Cardiac surgery elicits pericardial inflammatory responses that are distinct compared with postcardiopulmonary bypass systemic inflammation

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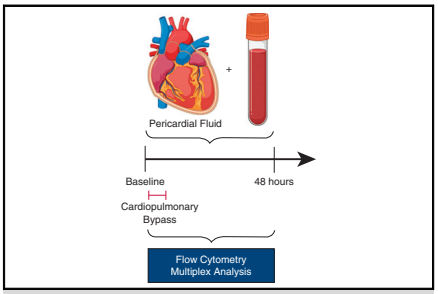
ABSTRACT

Objectives: Cardiac surgery using cardiopulmonary bypass contributes to a robust systemic inflammatory process. Local intrapericardial postsurgical inflammation is believed to trigger important clinical implications, such as postoperative atrial fibrillation and postsurgical intrathoracic adhesions. Immune mediators in the pericardial space may underlie such complications.

Methods: In this prospective pilot clinical study, 12 patients undergoing isolated coronary artery bypass graft surgery were enrolled. Native pericardial fluid and venous blood samples (baseline) were collected immediately after pericardiotomy. Postoperative pericardial fluid and venous blood samples were collected 48-hours after cardiopulmonary bypass and compared with baseline. Flow cytometry determined proportions of specific immune cells, whereas multiplex analysis probed for inflammatory mediators.

Results: Neutrophils are the predominant cells in both the pericardial space and peripheral blood postoperatively. There are significantly more CD163^{lo} macrophages in blood compared with pericardial effluent after surgery. Although there are significantly more CD163^{hi} macrophages in native pericardial fluid compared with baseline blood, after surgery there are significantly fewer of these cells present in the pericardial space compared with blood. Postoperatively, concentration of interleukin receptor antagonist 6, and interleukin 8 were significantly higher in the pericardial space compared with blood. After surgery, compared with blood, the pericardial space has a significantly higher concentration of matrix metalloproteinase 3, matrix metalloproteinase 8, and matrix metalloproteinase 9. The same trend was observed with transformational growth factor β.

Conclusions: Cardiac surgery elicits an inflammatory response in the pericardial space, which differs from systemic inflammatory responses. Future work should determine whether or not this distinct local inflammatory response contributes to postsurgical complications and could be modified to influence clinical outcomes. (JTCVS Open 2023;16:389-400)



Baseline and postoperative pericardial fluid and blood was probed for inflammatory factors.

CENTRAL MESSAGE

The pericardial postoperative inflammatory response is distinct compared with post-CPB systemic inflammation in patients undergoing CABG surgery.

PERSPECTIVE

Postoperative atrial fibrillation, postpericardiotomy syndrome, and postsurgical adhesions are associated with cardiac surgery. Local postsurgical inflammation may drive some of these complications. Determining whether or not the local postoperative inflammatory response differs from systemic inflammation may offer clues that can help us better understand these complications to prevent them.

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Ethical Statement: This study was approved by the Conjoint Health Research Ethics Board at the University of Calgary underlying the Declaration of Helsinki (Ethics ID: REB16-1906, approved 12 February 2021).

Informed Consent Statement: Patients enrolled in this study provided written informed consent to be included in the study and to have findings published in a peer-reviewed manuscript.

Drs Hassanabad and Schoettler contributed equally to this article.

Abbreviations and Acronyms

ACS = acute coronary syndrome
CABG = coronary artery bypass graft
CD = cluster of differentiation
CPB = cardiopulmonary bypass
IFN = interferon
IL = interleukin
IL-1Ra = interleukin 1 receptor antagonist
MCP-1 = monocyte chemoattractant protein-1
MMP = matrix metalloproteinase
NK = natural killer
PAOF = post-operative atrial fibrillation
PF = pericardial fluid
PPS = postpericardiotomy syndrome
TGFβ = transformation growth factor β
TIMP = tissue inhibitor of metalloproteinases
TNF-α = tumor necrosis factor-alpha

