conflicting results. The SIRS trial showed no difference in risk of death or major morbidity between those randomized to receive methylprednisolone or placebo at 30 days postoperatively, while the most common adverse effects in both experimental arms were infectious or delirium-related (306). Overall, a clear benefit attributed to corticosteroid treatment in the setting of CPB remains to be demonstrated (293, 302, 306–310). The need for further studies investigating optimal dosage regimens, characterizing adverse events, and optimizing clinical outcomes cannot be overstated.

3.2.4 Antioxidants and free radical scavengers

Myocardial antioxidant enzymes (including glutathione reductase, superoxide dismutase, and catalase) become activated in proportion to the degree of myocardial ischemia and reperfusion injury, but host antioxidants may become depleted after CPB (311–313). When ROS production exceeds host defense scavenging capacity, cellular injury results (314, 315). Increased preoperative total plasma antioxidant status has been associated with decreased levels of lipid peroxidation, which is directly correlated with indices of myocardial injury (313).

Vitamin C and vitamin E levels decline intraoperatively and remain low over two days postoperatively (316). High-dose vitamin C is an effective scavenger of free radicals and has been associated with decreased membrane lipid peroxidation, indices of myocardial injury, improved hemodynamics, and shorter ICU and hospital length of stay (314, 317). High-dose vitamin E has been associated with decreased plasma hydrogen peroxide concentrations and decreased membrane lipid peroxidation after CPB (26, 314). Prophylactic coadministration of vitamin C, vitamin E, and n-PUFAs (eicosapentaenoic acid:docosahexaenoic acid ratio 1:2) has been associated with reduced incidence of postoperative atrial fibrillation (318).

Allopurinol inhibits xanthine oxidase, a pivotal generator of free radicals during reperfusion injury. Some studies have demonstrated allopurinol reduced myocardial formation of cytotoxic free radicals, decreased myocardial injury, and improved myocardial recovery following CPB (315, 319–322). Other studies showed conflicting results, showing no benefit in myocardial injury or function with allopurinol alone (319, 323, 324). Preoperative supplementation of allopurinol in combination with vitamin C and vitamin E reduced cardiovascular dysfunction in both stable and unstable patients undergoing CABG, with unstable patients sustained lesser degree of myocardial injury and lower incidence of perioperative myocardial infarction (325). Results of other studies, however, have refuted effects of vitamin C and vitamin E supplementation on myocardial injury (326). Use of allopurinol in neonates to improve neurodevelopment following cardiac operations for congenital heart disease is an active area of research as it has already demonstrated benefit in infants with hypoplastic left heart syndrome (327, 328).

Preoperative or intraoperative administration of high-dose N-acetylcysteine, another scavenger of free radicals has been shown to reduced neutrophil oxidative burst response and elastase activity (329–331). N-acetylcysteine improved oxygenation and lung mechanics in patients with known acute lung injury, though no change in rates of progression to acute respiratory distress syndrome was noted (332). Clinical outcomes were not significantly affected with regard to mortality, myocardial infarction, bleeding, transfusion requirements, intubation time, and hospital length of stay (333). Low-dose N-acetylcysteine as an adjunct to cardioplegia reduced myocardial oxidative stress in patients undergoing CABG (334). Modified N-acetylcysteine via preparation with activated carbons to create sustained-release microcapsules demonstrated greater cardioprotection than N-acetylcysteine alone in a rat model of myocardial ischemia-reperfusion (335). In another rat model of CPB, N-acetylcysteine was shown to ameliorate CPB-associated intestinal injury via reduction in inflammation and oxidative stress as measured by decreased levels of intestinal malondialdehyde, TNF-α, IL-6, and serum diamine oxidase (336). A number of studies have recently shown N-acetylcysteine to improve pulmonary, hepatic, and renal outcomes in patients undergoing CPB with and without preexisting pulmonary and renal insufficiency (337–342).

Mannitol pretreatment has been associated with decreased myocardial formation of cytotoxic free radicals following CPB (315).

Post-CPB endothelial dysfunction is in large part mediated by ROS. In this way, free-radical scavengers, antioxidants, and iron chelators represent a potential therapeutic adjunct to mitigate deleterious effects of CPB-associated inflammation.

3.2.5 Monoclonal antibodies, complement inhibition, and inhibition of endothelial cell activation

Another approach for decreasing contact activation and downstream inflammation may be utilizing endogenous soluble complement inhibitors. An RCT investigating a monoclonal antibody to human C5 demonstrated its efficacy and safety in the setting of CPB. Inhibition of synthesis of mediators in complement activation and formation of adhesion molecules in a dose-dependent fashion clinically translated to a reduction in coagulopathy, myocardial injury, and postoperative

neurocognitive deficits (343). Compstatin, a peptide inhibitor of complement, completely inhibited heparin-protamine-induced complement activation *in vivo* in non-human primates without associated adverse events (344).

Other promising strategies for complement inhibition therapy include C1 inhibitor, recombinant soluble inhibitor-1 or soluble complement receptor 1, monoclonal antibodies to C3 and C5a, neutrophil elastase inhibitor, membrane-bound regulators of complement, and attenuation of complement receptor-3-mediated adhesion of inflammatory cells to vascular endothelium (7). In unstable patients with acute myocardial infarction undergoing emergency CABG, administration of C1 esterase inhibitor effectively limited complement activation and reduced myocardial ischemia-reperfusion injury as measured by significant reduction in cardiac troponin I. Its use was associated with improved cardiac function and hemodynamic performance without an impact on early mortality (345, 346). Soluble human complement receptor 1 effectively inhibited complement activation during CPB and significantly decreased mortality and myocardial infarction in male patients (347). Neutrophil elastase inhibitor, sivelestat, reduced levels of neutrophil elastase, IL-6, and IL-8 while also attenuating the pattern of physiological deterioration of gas exchange as measured by relative effect on alveolar-arterial oxygen index (348).

C5 complement inhibitor, pexelizumab, may offer mortality benefit. Results of an RCT enrolling over 3,000 patients showed a significant risk reduction of death or postoperative myocardial infarction within 30 days postoperatively in patients undergoing CABG with or without valve surgery. However, the study was not powered to detection reduction in mortality alone (349). These results were reproduced in a more recent RCT. Additionally, an exploratory analysis showed a significant mortality benefit in high-risk patients (350).

Selective inhibition of vascular endothelial activation may reduce deleterious effects of uncontrolled inflammation. Adhesion molecular blockade may interfere with adherence within 24 h following CPB, thereby preventing neutrophil-mediated widespread organ damage. Blockade of neutrophil and endothelial selectin molecules resulted in notable attenuation of cerebral injury in an animal model of CPB and DHCA, while inhibition of neutrophil adhesion markedly decreased pulmonary injury in a swine model of CPB (351, 352). A caveat to adhesion molecule blockade is increased susceptibility to infection due to impaired neutrophil demargination and recruitment to sites of infection (353). Strategies to prevent nuclear localization of transcriptional activator NFκB, a key mediator of pro-inflammatory signaling, have also showed promise but studies demonstrating efficacy and safety are pending (151).

3.2.6 Cyclooxygenase inhibitors

Constitutive cyclooxygenase 1 (COX-1) and its inducible isoform, cyclooxygenase 2 (COX-2), are sensible targets for modulating immune activation in response to CPB. Antiplatelet agents, including inhibitors of COX-1 and COX-2, prevent platelet aggregation. Acetylsalicylic acid, or aspirin (ASA), is one of the most commonly prescribed medications for the prevention of cardiovascular diseases. It irreversibly acetylates a serine residue of COX-1, thereby preventing release of thromboxane A2 and its downstream effects on platelet aggregation. The beneficial effects of ASA are not confined to platelet aggregation, as other mechanisms including attenuating inflammation, reducing oxidative stress, inhibiting prostaglandin formation, and inhibition of thromboxane-mediated vasoconstriction may be modulated for clinical benefit. Continued ASA treatment until the time of CABG has been shown to reduce inflammation as demonstrated by lower levels of plasma high-sensitivity CRP at all time points, though no change in cytokines was observed (354). Aspirin and clopidogrel in combination with aprotinin did not significantly affect clinical outcomes (355).

4 Limitations and conclusion

Over the past several decades since modern extracorporeal circulation was first conceived of by Gibbon, strategies for controlling CPB-associated inflammation had some success but have fallen short of controlling SIRS. Surface modification of the extracorporeal circuit, technical advances including control of flow dynamics in the CPB circuit, and mechanical refinements in pumps, oxygenators, tubing, filters, and other material components of the CPB circuit have reduced adverse sequelae and shown clinical benefit. Initial studies completed several decades prior investigating strategies that reduced circulating interleukins and other pro-inflammatory mediators showed limited translational benefit or showed contradictory results; more recent studies investigating these strategies are few as new technologies and therapies have emerged. Appropriate application of adsorptive blood purification techniques or use of immunomodulatory pharmacologics to mitigate hyperinflammatory states following CPB still remains uncertain given inconclusive efficacy and safety results from several studies. Though initially promising, aprotinin has been associated with a significant adverse event profile in target populations reported in several large studies, leading to restricted use in several countries. Postoperative SIRS may delay diagnosis of sepsis and septic shock following cardiac surgery, particularly in high-risk patients. The Sequential Organ Failure Assessment (SOFA) score may be more sensitive for predicting physiologic effects of infection, while Sepsis-3 criteria may be a useful tool for early identification and management of sepsis in patients following cardiac surgery (356). Overall, improving biocompatibility of the CPB circuit and more minimally invasive techniques may lead to improved myocardial preservation. Investigations into pharmacological adjuncts to more specifically and effectively attenuate inflammation continue. A multimodal approach incorporating technical, circuit-specific, and pharmacologic strategies will likely yield maximal clinical benefit.

Author contributions

DB contributed to drafting, design, and revision of the manuscript. JF contributed to revision of the manuscript. FS contributed to revision of the manuscript. All authors contributed to the article and approved the submitted version.

Funding

This research was supported by NIH T32GM065085-18 (Trauma and Inflammation Research Training Grant, Division of Surgical Research at Brown University) to DB; RO1-HL46716 and RO1HL128831 to FS; 1RO1HL127072-01A1, 1RO1HL136347-01, and RO1HL136347-04S1 to JF.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

1. Warren OJ, Smith AJ, Alexiou C, Rogers PL, Jawad N, Vincent C, et al. The inflammatory response to cardiopulmonary bypass: part 1–mechanisms of pathogenesis. *J Cardiothorac Vasc Anesth*. (2009) 23(2):223–31. doi: 10.1053/j.jvca.2008.08.007

2. Kirklin JK, Westaby S, Blackstone EH, Kirklin JW, Chenoweth DE, Pacifico AD. Complement and the damaging effects of cardiopulmonary bypass. *J Thorac Cardiovasc Surg*. (1983) 86(6):845–57. doi: 10.1016/S0022-5223(19)39061-0

3. McBride WT, Armstrong MA, Crockard AD, McMurray TJ, Rea JM. Cytokine balance and immunosuppressive changes at cardiac surgery: contrasting response between patients and isolated CPB circuits. *Br J Anaesth*. (1995) 75(6):724–33. doi: 10.1093/bja/75.6.724

4. Gasz B, Lenard L, Racz B, Benko L, Borsiczky B, Cserepes B, et al. Effect of cardiopulmonary bypass on cytokine network and myocardial cytokine production. *Clin Cardiol*. (2006) 29(7):311–5. doi: 10.1002/clc.4960290708

5. Bone RC. Sir Isaac Newton, sepsis, SIRS, and CARS. *Crit Care Med*. (1996) 24 (7):1125–8. doi: 10.1097/00003246-199607000-00010

6. Larmann J, Theilmeier G. Inflammatory response to cardiac surgery: cardiopulomonary bypass versus non-cardiopulmonary bypass surgery. *Best Pract Res Clin Anaesthesiol*. (2004) 18(3):425–38. doi: 10.1016/j.bpa.2003.12.004

7. Raja SG, Dreyfus GD. Modulation of systemic inflammatory response after cardiac surgery. *Asian Cardiovasc Thorac Ann*. (2005) 13(4):382–95. doi: 10.1177/021849230501300422

8. Cate JW, van der Poll T, Levi M, ten Cate H, van Deventer SJ. Cytokines: triggers of clinical thrombotic disease. *Thromb Haemost*. (1997) 78(1):415–9. doi: 10.1055/s-0038-1657562

9. Hill GE, Robbins RA. Aprotinin but not tranexamic acid inhibits cytokine-induced inducible nitric oxide synthase expression. *Anesth Analg*. (1997) 84 (6):1198–202. doi: 10.1097/00000539-199706000-00005

10. Paparella D, Brister SJ, Buchanan MR. Coagulation disorders of cardiopulmonary bypass: a review. *Intensive Care Med*. (2004) 30(10):1873–81. doi: 10.1007/s00134-004-2388-0

11. Chung JH, Gikakis N, Rao AK, Drake TA, Colman RW, Edmunds LH Jr. Pericardial blood activates the extrinsic coagulation pathway during clinical cardiopulmonary bypass. *Circulation*. (1996) 93(11):2014–8. doi: 10.1161/01.cir.93.11.2014

12. Nemerson Y. Tissue factor and hemostasis. *Blood*. (1988) 71(1):1–8. Erratum in: Blood 1988 71(4):1178. doi: 10.1182/blood.V71.1.1.1

13. De Somer F, Van Belleghem Y, Caes F, François K, Van Overbeke H, Arnout J, et al. Tissue factor as the main activator of the coagulation system during cardiopulmonary bypass. *J Thorac Cardiovasc Surg*. (2002) 123(5):951–8. doi: 10.1067/mtc.2002.120334

14. Brister SJ, Ofosu FA, Heigenhauser GJ, Gianese F, Buchanan MR. Is heparin the ideal anticoagulant for cardiopulmonary bypass? Dermatan sulphate may be an alternate choice. *Thromb Haemost*. (1994) 71(4):468–73. doi: 10.1055/s-0038-1642462

15. Brister SJ, Ofosu FA, Buchanan MR. Thrombin generation during cardiac surgery: is heparin the ideal anticoagulant? *Thromb Haemost*. (1993) 70(2):259–62. doi: 10.1055/s-0038-1649561

16. Boisclair MD, Lane DA, Philippou H, Sheikh S, Hunt B. Thrombin production, inactivation and expression during open heart surgery measured by assays for activation fragments including a new ELISA for prothrombin fragment F1+2. *Thromb Haemost*. (1993) 70(2):253–8. doi: 10.1055/s-0038-1649480

17. Parolari A, Colli S, Mussoni L, Eligini S, Naliato M, Wang X, et al. Coagulation and fibrinolytic markers in a two-month follow-up of coronary bypass surgery. *J Thorac Cardiovasc Surg*. (2003) 125(2):336–43. doi: 10.1067/mtc.2003.2

18. Knudsen L, Hasenkam JM, Kure HH, Hughes P, Bellaiche L, Ahlburg P, et al. Monitoring thrombin generation with prothrombin fragment 1.2 assay during cardiopulmonary bypass surgery. *Thromb Res*. (1996) 84(1):45–54. doi: 10.1016/0049-3848(96)00160-0

19. Chenoweth DE, Cooper SW, Hugli TE, Stewart RW, Blackstone EH, Kirklin JW. Complement activation during cardiopulmonary bypass: evidence for generation of C3a and C5a anaphylatoxins. *N Engl J Med*. (1981) 304(9):497–503. doi: 10.1056/NEJM198102263040901

