Comprehensive characterization of the postoperative pericardial inflammatory response: Potential implications for clinical outcomes

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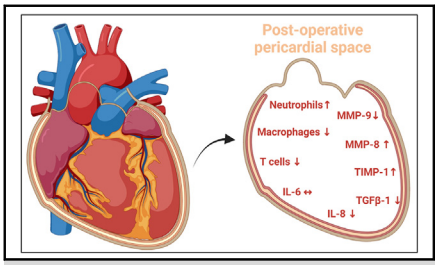
ABSTRACT

Objective: There is a paucity of data on the inflammatory response that takes place in the pericardial space after cardiac surgery. This study provides a comprehensive assessment of the local postoperative inflammatory response.

Methods: Forty-three patients underwent cardiotomy, where native pericardial fluid was aspirated and compared with postoperative pericardial effluent collected at 4, 24, and 48 hours' postcardiopulmonary bypass. Flow cytometry was used to define the levels and proportions of specific immune cells. Samples were also probed for concentrations of inflammatory cytokines, matrix metalloproteinases (MMPs), and tissue inhibitors of metalloproteinases (TIMPs).

Results: Preoperatively, the pericardial space mainly contains macrophages and T cells. However, the postsurgical pericardial space was populated predominately by neutrophils, which constituted almost 80% of immune cells present, and peaked at 24 hours. When surgical approaches were compared, minimally invasive surgery was associated with fewer neutrophils in the pericardial space at 4 hours' postsurgery. Analysis of the intrapericardial concentrations of inflammatory mediators showed interleukin-6, MMP-9, and TIMP-1 to be highest postsurgery. Over time, MMP-9 concentrations decreased significantly, whereas TIMP-1 levels increased, resulting in a significant reduction of the ratio of MMP:TIMP after surgery, suggesting that active inflammatory processes may influence extracellular matrix remodeling.

Conclusions: These results show that cardiac surgery elicits profound alterations in the immune cell profile in the pericardial space. Defining the cellular and molecular mediators that drive pericardial-specific postoperative inflammatory processes may allow for targeted therapies to reduce immune-mediated complications. (JTCVS Open 2022;12:118-36)



The inflammatory mediators that are present in the pericardial space after surgery.

CENTRAL MESSAGE

Specific inflammatory markers, which may result in immune-mediated complications such as postpericardiotomy syndrome, are present in the pericardial space postsurgery.

PERSPECTIVE

Postpericardiotomy syndrome, postsurgical adhesions, and postoperative atrial fibrillation are associated with cardiac surgery. Inflammation can drive these complications, but the inflammatory mediators that occupy the postoperative pericardial space are poorly understood. Establishing the postoperative pericardial inflammatory response may offer clues that can explain these complications.

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Abbreviations and Acronyms

AVR	= aortic valve replacement
CABG	= coronary artery bypass graft
CD	= cluster of differentiation
cDC	= classical dendritic cell
CPB	= cardiopulmonary bypass
DC	= dendritic cell
ECM	= extracellular matrix
FS	= full median sternotomy
IL	= interleukin
IL-1Ra	= interleukin-1 receptor antagonist
Inf DC	= inflammatory dendritic cell
MICS	= minimally invasive cardiac surgery
MMP	= matrix metalloproteinase
MMP_{tot}	= total matrix metalloproteinases
Mϕ	= macrophage
NK	= natural killer cell
PAOF	= postoperative atrial fibrillation
PPS	= postpericardiotomy syndrome
RAMT-AVR	= right anterior minithoracotomy aortic valve replacement
sAVR	= conventional full median sternotomy surgical aortic valve replacement
SSC	= side scatter
TGFβ	= transforming growth factor-beta
TIMP	= tissue inhibitor of metalloproteinases
$TIMP_{tot}$	= total tissue inhibitors of metalloproteinases

Postoperative atrial fibrillation (POAF)[1] and postpericardiotomy syndrome (PPS)[2] are common early complications after cardiac surgery, whereas postsurgical pericardial adhesions and pericardial constriction can have negative long-term consequences on outcomes.[3-10] Although inflammation is believed to play an important role in all these processes,[8,9,11-17] to date, we have lacked a clear understanding of their pathophysiology, resulting in limited therapeutic options.

Numerous studies have shown that the cardiopulmonary bypass (CPB) machine elicits a robust systemic inflammatory response, which is characterized by early and late stages involving immune-mediated pathways, such as activation of the complement system and the coagulation cascade.[16-26] These studies have also found that the activation of systemic inflammatory pathways can adversely impact clinical outcomes by resulting in acute kidney injury, lung injury, and neurocognitive disorders such as delirium.[22-24,27] While some groups have provided a rudimentary description of a subset of immune factors that are present in the pericardial space after an operation,[28] there is a paucity of comprehensive data on how surgical interventions impact the composition of the postoperative pericardial inflammatory profile. Moreover, to date, we do not know how different surgical approaches influence the pericardial space postoperatively. This is relevant because minimally invasive cardiac surgery (MICS) requires less pericardial manipulation (ie, handling, exposure, and disruption). In MICS cases, the length and location of the pericardial incision differs from conventional full median sternotomy (FS) cases. We have recently shown that the preoperative pericardial space is populated with immune cells and acute myocardial ischemia can alter their composition.[29] Elucidating the local postoperative inflammatory response may provide a therapeutic opportunity in which specific markers that potentiate POAF, PPS, and postsurgical pericardial adhesions can be targeted in a precise manner to reduce their incidence.

The objectives of this study are to (1) characterize the postsurgical inflammatory profile of the pericardial space (Figure 1); (2) to describe differences that can exist in this profile over time after surgery; and (3) to determine whether this profile is different between MICS and conventional FS surgical approaches. The immune cells that are present in the pericardial space after surgery are defined. The cytokines, matrix metalloproteinases (MMPs), and tissue inhibitors of metalloproteinases (TIMPs) that populate the pericardial space postsurgery are also identified. Finally, by comparing between MICS and FS approaches, we elucidate how different types of pericardial handling can drive the local postoperative inflammatory response. Our findings provide insight into the inflammatory factors that have postsurgical sequalae in the pericardial space.

METHODS

Patient Sample Acquisition

Patients undergoing elective cardiac surgery were prospectively selected for the study. Those enrolled in this study provided written informed consent.

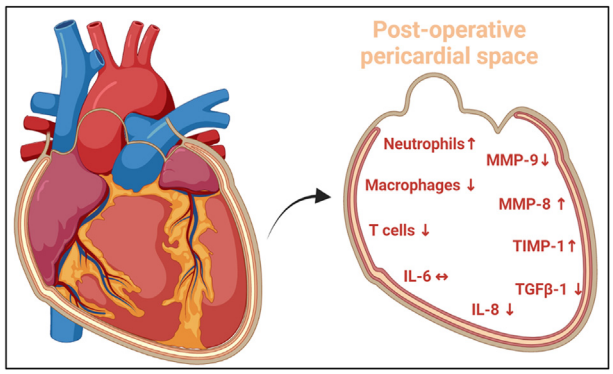

FIGURE 1. The inflammatory mediators that are present in the pericardial space after surgery. *MMP*, Matrix metalloproteinase; *TIMP*, tissue inhibitor of metalloproteinase; *TGFβ*, transforming growth factor beta; *IL*, interleukin.

TABLE 1. Baseline characteristics for all patients

Characteristic	n = 43
Age, y, mean ± SD	62.4 ± 12.9
Sex	
Female	15
Male	28
Intervention	
CABG	14
Full median sternotomy	
Aortic valve replacement	8
Full median sternotomy	
Right anterior mini thoracotomy	8
Mitral valve repair/replacement	9
Right mini thoracotomy	
CABG + AVR	1
Full median sternotomy	
ASD repair	3
Right anterolateral mini thoracotomy	
Coronary artery disease	17
Hypertension	26
Diabetes mellitus type II	16
Dyslipidemia	25
Active tobacco use	10
Positive family history	1
Long standing persistent atrial fibrillation	1
Renal disease (stage III or worse; eGFR <60)	3
Pulmonary fibrosis	1
Chronic obstructive pulmonary disease	2
Benign pulmonary nodule	1
Obstructive sleep apnea	3
Asthma	2
Peripheral arterial disease	1
Previous stroke	3
Cardiovascular event	5
Chronic steroid use	1
Immune-suppressed	0
Preoperative ASA use	16
Preoperative NSAID use	1
Orthopnea	1
Presyncope	4
Syncope	2
NYHA class dyspnea	
Class I	9
Class II	15
Class III	6
Class IV	0
Preoperative LV dysfunction (by echocardiography)	
Mild	5
Moderate	2
Severe	0

SD, Standard deviation; *CABG*, coronary artery bypass graft; *AVR*, aortic valve replacement; *ASD*, atrial septal defect; *eGFR*, estimated glomerular filtration rate; *ASA*, acetylsalicylic acid; *NSAID*, nonsteroidal anti-inflammatory drug; *NYHA*, New York Heart Association; *LV*, left ventricular.

A flowchart summarizing the patient selection process according to the Strengthening the Reporting of Observational Studies in Epidemiology recommendations is provided in Figure E1. The experiments were conducted under approval of the Conjoint Health Research Ethics Board at the University of Calgary underlying the Declaration of Helsinki (Ethics ID: REB16-1906, approved February 12, 2021). Native pericardial fluid samples were obtained from patients undergoing elective on-pump cardiac surgery at the Foothills Medical Centre Samples were collected before the institution of CPB. Postoperative pericardial fluid was collected from a drain that was left in the pericardial space. Inclusion criteria were age greater than 18 years and patients undergoing conventional FS surgery or minimally invasive cardiac surgery (MICS). Exclusion criteria were patients who were prescribed insulin or immunosuppressive medications, patients with a history of inflammatory or rheumatic disease, patients requiring dialysis, patients with active infective endocarditis, and those who were undergoing emergent surgery, redo surgery, or hemi-sternotomy cardiac surgery. We also excluded patients in whom MICS was converted to FS (n = 2), and patients in whom the pericardial drain had to be removed before reaching the 48-hour time point due to reasons unrelated to the study (n = 1). The FS group included patients undergoing coronary artery bypass graft (CABG) surgery, aortic valve replacement (AVR), and combined CABG and AVR. In this cohort, the pericardium was incised at full length anteriorly and teed off, creating the pericardial cradle. The MICS cohort included patients undergoing right anterior minithoracotomy for aortic valve replacement (RAMT-AVR), right minithoracotomy for mitral valve surgery, and right anterolateral mini thoracotomy for atrial septal defect closure surgery. For the MICS cases, the pericardium was incised and retracted laterally along the course of the phrenic nerve. Except for patients undergoing CABG surgery, the pericardium was reapproximated at the conclusion of the operation in all cases. In all patients, native pericardial fluid was completely removed intraoperatively. Postsurgical pericardial fluid was collected at 4, 24, and 48 hours after the patient was weaned off CPB. After collection, all fluid samples were transferred into a sodium heparin tube (Greiner Bio-Oney) and transported on ice to the laboratory for immediate workup. While initial analysis focused on samples collected from all patients, we also directly compared patients undergoing conventional full median sternotomy surgical aortic valve replacement (sAVR) and RAMT-AVR.