Postoperative atrial fibrillation (POAF)[1] and postpericardiotomy syndrome (PPS)[2] are common early complications after cardiac surgery, whereas postsurgical pericardial adhesions can have major implications for redo cardiac surgery.[3] Although inflammation has been shown to drive these processes,[3-11] to date we lack a clear understanding of their pathophysiology, resulting in limited therapeutic options. Moreover, several studies demonstrate that the cardiopulmonary bypass (CPB) machine elicits a robust systemic inflammatory response.[10-20] This inflammatory response, which is characterized by immune-mediated pathways, such as activation of the complement system, is associated with adverse clinical outcomes, including acute kidney injury, lung injury, and neurocognitive disorders.[16-18]

In addition to the systemic postoperative inflammatory response, it is important to elucidate the inflammatory processes that occur in the pericardial space after surgery, and to determine whether or not specific immune mediators that are present in this space contribute to the pathogenesis of POAF, PPS, and postsurgical pericardial adhesions. We previously showed that acute ischemia alters the immune cell profile of pericardial fluid.[21] Our observations suggest that the pericardial space is a dynamic environment that can respond to myocardial injury in the absence of invasive surgery. Our group and others have shown that inflammatory processes are active in the pericardial space after surgery.[22-25] We characterized the immune cells and inflammatory mediators, including cytokines, chemokines, matrix metalloproteinases (MMPs) and tissue inhibitors of matrix metalloproteinases (TIMPs), that are present in the pericardial space after cardiac surgery. We described how the postoperative pericardial inflammatory profile evolves over 48-hours after surgery. Finally, we determined whether the local postsurgical inflammatory response is different between conventional cardiac surgery and minimally invasive cardiac surgery.

Establishing the local postoperative inflammatory response may provide a therapeutic opportunity where specific markers can be targeted in a precise manner to reduce the incidence of POAF, PPS, and pericardial adhesions. An inflammatory response has been shown to be present in the pericardial space after surgery.[22,26] However, few studies have comprehensively compared the local versus systemic postoperative inflammatory response in patients undergoing cardiac surgery. Thus, most treatment strategies have focused on circulating immune mediators. The objective of the present study is to evaluate whether or not a difference exists between the local and systemic postoperative inflammatory response in patients undergoing coronary artery bypass graft (CAGB) surgery. We demonstrate that the local postoperative inflammatory response is distinct compared with post-CPB systemic inflammation. These novel findings offer insights that can be used to assess whether specific postsurgical pericardial factors can result in immune-driven complications and validate if targeting such mediators may reduce the incidence of those complications and improve clinical outcomes.

METHODS

Patient Sample Acquisition

Twelve patients undergoing isolated CABG surgery at Foothills Medical Centre were prospectively enrolled in the study after providing written informed consent for publication of study data (Figure 1). 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 and venous blood samples were obtained from all 12 patients. Baseline samples were collected before the institution of CPB and the administration of steroids. A drain was left in the pericardial space to facilitate collecting pericardial fluid after surgery, whereas venous blood was drawn from a venipuncture. Inclusion criteria were age older than 18 years and patients undergoing CABG surgery through a conventional full median sternotomy. We excluded patients who received insulin or immune-suppressive medications; patients with a

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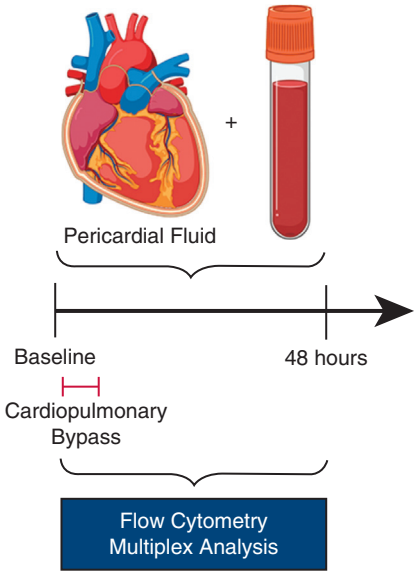

FIGURE 1. Baseline and postoperative pericardial fluid and blood was probed for inflammatory factors. Created with BioRender.com.