20. Ruddy S, Gigle I, Austen KF. The complement system of man (first of four parts). *N Engl J Med*. (1972) 287:489–94. doi: 10.1056/NEJM197209072871005

21. Matata BM, Sosnowski AW, Galiñanes M. Off-pump bypass graft operation significantly reduces oxidative stress and inflammation. *Ann Thorac Surg*. (2000) 69 (3):785–91. doi: 10.1016/s0003-4975(99)01420-4

22. Gu YJ, Mariani MA, van Oeveren W, Grandjean JG, Boonstra PW. Reduction of the inflammatory response in patients undergoing minimally invasive coronary artery bypass grafting. *Ann Thorac Surg*. (1998) 65(2):420–4. doi: 10.1016/s0003-4975(97)01127-2

23. Collett B, Alhaq A, Abdullah NB, Korjtsas L, Ware RJ, Dodd NJ, et al. Pathways to complement activation during cardiopulmonary bypass. *Br Med J (Clin Res Ed)*. (1984) 289(6454):1251–4. doi: 10.1136/bmj.289.6454.1251

24. Cavarocchi NC, Pluth JR, Schaff HV, Orszulak TA, Homburger HA, Solis E, et al. Complement activation during cardiopulmonary bypass. Comparison of bubble and membrane oxygenators. *J Thorac Cardiovasc Surg*. (1986) 91(2):252–8. doi: 10.1016/S0022-5223(19)36089-1

25. Jansen NJ, van Oeveren W, Gu YJ, van Vliet MH, Eijsman L, Wildevuur CR. Endotoxin release and tumor necrosis factor formation during cardiopulmonary bypass. *Ann Thorac Surg*. (1992) 54(4):744–7; discussion 747–8. doi: 10.1016/0003-4975(92)91021-z

26. Cavarocchi NC, England MD, O'Brien JF, Solis E, Russo P, Schaff HV, et al. Superoxide generation during cardiopulmonary bypass: is there a role for vitamin E? *J Surg Res*. (1986) 40(6):519–27. doi: 10.1016/0022-4804(86)90093-4

27. Moore FD Jr, Warner KG, Assousa S, Valeri CR, Khuri SF. The effects of complement activation during cardiopulmonary bypass. Attenuation by hypothermia, heparin, and hemodilution. *Ann Surg*. (1988) 208(1):95–103. doi: 10.1097/00000658-198807000-00014

28. Steinberg JB, Kapelanski DP, Olson JD, Weiler JM. Cytokine and complement levels in patients undergoing cardiopulmonary bypass. *J Thorac Cardiovasc Surg*. (1993) 106(6):1008–16. doi: 10.1016/S0022-5223(19)33971-6

29. Chenoweth DE, Hugli TE. Demonstration of specific C5a receptor on intact human polymorphonuclear leukocytes. *Proc Natl Acad Sci U S A*. (1978) 75 (8):3943–7. doi: 10.1073/pnas.75.8.3943

30. Ward PA, Cochrane CG, Mueller-Eberhard HJ. The role of Serum complement in chemotaxis of leukocytes in vitro. *J Exp Med*. (1965) 122(2):327–46. doi: 10.1084/jem.122.2.327

31. Craddock PR, Hammerschmidt D, White JG, Dalmosso AP, Jacob HS. Complement (C5-a)-induced granulocyte aggregation in vitro. A possible mechanism of complement-mediated leukostasis and leukopenia. *J Clin Invest*. (1977) 60(1):260–4. doi: 10.1172/JCI108763

32. Hammerschmidt DE, Harris PD, Wayland JH, Craddock PR, Jacob HS. Complement-induced granulocyte aggregation in vivo. *Am J Pathol*. (1981) 102 (2):146–50.

33. Tonnesen MG, Smedly LA, Henson PM. Neutrophil-endothelial cell interactions. Modulation of neutrophil adhesiveness induced by complement fragments C5a and C5a des arg and formyl-methionyl-leucyl-phenylalanine in vitro. *J Clin Invest*. (1984) 74(5):1581–92. doi: 10.1172/JCI111574

34. Nilsson L, Brunnkvist S, Nilsson U, Nyström SO, Tydén H, Venge P, et al. Activation of inflammatory systems during cardiopulmonary bypass. *Scand J Thorac Cardiovasc Surg*. (1988) 22(1):51–3. doi: 10.3109/14017438809106051

35. Jacob HS, Craddock PR, Hammerschmidt DE, Moldow CF. Complement-induced granulocyte aggregation: an unsuspected mechanism of disease. *N Engl J Med*. (1980) 302(14):789–94. doi: 10.1056/NEJM198004033021407

36. Howard RJ, Crain C, Franzini DA, Hood CI, Hugli TE. Effects of cardiopulmonary bypass on pulmonary leukostasis and complement activation. *Arch Surg*. (1988) 123(12):1496–501. doi: 10.1001/archsurg.1988.01400360066010

37. Tao W, Zwischenberger JB, Nguyen TT, Vertrees RA, McDaniel LB, Nutt LK, et al. Gut mucosal ischemia during normothermic cardiopulmonary bypass results from blood flow redistribution and increased oxygen demand. *J Thorac Cardiovasc Surg*. (1995) 110(3):819–28. doi: 10.1016/S0022-5223(95)70116-8

38. Baue AE. The role of the gut in the development of multiple organ dysfunction in cardiothoracic patients. *Ann Thorac Surg*. (1993) 55(4):822–9. doi: 10.1016/0003-4975(93)90098-3

39. Sinclair DG, Haslam PL, Quinlan GJ, Pepper JR, Evans TW. The effect of cardiopulmonary bypass on intestinal and pulmonary endothelial permeability. *Chest*. (1995) 108(3):718–24. doi: 10.1378/chest.108.3.718

40. Riddington DW, Venkatesh B, Boivin CM, Bonser RS, Elliott TS, Marshall T, et al. Intestinal permeability, gastric intramucosal pH, and systemic endotoxemia in patients undergoing cardiopulmonary bypass. *JAMA*. (1996) 275(13):1007–12. doi: 10.1001/jama.1996.03530370045029

41. Ohri SK, Bjarnason I, Pathi V, Somasundaram S, Bowles CT, Keogh BE, et al. Cardiopulmonary bypass impairs small intestinal transport and increases gut permeability. *Ann Thorac Surg*. (1993) 55(5):1080–6. doi: 10.1016/0003-4975(93)90011-6

42. Andersen LW, Landow L, Baek L, Jansen E, Baker S. Association between gastric intramucosal pH and splanchnic endotoxin, antibody to endotoxin, and tumor necrosis factor-alpha concentrations in patients undergoing cardiopulmonary bypass. *Crit Care Med*. (1993) 21(2):210–7. doi: 10.1097/00003246-199302000-00011

43. Jirik FR, Podor TJ, Hirano T, Kishimoto T, Loskutoff DJ, Carson DA, et al. Bacterial lipopolysaccharide and inflammatory mediators augment IL-6 secretion by human endothelial cells. *J Immunol*. (1989) 142(1):144–7. doi: 10.4049/jimmunol.142.1.144

44. Taggart DP, Sundaram S, McCartney C, Bowman A, McIntyre H, Courtney JM, et al. Endotoxemia, complement, and white blood cell activation in cardiac surgery: a randomized trial of laxatives and pulsatile perfusion. *Ann Thorac Surg*. (1994) 57 (2):376–82. doi: 10.1016/0003-4975(94)91000-6

45. Giroir BP. Mediators of septic shock: new approaches for interrupting the endogenous inflammatory cascade. *Crit Care Med*. (1993) 21(5):780–9. doi: 10.1097/00003246-199305000-00024

46. te Velthuis H, Jansen PG, Oudemans-van Straaten HM, Sturk A, Eijsman L, Wildevuur CR. Myocardial performance in elderly patients after cardiopulmonary bypass is suppressed by tumor necrosis factor. *J Thorac Cardiovasc Surg*. (1995) 110 (6):1663–9. doi: 10.1016/S0022-5223(95)70028-5

47. Andersen LW, Baek L, Degn H, Lehd J, Krasnik M, Rasmussen JP. Presence of circulating endotoxins during cardiac operations. *J Thorac Cardiovasc Surg*. (1987) 93 (1):115–9. doi: 10.1016/S0022-5223(19)36483-9

48. Rocke DA, Gaffin SL, Wells MT, Koen Y, Brock-Utine JG. Endotoxemia associated with cardiopulmonary bypass. *J Thorac Cardiovasc Surg*. (1987) 93 (6):832–7. doi: 10.1016/S0022-5223(19)37043-6

49. Kharazmi A, Andersen LW, Baek L, Valerius NH, Laub M, Rasmussen JP. Endotoxemia and enhanced generation of oxygen radicals by neutrophils from patients undergoing cardiopulmonary bypass. *J Thorac Cardiovasc Surg*. (1989) 98 (3):381–5. doi: 10.1016/S0022-5223(19)34384-3

50. Nilsson L, Kulander L, Nyström SO, Eriksson O. Endotoxins in cardiopulmonary bypass. *J Thorac Cardiovasc Surg*. (1990) 100(5):777–80. doi: 10.1016/S0022-5223(19)35477-7

51. Lequier LL, Nikaidoh H, Leonard SR, Bokovoy JL, White ML, Scannon PJ, et al. Preoperative and postoperative endotoxemia in children with congenital heart disease. *Chest*. (2000) 117(6):1706–12. doi: 10.1378/chest.117.6.1706

52. Cavarocchi NC, Schaff HV, Orszulak TA, Homburger HA, Schnell WA Jr, Pluth JR. Evidence for complement activation by protamine-heparin interaction after cardiopulmonary bypass. *Surgery*. (1985) 98(3):525–31.

53. Kirklin JK, Chenoweth DE, Naftel DC, Blackstone EH, Kirklin JW, Bitran DD, et al. Effects of protamine administration after cardiopulmonary bypass on complement, blood elements, and the hemodynamic state. *Ann Thorac Surg*. (1986) 41(2):193–9. doi: 10.1016/s0003-4975(10)62668-9

54. Zupancich E, Paparella D, Turani F, Munch C, Rossi A, Massaccesi S, et al. Mechanical ventilation affects inflammatory mediators in patients undergoing cardiopulmonary bypass for cardiac surgery: a randomized clinical trial. *J Thorac Cardiovasc Surg*. (2005) 130(2):378–83. doi: 10.1016/j.jtcvs.2004.11.061

55. Durukan AB, Gurbuz HA, Salman N, Unal EU, Ucar HI, Yorgancioglu CE. Ventilation during cardiopulmonary bypass did not attenuate inflammatory response or affect postoperative outcomes. *Cardiovasc J Afr*. (2013) 24(6):224–30. doi: 10.5830/CVJA-2013-041

56. Kankılıç N, Aydın MS, Göz M. The effect of low tidal volume ventilation on inflammatory cytokines during cardiopulmonary bypass. *Braz J Cardiovasc Surg*. (2022) 37(5):694–701. doi: 10.21470/1678-9741-2020-0466

57. Paparella D, Yau TM, Young E. Cardiopulmonary bypass induced inflammation: pathophysiology and treatment. An update. *Eur J Cardiothorac Surg*. (2002) 21:232–44. doi: 10.1016/S1010-7940(01)01099-5

58. Dekker RJ, van Soest S, Fontijn RD, Salamanca S, de Groot PG, VanBavel E, et al. Prolonged fluid shear stress induces a distinct set of endothelial cell genes, most specifically lung Krüppel-like factor (KLF2). *Blood*. (2002) 100(5):1689–98. doi: 10.1182/blood-2002-01-0046

59. Inoue N, Ramasamy S, Fukai T, Nerem RM, Harrison DG. Shear stress modulates expression of Cu/Zn superoxide dismutase in human aortic endothelial cells. *Circ Res*. (1996) 79(1):32–7. doi: 10.1161/01.res.79.1.32

60. Siepe M, Goebel U, Mecklenburg A, Doenst T, Benk C, Stein P, et al. Pulsatile pulmonary perfusion during cardiopulmonary bypass reduces the pulmonary inflammatory response. *Ann Thorac Surg*. (2008) 86(1):115–22. doi: 10.1016/j.athoracsur.2008.03.062

61. Orime Y, Shiono M, Hata H, Yagi S, Tsukamoto S, Okumura H, et al. Cytokine and endothelial damage in pulsatile and nonpulsatile cardiopulmonary bypass. *Artif Organs*. (1999) 23(6):508–12. doi: 10.1046/j.1525-1594.1999.06392.x

62. Sezai A, Shiono M, Nakata K, Hata M, Iida M, Saito A, et al. Effects of pulsatile CPB on interleukin-8 and endothelin-1 levels. *Artif Organs*. (2005) 29(9):708–13. doi: 10.1111/j.1525-1594.2005.29112.x

63. Onorati F, Santarpino G, Tangredi G, Palmieri G, Rubino AS, Foti D, et al. Intra-aortic balloon pump induced pulsatile perfusion reduces endothelial activation and inflammatory response following cardiopulmonary bypass. *Eur J Cardiothorac Surg*. (2009) 35(6):1012–9; discussion 1019. doi: 10.1016/j.ejcts.2008.12.037

64. Jordan JE, Zhao ZQ, Vinten-Johansen J. The role of neutrophils in myocardial ischemia-reperfusion injury. *Cardiovasc Res*. (1999) 43(4):860–78. doi: 10.1016/s0008-6363(99)00187-x

65. Rossi F. The O2- -forming NADPH oxidase of the phagocytes: nature, mechanisms of activation and function. *Biochim Biophys Acta*. (1986) 853(1):65–89. doi: 10.1016/0304-4173(86)90005-4

66. Kawahito K, Kobayashi E, Ohmori M, Harada K, Kitoh Y, Fujimura A, et al. Enhanced responsiveness of circulatory neutrophils after cardiopulmonary bypass: increased aggregability and superoxide producing capacity. *Artif Organs*. (2000) 24 (1):37–42. doi: 10.1046/j.1525-1594.2000.06381.x

67. Saugstad OD. Bronchopulmonary dysplasia-oxidative stress and antioxidants. *Semin Neonatol*. (2003) 8(1):39–49. doi: 10.1016/s1084-2756(02)00194-x

68. Nakamura H, Vaage J, Valen G, Padilla CA, Björnstedt M, Holmgren A. Measurements of plasma glutaredoxin and thioredoxin in healthy volunteers and during open-heart surgery. *Free Radic Biol Med*. (1998) 24(7-8):1176–86. doi: 10.1016/s0891-5849(97)00429-2

69. Zitta K, Meybohm P, Gruenewald M, Cremer J, Zacharowski KD, Scholz J, et al. Profiling of cell stress protein expression in cardiac tissue of cardiosurgical patients undergoing remote ischemic preconditioning: implications for thioredoxin in cardioprotection. *J Transl Med*. (2015) 13:34. doi: 10.1186/s12967-015-0403-6

70. Cherry AD. Mitochondrial dysfunction in cardiac surgery. *Anesthesiol Clin*. (2019) 37(4):769–85. doi: 10.1016/j.anclin.2019.08.003

71. Prasad K, Kalra J, Bharadwaj B, Chaudhary AK. Increased oxygen free radical activity in patients on cardiopulmonary bypass undergoing aortocoronary bypass surgery. *Am Heart J*. (1992) 123(1):37–45. doi: 10.1016/0002-8703(92)90744-g