Cell Isolation and Flow Cytometry

Pericardial fluid samples were filtered through a 40-μm Nylon cell strainer (Falcon) and centrifuged at 1500 rpm (450 *g*) for 5 minutes at 4 °C. Acellular components were stored at –80 °C for further analysis. For red blood cell sedimentation, postoperative samples were incubated with 6% dextran in phosphate-buffered saline for 30 minutes in a 2:1 ratio at room temperature before flow cytometry workup. Cell pellets were then processed for flow cytometry. After cell counts, 1 million cells were blocked with a human F_c gamma receptor binding inhibitor (eBioscience) and incubated with GhostDye Red 710 viability dye (Tonbo Biosciences) for 20 minutes. Cell staining followed for 20 minutes with specific markers (Table E1). Fixed samples were run on a BD FACS Canto flow cytometer and analyzed with FlowJo 10 Software (Becton Dickinson

TABLE 2. Baseline characteristics, intraoperative details, and clinical outcomes for patients who underwent sAVR and RAMT-AVR

Postoperative outcomes	sAVR, n = 8	RAMT-AVR, n = 8
Age, mean ± SD	68.0 ± 6.68	68.9 ± 6.49
Sex		
Female	2	4
Male	6	4
Coronary artery disease	1	2
Hypertension	5	3
Diabetes mellitus type II	1	3
Dyslipidemia	3	6
Active smoking history	2	0
Positive family history	0	0
Long standing persistent atrial fibrillation	1	0
Renal disease (stage III or worse; eGFR <60)	1	1
Lung Disease (any type)	0	0
Peripheral arterial disease	0	0
Previous stroke	1	1
Cardiovascular event	1	1
Chronic steroid use	0	0
Immune-suppressed	0	0
Preoperative aspirin use	3	4
Preoperative NSAID use	0	0
Orthopnea	0	0
Presyncope	0	3
Syncope	0	2
NYHA class dyspnea		
Class I	1	2
Class II	2	4
Class III	4	2
Class IV	0	0
Preoperative LV dysfunction (by echocardiography)		
Mild	1	0
Moderate	0	0
Severe	0	0
Intraoperative details		
Cardiopulmonary bypass time, min, mean ± SD	90.9 ± 22.3	76.9 ± 19.5
Aortic crossclamp time, min, mean ± SD	70.1 ± 20.2	60.3 ± 20.7
Postoperative outcomes		
Death	0	0
Stroke	0	0
Dialysis	0	0
Infection	0	0
Prescribed aspirin	8	8
Mechanical ventilatory time, h	13.62 ± 13.02	4.93 ± 1.37
Postoperative atrial fibrillation	2	2

sAVR, Conventional full median sternotomy surgical aortic valve replacement; *RAMT-AVR*, right anterior minithoracotomy aortic valve replacement; *SD*, standard deviation; *eGFR*, estimated glomerular filtration rate; *NSAID*, nonsteroidal anti-inflammatory drug; *NYHA*, New York Heart Association; *LV*, left ventricular.

& Company). Cell counts were performed by using counting beads (Thermo Fisher Scientific) and normalized to individual sample flow cytometry run times.

Proportional cell quantifications are shown as percentage of cluster of differentiation (CD)45^+ cells. T cells were identified as $CD3^+$. Neutrophils were identified as $CD3^-$ $CD64^-$ $CD16^+$ side scatter (SSC^{hi}). Natural killer cells (NKs) were identified as $CD3^-$ $CD64^-$ $CD16^-$ SSC^{lo} $CD56^+$ population, including $CD16^-$ and $CD16^+$ NKs, respectively. B cells were identified as $CD3^-$ $CD64^-$ $CD16^-$ SSC^{lo} $CD56^-$ $CD45^+$ $CD19^+$. Classical dendritic cells (cDCs) type 2 were identified as $CD64^+$ $CD1c^+$ $CD14^-$. Inflammatory dendritic cells (Inf DCs) were identified as $CD64^+$ $CD1c^+$ $CD14^+$. Macrophages (Mϕ) were identified as $CD64^+$ $CD14^+$ cells, including a $CD16^-$ $CD163^{lo}$ macrophage population ($CD163^{lo}$ Mϕ) and a $CD16^+$ $CD163^{hi}$ ($CD163^{hi}$ Mϕ) macrophage population, respectively.

Cytokines, MMPs, TIMPs, and Transforming Growth Factor-Beta (TGFβ)

Cytokine, MMP, TIMP, and TGFβ concentrations in fluid supernatants were measured with blinded multiplex analysis (Eve Technologies). We probed for interleukin (IL)-1β, IL-1 receptor antagonist (IL-1Ra), IL-5, IL-6, IL-8, IL-10, IL-12p40, IL-13, interferon-gamma-γ, tumor necrosis factor alpha, and monocyte chemoattractant-1. We also assayed the fluid for MMP-1, MMP-2, MMP-3, MMP-7, MMP-8, MMP-9, MMP-10, MMP-12, and MMP-13. Further, we assessed for TIMP-1, TIMP-2, TIMP-3, and TIMP-4. MMP/TIMP ratios were calculated manually. Finally, we also probed for TGFβ-1, TGFβ-2, and TGFβ-3. To compare between different multiplex runs, we used control samples that were present in each run and calculated factors based on their concentration levels.

Statistical Analysis

Data are presented in box and whisker plots as median with minimum ± maximum. Line graphs are shown as mean ± standard error of mean. Patient characteristics are expressed as mean ± standard deviation where appropriate. GraphPad Prism 9 Software was used for statistical analysis. Under assumption of normal distribution, we performed an unpaired Student *t*-test where appropriate to compare demographics of the 2 groups. Two-way analysis of variance was used for comparison of multiple groups between time-points with the Tukey post hoc test. To determine differences between surgical approaches at the same time point, we performed unpaired Student *t*-tests under assumption of Gaussian distribution and the same standard deviation for both populations. We reported the number of independent replicates (n) in the figure legends.

RESULTS

Patient Demographics

Forty-three patients were included in the study, 28 were male, and the mean age was 62.4 ± 12.9 years.

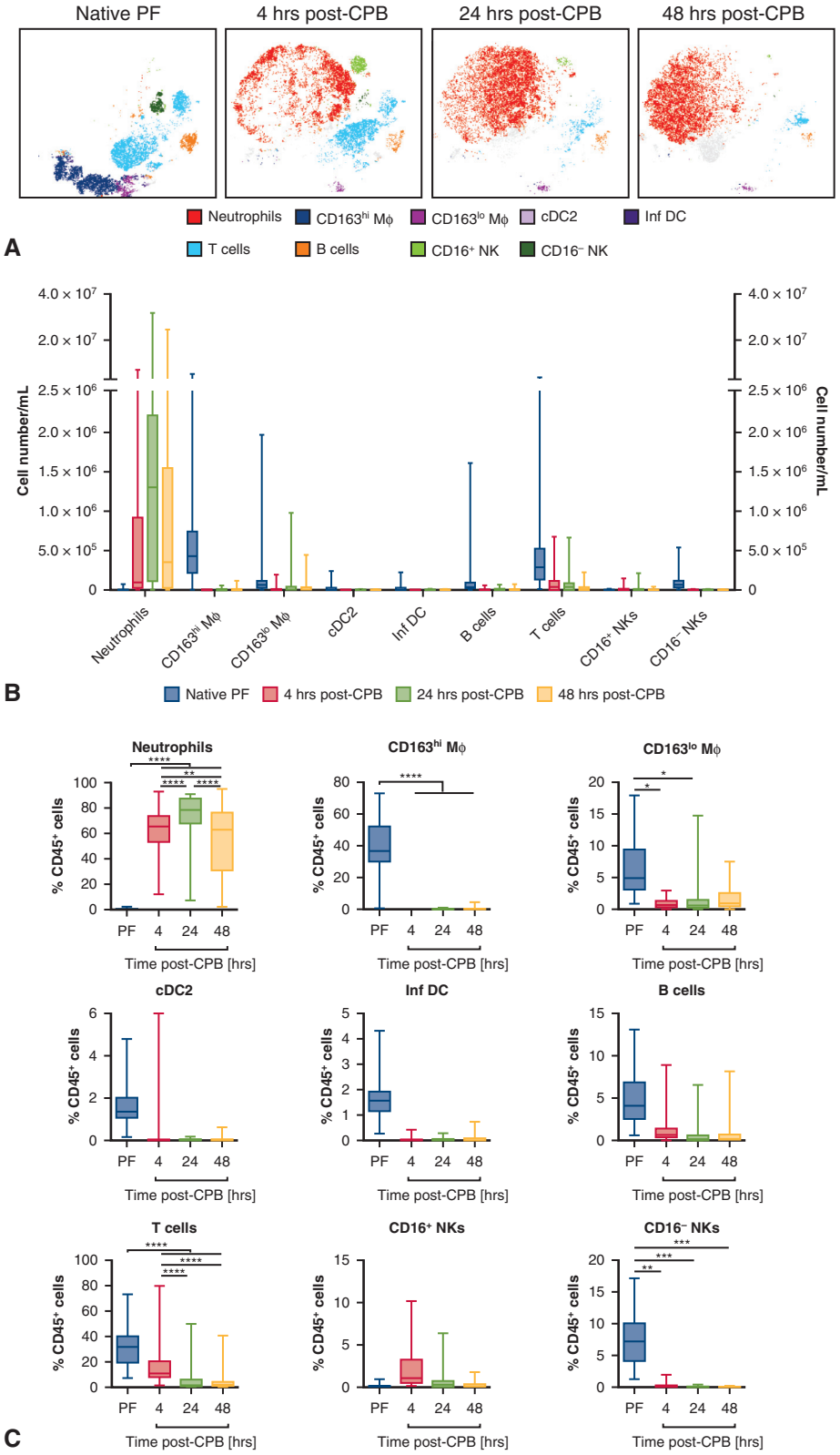

FIGURE 2. The immune cell profile that is present in the pericardial space changes postsurgery. A, Representative t-distributed stochastic neighbor embedding plots showing the immune cell profile present in native PF (*left*), at 4 (*middle left*), 24 (*middle right*), and 48 hours' post-CPB (*right*). The figure was prepared by using Adobe Illustrator 2022. B, Overview of the immune cells present in the pericardial space as total numbers per milliliter in native PF

Twenty-six patients had hypertension, 16 had diabetes, and 25 had dyslipidemia. Baseline patient characteristics are summarized in Table 1. Twenty-three patients underwent conventional FS cardiac surgery, where CABG was performed in 14, 8 had conventional sAVR, and 1 patient had CABG + AVR. A minimally invasive operation was performed in 20 patients, where 9 had surgery done on the mitral valve and minimally invasive aortic valve replacement (RAMT-AVR) was completed in 8 patients and minimally invasive atrial septal defect closure was done for 3 patients. All surgeries were performed by using the CPB machine, and an aortic crossclamp was applied in all cases. Blood cardioplegia or Del Nido cardioplegia was used for all cases. Intraoperative details for all patients are listed in Table E2.