history of inflammatory or rheumatic disease; patients who required dialysis; patients with active infectious endocarditis; and those who underwent emergency surgery, redo surgery, or hemisternotomy cardiac surgery. Intraoperatively, the pericardium was incised at full length anteriorly and teed off with creation of the pericardial cradle. In all patients, native pericardial fluid was completely removed after pericardiotomy. The pericardium was left open at the conclusion of the surgery. Moreover, all cases were performed by administering cold blood cardioplegia to arrest the heart and establishing CPB via conventional central cannulation. Postsurgical pericardial fluid and venous blood was collected at 48 hours after the patient was weaned off CPB. Therefore, 4 samples were collected from each patient: native pericardial fluid, baseline venous blood, postoperative pericardial fluid, and postoperative venous blood. After collection, all fluid samples were transported on ice to the laboratory in a sodium heparin tube (Greiner Bio-One) and immediately processed.

Cell Isolation and Flow Cytometry

Native pericardial fluid samples were immediately filtered through a 40 μm Nylon cell strainer (Falcon) and centrifuged at 1500 rpm for 5 minutes at 4 °C. Acellular components were stored at −80 °C for further analysis. Venous blood and postoperative pericardial fluid 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 to allow for red blood cell sedimentation. After incubation, the cellular components were filtered through a 40-μm cell strainer (Falcon) and centrifuged at 1500 rpm for 5 minutes at 4 °C. We processed the cell pellets for flow cytometry and blocked 1 million cells with a human $F_c\gamma R$ binding inhibitor (eBioscience) and incubated with GhostDye Red 710 viability dye (TONBO Biosciences) for 20 minutes. Cell staining was performed for 20 minutes with specific markers (Table E1). After fixation, samples were run on a FACS Canto flow cytometer and analyzed with FlowJo10 Software (Becton Dickinson & Company). Cell counts were conducted with counting beads (Thermo Fisher Scientific) and calculated by normalization to the individual sample run times in the flow cytometer. Specific immune cell quantifications are presented as percentage of cluster of differentiation (CD) 45^+ cells. Individual immune cell gating strategies are shown in Table E2.

TABLE 1. Baseline characteristics for all patients

Baseline patient characteristics (n = 12)	Result
Age (y)	62 ± 8.5
Sex	
Female	2
Male	10
Coronary artery disease	11
Hypertension	10
Diabetes mellitus type II	8
Dyslipidemia	10
Active smoking history	2
Positive Family History	1
Long-standing persistent atrial fibrillation	0
Renal disease*	1
Lung disease, any type	3
Peripheral arterial disease	1
Previous stroke	0
Cardiovascular event	0
Chronic steroid use	0
Immune-suppressed	0
Preoperative aspirin use	8
Preoperative nonsteroidal anti-inflammatory drug use	1
Orthopnea	0
Presyncope	0
Syncope	0
New York Heart Association functional class dyspnea	
Class I	2
Class II	1
Class III	0
Class IV	0
Preoperative left ventricle dysfunction by echocardiography	
Mild	4
Moderate	1
Severe	0

Values are presented as mean ± SD or n. *Stage III or worse, estimated glomerular filtration rate <60.

Cytokines, Matrix Metalloproteinases, Tissue Inhibitors of Metalloproteinases, and Transformation Growth Factor-β

Concentrations of cytokines, matrix metalloproteinases (MMPs), tissue inhibitors of metalloproteinases (TIMPs), and transformation growth factor β (TGFβ) in fluid supernatants and plasma were measured with blinded multiplex analysis (EveTechnologies, Calgary, Canada). Interleukin (IL)-1β, IL-1 receptor antagonist (IL-1Ra), IL-5, IL-6, IL-8, IL-10, IL-13, interferon-gamma (IFN)-γ, tumor necrosis factor alpha (TNF-α), and monocyte chemoattractant (MCP)-1 were included in our assays. Further, we probed for MMP-1, MMP-2, MMP-3, MMP-7, MMP-8, MMP-9, MMP-10, MMP-12, and MMP-13. We also assessed for TIMP-1, TIMP-2, and TIMP-4. Finally, we also probed for TGFβ-1 and TGFβ-2 concentrations.