72. Davies SW, Duffy JP, Wickens DG, Underwood SM, Hill A, Alladine MF, et al. Time-course of free radical activity during coronary artery operations with cardiopulmonary bypass. *J Thorac Cardiovasc Surg*. (1993) 105(6):979–87. doi: 10.1016/S0022-5223(19)33769-9

73. Toivonen HJ, Ahotupa M. Free radical reaction products and antioxidant capacity in arterial plasma during coronary artery bypass grafting. *J Thorac Cardiovasc Surg*. (1994) 108(1):140–7. doi: 10.1016/S0022-5223(94)70230-6

74. Pesonen EJ, Korpela R, Peltola K, Leijala M, Sairanen H, Raivio KO, et al. Regional generation of free oxygen radicals during cardiopulmonary bypass in children. *J Thorac Cardiovasc Surg*. (1995) 110(3):768–73. doi: 10.1016/S0022-5223(95)70110-9

75. Clermont G, Vergely C, Jazayeri S, Lahet JJ, Goudeau JJ, Lecour S, et al. Systemic free radical activation is a major event involved in myocardial oxidative stress related to cardiopulmonary bypass. *Anesthesiology*. (2002) 96(1):80–7. doi: 10.1097/00000542-200201000-00019

76. Seccombe JF, Pearson PJ, Schaff HV. Oxygen radical-mediated vascular injury selectively inhibits receptor-dependent release of nitric oxide from canine coronary arteries. *J Thorac Cardiovasc Surg*. (1994) 107(2):505–9. doi: 10.1016/S0022-5223(94)70096-6

77. Pearson PJ, Lin PJ, Schaff HV. Global myocardial ischemia and reperfusion impair endothelium-dependent relaxations to aggregating platelets in the canine coronary artery. A possible cause of vasospasm after cardiopulmonary bypass. *J Thorac Cardiovasc Surg*. (1992) 103(6):1147–54. doi: 10.1016/S0022-5223(19)34880-9

78. Matheis G, Sherman MP, Buckberg GD, Haybron DM, Young HH, Ignarro LJ. Role of L-arginine-nitric oxide pathway in myocardial reoxygenation injury. *Am J Physiol*. (1992) 262(2 Pt 2):H616–20. doi: 10.1152/ajpheart.1992.262.2.H616

79. Haniuda M, Dresler CM, Mizuta T, Cooper JD, Patterson GA. Free radical-mediated vascular injury in lungs preserved at moderate hypothermia. *Ann Thorac Surg*. (1995) 60(5):1376–81. doi: 10.1016/0003-4975(95)00620-Z

80. Menasché P, Peynet J, Larivière J, Tronc F, Piwnica A, Bloch G, et al. Does normothermia during cardiopulmonary bypass increase neutrophil-endothelium interactions? *Circulation*. (1994) 90(5 Pt 2):II275–9.

81. Menasché P, Peynet J, Haeffner-Cavaillon N, Carreno MP, de Chaumaray T, Dillisse V, et al. Influence of temperature on neutrophil trafficking during clinical cardiopulmonary bypass. *Circulation*. (1995) 92(9 Suppl):II334–40. doi: 10.1161/01.cir.92.9.334

82. Le Deist F, Menasché P, Kucharski C, Bel A, Piwnica A, Bloch G. Hypothermia during cardiopulmonary bypass delays but does not prevent neutrophil-endothelial cell adhesion. A clinical study. *Circulation*. (1995) 92(9 Suppl):II354–8. doi: 10.1161/01.cir.92.9.354

83. Soslau G, Morgan DA, Jaffe JS, Brodsky I, Wang Y. Cytokine mRNA expression in human platelets and a megakaryocytic cell line and cytokine modulation of platelet function. *Cytokine*. (1997) 9(6):405–11. doi: 10.1006/cyto.1996.0182

84. Krishnaswamy G, Smith JK, Mukkamala R, Hall K, Joyner W, Yerra L, et al. Multifunctional cytokine expression by human coronary endothelium and regulation by monokines and glucocorticoids. *Microvasc Res*. (1998) 55(3):189–200. doi: 10.1006/mvre.1998.2079

85. Ala Y, Palluy O, Favero J, Bonne C, Modat G, Dornand J. Hypoxia/reoxygenation stimulates endothelial cells to promote interleukin-1 and interleukin-6 production. Effects of free radical scavengers. *Agents Actions*. (1992) 37(1–2):134–9. doi: 10.1007/BF01987902

86. Sterpetti AV, Cucina A, Morena AR, Di Donna S, D'Angelo LS, Cavalarro A, et al. Shear stress increases the release of interleukin-1 and interleukin-6 by aortic endothelial cells. *Surgery*. (1993) 114(5):911–4.

87. Colotta F, Sironi M, Borrè A, Luini W, Maddalena F, Mantovani A. Interleukin 4 amplifies monocyte chemotactic protein and interleukin 6 production by endothelial cells. *Cytokine*. (1992) 4(1):24–8. doi: 10.1016/1043-4666(92)90032-m

88. Delneste Y, Lassalle P, Jeannin P, Joseph M, Tonnel AB, Gosset P. Histamine induces IL-6 production by human endothelial cells. *Clin Exp Immunol*. (1994) 98(2):344–9. doi: 10.1111/j.1365-2249.1994.tb06148.x

89. von Asmuth EJ, Leeuwenberg JF, Ceska M, Buurman WA. LPS and cytokine-induced endothelial cell IL-6 release and ELAM-1 expression; involvement of serum. *Eur Cytokine Netw*. (1991) 2(4):291–7.

90. Verma S, Nakaoke R, Dohgu S, Banks WA. Release of cytokines by brain endothelial cells: a polarized response to lipopolysaccharide. *Brain Behav Immun*. (2006) 20(5):449–55. doi: 10.1016/j.bbi.2005.10.005

91. Torre-Amione G, Kapadia S, Lee J, Bies RD, Lebovitz R, Mann DL. Expression and functional significance of tumor necrosis factor receptors in human myocardium. *Circulation*. (1995) 92(6):1487–93. doi: 10.1161/01.cir.92.6.1487

92. Yu Y, Gao M, Li H, Zhang F, Gu C. Pulmonary artery perfusion with anti-tumor necrosis factor alpha antibody reduces cardiopulmonary bypass-induced inflammatory lung injury in a rabbit model. *PLoS One*. (2013) 8(12):e83236. doi: 10.1371/journal.pone.0083236

93. Meldrum DR, Donnahoo KK. Role of TNF in mediating renal insufficiency following cardiac surgery: evidence of a postbypass cardiorenal syndrome. *J Surg Res*. (1999) 85(2):185–99. doi: 10.1006/jsre.1999.5660

94. Ohkawa F, Ikeda U, Kanbe T, Kawasaki K, Shimada K. Effects of inflammatory cytokines on vascular tone. *Cardiovasc Res*. (1995) 30(5):711–5. doi: 10.1016/S0008-6363(95)00101-8

95. Menasché P, Haydar S, Peynet J, Du Buit C, Merval R, Bloch G, et al. A potential mechanism of vasodilation after warm heart surgery. The temperature-dependent release of cytokines. *J Thorac Cardiovasc Surg*. (1994) 107(1):293–9. doi: 10.1016/S0022-5223(94)70484-8

96. Cain BS, Meldrum DR, Dinarello CA, Meng X, Joo KS, Banerjee A, et al. Tumor necrosis factor-alpha and interleukin-1beta synergistically depress human myocardial function. *Crit Care Med*. (1999) 27(7):1309–18. doi: 10.1097/00003246-199907000-00018

97. Boehm J, Hauner K, Grammer J, Dietrich W, Wagenpfeil S, Braun S, et al. Tumor necrosis factor-α −863 C/A promoter polymorphism affects the inflammatory response after cardiac surgery. *Eur J Cardiothorac Surg*. (2011) 40(1):e50–4. doi: 10.1016/j.ejcts.2011.01.084

98. Hansen PR, Svendsen JH, Høyer S, Kharazmi A, Bendtzen K, Haunsø S. Tumor necrosis factor-alpha increases myocardial microvascular transport in vivo. *Am J Physiol*. (1994) 266(1 Pt 2):H60–7. doi: 10.1152/ajpheart.1994.266.1.H60

99. Youker K, Smith CW, Anderson DC, Miller D, Michael LH, Rossen RD, et al. Neutrophil adherence to isolated adult cardiac myocytes. Induction by cardiac lymph collected during ischemia and reperfusion. *J Clin Invest*. (1992) 89(2):602–9. doi: 10.1172/JCI115626

100. Umegaki H, Yamada K, Naito M, Kameyama T, Iguchi A, Nabeshima T. Protective effect of interleukin-6 against the death of PC12 cells caused by serum deprivation or by the addition of a calcium ionophore. *Biochem Pharmacol*. (1996) 52(6):911–6. doi: 10.1016/0006-2952(96)00422-4

101. Schwarze MM, Hawley RG. Prevention of myeloma cell apoptosis by ectopic bcl-2 expression or interleukin 6-mediated up-regulation of bcl-xL. *Cancer Res*. (1995) 55(11):2262–5.

102. Finkel MS, Oddis CV, Jacob TD, Watkins SC, Hattler BG, Simmons RL. Negative inotropic effects of cytokines on the heart mediated by nitric oxide. *Science*. (1992) 257(5068):387–9. doi: 10.1126/science.1631560

103. Ungureanu-Longrois D, Balligand JL, Kelly RA, Smith TW. Myocardial contractile dysfunction in the systemic inflammatory response syndrome: role of a cytokine-inducible nitric oxide synthase in cardiac myocytes. *J Mol Cell Cardiol*. (1995) 27(1):155–67. doi: 10.1016/s0022-2828(08)80015-6

104. Dreyer WJ, Phillips SC, Lindsey ML, Jackson P, Bowles NE, Michael LH, et al. Interleukin 6 induction in the canine myocardium after cardiopulmonary bypass. *J Thorac Cardiovasc Surg*. (2000) 120(2):256–63. doi: 10.1067/mtc.2000.108168

105. Butler J, Chong GL, Baigrie RJ, Pillai R, Westaby S, Rocker GM. Cytokine responses to cardiopulmonary bypass with membrane and bubble oxygenation. *Ann Thorac Surg*. (1992) 53(5):833–8. doi: 10.1016/0003-4975(92)91446-g

106. Kawamura T, Wakusawa R, Okada K, Inada S. Elevation of cytokines during open heart surgery with cardiopulmonary bypass: participation of interleukin 8 and 6 in reperfusion injury. *Can J Anaesth*. (1993) 40(11):1016–21. doi: 10.1007/BF03009470

107. Frering B, Philip I, Dehoux M, Rolland C, Langlois JM, Desmonts JM. Circulating cytokines in patients undergoing normothermic cardiopulmonary bypass. *J Thorac Cardiovasc Surg*. (1994) 108(4):636–41. doi: 10.1016/S0022-5223(94)70287-X

108. Whitten CW, Hill GE, Ivy R, Greilich PE, Lipton JM. Does the duration of cardiopulmonary bypass or aortic cross-clamp, in the absence of blood and/or blood product administration, influence the IL-6 response to cardiac surgery? *Anesth Analg*. (1998) 86(1):28–33. doi: 10.1097/00000539-199801000-00006

109. Eggum R, Ueland T, Mollnes TE, Videm V, Aukrust P, Fiane AE, et al. Effect of perfusion temperature on the inflammatory response during pediatric cardiac surgery. *Ann Thorac Surg*. (2008) 85(2):611–7. doi: 10.1016/j.athoracsur.2007.10.062

110. Howell KW, Cleveland JC Jr, Meng X, Ao L, Su X, Schwartz RS, et al. Interleukin 6 production during cardiac surgery correlates with increasing age. *J Surg Res*. (2016) 201(1):76–81. doi: 10.1016/j.jss.2015.10.016

111. Oka Y, Murata A, Nishijima J, Yasuda T, Hiraoka N, Ohmachi Y, et al. Circulating interleukin 6 as a useful marker for predicting postoperative complications. *Cytokine*. (1992) 4(4):298–304. Erratum in: Cytokine 1993 5(1):89-90. doi: 10.1016/1043-4666(92)90070-8

112. Deng MC, Dasch B, Erren M, Möllhoff T, Scheld HH. Impact of left ventricular dysfunction on cytokines, hemodynamics, and outcome in bypass grafting. *Ann Thorac Surg*. (1996) 62(1):184–90. doi: 10.1016/0003-4975(96)00230-5

113. Hennein HA, Ebba H, Rodriguez JL, Merrick SH, Keith FM, Bronstein MH, et al. Relationship of the proinflammatory cytokines to myocardial ischemia and dysfunction after uncomplicated coronary revascularization. *J Thorac Cardiovasc Surg*. (1994) 108(4):626–35. doi: 10.1016/S0022-5223(94)70286-1

114. Preiser JC, Schmartz D, Van der Linden P, Content J, Vanden Bussche P, Buurman W, et al. Interleukin-6 administration has no acute hemodynamic or hematologic effect in the dog. *Cytokine*. (1991) 3(1):1–4. doi: 10.1016/1043-4666(91)90002-u

115. Smith WB, Gamble JR, Clark-Lewis I, Vadas MA. Interleukin-8 induces neutrophil transendothelial migration. *Immunology*. (1991) 72:65–72.

116. Metinko AP, Kunkel SL, Standiford TJ, Strieter RM. Anoxia-hyperoxia induces monocyte-derived interleukin-8. *J Clin Invest*. (1992) 90:791–8. doi: 10.1172/JCI115953

117. Wan S, Izzat MB, Lee TW, Wan IY, Tang NL, Yim AP. Avoiding cardiopulmonary bypass in multivessel CABG reduces cytokine response and

myocardial injury. *Ann Thorac Surg*. (1999) 68(1):52–6; discussion 56–7. doi: 10.1016/s0003-4975(99)00315-x

118. Finn A, Naik S, Klein N, Levinsky RJ, Strobel S, Elliott M. Interleukin-8 release and neutrophil degranulation after pediatric cardiopulmonary bypass. *J Thorac Cardiovasc Surg*. (1993) 105(2):234–41. doi: 10.1016/S0022-5223(19)33806-1

119. Burns SA, Newburger JW, Xiao M, Mayer JE Jr, Walsh AZ, Neufeld EJ. Induction of interleukin-8 messenger RNA in heart and skeletal muscle during pediatric cardiopulmonary bypass. *Circulation*. (1995) 92(9 Suppl):II315–21. doi: 10.1161/01.cir.92.9.315

120. Wan S, DeSmet JM, Barvais L, Goldstein M, Vincent JL, LeClerc JL. Myocardium is a major source of proinflammatory cytokines in patients undergoing cardiopulmonary bypass. *J Thorac Cardiovasc Surg*. (1996) 112 (3):806–11. doi: 10.1016/S0022-5223(96)70068-5

121. Wan S, DeSmet JM, Antoine M, Goldman M, Vincent JL, LeClerc JL. Steroid administration in heart and heart-lung transplantation: is the timing adequate? *Ann Thorac Surg*. (1996) 61(2):674–8. doi: 10.1016/0003-4975(95)01059-9

122. Kapadia S, Lee J, Torre-Amione G, Birdsall HH, Ma TS, Mann DL. Tumor necrosis factor-alpha gene and protein expression in adult feline myocardium after endotoxin administration. *J Clin Invest*. (1995) 96(2):1042–52. doi: 10.1172/JCI118090

123. Liangos O, Kolyada A, Tighiouart H, Perianayagam MC, Wald R, Jaber BL. Interleukin-8 and acute kidney injury following cardiopulmonary bypass: a prospective cohort study. *Nephron Clin Pract*. (2009) 113(3):c148–54. doi: 10.1159/000232595

124. Wang P, Wu P, Anthes JC, Siegel MI, Egan RW, Billah MM. Interleukin-10 inhibits interleukin-8 production in human neutrophils. *Blood*. (1994) 83:2678–83. doi: 10.1182/blood.V83.9.2678.2678

125. Fiorentino DF, Zlotnik A, Mosmann TR, Howard M, O'Garra A. IL-10 inhibits cytokine production by activated macrophages. *J Immunol*. (1991) 147:3815–22. doi: 10.4049/jimmunol.147.11.3815

126. Cassatella MA, Meda L, Gasperini S, Calzetti F, Bonora S. Interleukin-10 (IL-10) upregulates IL-1 receptor antagonist production from lipopolysaccharide-stimulated human polymorphonuclear leukocytes by delaying mRNA degradation. *J Exp Med*. (1994) 179:1695–9. doi: 10.1084/jem.179.5.1695

127. Thompson K, Wright S, Sheron N. Chemotactic and suppressor cytokine networks. *Hepatogastroenterology*. (1996) 43:15–31.