In the AVR groups, the mean age was 68.0 ± 6.68 years and 68.9 ± 6.49 years in the sAVR and RAMT-AVR cohorts, respectively. The sAVR group consisted of 2 female and 6 male patients, whereas there were 4 male and 4 female patients in the RAMT-AVR cohort. Five and 3 patients had hypertension in the sAVR and RAMT-AVR groups, respectively. One patient had diabetes mellitus type II in the sAVR group and 3 patients with diabetes were present in the RAMT-AVR group. Three and 6 patients had dyslipidemia in the sAVR and RAMT-AVR groups. All patients presented with dyspnea, but only 1 patient, who was in the sAVR group, had mild left ventricular dysfunction. None of the patients were actively prescribed steroids or immunosuppressive medications. There were no statistically significant differences between the 2 groups and operative times. While CPB time was 90.9 ± 22.3 minutes and 76.9 ± 19.5 minutes (*P*-value: .20), aortic crossclamp time was 70.1 ± 20.2 minutes and 60.3 ± 20.7 minutes (*P*-value: .35) in the sAVR and RAMT-AVR groups, respectively. Postoperatively, there were no deaths or strokes, but 1 patient from the sAVR was rehospitalized for pleural effusion. Baseline demographics, intraoperative details, and postoperative outcomes for the sAVR and RAMT-AVR groups are presented in Table 2.

Neutrophils and T Cells Are the Predominant Postoperative Pericardial Immune Cells

The cellular components of native pericardial fluid and the postoperative pericardial fluid were analyzed to determine the composition of immune cells (Figure 2, *A*). Neutrophils, macrophages, cDCs, Inf DCs, B cells, T cells, and NK cells were present in the pericardial space after surgery (Figure 2, *B*). $CD16^+$ and $CD16^-$ NK cells were also present in the pericardial cavity after surgery. The different cell types that were identified were quantified for cell count and percentage of $CD45^+$ cells (Figure 2, *C*). At 4 hours' post-CPB, neutrophils were the most abundant cell type. Levels of neutrophils increased at 24 hours' post-CPB but reduced at the 48-hour time point. The increase in the neutrophil levels coincided with a decrease in macrophage populations, T cells, and $CD16^-$ NK cells. Proportions of DC populations, B cells, and $CD16^+$ NK cells remained at low levels at the 48-hour time point.

Cytokines Populate the Pericardial Space After Surgery, Where IL-6 Is the Predominant Cytokine

To further characterize the local postoperative inflammatory response, the acellular portion of postsurgical pericardial fluid samples was assessed for the concentration of cytokines, MMPs, and TIMPs. The postoperative pericardial concentration of IL-6 was found to be highest, followed by IL-8, and IL-10 (Figure 3, *A*). When compared with 4 hours' post-CPB, the concentration of IL-1Ra increased at 24 hours' post-CPB whereas IL-5 increased significantly in the pericardial space at 48 hours' post-CPB. In contrast, when comparing between 4 and 24 hours' post-CPB time points, IL-10 and IL-1Ra concentrations decreased in the pericardial space, whereas IL-8 and IL-10 concentrations were significantly lower at 48 hours' versus 4 hours' post-CPB. Pericardial concentration of IL-13 also decreased significantly where there were differences between both 48 hours' versus 4 hours' post-CPB and 48 hours' versus 24 hours' post-CPB. There was a significant difference in the pericardial concentrations of IL-12p40 between 48 and 4 hours' post-CPB. With respect to different time points post-CPB, there were no differences in the pericardial concentrations of IL-1β, IL-6, monocyte chemoattractant protein-1, interferon-γ, and tumor necrosis factor-α.

The Concentration of MMP-9 and MMP-8 is Highest in the Pericardial Space Postsurgery

MMP-9 and MMP-8 concentrations were highest in the pericardial space after surgery (Figure 3, *B*). When compared with different post-CPB time points, MMP-1, MMP-3, MMP-8, and MMP-10 concentrations increased

and at the postoperative time points. C, Proportional quantification of the immune cells that are present in native and postsurgical PF, presented as percentage of $CD45^+$ cells. Comparisons for neutrophils, $CD163^{hi}$ and ^{lo} Mϕ, cDC2, Inf DC, B cells, T-cells, $CD16^+$ and $CD16^-$ NKs are included. Each comparison represents n = 36 for native PF, n = 43 for 4, 24, and 48 hours' post-CPB. *Box and whisker plots* indicate median with minimum ± maximum. The *lower and upper borders* of the box represent the lower and upper quartiles (25th percentile and 75th percentile). The *middle horizontal line* represents the median. The *lower and upper whiskers* represent the minimum and maximum values of nonoutliers. For statistical analysis 2-way analysis of variance with the Tukey post hoc test was performed to determine differences between time points. *$P \leq .05$. **$P \leq .01$. ***$P \leq .001$. ****$P \leq .0001$. *PF*, Pericardial fluid; *CPB*, cardiopulmonary bypass; *CD*, cluster of differentiation, *Mϕ*, macrophage, *cDC2*, classical dendritic cell type 2; *Inf DC*, inflammatory dendritic cell; *NK*, neutral killer cell.