TABLE 2. Intraoperative details for all patients (n = 12)

Intraoperative details	Result
No. of coronary artery bypass grafts*	
2	2
3	9
4	1
Cardiopulmonary bypass time (min)	91.83 ± 23.66
Aortic crossclamp time (min)	75.00 ± 19.49

Values are presented as n or mean ± SD. *All patients had left internal thoracic artery and saphenous vein grafts harvested and used.

Statistical Analysis

Data are shown in box and whisker plots as median with minimum ± maximum. Patient characteristics are expressed as mean ± SD where appropriate. Unpaired Student *t* test was performed under assumption of Gaussian distribution and the same SD to compare pericardial fluid (PF) and blood samples at each time point, respectively. Statistical analysis was conducted using GraphPad Prism 9 Software. The number of independent replicates is 12 for each individual comparison.

RESULTS

Patient Demographic Characteristics

Twelve patients were enrolled in the study, 10 were men, and the mean age was 62.0 ± 8.5 years. Ten patients had hypertension, 8 had diabetes, and 10 had dyslipidemia. All patients presented with acute coronary syndrome (ACS) and underwent CABG surgery via a full median sternotomy approach. All patients had their operation within 12 days of being admitted with an ACS event. One of the patients enrolled had prior documented myocardial infarction (in 2014), none were actively prescribed steroids or immunosuppressive medications, or had contracted COVID-19 in the preceding 3 months. Baseline patient characteristics are summarized in Table 1. The surgeries were performed by using CPB and an aortic crossclamp with cardioplegic arrest was applied in all cases. The internal thoracic artery was harvested in all cases, and the number of bypass grafts varied from 2 to 4. Intraoperative details are summarized in Table 2. Whereas 3 patients experienced new onset POAF, there were no deaths or major neurological events. In all patients, protamine sulfate was the only agent given to reverse heparin upon weaning from the CPB machine. Given bleeding was not an issue, no systemic or local hemostatic agents were used. There was no incidence of postoperative bleeding necessitating the transfusion of blood products. Postsurgical pain control was achieved by intravenous opioids in all patients while in the cardiovascular intensive care unit and oral analgesics once they were transferred to the ward. Furthermore, all patients were encouraged to mobilize on postoperative day 1. There were no complications secondary to the placement of the pericardial drain. Postoperative findings are summarized in Table 3.

TABLE 3. Postoperative outcomes for all patients (N = 12)

Postoperative outcomes	Result
Death	0
Stroke	0
Dialysis	0
Wound infection	0
Prescribed aspirin	12
Readmission within 30 d of surgery	1
Permanent pacemaker implant	0
Postoperative atrial fibrillation	3

Postsurgical PF Adopts a Neutrophil-rich Profile

Four samples were collected from each patient and assayed for their immune cell composition, which was calculated as percentage of $CD45^+$ cells (Figure 2). The immune cells of interest included neutrophils, monocytes/macrophages, dendritic cells, natural killer (NK) cells, T cells, and B cells. Proportional quantification of these populations revealed dramatic changes in the pericardial fluid following surgery, adopting a similar neutrophilic profile observed in the systemic blood (Figure 2, *A*). Specifically at baseline, the pericardial space contains a higher proportion of $CD163^{hi}$ macrophages, NK cells, and T cells compared with the blood (Figure 2, *B*). Conversely, the baseline blood comprises a high neutrophil content that is absent in the pericardial space (Figure 2, *B*). However, at 48 hours post-CPB, a high neutrophil influx into the pericardial space led to matching profile with the systemic blood (Figure 2, *B*). In addition, systemic blood contained higher levels of $CD163^{hi}$ macrophages, $CD163^{lo}$ monocytes/macrophages, NK cells, B cells, and T cells at this time point compared with the pericardial space.