128. Ayala A, Deol DK, Lehman DL, Herdon CD, Chaudry IH. Polymicrobial sepsis but not low-dose endotoxin infusion causese decreased splenocyte IL-1/IFN-gamma release while increasing IL-4/IL-10 production. *J Surg Res*. (1994) 56:579–85. doi: 10.1006/jsre.1994.1092

129. Oz MC, Liao H, Naka Y, Seldomridge A, Becker DN, Michler RE, et al. Ischemia-induced interleukin-8 release after human heart transplantation: a potential role for endothelial cells. *Circulation*. (1995) 92(Suppl II):II-428–32. doi: 10.1161/01.cir.92.9.428

130. Drazan KE, Wu L, Olthoff KM, Jurim O, Busuttil RW, Shaked A. Transduction of hepatic allografts achieves local levels of viral IL-10 which suppress alloreactivity in vitro. *J Surg Res*. (1995) 59:219–23. doi: 10.1006/jsre.1995.1157

131. Le Moine O, Marchant A, Durand F, Ickx B, Pradier O, Belghiti J, et al. Systemic release of interleukin-10 during orthotopic liver transplantation. *Hepatology*. (1994) 20:889–92. doi: 10.1002/hep.1840200417

132. Tabardel Y, Duchateau J, Schmartz D, Marécaux G, Shahla M, Barvais L, et al. Corticosteroids increase blood interleukin-10 levels during cardiopulmonary bypass in men. *Surgery*. (1996) 119(1):76–80. doi: 10.1016/s0039-6060(96)80217-0

133. Dehoux M, Philip I, Chollet-Martin S, Boutten A, Hvass U, Desmots JM, et al. Early production of interleukin-10 during normothermic cardiopulmonary bypass. *J Thorac Cardiovasc Surg*. (1995) 110:286–7. doi: 10.1016/S0022-5223(05)80052-2

134. Marchant A, Bruyns C, Vandenabeele P, Ducarme M, Gérard C, Delvaux A, et al. Interleukin-10 controls interferon-gamma and tumor necrosis factor production during experimental endotoxemia. *Eur J Immunol*. (1994) 24:1167–71. doi: 10.1002/eji.1830240524

135. de Waal Malefyt R, Abrams J, Bennett B, Figdor CG, de Vries JE. Interleukin-10 inhibits cytokine synthesis by human monocytes: an autoregulatory role of IL-10 produced by monocytes. *J Exp Med*. (1991) 174:1209–20. doi: 10.1084/jem.174.5.1209

136. Brull DJ, Montgomery HE, Sanders J, Dhamrait S, Luong L, Rumley A, et al. Interleukin-6 gene −174g>c and −572g>c promoter polymorphisms are strong predictors of plasma interleukin-6 levels after coronary artery bypass surgery. *Arterioscler Thromb Vasc Biol*. (2001) 21(9):1458–63. doi: 10.1161/hq0901.094280

137. Gaudino M, Di Castelnuovo A, Zamparelli R, Andreotti F, Burzotta F, Iacoviello L, et al. Genetic control of postoperative systemic inflammatory reaction and pulmonary and renal complications after coronary artery surgery. *J Thorac Cardiovasc Surg*. (2003) 126(4):1107–12. doi: 10.1016/s0022-5223(03)00396-9

138. Grocott HP, White WD, Morris RW, Podgoreanu MV, Mathew JP, Nielsen DM, et al. Genetic polymorphisms and the risk of stroke after cardiac surgery. *Stroke*. (2005) 36(9):1854–8. doi: 10.1161/01.STR.0000177482.23478.dc

139. Podgoreanu MV, White WD, Morris RW, Mathew JP, Stafford-Smith M, Welsby IJ, et al. Inflammatory gene polymorphisms and risk of postoperative myocardial infarction after cardiac surgery. *Circulation*. (2006) 114(1 Suppl): I275–81. doi: 10.1161/CIRCULATIONAHA.105.001032

140. Burzotta F, Iacoviello L, Di Castelnuovo A, Glieca F, Luciani N, Zamparelli R, et al. Relation of the −174 G/C polymorphism of interleukin-6 to interleukin-6 plasma level s and to length of hospitalization after surgical coronary revascularization. *Am J Cardiol*. (2001) 88(10):1125–8. doi: 10.1016/s0002-9149(01)02046-x

141. Yoon SZ, Jang IJ, Choi YJ, Kang MH, Lim HJ, Lim YJ, et al. Association between tumor necrosis factor alpha 308G/A polymorphism and increased proinflammatory cytokine release after cardiac surgery with cardiopulmonary bypass in the Korean population. *J Cardiothorac Vasc Anesth*. (2009) 23(5):646–50. doi: 10.1053/j.jvca.2009.03.004

142. Isbir CS, Ergen A, Tekeli A, Zeybek U, Gormus U, Arsan S. The effect of NQO1 polymorphism on the inflammatory response in cardiopulmonary bypass. *Cell Biochem Funct*. (2008) 26(4):534–8. doi: 10.1002/cbf.1456

143. MacKensen GB, Swaminathan M, Ti LK, Grocott HP, Phillips-Bute BG, Mathew JP, et al. Preliminary report on the interaction of apolipoprotein E polymorphism with aortic atherosclerosis and acute nephropathy after CABG. *Ann Thorac Surg*. (2004) 78(2):520–6. doi: 10.1016/j.athoracsur.2004.02.106

144. Drabe N, Zünd G, Grünenfelder J, Sprenger M, Hoerstrup SP, Bestmann L, et al. Genetic predisposition in patients undergoing cardiopulmonary bypass surgery is associated with an increase of inflammatory cytokines. *Eur J Cardiothorac Surg*. (2001) 20(3):609–13. doi: 10.1016/s1010-7940(01)00842-9

145. Grünenfelder J, Umbehr M, Plass A, Bestmann L, Maly FE, Zünd G, et al. Genetic polymorphisms of apolipoprotein E4 and tumor necrosis factor beta as predisposing factors for increased inflammatory cytokines after cardiopulmonary bypass. *J Thorac Cardiovasc Surg*. (2004) 128(1):92–7. doi: 10.1016/j.jtcvs.2004.02.022

146. Patrick DA, Moore EE, Fullerton DA, Barnett CC Jr, Meldrum DR, Silliman CC. Cardiopulmonary bypass renders patients at risk for multiple organ failure via early neutrophil priming and late neutrophil disability. *J Surg Res*. (1999) 86 (1):42–9. doi: 10.1006/jsre.1999.5702

147. Elliott MJ, Finn AH. Interaction between neutrophils and endothelium. *Ann Thorac Surg*. (1993) 56(6):1503–8. doi: 10.1016/0003-4975(93)90741-y

148. Foreman KE, Vaporciyan AA, Bonish BK, Jones ML, Johnson KJ, Glovsky MM, et al. C5a-induced expression of P-selectin in endothelial cells. *J Clin Invest*. (1994) 94 (3):1147–55. doi: 10.1172/JCI117430

149. Boyle EM Jr, Pohlman TH, Johnson MC, Verrier ED. Endothelial cell injury in cardiovascular surgery: the systemic inflammatory response. *Ann Thorac Surg*. (1997) 63(1):277–84. doi: 10.1016/s0003-4975(96)01061-2

150. Gilliland HE, Armstrong MA, Uprichard S, Clarke G, McMurray TJ. The effect of aprotinin on interleukin-8 concentration and leukocyte adhesion molecule expression in an isolated cardiopulmonary bypass system. *Anaesthesia*. (1999) 54 (5):427–33. doi: 10.1046/j.1365-2044.1999.00656.x

151. Tönz M, Mihaljevic T, von Segesser LK, Fehr J, Schmid ER, Turina MI. Acute lung injury during cardiopulmonary bypass. Are the neutrophils responsible? *Chest*. (1995) 108(6):1551–6. doi: 10.1378/chest.108.6.1551

152. Boyle EM Jr, Morgan EN, Kovacich JC, Canty TG Jr, Verrier ED. Microvascular responses to cardiopulmonary bypass. *J Cardiothorac Vasc Anesth*. (1999) 13(4 Suppl 1):30–5; discussion 36–7. doi: 10.1016/S1053-0770(21)00595-4

153. Shimono T, Yada I, Kanamori Y, Sato T, Kondo C, Tanaka K, et al. Reticuloendothelial function following cardiopulmonary bypass: laboratory and electron microscopy findings. *J Surg Res*. (1994) 56(5):446–51. doi: 10.1006/jsre.1994.1070

154. Shimono T, Yada I, Kusagawa M, Nosé Y. Oversaturation status of reticuloendothelial system following cardiopulmonary bypass. *Artif Organs*. (1994) 18(8):596–602. doi: 10.1111/j.1525-1594.1994.tb03384.x

155. Ratliff NB, Young WG Jr, Hackel DB, Mikat E, Wilson JW. Pulmonary injury secondary to extracorporeal circulation. An ultrastructural study. *J Thorac Cardiovasc Surg*. (1973) 65(3):425–32. doi: 10.1016/S0022-5223(19)40771-X

156. Royston D, Fleming JS, Desai JB, Westaby S, Taylor KM. Increased production of peroxidation products associated with cardiac operations. Evidence for free radical generation. *J Thorac Cardiovasc Surg*. (1986) 91(5):759–66. doi: 10.1016/S0022-5223(19)35998-7

157. Wilson I, Gillinov AM, Curtis WE, DiNatale J, Burch RM, Gardner TJ, et al. Inhibition of neutrophil adherence improves postischemic ventricular performance of the neonatal heart. *Circulation*. (1993) 88(5 Pt 2):II372–9.

158. Mayers I, Hurst T, Johnson D, Cujec B, Ang LC, Thomson D, et al. Anti-CD18 antibodies improve cardiac function following cardiopulmonary bypass in dogs. *J Crit Care*. (1996) 11(4):189–96. doi: 10.1016/s0883-9441(96)90030-1

159. Friedman M, Wang SY, Sellke FW, Cohn WE, Weintraub RM, Johnson RG. Neutrophil adhesion blockade with NPC 15669 decreases pulmonary injury after total cardiopulmonary bypass. *J Thorac Cardiovasc Surg*. (1996) 111(2):460–8. doi: 10.1016/s0022-5223(96)70457-9

160. Roth M, Kraus B, Scheffold T, Reuthebuch O, Klövekorn WP, Bauer EP. The effect of leukocyte-depleted blood cardioplegia in patients with severe left ventricular dysfunction: a randomized, double-blind study. *J Thorac Cardiovasc Surg*. (2000) 120(4):642–50. doi: 10.1067/mtc.2000.109707

161. Hill GE, Whitten CW, Landers DF. The influence of cardiopulmonary bypass on cytokines and cell-cell communication. *J Cardiothorac Vasc Anesth*. (1997) 11(3):367–75. doi: 10.1016/s1053-0770(97)90107-5

162. Cicala C, Cirino G. Linkage between inflammation and coagulation: an update on the molecular basis of the crosstalk. *Life Sci*. (1998) 62(20):1817–24. doi: 10.1016/s0024-3205(97)01167-3

163. Mulivor AW, Lipowsky HH. Role of glycocalyx in leukocyte-endothelial cell adhesion. *Am J Physiol Heart Circ Physiol*. (2002) 283(4):H1282–91. doi: 10.1152/ajpheart.00117.2002

164. Rehm M, Bruegger D, Christ F, Conzen P, Thiel M, Jacob M, et al. Shedding of the endothelial glycocalyx in patients undergoing major vascular surgery with global and regional ischemia. *Circulation*. (2007) 116(17):1896–906. doi: 10.1161/CIRCULATIONAHA.106.684852

165. Myers GJ, Wegner J. Endothelial glycocalyx and cardiopulmonary bypass. *J Extra Corpor Technol*. (2017) 49(3):174–81. doi: 10.1051/ject/201749174

166. Yao Y, Rabodzey A, Dewey CF Jr. Glycocalyx modulates the motility and proliferative response of vascular endothelium to fluid shear stress. *Am J Physiol Heart Circ Physiol*. (2007) 293(2):H1023–30. doi: 10.1152/ajpheart.00162.2007

167. Becker BF, Jacob M, Leipert S, Salmon AH, Chappell D. Degradation of the endothelial glycocalyx in clinical settings: searching for the sheddases. *Br J Clin Pharmacol*. (2015) 80(3):389–402. doi: 10.1111/bcp.12629

168. Madjid M, Willerson JT. Inflammatory markers in coronary heart disease. *Br Med Bull*. (2011) 100:23–38. doi: 10.1093/bmb/ldr043

169. Endo K, Takino T, Miyamori H, Kinsen H, Yoshizaki T, Furukawa M, et al. Cleavage of syndecan-1 by membrane type matrix metalloproteinase-1 stimulates cell migration. *J Biol Chem*. (2003) 278:40764–70. doi: 10.1074/jbc.M306736200

170. Robich M, Ryzhov S, Kacer D, Palmeri M, Peterson SM, Quinn RD, et al. Prolonged cardiopulmonary bypass is associated with endothelial glycocalyx degradation. *J Surg Res*. (2020) 251:287–95. doi: 10.1016/j.jss.2020.02.011

171. Zhang Y, Wang Z, Liu J, Zhang S, Fei J, Li J, et al. Cell surface-anchored syndecan-1 ameliorates intestinal inflammation and neutrophil transmigration in ulcerative colitis. *J Cell Mol Med*. (2017) 21:13–25. doi: 10.1111/jcmm.12934

172. Gill SE, Nadler ST, Li Q, Frevert CW, Park PW, Chen P, et al. Shedding of syndecan-1/CXCL1 complexes by matrix metalloproteinase 7 functions as an epithelial checkpoint of neutrophil activation. *Am J Respir Cell Mol Biol*. (2016) 55:243–51. doi: 10.1165/rcmb.2015-0193OC