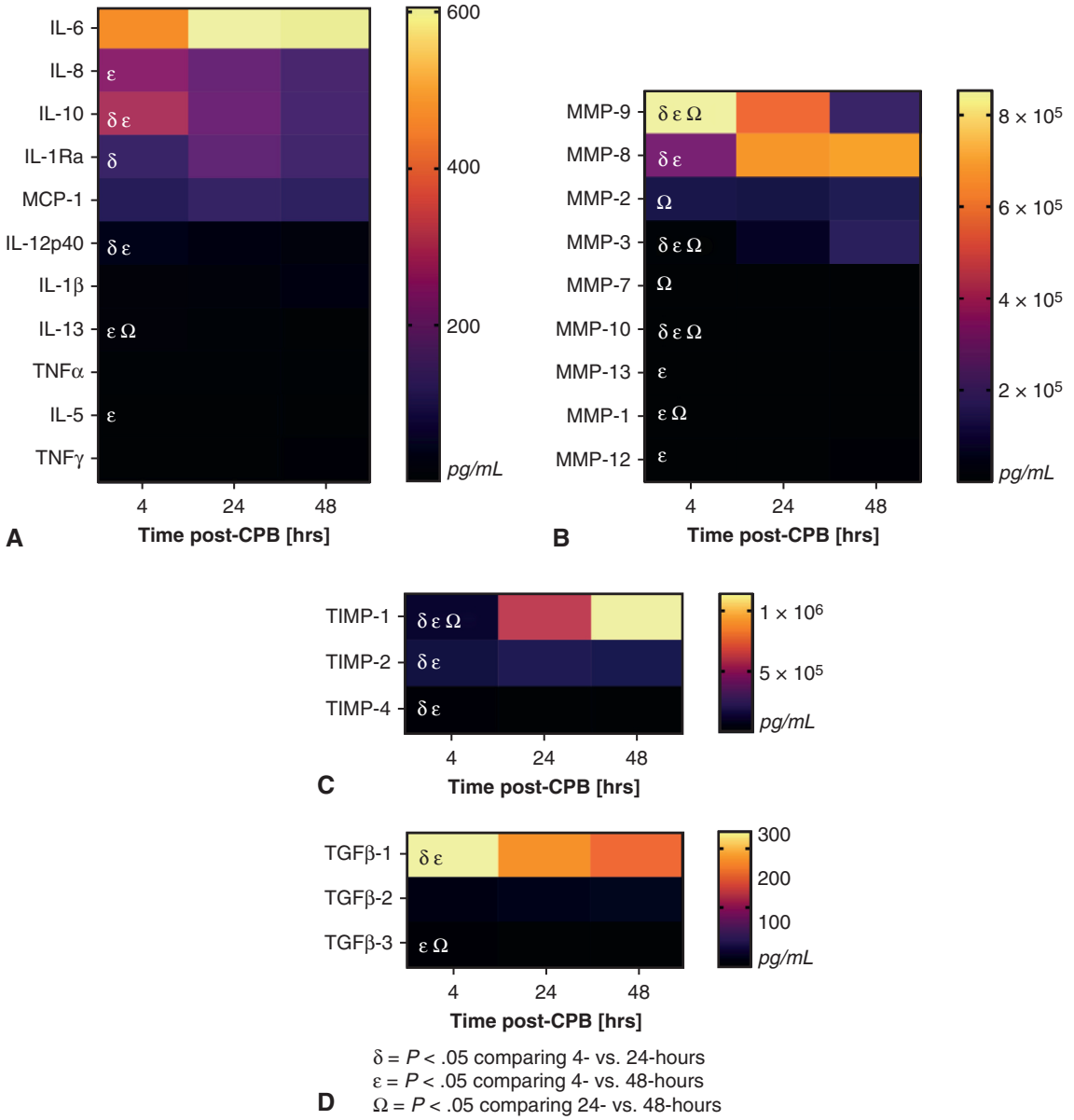

FIGURE 3. Cytokines, MMPs, TIMPs, and TGFβ are present in the pericardial space post-surgery and their relative concentrations change over time. A, Heatmap representation of the cytokines that are present in the pericardial space at 4, 24, and 48 hours' post-CPB in pg/mL. The cytokine with the highest expressed level is at the top, and the lowest is at the bottom. Each comparison includes n = 27 for 4, 24, and 48 hours' post-CPB. B, Heatmap representation of the MMPs that are present in the pericardial space at 4, 24, and 48 hours' post-CPB in pg/mL. The highest MMP is at the top, the lowest at the bottom. Each comparison includes n = 27 for 4, 24, and 48 hours' post-CPB, except MMP-12 with n = 20 for each time point. C, Heatmap representation of the TIMPs that are present in the pericardial cavity at 4, 24, and 48 hours post-CPB in pg/mL. The highest TIMP is at the top, the lowest at the bottom. D, Heatmap representation of the TGFβs that are present in the pericardial cavity at 4, 24, and 48 hours post-CPB in pg/mL. The highest TGFβ is at the top, the lowest at the bottom. For statistical analysis 2-way variance analysis with the Tukey post hoc test was performed to determine difference between time points. $\delta P < .05$ comparing 4 versus 24 hours. $\varepsilon P < .05$ comparing 4 versus 48 hours. $\Omega P < .05$ comparing 24 versus 48 hours. The figure was prepared by using Adobe Illustrator 2022. *IL*, Interleukin; *IL-1R*α, interleukin-1 receptor-α; *MCP-1*, monocyte chemoattractant protein-1; *TNF*α, tumor necrosis factor-α; *INF*γ, interferon-γ; *MMP*, matrix metalloproteinase; *TIMP*, tissue inhibitor of metalloproteinase; *TGF*β, transforming growth factor beta; *CPB*, cardiopulmonary bypass.

in the pericardial space after surgery. Pericardial concentrations of MMP2- and MMP-7 remained relatively unchanged, whereas MMP-9, MMP-12, and MMP-13 significantly decreased over time after surgery. The decrease in MMP-9 concentrations was significant between each time interval. There were significant increases between each time point and MMP-3 and MMP-10 concentrations.

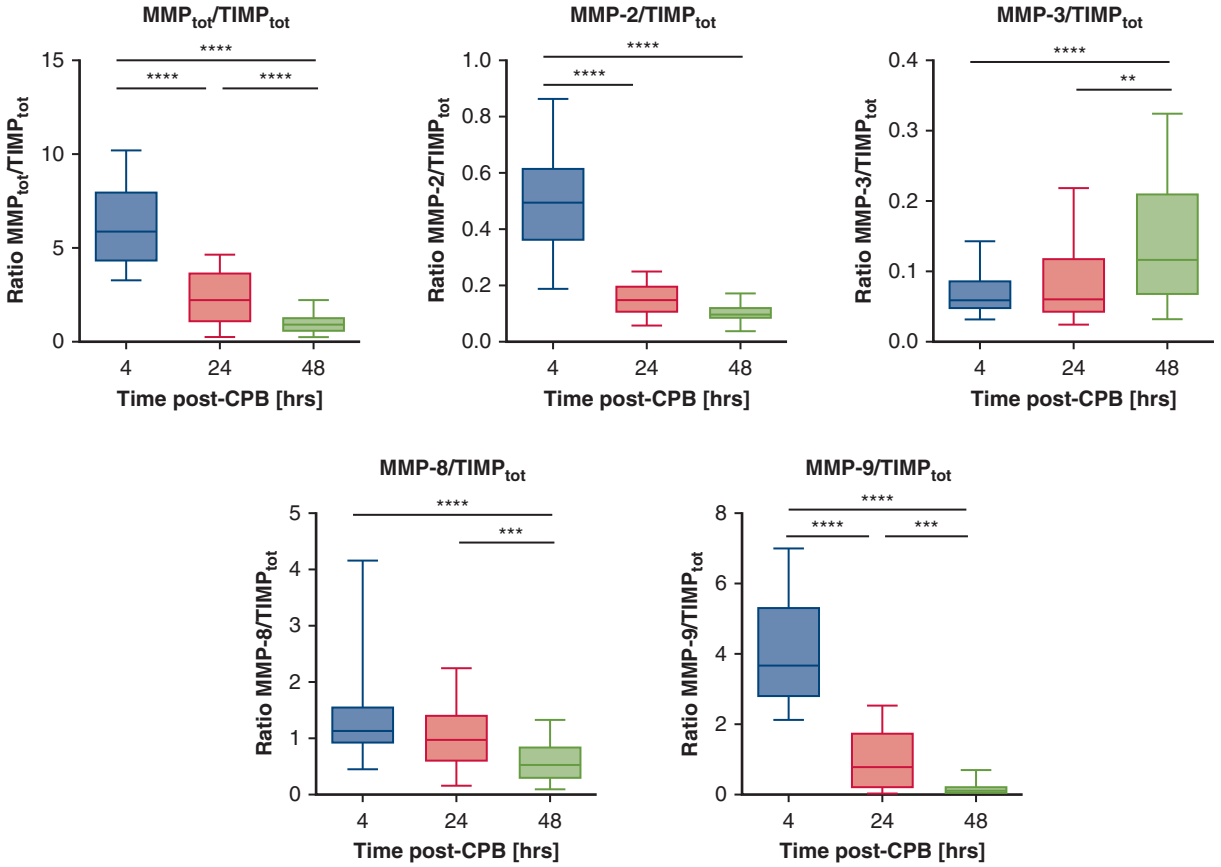

FIGURE 4. Assessment of postoperative pericardial MMP/TIMP ratios. Quantitative comparison of postsurgical pericardial MMP/TIMP ratios at 4, 24, and 48 hours' post-CPB with respect to each time point. Analysis and comparison of MMP_{tot} to $TIMP_{tot}$ ratio when considering the time point after surgery (*left*), MMP-2/$TIMP_{tot}$ (*second left*), MMP-3/$TIMP_{tot}$ (*middle*), MMP-8/$TIMP_{tot}$ (*second right*), and MMP-9/$TIMP_{tot}$ (*right*). Time-point comparisons contain n = 27 for 4, 24, and 48 hours' post-CPB. For statistical analysis 2-way variance analysis with the Tukey post hoc test was performed to determine difference between time points. *Box and whisker plots* indicate median with minimum ± maximum. The *lower and upper borders* of *the box* represent the lower and upper quartiles (25th percentile and 75th percentile). The *middle horizontal line* represents the median. The *lower and upper whiskers* represent the minimum and maximum values of nonoutliers. $^{**}P \leq .01$. $^{***}P \leq .001$. $^{****}P \leq .0001$. *MMP*, Matrix metalloproteinase; *TIMP*, tissue inhibitor of metalloproteinase; *CPB*, cardiopulmonary bypass; *tot*, total.

TIMP-1 and TIMP-2 Have the Highest Concentration in the Postoperative Pericardial Space

Although we probed for it, our multiplex analysis did not reveal appreciable levels of TIMP-3, whereas TIMP-1 and TIMP-2 concentrations were noted to be highest in the pericardial space after surgery (Figure 3, *C*). Comparing between postoperative time points, there was a significant increase in pericardial TIMP-1 concentrations between 24 hours' versus 4 hours' post-CPB, between 48 hours' versus 4 hours' post-CPB, and between 48 hours' and 24 hours' post-CPB. Pericardial TIMP-2 and TIMP-4 concentrations increased significantly from 4 to 24 hours' post-CPB and 4 to 48 hours' post-CPB.

Concentration of TGFβ-1 Is Twice as High at 4 Hours Compared With 48 Hours Postcardiotomy

Finally, we assessed for the postoperative pericardial concentration of TGFβ and found TGFβ-1 to have the highest concentration (Figure 3, *D*). The concentration of TGFβ-1 was lower at both the 24- and 48-hours post-CPB when compared with the 4-hour time point.

There Is a Significant Decrease in the MMP_{tot}/$TIMP_{tot}$ in the Pericardial Space After Surgery

The proteolytic capacity was evaluated by comparing ratios of MMPs to TIMPs. First, the total pericardial concentration of MMPs (MMP_{tot}) and TIMPs ($TIMP_{tot}$) was calculated. The MMP_{tot}/$TIMP_{tot}$ ratio decreased significantly in the pericardial space over time (Figure 4, first panel). We then measured the ratio between the MMPs that were present at the highest concentrations in the pericardial space after surgery and $TIMP_{tot}$. Specifically, we focused on MMP-2, MMP-3, MMP-8, and MMP-9 (Figure 4). Except for MMP-3/$TIMP_{tot}$, which increased over time, MMP-2/$TIMP_{tot}$, MMP-8/$TIMP_{tot}$, and MMP-9/$TIMP_{tot}$ all decreased in the pericardial space after surgery.

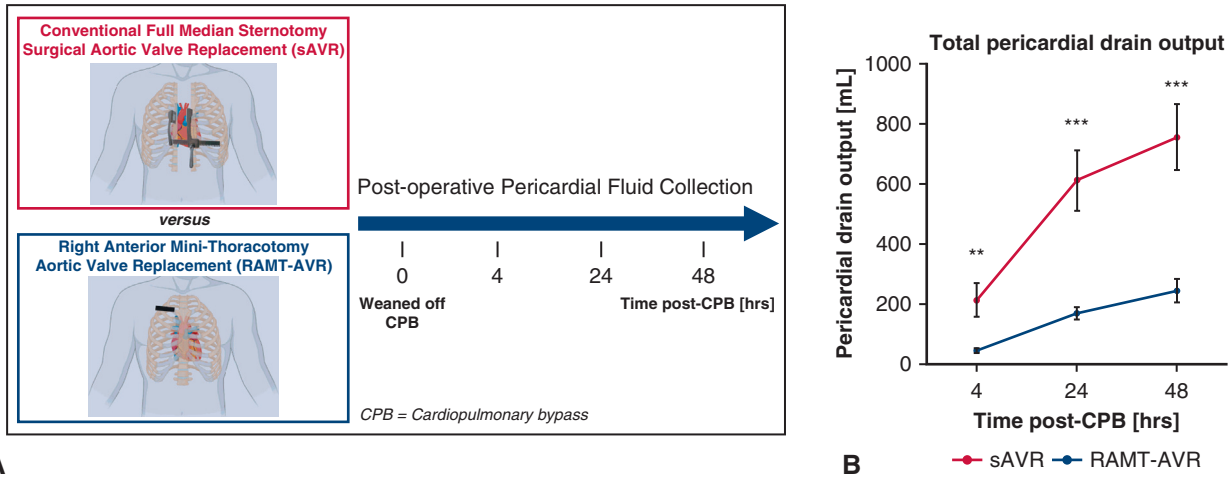

FIGURE 5. Comparison between sAVR and RAMT-AVR output volume of the fluid that is present in the pericardial space postoperatively. A, Schematic diagram showing the 2 surgical approaches: sAVR is at the top (in *red*) and RAMT-AVR is at the bottom (in *blue*). Postoperative pericardial fluid was collected at 4, 24, and 48 hours after the patient was weaned off CPB. B, The total postsurgical pericardial drain output in mL is shown at 4, 24, and 48 hours' post-CPB. Comparison includes n = 8 for each group at each time-point. Statistical analysis was performed at each time point using an unpaired Student *t*-test. Lines show mean ± standard error of mean. $^{**}P \leq .01$. $^{***}P \leq .001$. *sAVR*, Conventional full median sternotomy surgical aortic valve replacement; *RAMT-AVR*, right anterior mini-thoracotomy aortic valve replacement; *CPB*, cardiopulmonary bypass.