Surgery Stimulates Specific Local Proinflammatory and Anti-inflammatory Mediator Production in the Pericardial Space Postsurgery

To more comprehensively compare the local to systemic postoperative inflammatory response, the acellular portion of postsurgical pericardial effluent and plasma samples were assessed for the concentration of cytokines and chemokines (Figure 3). The postoperative pericardial space is populated with proinflammatory/Th1 (IL-6, IL-1β, TNF-α, and IFN-γ) and anti-inflammatory/Th2 (IL-1Ra, IL-10, IL-5, and IL-13) cytokines. Athough there was no significant difference in the pericardial versus systemic concentration of IL-1 β, TNF-α, and IFN-γ before and after surgery, the concentration of IL-6 was higher in the pericardial space compared with blood at baseline ($P = .003$) and further elevated after surgery ($P = .003$).

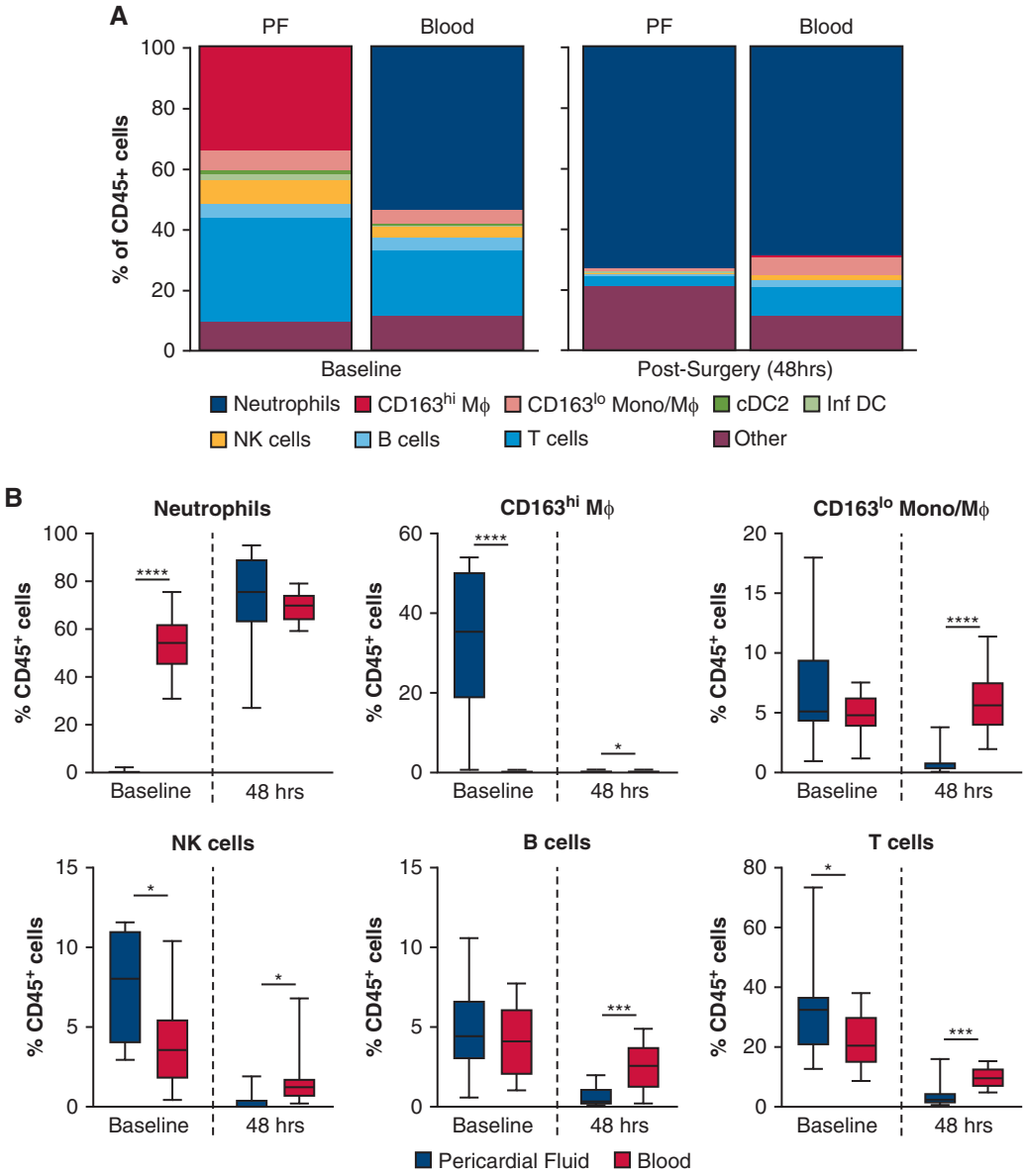

FIGURE 2. Comparison between the immune cells that populate the native pericardial space versus baseline blood, and postoperative pericardial fluid (*PF*) versus blood. Immune cells were detected with flow cytometry and are presented as percentage of $CD45^+$ cells. A, Proportional quantification of immune cells that are present at baseline (*left*) and 48 hours postsurgery (*right*) in PF and blood, respectively. Shown is the distribution of neutrophils, $CD163^{hi}$ macrophages (*$CD163^{hi}$ Mϕ*) and $CD163^{lo}$ monocytes/macrophages (*$CD163^{lo}$ Mono/Mϕ*), classical dendritic cells type 2 (cDC2), inflammatory dendritic cells (*Inf DC*), natural killer cells (*NK cells*), B cells, T cells, and others among $CD45^+$ cells. B, Neutrophils (*top left*), $CD163^{hi}$ Mϕ (*top middle*), $CD163^{lo}$ Mono/Mϕ (*top right*), NK cells (*bottom left*), B cells (*bottom middle*), and T cells (*bottom right*) represented at baseline and 48 hours postsurgery in PF (*blue*) and blood (*red*), respectively (n = 12 for each comparison). The *lower and upper whiskers* represent the minimum and maximum values of nonoutliers. For statistical analysis unpaired Student *t* test was performed to determine differences between PF and blood. *CD*, Cluster of differentiation. $*P \leq .05$. $***P \leq .001$. $****P \leq .0001$.

With respect to anti-inflammatory cytokines, although there was no difference in the concentration of IL-1Ra and IL10 between native pericardial fluid and baseline blood, they were significantly higher in the pericardial space 48 hours post-CPB compared with blood ($P = .0004$). Although the concentration of IL-5 was significantly higher in native pericardial fluid compared with baseline blood ($P = .001$), no appreciable difference was observed in postoperative pericardial effluent and plasma concentrations. There was no significant difference in the concentration of IL-13 in the pericardial space versus systemic circulation before or after surgery.

The pericardial space also contained elevated levels of chemokines: IL-8 and MCP-1. Although there was no significant