173. Mosheimer BA, Kaneider NC, Feistritzer C, Djanani AM, Sturn DH, Patsch JR, et al. Syndecan-1 is involved in osteoprotegerin-induced chemotaxis in human peripheral blood monocytes. *J Clin Endocrinol Metab*. (2005) 90:2964–71. doi: 10.1210/jc.2004-1895

174. Pesonen E, Passov A, Andersson S, Suojaranta R, Niemi T, Raivio P, et al. Glycocalyx degradation and inflammation in cardiac surgery. *J Cardiothorac Vasc Anesth*. (2019) 33(2):341–5. doi: 10.1053/j.jvca.2018.04.007

175. de Melo Bezerra Cavalcante CT, Castelo Branco KM, Pinto Júnior VC, Meneses GC, de Oliveira Neves FM, de Souza NM, et al. Syndecan-1 improves severe acute kidney injury prediction after pediatric cardiac surgery. *J Thorac Cardiovasc Surg*. (2016) 152(1):178–186.e2. doi: 10.1016/j.jtcvs.2016.03.079

176. 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. *J Cardiothorac Vasc Anesth*. (2009) 23(3):384–93. doi: 10.1053/j.jvca.2008.09.007

177. Landis RC, Brown JR, Fitzgerald D, Likosky DS, Shore-Lesserson L, Baker RA, et al. Attenuating the systemic inflammatory response to adult cardiopulmonary bypass: a critical review of the evidence base. *J Extra Corpor Technol*. (2014) 46(3):197–211. doi: 10.1051/ject/201446197

178. Videm V, Mollnes TE, Bergh K, Fosse E, Mohr B, Hagve TA, et al. Heparin-coated cardiopulmonary bypass equipment. II mechanism for reduced complement activation in vivo. *J Thorac Cardiovasc Surg*. (1999) 117(4):803–9. doi: 10.1016/S0022-5223(99)70302-8

179. Goudeau JJ, Clermont G, Guillery O, Lemaire-Ewing S, Musat A, Vernet M, et al. In high-risk patients, combination of antiinflammatory procedures during cardiopulmonary bypass can reduce incidences of inflammation and oxidative stress. *J Cardiovasc Pharmacol*. (2007) 49(1):39–45. doi: 10.1097/FJC.0b013e31802c0cd0

180. Gu YJ, van Oeveren W, Akkerman C, Boonstra PW, Huyzen RJ, Wildevuur CR. Heparin-coated circuits reduce the inflammatory response to cardiopulmonary bypass. *Ann Thorac Surg*. (1993) 55(4):917–22. doi: 10.1016/0003-4975(93)90117-z

181. Videm V, Mollnes TE, Fosse E, Mohr B, Bergh K, Hagve TA, et al. Heparin-coated cardiopulmonary bypass equipment. I biocompatability markers and development of complications in high-risk population. *J Thorac Cardiovasc Surg*. (1999) 117(4):794–802. doi: 10.1016/s0022-5223(99)70301-6

182. Wan S, LeClerc JL, Antoine M, DeSmet JM, Yim AP, Vincent JL. Heparin-coated circuits reduce myocardial injury in heart or heart-lung transplantation: a prospective, randomized trial. *Ann Thorac Surg*. (1999) 68(4):1230–5. doi: 10.1016/s0003-4975(99)00701-8

183. te Velthuis H, Baufreton C, Jansen PG, Thijs CM, Hack CE, Sturk A, et al. Heparin coating of extracorporeal circuits inhibits contact activation during cardiac operations. *J Thorac Cardiovasc Surg*. (1997) 114(1):117–22. doi: 10.1016/S0022-5223(97)70124-7

184. Moen O, Høgåsen K, Fosse E, Dregelid E, Brockmeier V, Venge P, et al. Attenuation of changes in leukocyte surface markers and complement activation with heparin-coated cardiopulmonary bypass. *Ann Thorac Surg*. (1997) 63(1):105–11. doi: 10.1016/s0003-4975(96)00743-6

185. Plötz FB, van Oeveren W, Hultquist KA, Miller C, Bartlett RH, Wildevuur CR. A heparin-coated circuit reduces complement activation and the release of leukocyte inflammatory mediators during extracorporeal circulation in a rabbit. *Artif Organs*. (1992) 16(4):366–70. doi: 10.1111/j.1525-1594.1992.tb00533.x

186. Weerwind PW, Maessen JG, van Tits LJ, Stad RK, Fransen EJ, de Jong DS, et al. Influence of Duraflo II heparin-treated extracorporeal circuits on the systemic inflammatory response in patients having coronary bypass. *J Thorac Cardiovasc Surg*. (1995) 110(6):1633–41. doi: 10.1016/S0022-5223(95)70024-2

187. Steinberg BM, Grossi EA, Schwartz DS, McLoughlin DE, Aguinaga M, Bizekis C, et al. Heparin bonding of bypass circuits reduces cytokine release during cardiopulmonary bypass. *Ann Thorac Surg*. (1995) 60(3):525–9. doi: 10.1016/0003-4975(95)00482-z

188. Lindholm L, Westerberg M, Bengtsson A, Ekroth R, Jensen E, Jeppsson A. A closed perfusion system with heparin coating and centrifugal pump improves cardiopulmonary bypass biocompatibility in elderly patients. *Ann Thorac Surg*. (2004) 78(6):2131–8. doi: 10.1016/j.athoracsur.2004.06.011

189. McCarthy PM, Yared JP, Foster RC, Ogella DA, Borsh JA, Cosgrove DM. A prospective randomized trial of Duraflo II heparin-coated circuits in cardiac reoperations. *Ann Thorac Surg*. (1999) 67(5):1268–73. doi: 10.1016/s0003-4975(99)00136-8

190. Mirow N, Zittermann A, Koertke H, Maleszka A, Knobl H, Coskun T, et al. Heparin-coated extracorporeal circulation in combination with low dose systemic heparinization reduces early postoperative blood loss in cardiac surgery. *J Cardiovasc Surg (Torino)*. (2008) 49(2):277–84.

191. Jansen PG, te Velthuis H, Huybregts RA, Paulus R, Bulder ER, van der Spoel HI, et al. Reduced complement activation and improved postoperative performance after cardiopulmonary bypass with heparin-coated circuits. *J Thorac Cardiovasc Surg*. (1995) 110(3):829–34. doi: 10.1016/S0022-5223(95)70117-6

192. Ranucci M, Mazzucco A, Pessotto R, Grillone G, Casati V, Porreca L, et al. Heparin-coated circuits for high risk patients: a multicenter, prospective, randomized trial. *Ann Thorac Surg*. (1999) 67(4):994–1000. doi: 10.1016/s0003-4975(99)00062-4

193. Mangoush O, Purkayastha S, Haj-Yahia S, Kinross J, Hayward M, Bartolozzi F, et al. Heparin-bonded circuits versus nonheparin-bonded circuits: an evaluation of their effect on clinical outcomes. *Eur J Cardiothorac Surg*. (2007) 31(6):1058–69. doi: 10.1016/j.ejcts.2007.01.029

194. Svenmarker S, Häggmark S, Jansson E, Lindholm R, Appelblad M, Sandström E, et al. Use of heparin-bonded circuits in cardiopulmonary bypass improves clinical outcome. *Scand Cardiovasc J*. (2002) 36(4):241–6. doi: 10.1080/14017430260180418

195. Thiara AS, Andersen VY, Videm V, Mollnes TE, Svennevig K, Hoel TN, et al. Comparable biocompatibility of phisio- and bioline-coated cardiopulmonary bypass circuits indicated by the inflammatory response. *Perfusion*. (2010) 25(1):9–16. doi: 10.1177/0267659110362822

196. Sohn N, Marcoux J, Mycyk T, Krahn J, Meng Q. The impact of different biocompatible coated cardiopulmonary bypass circuits on inflammatory response and oxidative stress. *Perfusion*. (2009) 24(4):231–7. doi: 10.1177/0267659109351218

197. Dekker NAM, Veerhoek D, van Leeuwen ALI, Vonk ABA, van den Brom CE, Boer C. Microvascular alterations during cardiac surgery using a heparin or phosphorylcholine-coated circuit. *J Cardiothorac Vasc Anesth*. (2020) 34(4):912–9. doi: 10.1053/j.jvca.2019.10.012

198. Chen L, Lv L, Long C, Lou S. Effects of circuit albumin coating on coagulation and inflammatory response for patients receiving aortic arch replacement: a randomized controlled trial. *Perfusion*. (2016) 31(7):576–83. doi: 10.1177/0267659116645662

199. Gunaydin S, McCusker K, Sari T, Onur MA, Zorlutuna Y. Clinical performance and biocompatibility of hyaluronan-based heparin-bonded extracorporeal circuits in different risk cohorts. *Interact CardioVasc Thorac Surg*. (2010) 10(3):371–6. doi: 10.1510/icvts.2009.220756

200. Hasegawa T, Oshima Y, Yokoyama S, Akimoto A, Misaka Y, Akiyama S. Clinical application of a new ternary polymer, SEC-1 coat^{TM}, for pediatric cardiopulmonary bypass circuits: a prospective randomized pilot study. *Perfusion*. (2020) 35(8):826–32. doi: 10.1177/0267659120915387

201. Giorni C, Pezzella C, Bojan M, Ricci Z, Pouard P, Raisky O, et al. Impact of heparin- or nonheparin-coated circuits on platelet function in pediatric cardiac surgery. *Ann Thorac Surg*. (2019) 107(4):1241–7. doi: 10.1016/j.athoracsur.2018.09.032

202. Tayama E, Hayashida N, Akasu K, Kosuga T, Fukunaga S, Akashi H, et al. Biocompatibility of heparin-coated extracorporeal bypass circuits: new heparin

bonded bioline system. *Artif Organs*. (2000) 24(8):618–23. doi: 10.1046/j.1525-1594.2000.06615.x

203. Fosse E, Thelin S, Svennevig JL, Jansen P, Mollnes TE, Hack E, et al. Duraflo II coating of cardiopulmonary bypass circuits reduces complement activastion but does not affect the release of granulocyte enzymes: a European multicenter study. *Eur J Cardiothorac Surg*. (1997) 11(2):320–7. doi: 10.1016/s1010-7940(96)01062-7

204. Journois D, Israel-Biet D, Pouard P, Rolland B, Silvester W, Vouhé P, et al. High-volume, zero-balanced hemofiltration to reduce delayed inflammatory response to cardiopulmonary bypass in children. *Anesthesiology*. (1996) 85(5):965–76. doi: 10.1097/00000542-199611000-00003

205. Huang H, Yao T, Wang W, Zhu D, Zhang W, Chen H, et al. Combination of balanced ultrafiltration with modified ultrafiltration attenuates pulmonary injury in patients undergoing open heart surgery. *Chin Med J (Engl)*. (2003) 116(10):1504–7.

206. Journois D, Pouard P, Greeley WJ, Mauriat P, Vouhé P, Safran D. Hemofiltration during cardiopulmonary bypass in pediatric cardiac surgery. Effects on hemostasis, cytokines, and complement components. *Anesthesiology*. (1994) 81(5):1181–9; discussion 26A–27A. doi: 10.1097/00000542-199411000-00011

207. Bando K, Vijay P, Turrentine MW, Sharp TG, Means LJ, Ensing GJ, Lalone BJ, Sekine Y, Szekely L, Brown JW. Dilutional and modified ultrafiltration reduces pulmonary hypertension after operations for congenital heart disease: a prospective randomized study. *J Thorac Cardiovasc Surg*. (1998) 115(3):517–25; discussion 525–7. doi: 10.1016/S0022-5223(98)70313-7

208. 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 Anestesiol*. (2019) 85(7):715–23. doi: 10.23736/S0375-9393.18.12898-7

209. Boodhwani M, Williams K, Babaev A, Gill G, Saleem N, Rubens FD. Ultrafiltration reduces blood transfusions following cardiac surgery: a meta-analysis. *Eur J Cardiothorac Surg*. (2006) 30(6):892–7. doi: 10.1016/j.ejcts.2006.09.014

210. Hiramatsu T, Imai Y, Kurosawa H, Takanashi Y, Aoki M, Shinoka T, et al. Effects of dilutional and modified ultrafiltration in plasma endothelin-1 and pulmonary vascular resistance after the Fontan procedure. *Ann Thorac Surg*. (2002) 73(3):861–5.

211. Kosour C, Dragosavac D, Antunes N, Almeida de Oliveira RA, Martins Oliveira PP, Wilson Vieira R. Effect of ultrafiltration on pulmonary function and interleukins in patients undergoing cardiopulmonary bypass. *J Cardiothorac Vasc Anesth*. (2016) 30(4):884–90. doi: 10.1053/j.jvca.2015.10.009

212. Naik SK, Knight A, Elliott MJ. A successful modification of ultrafiltration for cardiopulmonary bypass in children. *Perfusion*. (1991) 6(1):41–50. doi: 10.1177/026765919100600106

213. Davies MJ, Nguyen K, Gaynor JW, Elliott MJ. Modified ultrafiltration improves left ventricular systolic function in infants after cardiopulmonary bypass. *J Thorac Cardiovasc Surg*. (1998) 115(2):361–9; discussion 369–70. doi: 10.1016/S0022-5223(98)70280-6

214. Bierer J, Stanzel R, Henderson M, Sett S, Horne D. Ultrafiltration in pediatric cardiac surgery review. *World J Pediatr Congenit Heart Surg*. (2019) 10(6):778–88. doi: 10.1177/2150135119870176

215. Watanabe T, Sakai Y, Mayumi T, Shimomura T, Song MH, Tajima K, et al. Effect of ultrafiltration during cardiopulmonary bypass for pediatric cardiac surgery. *Artif Organs*. (1998) 22(12):1052–5. doi: 10.1046/j.1525-1594.1998.06192.x

216. Torina AG, Petrucci O, Oliveira PP, Severino ES, Vilarinho KA, Lavagnoli CF, et al. The effects of modified ultrafiltration on pulmonary function and transfusion requirements in patients underwent coronary artery bypass graft surgery. *Rev Bras Cir Cardiovasc*. (2010) 25(1):59–65. doi: 10.1590/s0102-76382010000100014

217. Luciani GB, Menon T, Vecchi B, Auriemma S, Mazzucco A. Modified ultrafiltration reduces morbidity after adult cardiac operations: a prospective, randomized clinical trial. *Circulation*. (2001) 104(12 Suppl 1):I253–9. doi: 10.1161/hc37t1.094931

218. Apostolakis E, Filos KS, Koletsis E, Dougenis D. Lung dysfunction following cardiopulmonary bypass. *J Card Surg*. (2010) 25(1):47–55. doi: 10.1111/j.1540-8191.2009.00823.x

219. Onorati F, Santini F, Mariscalco G, Bertolini P, Sala A, Faggian G, et al. Leukocyte filtration ameliorates the inflammatory response in patients with mild to moderate lung dysfunction. *Ann Thorac Surg*. (2011) 92(1):111–21; discussion 121. doi: 10.1016/j.athoracsur.2011.03.087

220. Rubino AS, Serraino GF, Mariscalco G, Marsico R, Sala A, Renzulli A. Leukocyte depletion during extracorporeal circulation allows better organ protection but does not change hospital outcomes. *Ann Thorac Surg*. (2011) 91(2):534–40. doi: 10.1016/j.athoracsur.2010.09.077

221. Zhang X, Zhou C, Zhuang J, Xiao X, Zheng S, Xiong W, et al. Effects of leukocyte depletion on cardiopulmonary protection and inflammation after valve surgery. *Int J Artif Organs*. (2010) 33(11):812–8. PMID: 21140357.