The Composition of Postoperative Pericardial Immune Cells Is Different Between Patients Undergoing sAVR and RAMT-AVR

The composition of immune cells present in the pericardial space after surgery was compared according to type of surgery: sAVR versus RAMT-AVR (Figure 5, *A*). The pericardial drain output for each surgical approach was first quantified, where a significantly higher output was noted in the sAVR group at all time-points (Figure 5, *B*). The drain output represented the volume of the fluid that accumulated in the pericardial space after surgery. Next, we quantified the proportion of postoperative pericardial immune cells as $CD45^{+}$ cell numbers/mL and found no statistically significant differences between sAVR and RAMT-AVR across all time points post-CPB (Figure 6, *A*). With respect to the postoperative pericardial neutrophils, there were significantly fewer cells in the RAMT-AVR cohort at 4 hours' post-CPB. At 48 hours' post-CPB, significantly more $CD163^{lo}$ macrophages were found in the pericardial cavity in the patients who underwent RAMT-AVR, whereas no differences were observed for $CD163^{hi}$ macrophages across time points and surgical approaches. The patients who underwent RAMT-AVR also had more cDC type 2 and Inf DC at the 24 and 48 hours' post-CPB time points, respectively. With respect to surgical approach, no significant differences were observed in the proportions of B cells, T cells, and $CD16^{-}$ NK cells. However, $CD16^{+}$ NK cells were found more abundantly at 48 hours' post-CPB in the sAVR group (Figure 6, *B*).

Surgical Approach Can Influence the Concentration of Cytokines and MMPs That Are Present in the Pericardial Space Postoperatively

Samples from patients who underwent sAVR were compared with patients who underwent RAMT-AVR to determine whether surgical approach can influence the postoperative concentrations of pericardial cytokines (Figure E2, *A*). The only significant difference was in the concentration of IL-8, which was higher in the RAMT-AVR group at 4 hours' post-CPB. With respect to the concentrations of MMPs, MMP-1 was higher in the patients who underwent sAVR at 4 and 48 hours' post-CPB, while MMP-10 was greater in the sAVR group at 4 hours' post-CPB and MMP-12 was higher in these patients at 48 hours after surgery (Figure E2, *B*). No significant differences were noted between sAVR versus RAMT-AVR and postoperative pericardial concentrations of TIMPs (Figure E2, *C*).

The $MMP_{tot}/TIMP_{tot}$ ratio was also compared with respect to surgical approaches, and no difference was seen (Figure E3, first panel). Regarding specific MMPs, MMP-$2/TIMP_{tot}$ was significantly higher in the sAVR cohort at 48 hours' post-CPB. Surgical approach did not appear to significantly influence the ratio of other MMPs (Figure E3).

DISCUSSION

During cardiac surgery, to access the heart and great vessels, the pericardium is opened, and native pericardial fluid is evacuated. This results in a significant perturbation of the

pericardial cavity, which is otherwise a homeostatic environment. The postoperative pericardial space may be repopulated by immune/inflammatory factors, such as cytokines, that predispose the heart to POAF and PPS. Such factors may also drive the formation of postsurgical pericardial adhesions and pericardial constriction. Circulating immune/inflammatory mediators have been shown to contribute to POAF.[9,15] Some inflammatory factors have also been found in the pericardial cavity after surgery.[28,30] However, there is a paucity of data on the composition of immune mediators that may be present in this space postoperatively. Moreover, to date, no study has explored the influence of surgical approaches on postsurgical pericardial inflammatory profiles. This study has 3 novel findings: (1) we provide a comprehensive profiling of the immune/inflammatory cells and mediators that are present in the pericardial space after surgery; (2) we elucidate how the levels of these immune/inflammatory cells and markers change over the first 2 days after surgery; and (3) we offer insight into how surgical approach can impact this profile.

Clinical Significance and Implications

The systemic inflammatory response after cardiac surgery has been extensively studied.[16-26] It is generally accepted that cardiopulmonary bypass and ischemia–reperfusion injury plays a major role in activating systemic inflammatory cascades. To date, little attention has been given to the local immune-mediated inflammation that occurs postoperatively. A better understanding of this local response may explain the precise mechanisms that drive POAF, postsurgical pericardial adhesion formation, PPS, and pericardial constriction, which are common complications associated with cardiac surgery. The present study is clinically relevant for 3 main reasons. First, we provide a comprehensive description of the local inflammatory response that takes place in the pericardial space after surgery. To do so, we identify the immune cells that are present in the cavity and quantify the concentration of cytokines, MMPs, TIMPs, and TGFβ. Importantly, we also demonstrate that the composition of native pericardial fluid immune cells before surgery is drastically different than its composition after surgery. Second, we outline how the concentration of these immune cells and inflammatory factors change with time after surgery. Third, we perform a subpopulation analysis and compare the local inflammatory response between the 2 different surgical approaches. Preliminary findings suggest that a minimally invasive surgical approach can affect the composition and kinetics of the immune response for certain factors but does not inhibit it. Since native pericardial fluid is routinely removed during cardiac surgery, this study offers insight into the inflammatory response that takes place in the pericardial space after pericardiotomy and cardiac surgery. Figure 7 summarizes the design and main findings of the study. This study confirms that, regardless of surgical approach, there is a local postoperative inflammatory response. Indeed, our work can be used to (1) appreciate the presence and nature of the inflammatory response that occurs in the pericardial space after conventional and minimally invasive cardiac surgery, and (2) to build upon in future work to determine whether the inflammatory mediators that populate the pericardial space after surgery contribute to POAF, postsurgical pericardial adhesions, and pericardial adhesions. Given the importance of these common complications, and their association with inflammation, we hope this study provides a detailed description of the local inflammatory response postcardiotomy. Follow-up studies with larger sample size and longer follow-up may reveal a link between specific postsurgical pericardial inflammatory mediators and the aforementioned complications. Moreover, building on our study, future work can (1) better delineate whether pericardial handling in conventional cardiac surgery or through minimally invasive approaches perturb the local postoperative inflammatory response to an extent that influences the incidence of the aforementioned postsurgical complications, and (2) determine whether postoperative factors, such as duration of mechanical ventilation, can impact the pericardial inflammatory profile.

Postoperative Pericardial Immune Cells

In the present study, for the first time, we provide a comprehensive description of the immune cells that repopulate the pericardial space after cardiac surgery. We show that neutrophils are the predominant cell type that is present in this space after an operation. This contrasts with native pericardial fluid, which has negligible levels of neutrophils. The heavy influx of neutrophils in the pericardial space is likely due to a combination of local surgical bleeding and active recruitment. Interestingly, neutrophil levels were lower in patients who underwent RAMT-AVR when compared with the sAVR cohort. This may be a consequence of less postsurgical bleeding in the RAMT-AVR group, as demonstrated by the smaller volume of postoperative fluid that was present in the pericardial drains in this cohort. Neutrophils are known as important sources of proteases, reactive oxygen species, neutrophil extracellular traps that is effective in neutralizing pathogens but can also contribute to inadvertent tissue damage. However, an evolving view is that neutrophils can use these effector mechanisms and phagocytic capacity to promote the healing process through promotion of extracellular matrix (ECM) remodeling, angiogenesis, and cellular modulation.[31-33] Although beyond the current scope of our study, determining the direct contributions of neutrophils to the postsurgical pericardial/cardiac remodeling warrants further investigation. In contrast, the predominant immune cells that populate the native pericardial fluid including macrophages and T cells, were found at