222. Koskenkari J, Rimpiläinen J, Biancari F, Surcel HM, Kaukoranta P, Kiviluoma K, et al. Leukocyte depleting filter attenuates myocardial injury during elective coronary artery bypass surgery. *Scand Cardiovasc J*. (2005) 39(6):358–68. doi: 10.1080/14017430510035943

223. Alexiou C, Tang AT, Sheppard SV, Haw MP, Gibbs R, Smith DC. A prospective randomized study to evaluate the effect of leukodepletion on the rate of alveolar production of exhaled nitric oxide during cardiopulmonary bypass. *Ann Thorac Surg*. (2004) 78(6):2139–45; discussion 2145. doi: 10.1016/j.athoracsur.2004.05.087

224. Alexiou C, Tang AA, Sheppard SV, Smith DC, Gibbs R, Livesey SA, et al. The effect of leucodepletion on leucocyte activation, pulmonary inflammation and respiratory index in surgery for coronary revascularization: a prospective randomized study. *Eur J Cardiothorac Surg*. (2004) 26(2):294–300. doi: 10.1016/j.ejcts.2004.04.017

225. Chen YF, Tsai WC, Lin CC, Tsai LY, Lee CS, Huang CH, Pan PC, Chen ML. Effect of leukocyte depletion on endothelial cell activation and transendothelial migration of leukocytes during cardiopulmonary bypass. *Ann Thorac Surg*. (2004) 78(2):634–42; discussion 642–3. doi: 10.1016/j.athoracsur.2004.02.091

226. de Amorim CG, Malbouisson LM, da Silva FC Jr, Fiorelli AI, Murakami CK, Carmona MJ. Leukocyte depletion during CPB: effects on inflammation and lung function. *Inflammation*. (2014) 37(1):196–204. doi: 10.1007/s10753-013-9730-z

227. Karaiskos TE, Palatianos GM, Triantafillou CD, Kantidakis GH, Astras GM, Papadakis EG, et al. Clinical effectiveness of leukocyte filtration during cardiopulmonary bypass in patients with chronic obstructive pulmonary disease. *Ann Thorac Surg*. (2004) 78(4):1339–44. doi: 10.1016/j.athoracsur.2004.04.040

228. Sheppard SV, Gibbs RV, Smith DC. Does leucocyte depletion during cardiopulmonary bypass improve oxygenation indices in patients with mild lung dysfunction? *Br J Anaesth*. (2004) 93(6):789–92. doi: 10.1093/bja/aeh267

229. Hurst T, Johnson D, Cujec B, Thomson D, Mycyk T, Burbridge B, et al. Depletion of activated neutrophils by a filter during cardiac valve surgery. *Can J Anaesth*. (1997) 44(2):131–9. doi: 10.1007/BF03013000

230. De Vecchi E, Paroni R, Pala MG, Di Credico G, Agape V, Gobbi C, et al. Role of leucocytes in free radical production during myocardial revascularization. *Heart*. (1997) 77(5):449–55. doi: 10.1136/hrt.77.5.449

231. Sawa Y, Taniguchi K, Kadoba K, Nishimura M, Ichikawa H, Amemiya A, et al. Leukocyte depletion attenuates reperfusion injury in patients with left ventricular hypertrophy. *Circulation*. (1996) 93(9):1640–6. doi: 10.1161/01.cir.93.9.1640

232. Hayashi Y, Sawa Y, Fukuyama N, Miyamoto Y, Takahashi T, Nakazawa H, et al. Leukocyte-depleted terminal blood cardioplegia provides superior myocardial protective effects in association with myocardium-derived nitric oxide and peroxynitrite production for patients undergoing prolonged aortic crossclamping for more than 120 min. *J Thorac Cardiovasc Surg*. (2003) 126(6):1813–21. doi: 10.1016/s0022-5223(03)01282-0

233. van de Watering LM, Hermans J, Houbiers JG, van den Broek PJ, Bouter H, Boer F, Harvey MS, Huysmans HA, Brand A. Beneficial effects of leukocyte depletion of transfused blood on postoperative complications in patients undergoing cardiac surgery: a randomized clinical trial. *Circulation*. (1998) 97(6):562–8. doi: 10.1161/01.cir.97.6.562

234. Soo AW, Maher BM, Daly L, Wood AE, Watson WR. Preoperative neutrophil response as a predictive marker of clinical outcome following open heart surgery and the impact of leukocyte filtration. *Interact Cardiovasc Thorac Surg*. (2010) 11(5):604–11. doi: 10.1510/icvts.2009.228056

235. Tang AT, Alexiou C, Hsu J, Sheppard SV, Haw MP, Ohri SK. Leukodepletion reduces renal injury in coronary revascularization: a prospective randomized study. *Ann Thorac Surg*. (2002) 74(2):372–7; discussion 377. doi: 10.1016/s0003-4975(02)03715-3.

236. Yang B, Hosgood SA, Harper SJ, Nicholson ML. Leucocyte depletion improves renal function in porcine kidney hemoreperfusion through reduction of myeloperoxidase+ cells, caspase-3, IL-1β, and tubular apoptosis. *J Surg Res*. (2010) 164(2):e315–24. doi: 10.1016/j.jss.2010.07.044

237. Bernardi MH, Rinoesl H, Dragosits K, Ristl R, Hoffelner F, Opfermann P, et al. Effect of hemoadsorption during cardiopulmonary bypass surgery—a blinded, randomized, controlled pilot study using a novel adsorbent. *Crit Care*. (2016) 20:96. doi: 10.1186/s13054-016-1270-0

238. Poli EC, Alberio L, Bauer-Doerries A, Marcucci C, Roumy A, Kirsch M, et al. Cytokine clearance with CytoSorb® during cardiac surgery: a pilot randomized controlled trial. *Crit Care*. (2019) 23(1):108. doi: 10.1186/s13054-019-2399-4

239. Gleason TG, Argenziano M, Bavaria JE, Kane LC, Coselli JS, Engelman RM, et al. Hemoadsorption to reduce plasma-free hemoglobin during cardiac surgery: results of REFRESH I pilot study. *Semin Thorac Cardiovasc Surg*. (2019) 31(4):783–93. doi: 10.1053/j.semtcvs.2019.05.006

240. Taleska Stupica G, Sostaric M, Bozhinovska M, Rupert L, Bosnic Z, Jerin A, et al. Extracorporeal hemadsorption *versus* glucocorticoids during cardiopulmonary bypass: a prospective, randomized, controlled trial. *Cardiovasc Ther*. (2020) 2020:7834173. doi: 10.1155/2020/7834173

241. Bourbon A, Vionnet M, Leprince P, Vaissier E, Copeland J, McDonagh P, et al. The effect of methylprednisolone treatment on the cardiopulmonary bypass-induced systemic inflammatory response. *Eur J Cardiothorac Surg*. (2004) 26(5):932–8. doi: 10.1016/j.ejcts.2004.07.044

242. Doukas P, Hellfritsch G, Wendt D, Magliani M, Barbati ME, Jalaie H, et al. Intraoperative hemoadsorption (cytosorb™) during open thoracoabdominal aortic

repair: a pilot randomized controlled trial. *J Clin Med*. (2023) 12(2):546. doi: 10.3390/jcm12020546

243. Boldt J, Osmer C, Linke LC, Görlach G, Hempelmann G. Hypothermic versus normothermic cardiopulmonary bypass: influence on circulating adhesion molecules. *J Cardiothorac Vasc Anesth*. (1996) 10(3):342–7. doi: 10.1016/s1053-0770(96)80094-2

244. Birdi I, Caputo M, Underwood M, Bryan AJ, Angelini GD. The effects of cardiopulmonary bypass temperature on inflammatory response following cardiopulmonary bypass. *Eur J Cardiothorac Surg*. (1999) 16(5):540–5. doi: 10.1016/s1010-7940(99)00301-2

245. Ohata T, Sawa Y, Kadoba K, Kagisaki K, Suzuki K, Matsuda H. Role of nitric oxide in a temperature dependent regulation of systemic vascular resistance in cardiopulmonary bypass. *Eur J Cardiothorac Surg*. (2000) 18(3):342–7. doi: 10.1016/s1010-7940(00)00455-3

246. Lomivorotov VV, Shmirev VA, Efremov SM, Ponomarev DN, Moroz GB, Shahin DG, et al. Hypothermic versus normothermic cardiopulmonary bypass in patients with valvular heart disease. *J Cardiothorac Vasc Anesth*. (2014) 28(2):295–300. doi: 10.1053/j.jvca.2013.03.009

247. Oda T, Yamaguchi A, Yokoyama M, Shimizu K, Toyota K, Nikai T, et al. Plasma proteomic changes during hypothermic and normothermic cardiopulmonary bypass in aortic surgeries. *Int J Mol Med*. (2014) 34(4):947–56. doi: 10.3892/ijmm.2014.1855

248. Yuksel V, Canbaz S, Ege T. Comparison between normothermic and mild hypothermic cardiopulmonary bypass in myocardial revascularization of patients with left ventricular dysfunction. *Perfusion*. (2013) 28(5):419–23. doi: 10.1177/0267659113483798

249. Akeho K, Nakata H, Suehiro S, Shimizu K, Imai K, Yamaguchi A, et al. Hypothermic effects on gas exchange performance of membrane oxygenator and blood coagulation during cardiopulmonary bypass in pigs. *Perfusion*. (2020) 35(7):687–96. doi: 10.1177/0267659120901413

250. Caputo M, Pike K, Baos S, Sheehan K, Selway K, Ellis L, et al. Normothermic versus hypothermic cardiopulmonary bypass in low-risk paediatric heart surgery: a randomised controlled trial. *Heart*. (2019) 105(6):455–64. doi: 10.1136/heartjnl-2018-313567

251. Hannon CE, Osman Z, Grant C, Chung EML, Corno AF. Part II. Comparison of neurodevelopmental outcomes between normothermic and hypothermic pediatric cardiopulmonary bypass. *Front Pediatr*. (2019) 7:447. doi: 10.3389/fped.2019.00447

252. Yildirim F, Amanvermez Senarslan D, Yersel S, Bayram B, Taneli F, Tetik O. Systemic inflammatory response during cardiopulmonary bypass: axial flow versus radial flow oxygenators. *Int J Artif Organs*. (2022) 45(3):278–83. doi: 10.1177/03913988221075043

253. Caputo M, Mokhtari A, Miceli A, Ghorbel MT, Angelini GD, Parry AJ, Suleiman SM. Controlled reoxygenation during cardiopulmonary bypass decreases markers of organ damage, inflammation, and oxidative stress in single-ventricle patients undergoing pediatric heart surgery. *J Thorac Cardiovasc Surg*. (2014) 148(3):792–801.e8; discussion 800–1. doi: 10.1016/j.jtcvs.2014.06.001

254. Paparella D, Rotunno C, Guida P, Travascia M, De Palo M, Paradiso A, et al. Minimally invasive heart valve surgery: influence on coagulation and inflammatory response. *Interact Cardiovasc Thorac Surg*. (2017) 25(2):225–32. doi: 10.1093/icvts/ivx090

255. Farag M, Patil NP, Sabashnikov A, Arif R, Szabó G, Kallenbach K, et al. Comparison of two miniaturized cardiopulmonary bypass systems regarding inflammatory response. *Artif Organs*. (2017) 41(2):139–45. doi: 10.1111/aor.12750

256. Gygax E, Kaeser HU, Stalder M, Gahl B, Rieben R, Carrel T, et al. Type II minimal-invasive extracorporeal circuit for aortic valve replacement: a randomized controlled trial. *Artif Organs*. (2018) 42(6):620–9. doi: 10.1111/aor.13093

257. Beghi C, Nicolini F, Agostinelli A, Borrello B, Budillon AM, Bacciottini F, et al. Mini-cardiopulmonary bypass system: results of a prospective randomized study. *Ann Thorac Surg*. (2006) 81(4):1396–400. doi: 10.1016/j.athoracsur.2005.10.015

258. Starinieri P, Declercq PE, Robic B, Yilmaz A, Van Tornout M, Dubois J, Mees U, Hendrikx M. A comparison between minimized extracorporeal circuits and conventional extracorporeal circuits in patients undergoing aortic valve surgery: is "minimally invasive extracorporeal circulation" just low prime or closed loop perfusion? *Perfusion*. (2017) 32(5):403–8. doi: 10.1177/0267659117691814

259. Pasrija C, Shah A, Sultanik E, Rouse M, Ghoreishi M, Bittle GJ, et al. Minimally invasive surgical pulmonary embolectomy: a potential alternative to conventional sternotomy. *Innovations (Phila)*. (2017) 12(6):406–10. doi: 10.1097/IMI.0000000000000439

260. Jiang Q, Wang Z, Guo J, Yu T, Zhang X, Hu S. Retrospective comparison of endoscopic versus open procedure for mitral valve disease. *J Invest Surg*. (2021) 34(9):1000–6. doi: 10.1080/08941939.2020.1726531

261. Ahangar AG, Charag AH, Wani ML, Ganie FA, Singh S, Ahmad Qadri SA, et al. Comparing aortic valve replacement through right anterolateral thoracotomy with median sternotomy. *Int Cardiovasc Res J*. (2013) 7(3):90–4.

262. Calderon J, Richebe P, Guibaud JP, Coiffic A, Branchard O, Asselineau J, et al. Prospective randomized study of early pulmonary evaluation of patients scheduled for aortic valve surgery performed by ministernotomy or total median sternotomy. *J Cardiothorac Vasc Anesth*. (2009) 23(6):795–801. doi: 10.1053/j.jvca.2009.03.011

263. Dogan S, Dzemali O, Wimmer-Greinecker G, Derra P, Doss M, Khan MF, et al. Minimally invasive versus conventional aortic valve replacement: a prospective randomized trial. *J Heart Valve Dis*. (2003) 12(1):76–80.

264. Moustafa MA, Abdelsamad AA, Zakaria G, Omarah MM. Minimal vs median sternotomy for aortic valve replacement. *Asian Cardiovasc Thorac Ann*. (2007) 15(6):472–5. doi: 10.1177/021849230701500605

265. Jongman RM, Zijlstra JG, Kok WF, van Harten AE, Mariani MA, Moser J, et al. Off-pump CABG surgery reduces systemic inflammation compared with on-pump surgery but does not change systemic endothelial responses: a prospective randomized study. *Shock*. (2014) 42(2):121–8. doi: 10.1097/SHK.0000000000000190

266. Lehmann S, Dieterlen MT, Flister A, Klaeske K, Jawad K, Garbade J, et al. Differences of early immunological responses in on-pump versus off-pump cardiac surgery. *Perfusion*. (2019) 34(5):399–407. doi: 10.1177/0267659118823137

267. Vallely MP, Bannon PG, Kritharides L. The systemic inflammatory response syndrome and off-pump cardiac surgery. *Heart Surg Forum*. (2001) 4(Suppl 1):S7–13.

268. Khan TA, Bianchi C, Araujo E, Voisine P, Xu SH, Feng J, et al. Aprotinin preserves cellular junctions and reduces myocardial edema after regional ischemia and cardioplegic arrest. *Circulation*. (2005) 112(9 Suppl):I196–201. doi: 10.1161/CIRCULATIONAHA.104.526053

269. Lisman T, Adelmeijer J, Huskens D, Meijers JCM. Aprotinin inhibits thrombin generation by inhibition of the intrinsic pathway, but is not a direct thrombin inhibitor. *TH Open*. (2021) 5(3):e363–75. doi: 10.1055/s-0041-1735154

270. Levi M, Cromheecke ME, de Jonge E, Prins MH, de Mol BJ, Briët E, et al. Pharmacological strategies to decrease excessive blood loss in cardiac surgery: a meta-analysis of clinically relevant endpoints. *Lancet*. (1999) 354(9194):1940–7. doi: 10.1016/S0140-6736(99)01264-7

271. Mössinger H, Dietrich W, Braun SL, Jochum M, Meisner H, Richter JA. High-dose aprotinin reduces activation of hemostasis, allogeneic blood requirement, and duration of postoperative ventilation in pediatric cardiac surgery. *Ann Thorac Surg*. (2003) 75(2):430–7. doi: 10.1016/s0003-4975(02)04412-0

272. Ray MJ, Marsh NA. Aprotinin reduces blood loss after cardiopulmonary bypass by direct inhibition of plasmin. *Thromb Haemost*. (1997) 78(3):1021–6. doi: 10.1055/s-0038-1657680

273. Wachtfogel YT, Kucich U, Hack CE, Gluszko P, Niewiarowski S, Colman RW, et al. Aprotinin inhibits the contact, neutrophil, and platelet activation systems during simulated extracorporeal perfusion. *J Thorac Cardiovasc Surg*. (1993) 106(1):1–9; discussion 9–10. doi: 10.1016/S0022-5223(19)33735-3

274. Mojcik CF, Levy JH. Aprotinin and the systemic inflammatory response after cardiopulmonary bypass. *Ann Thorac Surg*. (2001) 71(2):745–54. doi: 10.1016/s0003-4975(00)02218-9

275. Hill GE, Snider S, Galbraith TA, Forst S, Robbins RA. Glucocorticoid reduction of bronchial epithelial inflammation during cardiopulmonary bypass. *Am J Respir Crit Care Med*. (1995) 152(6 Pt 1):1791–5. doi: 10.1164/ajrccm.152.6.8520738

276. Harig F, Feyrer R, Mahmoud FO, Blum U, von der Emde J. Reducing the post-pump syndrome by using heparin-coated circuits, steroids, or aprotinin. *Thorac Cardiovasc Surg*. (1999) 47(2):111–8. doi: 10.1055/s-2007-1013121

277. Soeparwata R, Hartman AR, Frerichmann U, Stefano GB, Scheld HH, Bilfinger TV. Aprotinin diminishes inflammatory processes. *Int J Cardiol*. (1996) 53 Suppl:S55–63. doi: 10.1016/0167-5273(96)02573-9

278. Alonso A, Whitten CW, Hill GE. Pump prime only aprotinin inhibits cardiopulmonary bypass-induced neutrophil CD11b up-regulation. *Ann Thorac Surg*. (1999) 67(2):392–5. doi: 10.1016/s0003-4975(98)01132-1

279. Wendel HP, Heller W, Michel J, Mayer G, Ochsenfahrt C, Graeter U, et al. Lower cardiac troponin T levels in patients undergoing cardiopulmonary bypass and receiving high-dose aprotinin therapy indicate reduction of perioperative myocardial damage. *J Thorac Cardiovasc Surg*. (1995) 109(6):1164–72. doi: 10.1016/S0022-5223(95)70200-8

280. Gott JP, Cooper WA, Schmidt FE Jr, Brown WM 3rd, Wright CE, Merlino JD, Fortenberry JD, Clark WS, Guyton RA. Modifying risk for extracorporeal circulation: trial of four antiinflammatory strategies. *Ann Thorac Surg*. (1998) 66(3):747–53; discussion 753–4. doi: 10.1016/s0003-4975(98)00695-x

281. Toikkanen V, Rinne T, Nieminen R, Moilanen E, Laurikka J, Porkkala H, et al. Aprotinin impacts 8-isoprostane after coronary artery bypass grafting. *Scand J Surg*. (2018) 107(4):329–35. doi: 10.1177/1457496918766720

282. Lemmer JH Jr, Dilling EW, Morton JR, Rich JB, Robicsek F, Bricker DL, Hantler CB, Copeland JG 3rd, Ochsner JL, Daily PO, Whitten CW, Noon GP, Maddi R. Aprotinin for primary coronary artery bypass grafting: a multicenter trial of three dose regimens. *Ann Thorac Surg*. (1996) 62(6):1659–67; discussion 1667–8. doi: 10.1016/s0003-4975(96)00451-1

283. Mangano DT, Tudor IC, Dietzel C, Multicenter Study of Perioperative Ischemia Research Group, Ischemia Research and Education Foundation. The risk associated with aprotinin in cardiac surgery. *N Engl J Med*. (2006) 354(4):353–65. doi: 10.1056/NEJMoa051379

284. Fergusson DA, Hébert PC, Mazer CD, Fremes S, MacAdams C, Murkin JM, et al. A comparison of aprotinin and lysine analogues in high-risk cardiac surgery. *N Engl J Med*. (2008) 358(22):2319–31. Erratum in: N Engl J Med. 2010 363(13):1290. doi: 10.1056/NEJMoa0802395

285. Doherty GM, Jensen JC, Alexander HR, Buresh CM, Norton JA. Pentoxifylline suppression of tumor necrosis factor gene transcription. *Surgery*. (1991) 110(2):192–8.

286. Schade UF. Pentoxifylline increases survival in murine endotoxin shock and decreases formation of tumor necrosis factor. *Circ Shock*. (1990) 31(2):171–81.

287. Sullivan GW, Carper HT, Novick WJ Jr, Mandell GL. Inhibition of the inflammatory action of interleukin-1 and tumor necrosis factor (alpha) on neutrophil function by pentoxifylline. *Infect Immun*. (1988) 56(7):1722–9. doi: 10.1128/iai.56.7.1722-1729.1988

288. Tsang GM, Allen S, Pagano D, Wong C, Graham TR, Bonser RS. Pentoxifylline preloading reduces endothelial injury and permeability in cardiopulmonary bypass. *ASAIO J*. (1996) 42(5):M429–34. doi: 10.1097/00002480-199609000-00025

289. Türköz R, Yörükoğlu K, Akcay A, Yilik L, Baltalarli A, Karahan N, et al. The effect of pentoxifylline on the lung during cardiopulmonary bypass. *Eur J Cardiothorac Surg*. (1996) 10(5):339–46. doi: 10.1016/s1010-7940(96)80092-3

290. Mansourian S, Bina P, Fehri A, Karimi AA, Boroumand MA, Abbasi K. Preoperative oral pentoxifylline in case of coronary artery bypass grafting with left ventricular dysfunction (ejection fraction equal to/less than 30%). *Anatol J Cardiol*. (2015) 15(12):1014–9. doi: 10.5152/akd.2014.5883

291. Möllhoff T, Loick HM, Van Aken H, Schmidt C, Rolf N, Tjan TD, et al. Milrinone modulates endotoxemia, systemic inflammation, and subsequent acute phase response after cardiopulmonary bypass (CPB). *Anesthesiology*. (1999) 90 (1):72–80. doi: 10.1097/00000542-199901000-00012

292. McNicol L, Andersen LW, Liu G, Doolan L, Baek L. Markers of splanchnic perfusion and intestinal translocation of endotoxins during cardiopulmonary bypass: effects of dopamine and milrinone. *J Cardiothorac Vasc Anesth*. (1999) 13 (3):292–8. doi: 10.1016/s1053-0770(99)90266-5

293. Andersen LW, Baek L, Thomsen BS, Rasmussen JP. Effect of methylprednisolone on endotoxemia and complement activation during cardiac surgery. *J Cardiothorac Anesth*. (1989) 3(5):544–9. doi: 10.1016/0888-6296(89)90150-6

294. Dernek S, Tünerir B, Sevin B, Aslan R, Uyguç O, Kural T. The effects of methylprednisolone on complement, immunoglobulins and pulmonary neutrophil sequestration during cardiopulmonary bypass. *Cardiovasc Surg*. (1999) 7(4):414–8. doi: 10.1016/s0967-2109(98)00153-7

295. Wan S, LeClerc JL, Huynh CH, Schmartz D, DeSmet JM, Yim AP, et al. Does steroid pretreatment increase endotoxin release during clinical cardiopulmonary bypass? *J Thorac Cardiovasc Surg*. (1999) 117(5):1004–8. doi: 10.1016/S0022-5223(99)70382-X

296. Demir T, Demir H, Tansel T, Kalko Y, Tireli E, Dayioglu E, et al. Influence of methylprednisolone on levels of neuron-specific enolase in cardiac surgery: a corticosteroid derivative to decrease possible neuronal damage. *J Card Surg*. (2009) 24(4):397–403. doi: 10.1111/j.1540-8191.2009.00842.x

297. Liakopoulos OJ, Schmitto JD, Kazmaier S, Bräuer A, Quintel M, Schoendube FA, Dörge H. Cardiopulmonary and systemic effects of methylprednisolone in patients undergoing cardiac surgery. *Ann Thorac Surg*. 2007 84(1):110–8; discussion 118–9. doi: 10.1016/j.athoracsur.2007.01.003

298. Yared JP, Bakri MH, Erzurum SC, Moravec CS, Laskowski DM, Van Wagoner DR, et al. Effect of dexamethasone on atrial fibrillation after cardiac surgery: prospective, randomized, double-blind, placebo-controlled trial. *J Cardiothorac Vasc Anesth*. (2007) 21(1):68–75. doi: 10.1053/j.jvca.2005.10.014

299. Sobieski MA 2nd, Graham JD, Pappas PS, Tatooles AJ, Slaughter MS. Reducing the effects of the systemic inflammatory response to cardiopulmonary bypass: can single dose steroids blunt systemic inflammatory response syndrome? *ASAIO J*. (2008) 54(2):203–6. doi: 10.1097/MAT.0b013e3181640331

300. Kawamura T, Inada K, Okada H, Okada K, Wakusawa R. Methylprednisolone inhibits increase of interleukin 8 and 6 during open heart surgery. *Can J Anaesth*. (1995) 42(5 Pt 1):399–403. doi: 10.1007/BF03015485

301. Amr YM, Elmistekawy E, El-Serogy H. Effects of dexamethasone on pulmonary and renal functions in patients undergoing CABG with cardiopulmonary bypass. *Semin Cardiothorac Vasc Anesth*. (2009) 13(4):231–7. doi: 10.1177/1089253209351598

302. Jansen NJ, van Oeveren W, van Vliet M, Stoutenbeek CP, Eysman L, Wildevuur CR. The role of different types of corticosteroids on the inflammatory mediators in cardiopulmonary bypass. *Eur J Cardiothorac Surg*. (1991) 5(4):211–7. doi: 10.1016/1010-7940(91)90032-f

303. Tassani P, Richter JA, Barankay A, Braun SL, Haehnel C, Spaeth P, et al. Does high-dose methylprednisolone in aprotinin-treated patients attenuate the systemic inflammatory response during coronary artery bypass grafting procedures? *J Cardiothorac Vasc Anesth*. (1999) 13(2):165–72. doi: 10.1016/s1053-0770(99)90081-2

304. Halonen J, Halonen P, Järvinen O, Taskinen P, Auvinen T, Tarkka M, et al. Corticosteroids for the prevention of atrial fibrillation after cardiac surgery: a randomized controlled trial. *JAMA*. (2007) 297(14):1562–7. doi: 10.1001/jama.297.14.1562

305. Yilmaz M, Ener S, Akalin H, Sagdic K, Serdar OA, Cengiz M. Effect of low-dose methyl prednisolone on serum cytokine levels following extracorporeal circulation. *Perfusion*. (1999) 14(3):201–6. doi: 10.1177/026765919901400308

306. Whitlock RP, Devereaux PJ, Teoh KH, Lamy A, Vincent J, Pogue J, et al. Methylprednisolone in patients undergoing cardiopulmonary bypass (SIRS): a randomised, double-blind, placebo-controlled trial. *Lancet*. (2015) 386 (10000):1243–53. doi: 10.1016/S0140-6736(15)00273-1

307. Dieleman JM, van Paassen J, van Dijk D, Arbous MS, Kalkman CJ, Vandenbroucke JP, et al. Prophylactic corticosteroids for cardiopulmonary bypass in adults. *Cochrane Database Syst Rev*. (2011) 5:CD005566. doi: 10.1002/14651858.CD005566.pub3

308. Jorens PG, De Jongh R, De Backer W, Van Damme J, Van Overveld F, Bossaert L, et al. Interleukin-8 production in patients undergoing cardiopulmonary bypass. The influence of pretreatment with methylprednisolone. *Am Rev Respir Dis*. (1993) 148(4 Pt 1):890–5. doi: 10.1164/ajrccm/148.4_Pt_1.890

309. van Overveld FJ, De Jongh RF, Jorens PG, Walter P, Bossaert L, De Backer WA. Pretreatment with methylprednisolone in coronary artery bypass grafting influences the levels of histamine and tryptase in serum but not in bronchoalveolar lavage fluid. *Clin Sci (Lond)*. (1994) 86(1):49–53. doi: 10.1042/cs0860049

310. Lodge AJ, Chai PJ, Daggett CW, Ungerleider RM, Jaggers J. Methylprednisolone reduces the inflammatory response to cardiopulmonary bypass in neonatal piglets: timing of dose is important. *J Thorac Cardiovasc Surg*. (1999) 117(3):515–22. doi: 10.1016/s0022-5223(99)70331-4

311. Mezzetti A, Lapenna D, Pierdomenico SD, Di Giammarco G, Bosco G, Di Ilio C, et al. Myocardial antioxidant defenses during cardiopulmonary bypass. *J Card Surg*. (1993) 8(2):167–71. doi: 10.1111/j.1540-8191.1993.tb00368.x

312. Ballmer PE, Reinhart WH, Jordan P, Bühler E, Moser UK, Gey KF. Depletion of plasma vitamin C but not of vitamin E in response to cardiac operations. *J Thorac Cardiovasc Surg*. (1994) 108(2):311–20. Erratum in: J Thorac Cardiovasc Surg 1995 110(4 Pt 1):1072. doi: 10.1016/S0022-5223(94)70013-3

313. McColl AJ, Keeble T, Hadjinikolaou L, Cohen A, Aitkenhead H, Glenville B, Richmond W. Plasma antioxidants: evidence for a protective role against reactive oxygen species following cardiac surgery. *Ann Clin Biochem*. (1998) 35(Pt 5):616–23. doi: 10.1177/000456329803500504

314. Barta E, Pechán I, Cornák V, Luknárová O, Rendeková V, Verchovodko P. Protective effect of alpha-tocopherol and L-ascorbic acid against the ischemic-reperfusion injury in patients during open-heart surgery. *Bratisl Lek Listy*. (1991) 92 (3-4):174–83.

315. England MD, Cavarocchi NC, O'Brien JF, Solis E, Pluth JR, Orszulak TA, et al. Influence of antioxidants (mannitol and allopurinol) on oxygen free radical generation during and after cardiopulmonary bypass. *Circulation*. (1986) 74(5 Pt 2):III134–7.