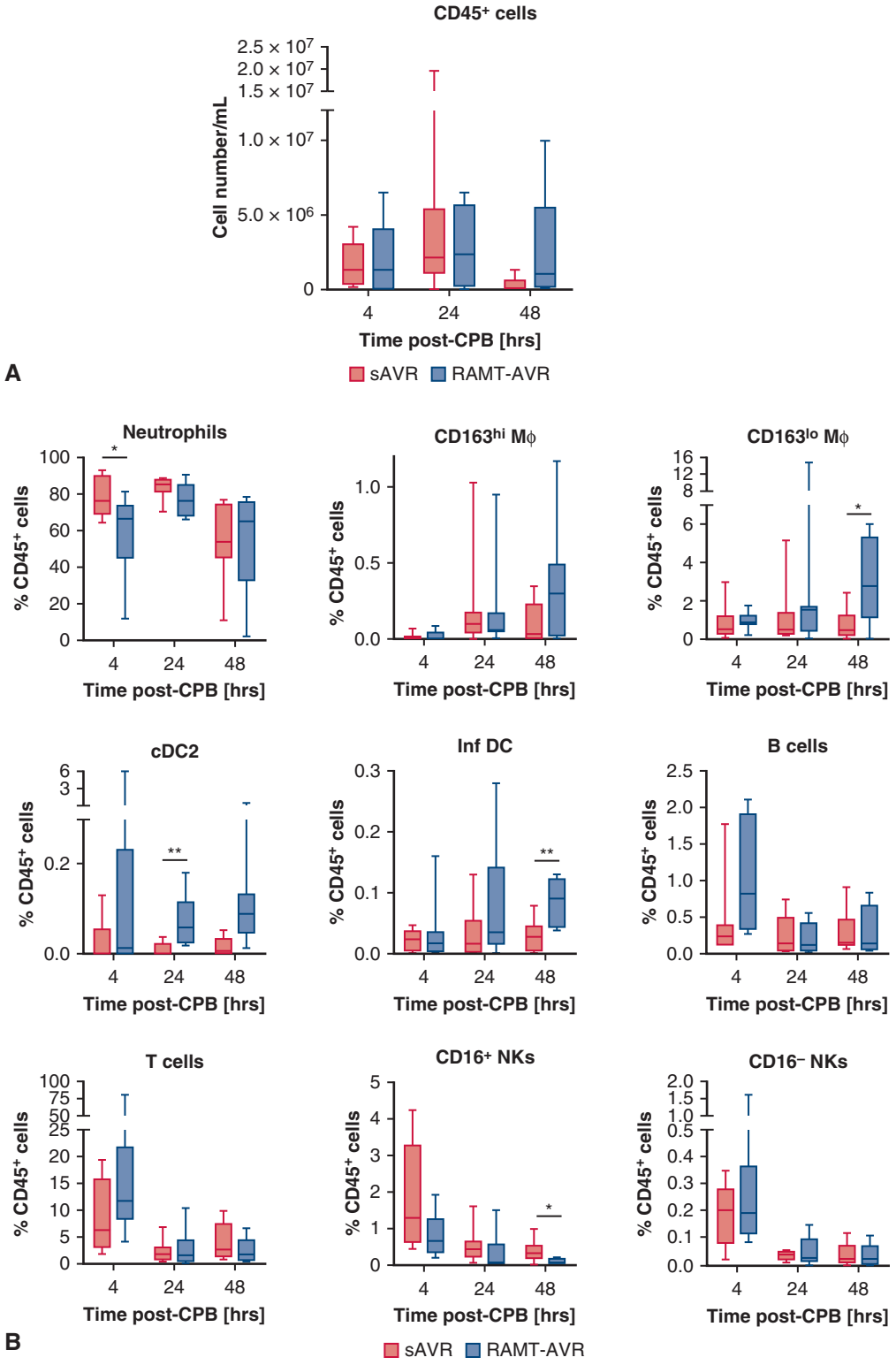

FIGURE 6. sAVR and RAMT-AVR elicit differences in the post-operative pericardial immune cell profile. A, Comparison of $CD45^{+}$ cell number per milliliter of postsurgical pericardial fluid in sAVR versus RAMT-AVR at 4, 24, and 48 hours' post-CPB. For statistical analysis an unpaired Student *t*-test was performed to determine differences between sAVR and RAMT-AVR at each time point. *Box and whisker plots* indicate median with minimum ± maximum. The *lower and upper borders of the box* represent the lower and upper quartiles (25th percentile and 75th percentile). The *middle horizontal line* represents the median. The *lower and upper whiskers* represent the minimum and maximum values of nonoutliers. B, Proportional quantifications of immune cell

significantly lower levels in the pericardial cavity postoperatively. This indicates that the immune cell compartment surrounding the heart dramatically changes following cardiac surgery and these resident cells are not re-established in the space within the first 2 days postsurgery. With respect to the postoperative immune cells that populate the pericardial space, it is important to emphasize that, although the proportion of immune cells may be similar between sAVR and RAMT-AVR, the significant difference that exists in the volume of post-surgical pericardial fluid in these patients should not be neglected. Given that numerous clinical studies have found MICS to be associated with lower rates of POAF,[34-37] future research with larger samples sizes, should assess whether the higher volume of postoperative pericardial fluid correlates with immune cell densities and clinical outcomes.

Postsurgical Pericardial Cytokines

We also assayed postoperative pericardial fluid for common pro- and anti-inflammatory cytokines with respect to time points after surgery and operative approaches. IL-6 levels were highest in the pericardial space postoperatively. IL-6 plays an important role in rapidly inducing acute inflammatory phase proteins such as C-reactive protein, serum amyloid A, fibrinogen, haptoglobin, and alpha-1-antichymotrypsin.[38] Interestingly, there were no statistically significant differences in the pericardial levels of IL-6 at the specific time points after surgery. IL-8 was also highly expressed in the postsurgical pericardial space, where its levels decreased significantly over time. It is important to highlight that, within the first 48 hours post-CPB, surgical approach did not elicit any differences in the pericardial concentrations of the other cytokines that were probed. Taken together, this would suggest that local surgical manipulation is most likely responsible for the cytokine response independent of incision made on the skin.

Postoperative Pericardial Concentration of MMPs and TGFβ

As mentioned, the postsurgical pericardial space is an altered environment. It is likely that pro- and anti-inflammatory pathways influence postoperative pericardial changes via different mechanisms. MMPs and TIMPs are major regulators of ECM remodeling and have been implicated in many cardiovascular diseases, such as coronary artery disease, aneurysm formation, and myocardial infarction.[39] Thus, we were interested to explore how the pericardial levels of MMPs and TIMPs change over time after an operation, and whether surgical approach can affect their levels.

We noted that the concentration of MMP-9 and MMP-8 were higher in the pericardial space after surgery. This can be explained by the neutrophils that are present in the pericardial space postoperatively since neutrophils express and secrete both MMP-8[40,41] and MMP-9.[42] Interestingly, although pericardial MMP-8 concentrations increased significantly post-CPB, pericardial MMP-9 concentrations reduced over the same time period. The reduction in MMP-9 may be explained by the lack of postoperative pericardial macrophages as they are predominantly responsible for MMP-9 expression.[43]

With respect to surgical approaches, there were significant differences in MMP-1, MMP-10, and MMP-12 levels. It is also intriguing to note that the kinetics of these differences vary for the different MMPs. Although probably multifactorial, there are 2 reasons that can potentially explain these differences. First, the higher amount of surgical bleeding that occurs in the patients who undergo sAVR compared with the RAMT-AVR group. Second, given their proportional representation in the pericardial space after surgery, neutrophils likely drive some of the differences we observe in the levels of MMPs. This is in keeping with what has been found in literature, where neutrophils are considered the main cellular source producing MMPs.[44]

While there were no differences between patients undergoing sAVR and RAMT-AVR (data not shown), we established the presence of TGFβ in the pericardial space after surgery. This is an important finding, since many studies have shown that TGFβ plays a critical role in inflammation and ECM remodeling.[45-47]

Postsurgical Pericardial TIMPs and the Ratio of Postoperative Pericardial MMPs to TIMPs

A family of 4 members, TIMPs regulate ECM degradation and remodeling by inhibiting proteolytic factors; inhibiting MMPs; and inhibiting angiogenesis by antagonizing vascular endothelial growth factor.[48-50] Thus, it is

populations at 4, 24, and 48 hours' post-CPB comparing sAVR versus RAMT-AVR, including neutrophils (*top row left*), macrophages with CD163^{hi} Mϕ (*top row middle*) and CD163^{lo} Mϕ (*top row right*), dendritic cells with cDC2 (*middle row left*) and Inf DC (*middle row middle*), B cells (*middle row right*), T cells (*bottom row left*), and NK cells with CD16^{+} NKs (*bottom row middle*) and CD16^{-} NKs (*bottom row right*). Each comparison includes n = 8 for sAVR and n = 8 for RAMT-AVR. For statistical analysis, an unpaired Student *t*-test was performed to determine differences between sAVR and RAMT-AVR at each time point. *Box and whisker plots* indicate median with minimum ± maximum. The *lower and upper borders of the box* represent the lower and upper quartiles (25th percentile and 75th percentile). The *middle horizontal line* represents the median. The *lower and upper whiskers* represent the minimum and maximum values of nonoutliers. *$P \leq .05$. **$P \leq .01$ for sAVR versus RAMT-AVR. *CD*, Cluster of differentiation; *sAVR*, conventional full median sternotomy surgical aortic valve replacement; *RAMT-AVR*, right anterior mini-thoracotomy aortic valve replacement; *CPB*, cardiopulmonary bypass; *Mϕ*, macrophage, *cDC2*, classical dendritic cell type 2; *Inf DC*, inflammatory dendritic cell; *NK*, neutral killer cell.