316. Hill A, Borgs C, Fitzner C, Stoppe C. Perioperative vitamin C and E levels in cardiac surgery patients and their clinical significance. *Nutrients*. (2019) 11(9):2157. doi: 10.3390/nu11092157

317. Dingchao H, Zhiduan Q, Liye H, Xiaodong F. The protective effects of high-dose ascorbic acid on myocardium against reperfusion injury during and after cardiopulmonary bypass. *Thorac Cardiovasc Surg*. (1994) 42(5):276–8. doi: 10.1055/s-2007-1016504

318. Rodrigo R, Korantzopoulos P, Cereceda M, Asenjo R, Zamorano J, Villalabeitia E, et al. A randomized controlled trial to prevent post-operative atrial fibrillation by antioxidant reinforcement. *J Am Coll Cardiol*. (2013) 62(16):1457–65. doi: 10.1016/j.jacc.2013.07.014

319. Zoran P, Juraj F, Ivana D, Reik H, Dusan N, Mihailo V. Effects of allopurinol on oxygen stress status during open heart surgery. *Int J Cardiol*. (1994) 44(2):123–9. doi: 10.1016/0167-5273(94)90015-9

320. Tabayashi K, Suzuki Y, Nagamine S, Ito Y, Sekino Y, Mohri H. A clinical trial of allopurinol (zyloric) for myocardial protection. *J Thorac Cardiovasc Surg*. (1991) 101 (4):713–8. doi: 10.1016/S0022-5223(19)36703-0

321. Castelli P, Condemi AM, Brambillasca C, Fundarò P, Botta M, Lemma M, et al. Improvement of cardiac function by allopurinol in patients undergoing cardiac surgery. *J Cardiovasc Pharmacol*. (1995) 25(1):119–25. doi: 10.1097/00005344-199501000-00019

322. Bochenek A, Religa Z, Spyt TJ, Mistarz K, Bochenek A, Zembala M, et al. Protective influence of pretreatment with allopurinol on myocardial function in patients undergoing coronary artery surgery. *Eur J Cardiothorac Surg*. (1990) 4 (10):538–42. doi: 10.1016/1010-7940(90)90142-m

323. Coetzee A, Roussouw G, Macgregor L. Failure of allopurinol to improve left ventricular stroke work after cardiopulmonary bypass surgery. *J Cardiothorac Vasc Anesth*. (1996) 10(5):627–33. doi: 10.1016/s1053-0770(96)80141-8

324. Taggart DP, Young V, Hooper J, Kemp M, Walesby R, Magee P, et al. Lack of cardioprotective efficacy of allopurinol in coronary artery surgery. *Br Heart J*. (1994) 71(2):177–81. doi: 10.1136/hrt.71.2.177

325. Sisto T, Paajanen H, Metsä-Ketelä T, Harmoinen A, Nordback I, Tarkka M. Pretreatment with antioxidants and allopurinol diminishes cardiac onset events in coronary artery bypass grafting. *Ann Thorac Surg*. (1995) 59(6):1519–23. doi: 10.1016/0003-4975(95)00197-s

326. Westhuyzen J, Cochrane AD, Tesar PJ, Mau T, Cross DB, Frenneaux MP, et al. Effect of preoperative supplementation with alpha-tocopherol and ascorbic acid on myocardial injury in patients undergoing cardiac operations. *J Thorac Cardiovasc Surg*. (1997) 113(5):942–8. doi: 10.1016/s0022-5223(97)70268-x

327. Stegeman R, Lamur KD, van den Hoogen A, Breur JMPJ, Groenendaal F, Jansen NJG, et al. Neuroprotective drugs in infants with severe congenital heart disease: a systematic review. *Front Neurol*. (2018) 9:521. doi: 10.3389/fneur.2018.00521

328. Stegeman R, Nijman M, Breur JMPJ, Groenendaal F, Haas F, Derks JB, et al. Cerebrum and cardiac protection with allopurinol in neonates with critical congenital heart disease requiring cardiac surgery with cardiopulmonary bypass (CRUCIAL): study protocol of a phase III, randomized, quadruple-blinded, placebo-controlled, Dutch multicenter trial. *Trials*. (2022) 23(1):174. doi: 10.1186/s13063-022-06098-y

329. Andersen LW, Thiis J, Kharazmi A, Rygg I. The role of N-acetylcystein administration on the oxidative response of neutrophils during cardiopulmonary bypass. *Perfusion*. (1995) 10(1):21–6. doi: 10.1177/026765919501000105

330. De Backer WA, Amsel B, Jorens PG, Bossaert L, Hiemstra PS, van Noort P, et al. N-acetylcysteine pretreatment of cardiac surgery patients influences plasma neutrophil elastase and neutrophil influx in bronchoalveolar lavage fluid. *Intensive Care Med*. (1996) 22(9):900–8. doi: 10.1007/BF02044114

331. Tossios P, Bloch W, Huebner A, Raji MR, Dodos F, Klass O, et al. N-acetylcysteine prevents reactive oxygen species-mediated myocardial stress in patients undergoing cardiac surgery: results of a randomized, double-blind, placebo-controlled clinical trial. *J Thorac Cardiovasc Surg*. (2003) 126(5):1513–20. doi: 10.1016/s0022-5223(03)00968-1

332. Suter PM, Domenighetti G, Schaller MD, Laverrière MC, Ritz R, Perret C. N-acetylcysteine enhances recovery from acute lung injury in man. A randomized, double-blind, placebo-controlled clinical study. *Chest*. (1994) 105(1):190–4. doi: 10.1378/chest.105.1.190

333. El-Hamamsy I, Stevens LM, Carrier M, Pellerin M, Bouchard D, Demers P, et al. Effect of intravenous N-acetylcysteine on outcomes after coronary artery bypass surgery: a randomized, double-blind, placebo-controlled clinical trial. *J Thorac Cardiovasc Surg*. (2007) 133(1):7–12. doi: 10.1016/j.jtcvs.2006.05.070

334. Köksal H, Rahman A, Burma O, Halifeoğlu I, Bayar MK. The effects of low dose N-acetylcysteine (NAC) as an adjunct to cardioplegia in coronary artery bypass surgery. *Anadolu Kardiyol Derg*. (2008) 8(6):437–43.

335. Cai Z, Shi T, Zhuang R, Fang H, Jiang X, Shao Y, et al. Protective effect of N-acetylcysteine activated carbon release microcapsule on myocardial ischemia-reperfusion injury in rats. *Exp Ther Med*. (2018) 15(2):1809–18. doi: 10.3892/etm.2017.5653

336. Xu Z, Jiang G, Lin S, Guan J, Chen G, Chen G. Effect of N-acetylcysteine on intestinal injury induced by cardiopulmonary bypass in rats. *Nan Fang Yi Ke Da Xue Xue Bao*. (2014) 34(8):1171–5. Chinese.

337. Kumar R, Bansal M, Nath SS, Kumar V, Malviya D, Srivastava D. N-Acetylcysteine supplementation for the prevention of postoperative liver dysfunction after on-pump cardiac surgery. *Turk J Anaesthesiol Reanim*. (2021) 49(6):460–9. doi: 10.5152/TJAR.2021.21370

338. Onk D, Özçelik F, Onk OA, Günay M, Akarsu Ayazoğlu T, Ünver E. Assessment of renal and hepatic tissue-protective effects of N-acetylcysteine via ammonia metabolism: a prospective randomized study. *Med Sci Monit*. (2018) 24:1540–6. doi: 10.12659/msm.908172

339. Savluk OF, Guzelmeric F, Yavuz Y, Cevirme D, Gurcu E, Ogus H, et al. N-acetylcysteine versus dopamine to prevent acute kidney injury after cardiac surgery in patients with preexisting moderate renal insufficiency. *Braz J Cardiovasc Surg*. (2017) 32(1):8–14. doi: 10.21470/1678-9741-2016-0028

340. Tan SI, Brewster DJ, Horrigan D, Sarode V. Pharmacological and non-surgical renal protective strategies for cardiac surgery patients undergoing cardiopulmonary bypass: a systematic review. *ANZ J Surg*. (2019) 89(4):296–302. doi: 10.1111/ans.14800

341. Erdil N, Eroglu T, Akca B, Disli OM, Yetkin O, Colak MC, et al. The effects of N-acetylcysteine on pulmonary functions in patients Undergoing on-pump coronary artery surgery: a double blind placebo controlled study. *Eur Rev Med Pharmacol Sci*. (2016) 20(1):180–7.

342. Santana-Santos E, Gowdak LH, Gaiotto FA, Puig LB, Hajjar LA, Zeferino SP, et al. High dose of N-acetylcystein prevents acute kidney injury in chronic kidney disease patients undergoing myocardial revascularization. *Ann Thorac Surg*. (2014) 97(5):1617–23. doi: 10.1016/j.athoracsur.2014.01.056

343. Fitch JC, Rollins S, Matis L, Alford B, Aranki S, Collard CD, et al. Pharmacology and biological efficacy of a recombinant, humanized, single-chain antibody C5 complement inhibitor in patients undergoing coronary artery bypass graft surgery with cardiopulmonary bypass. *Circulation*. (1999) 100(25):2499–506. doi: 10.1161/01.cir.100.25.2499

344. Soulika AM, Khan MM, Hattori T, Bowen FW, Richardson BA, Hack CE, et al. Inhibition of heparin/protamine complex-induced complement activation by compstatin in baboons. *Clin Immunol*. (2000) 96(3):212–21. doi: 10.1006/clim.2000.4903

345. Thielmann M, Marggraf G, Neuhäuser M, Forkel J, Herold U, Kamler M, et al. Administration of C1-esterase inhibitor during emergency coronary artery bypass surgery in acute ST-elevation myocardial infarction. *Eur J Cardiothorac Surg*. (2006) 30(2):285–93. doi: 10.1016/j.ejcts.2006.04.022

346. Fattouch K, Bianco G, Speziale G, Sampognaro R, Lavalle C, Guccione F, et al. Beneficial effects of C1 esterase inhibitor in ST-elevation myocardial infarction in patients who underwent surgical reperfusion: a randomised double-blind study. *Eur J Cardiothorac Surg*. (2007) 32(2):326–32. doi: 10.1016/j.ejcts.2007.04.038

347. Lazar HL, Bokesch PM, van Lenta F, Fitzgerald C, Emmett C, Marsh HC Jr, Ryan U. Soluble human complement receptor 1 limits ischemic damage in cardiac surgery patients at high risk requiring cardiopulmonary bypass. *Circulation*. (2004) 110(11 Suppl 1):II274–9. doi: 10.1161/01.CIR.0000138315.99788.eb

348. Fujii M, Miyagi Y, Bessho R, Nitta T, Ochi M, Shimizu K. Effect of a neutrophil elastase inhibitor on acute lung injury after cardiopulmonary bypass. *Interact Cardiovasc Thorac Surg*. (2010) 10(6):859–62. doi: 10.1510/icvts.2009.225243

349. Verrier ED, Shernan SK, Taylor KM, Van de Werf F, Newman MF, Chen JC, et al. Terminal complement blockade with pexelizumab during coronary artery bypass graft surgery requiring cardiopulmonary bypass: a randomized trial. *JAMA*. (2004) 291(19):2319–27. Erratum in: JAMA. 2006 295(2):164. doi: 10.1001/jama.291.19.2319

350. Smith PK, Shernan SK, Chen JC, Carrier M, Verrier ED, Adams PX, et al. Effects of C5 complement inhibitor pexelizumab on outcome in high-risk coronary artery bypass grafting: combined results from the PRIMO-CABG I and II trials. *J Thorac Cardiovasc Surg*. (2011) 142(1):89–98. doi: 10.1016/j.jtcvs.2010.08.035

351. Shin'oka T, Nagashima M, Nollert G, Shum-Tim D, Laussen PC, Lidov HG, et al. A novel sialyl Lewis X analog attenuates cerebral injury after deep hypothermic circulatory arrest. *J Thorac Cardiovasc Surg*. (1999) 117(6):1204–11. doi: 10.1016/s0022-5223(99)70260-6

352. Gillinov AM, Redmond JM, Zehr KJ, Wilson IC, Curtis WE, Bator JM, et al. Inhibition of neutrophil adhesion during cardiopulmonary bypass. *Ann Thorac Surg*. (1994) 57(1):126–33. doi: 10.1016/0003-4975(94)90380-8

353. Sharar SR, Winn RK, Murry CE, Harlan JM, Rice CL. A CD18 monoclonal antibody increases the incidence and severity of subcutaneous abscess formation after high-dose Staphylococcus aureus injection in rabbits. *Surgery*. (1991) 110 (2):213–9; discussion 219–20.

354. Berg K, Langaas M, Ericsson M, Pleym H, Basu S, Nordrum IS, et al. Acetylsalicylic acid treatment until surgery reduces oxidative stress and inflammation in patients undergoing coronary artery bypass grafting. *Eur J Cardiothorac Surg*. (2013) 43(6):1154–63. doi: 10.1093/ejcts/ezs591

355. Akowuah E, Shrivastava V, Jamnadas B, Hopkinson D, Sarkar P, Storey R, et al. Comparison of two strategies for the management of antiplatelet therapy during urgent surgery. *Ann Thorac Surg*. (2005) 80(1):149–52. doi: 10.1016/j.athoracsur.2005.01.009

356. Littlejohn JE. Postoperative septic shock. In: Flynn B, et al., editors. *Cardiothoracic Critical Care*. Online ed. Oxford, United Kingdom: Oxford Academic (2020). p. 111–C10.P31.

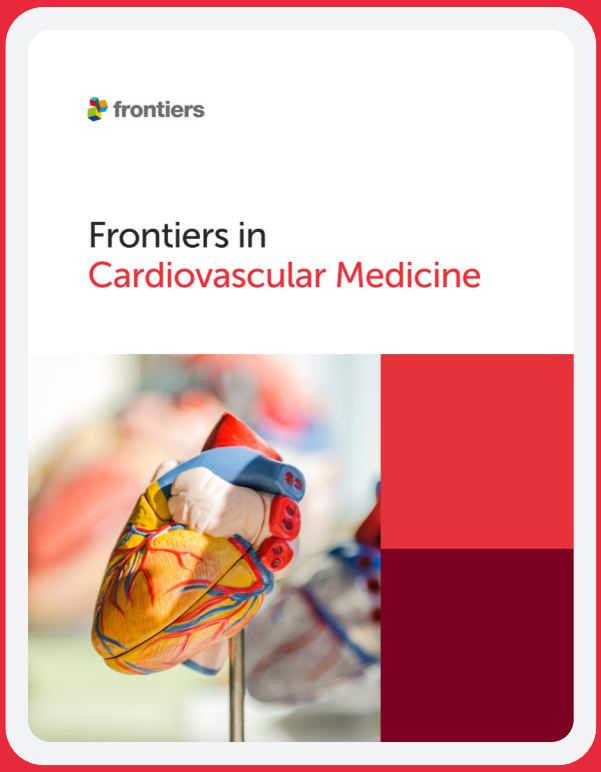
Frontiers in
Cardiovascular Medicine

Innovations and improvements in cardiovascular treatment and practice

Focuses on research that challenges the status quo of cardiovascular care, or facilitates the translation of advances into new therapies and diagnostic tools.

Discover the latest Research Topics

See more →



Frontiers

Avenue du Tribunal-Fédéral 34
1005 Lausanne, Switzerland
frontiersin.org

Contact us

+41 (0)21 510 17 00
frontiersin.org/about/contact