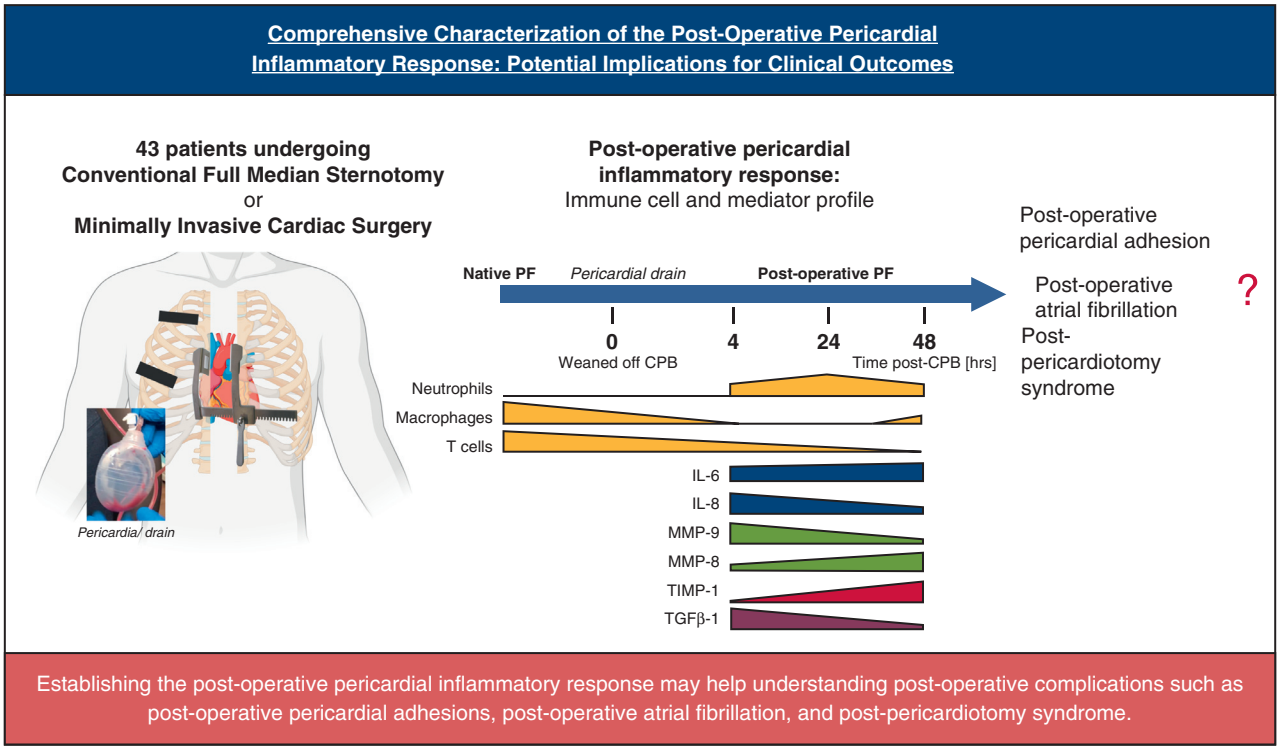

FIGURE 7. Forty-three patients undergoing cardiac surgery were enrolled in the study. After removing each patient's native pericardial fluid, a drain was placed in the pericardial space to allow for collecting postoperative pericardial fluid. The collected fluid was analyzed for immune cells and inflammatory mediators, including cytokines, matrix metalloproteinases, tissue inhibitors of metalloproteinases, and growth factors. Furthermore, to determine how pericardial handling affects the local postoperative inflammatory response, postoperative pericardial fluid was compared between patients undergoing conventional aortic valve replacement and right anterior mini thoracotomy aortic valve replacement. The local postoperative inflammatory profile may have important clinical implications, where certain factors can contribute to postoperative atrial fibrillation, postsurgical pericardial adhesion formation, and post-pericardiotomy syndrome. *PF*, Pericardial fluid; *CPB*, cardiopulmonary bypass; *IL*, Interleukin; *MMP*, matrix metalloproteinase; *TIMP*, tissue inhibitor of metalloproteinase; *TGFβ*, transforming growth factor beta.

important to assess whether their postoperative levels in the pericardial space change over time and with respect to surgical approach. We were able to show that TIMP-1, TIMP-2, and TIMP-4 are present in the pericardial space after surgery, where their concentrations increased over time after surgery, suggesting an active local inflammatory process.

Moreover, the balance between MMPs and TIMPs is an important determinant of ECM regulation and dysregulation, where alterations in their ratio may contribute to different pathologies.[51] Thus, we asked whether the ratio between postoperative pericardial MMPs and TIMPs varied over time and with respect to the surgical approaches. Interestingly, when we compared between MMP_{tot} and $TIMP_{tot}$, we found that $MMP_{tot}/TIMP_{tot}$ levels significantly decreased in the pericardial space over time after surgery. It is interesting that the ratio was not affected by the surgical approach. This would suggest an induction of TIMP-mediated anti-inflammatory processes in the pericardial cavity that was independent from the surgical approach. We then considered the ratio between individual MMPs and TIMPs but focused on the most abundantly expressed MMPs and $TIMP_{tot}$. Except for MMP-3/$TIMP_{tot}$, the other ratios all decreased significantly after surgery.

Limitations

Although this study provides original insights into the postsurgical local inflammatory response, there are a few limitations. First, for the patients who underwent RAMT-AVR, we were able to collect postoperative pericardial fluid only up to 48 hours' post-CPB, as these patients were otherwise ready for discharge. Second, to better assess for differences in the incidence of POAF, more patients are being enrolled in an ongoing study. We will be enrolling a total of 100 patients (50 in each group), which will provide enough power to assess for statistical significance. Finally, since this study focuses on reporting the early cellular and molecular changes that occur in the pericardial space, intermediate- and long-term clinical outcomes have not been collected.

CONCLUSIONS

It is well known that cardiac surgery, especially by the way of the cardiopulmonary pulmonary bypass machine,

induces a robust systemic inflammatory response. Such a response has been shown to be associated with adverse outcomes, including delirium and end-organ injury. Nevertheless, to date, literature has yet to fully characterize the local inflammatory response after cardiac surgery. In this prospective pilot clinical study, we have comprehensively characterized the postsurgical pericardial inflammatory profile. We have also considered how this profile evolves over the first 48 hours after surgery. Finally, to query whether pericardial handling affects the composition of the postsurgical inflammatory profile, we have compared between conventional and minimally invasive surgical approaches. Although this is a relatively small study, our findings offer insight into the inflammatory changes that occur in the pericardial space after surgery. Our observations can be extrapolated in future studies to better understand whether postoperative pericardial inflammatory mediators contribute to complications common to cardiac surgery, such as POAF and PPS. The ultimate clinical application of this study would be to identify such markers and target them in a precise and personalized manner to reduce the incidence of such complications and improve clinical outcomes for patients undergoing cardiac surgery.

Conflict of Interest Statement

The authors reported no conflicts of interest.

The *Journal* policy requires editors and reviewers to disclose conflicts of interest and to decline handling or reviewing manuscripts for which they may have a conflict of interest. The editors and reviewers of this article have no conflicts of interest.

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Key Words: inflammation, pericardial space, postoperative pericardial fluid, conventional cardiac surgery, minimally invasive cardiac surgery

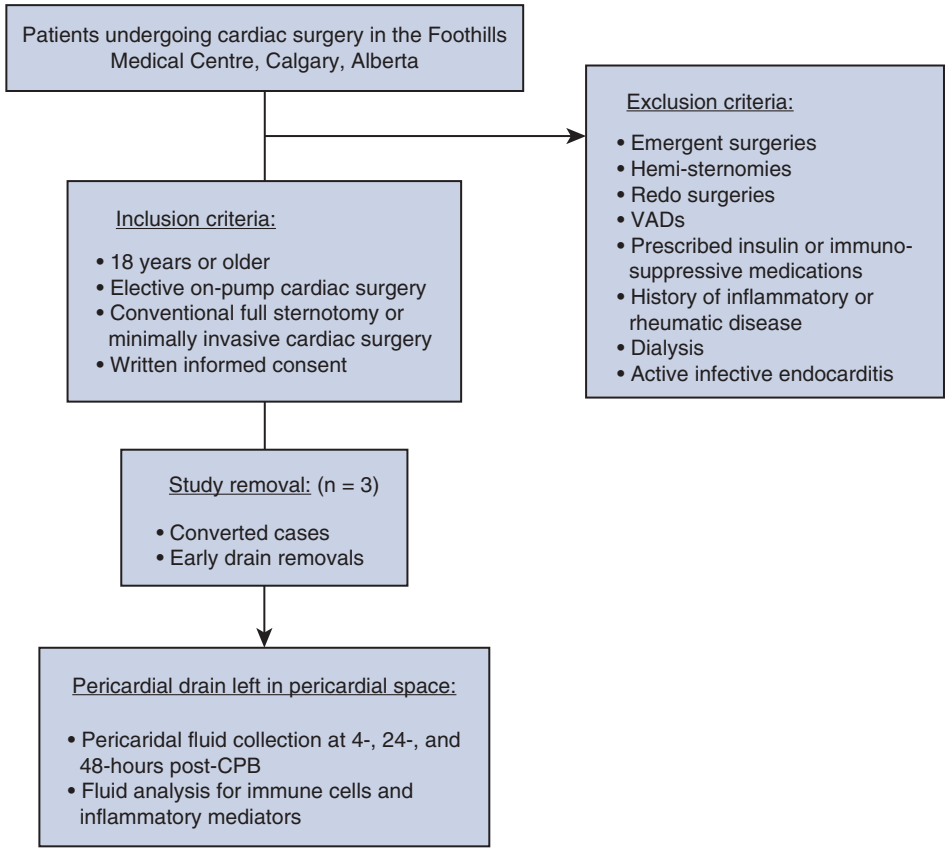

FIGURE E1. Flowchart depicting the patient selection process as per Strengthening the Reporting of Observational Studies in Epidemiology recommendation. *VADs*, Ventricular assist devices; *CPB*, cardiopulmonary bypass.

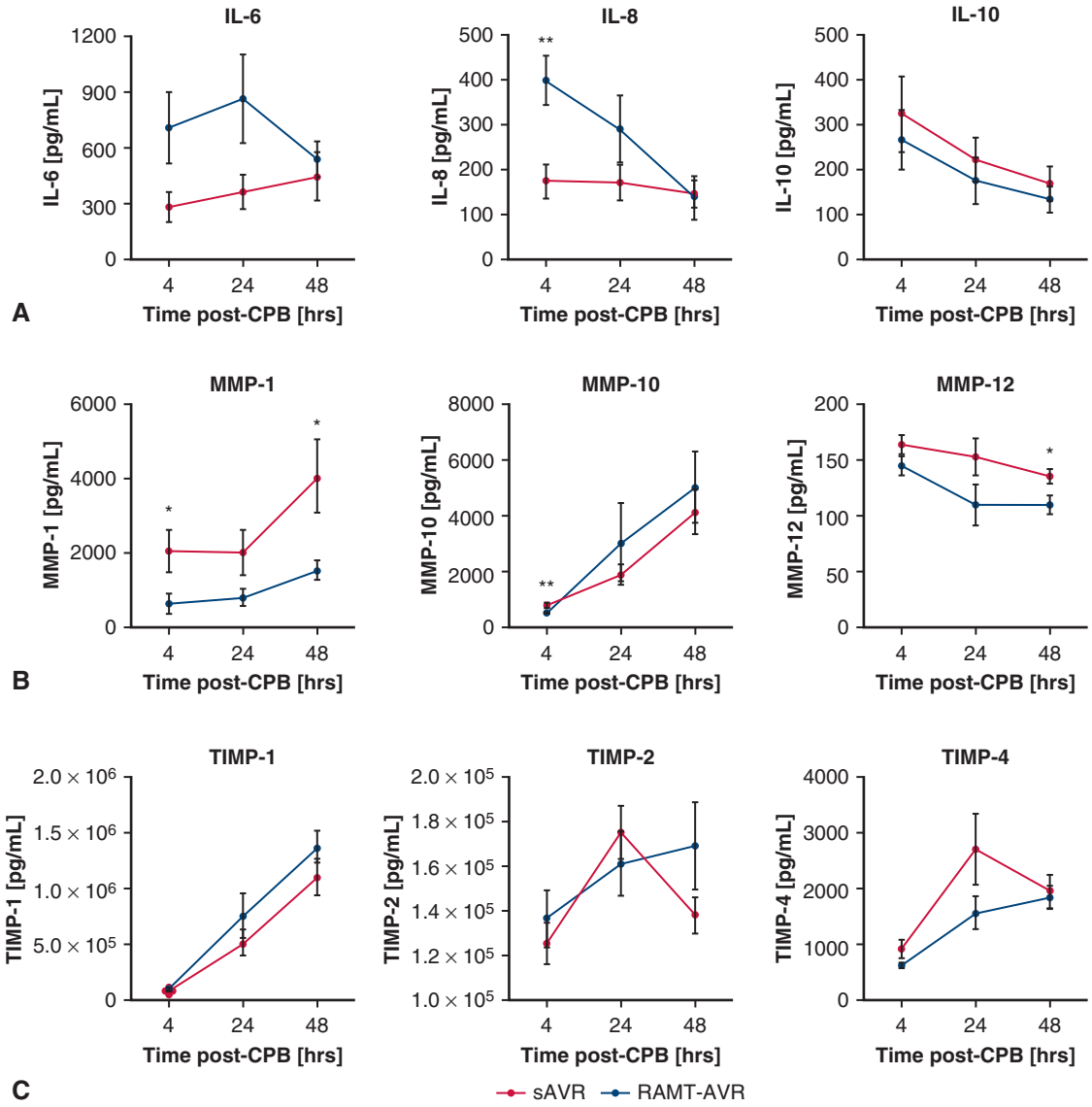

FIGURE E2. Comparison between sAVR and RAMT-AVR and the concentrations of cytokines, MMPs, and TIMPs. A, Comparison of the concentration of 3 cytokines in sAVR versus RAMT-AVR groups at 4, 24, and 48 hours' post-CPB. Cytokines being presented include IL-6 (*left*), IL-8 (*middle*), and IL-10 (*right*) where concentrations are shown in pg/mL. Each panel represents n = 8 for sAVR and n = 8 for RAMT-AVR. For statistical analysis, an unpaired Student *t*-test was performed to determine differences between sAVR and RAMT-AVR at each time point. *Lines* represent mean ± standard error. $^{**}P \leq .01$. B, Comparison of MMP levels in sAVR versus RAMT-AVR at 4, 24, and 48 hours post-CPB. MMP-1 (*left*), MMP-10 (*middle*), and MMP-12 (*right*) are shown where concentrations are shown in pg/mL. MMP-1 (*left*) and MMP-10 (*middle*) represent n = 8 for sAVR and n = 8 for RAMT-AVR, while MMP-12 (*right*) shows n = 5 for sAVR and n = 4 for RAMT. For statistical analysis, an unpaired Student *t*-test was performed to determine differences between sAVR and RAMT-AVR at each time point. *Lines* represent mean ± standard error. $^{*}P \leq .05$. C, Comparison of TIMP-1 (*left*), TIMP-2 (*middle*), and TIMP-4 (*right*) concentration levels in postsurgical pericardial fluid in sAVR versus RAMT-AVR at 4, 24, and 48 hours' post-CPB. Each panel represents n = 8 for sAVR and n = 8 for RAMT-AVR. For statistical analysis, an unpaired Student *t*-test was performed to determine differences between sAVR and RAMT-AVR at each time point. *Lines* present mean ± standard error. *IL*, Interleukin*s*; *CPB*, cardiopulmonary bypass; *MMP*, matrix metalloproteinase; *TIMP*, tissue inhibitor of metalloproteinase; *sAVR*, conventional full median sternotomy surgical aortic valve replacement; *RAMT-AVR*, right anterior mini-thoracotomy aortic valve replacement.

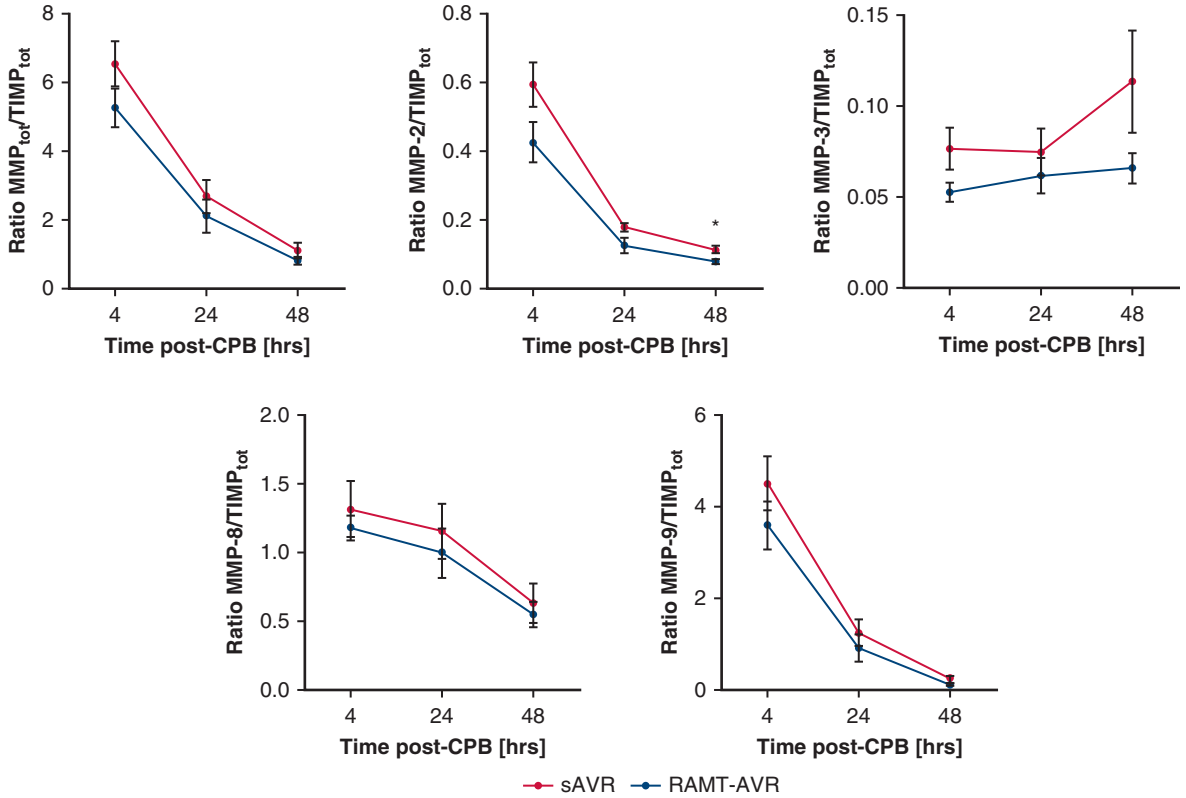

FIGURE E3. Assessment of postoperative pericardial MMP/TIMP ratios in patients undergoing sAVR versus RAMT-AVR. Quantitative comparison of postsurgical pericardial MMP/TIMP ratios at 4-, 24-, and 48-hours post-CPB with respect to surgical approach. Analysis and comparison of MMP_{tot} to $TIMP_{tot}$ ratio when considering sAVR versus RAMT-AVR (*left*), MMP-2/$TIMP_{tot}$ (*second left*), MMP-3/$TIMP_{tot}$ (*middle*), MMP-8/$TIMP_{tot}$ (*second right*), and MMP-9/$TIMP_{tot}$ (*right*). Comparisons represent n = 8 for sAVR and n = 8 for RAMT-AVR. For statistical analysis unpaired Student t-test was performed to determine differences between sAVR and RAMT-AVR at each time-point. Lines present mean ± standard error. *$P \leq .05$. *MMP*, Matrix metalloproteinase; *TIMP*, tissue inhibitor of metalloproteinase; *sAVR*, conventional full median sternotomy surgical aortic valve replacement; *RAMT-AVR*, right anterior mini thoracotomy aortic valve replacement; *CPB*, cardiopulmonary bypass; $_{tot}$, total.

TABLE E1. Reagents and resources

Reagent or resource	Source	Identifier
Antibodies		
eFluor450 anti-human CD3	Thermo Fisher Scientific	Cat#: 48-0037-42
BV510 anti-human CD45	BioLegend	Cat#: 304036
BV650 anti-human CD1c	BioLegend	Cat#: 331542
FITC anti-human CD163	BioLegend	Cat#: 333618
PE anti-human CD19	BioLegend	Cat#: 392506
PerCP-Cy5.5 anti-human CD14	BioLegend	Cat#: 367110
PE-Cy7 anti-human CD64	BioLegend	Cat#: 305021
APC anti-human CD56	BioLegend	Cat#: 362504
APC-Cy7 anti-human CD16	BioLegend	Cat#: 302018
Reagents		
GhostDye Red710 Viability Dye	Tonbo Biosciences	Cat#: 13-0871-T100
Anti-human FcγR Binding Inhibitor	Thermo Fisher Scientific	Cat#: 14-9161-73
Dextran powder	Spectrum Chemical	Cat#: 9004-54-0
123count eBeads Counting Beads	Thermo Fisher Scientific	Cat#: 01-1234-42
Compensation Beads	Invitrogen	Cat#: Q1-2222-42
Vacuette NH Sodium Heparin tubes	Greiner bio-one	Cat#: 456028 (6 mL) Cat#: 455051 (9 mL)
Software		
FlowJo10	Becton Dickinson & Company (BD)	www.flowjo.com
GraphPad Prism v9.0	GraphPad Software	www.graphpad.com
BioRender	BioRender	www.biorender.com
Adobe Illustrator 2022	Adobe	www.adobe.com/ca/products/illustrator

CD, Cluster of differentiation; *BV*, Brillian Violet; *FITC*, fluorescein isothiocyanate; *PE*, Phycoerythrin; *PerCP-Cy*, Peridinin chlorophyll protein-cyanine; *PE-Cy*, Phycoerythrin-cyanine; *APC*, Allophycocyanin; *APC-Cy*, Allophycocyanin-cyanine; *FcγR*, F_c gamma receptor; *NH*, Sodium Heparin.

TABLE E2. Intraoperative details for all patients

	n = 43
Coronary artery bypass grafts	
×2	2
×3	11
×4	1
Valve replacements	
Aortic valve	16
Mitral valve	1
Valve repairs	
Aortic valve	0
Mitral valve	8
ASD patch	3
Cardiopulmonary bypass time, min, mean ± SD	100.8 ± 37.6
Aortic crossclamp time, min, mean ± SD	78.9 ± 31.7

ASD, Atrial septal defect; *SD*, standard deviation.