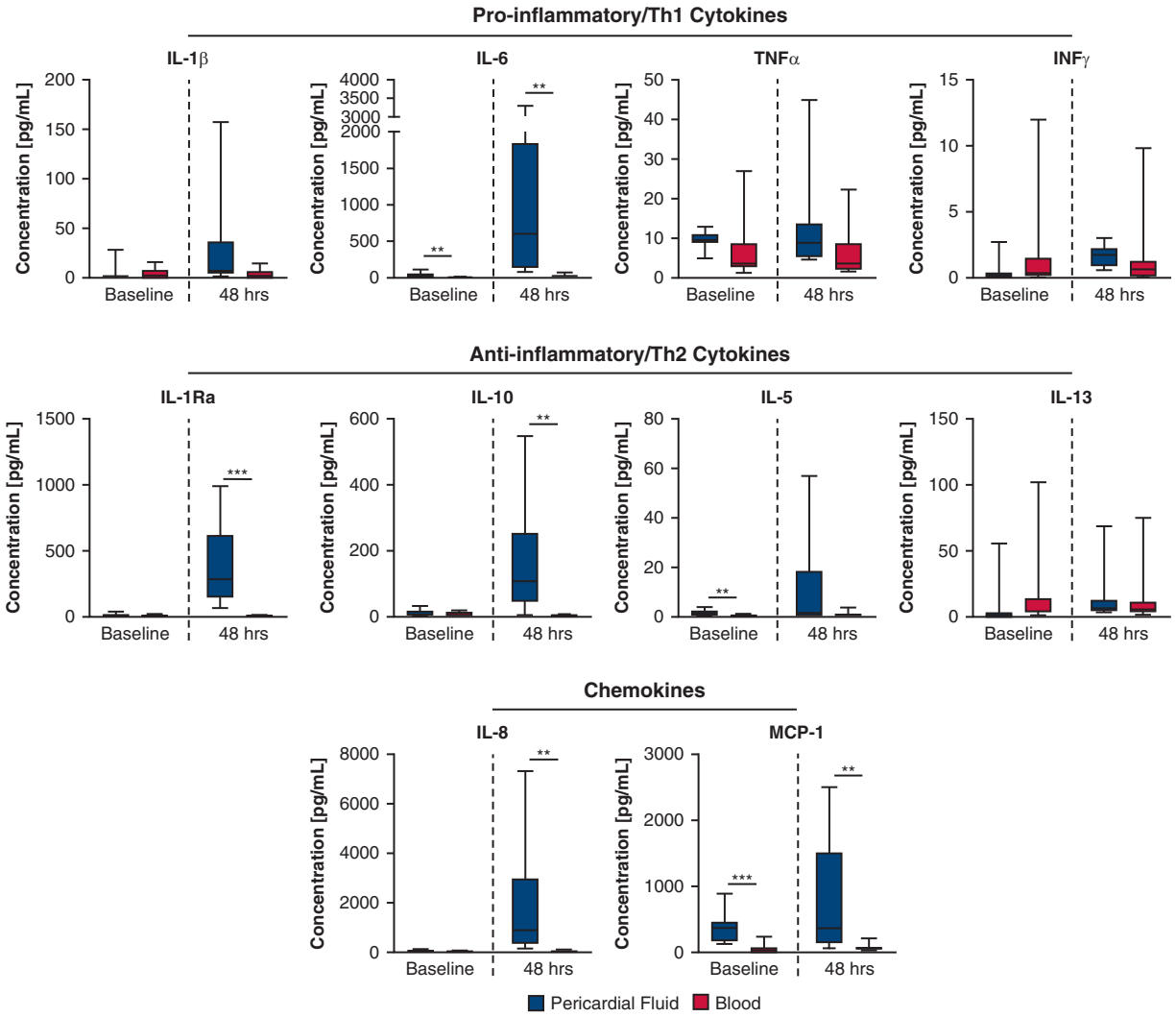

FIGURE 3. The concentrations of proinflammatory/Th1 cytokines, anti-inflammatory/Th2 cytokines, and chemokines distinguish in pericardial fluid (PF) and blood. Comparison of proinflammatory/Th1 cytokines (*top*), anti-inflammatory/Th2 cytokines (*middle*), and chemokines (*bottom*) at baseline and 48 hours postsurgery in PF (*blue*) and blood (*red*). Proinflammatory/Th1 cytokines include interleukin (*IL*) 1*β* (*left*), IL-6 (*middle left*), tumor necrosis factor alpha (*TNFα*) (*middle right*), and interferon-gamma (INFγ) (*right*). Anti-inflammatory/Th2 cytokines show interleukin 1 receptor antagonist (IL-1Ra) (*left*), IL-10 (*middle left*), IL-5 (*middle right*), and IL-13 (*right*). Chemokines present IL-8 (*left*) and monocyte chemoattractant-1 (*MCP-1*) (*right*). Concentrations are measured by multiplex analysis and shown as picograms per milliliter. The *lower and upper whiskers* represent the minimum and maximum values of nonoutliers. For statistical analysis unpaired Student t test was performed to determine differences between PF and blood, respectively, at each time point. $^{**}P \leq .01$. $^{***}P \leq .001$.

difference between native pericardial versus systemic concentrations of IL-8 before surgery, IL-8 had a significantly higher concentration in the pericardial space compared with systemic circulation after surgery ($P = .007$). The concentration of MCP-1 was significantly higher in the pericardial space compared with systemic circulation before and after surgery.

The Concentration of MMP Is Significantly Higher in the Pericardial Space Compared With Systemic Circulation After Surgery

Given the important role MMPs play in tissue remodeling and degradation of the extracellular matrix, the four samples collected from each patient were probed for their concentration of MMPs (Figure 4). Under baseline conditions, total MMP levels were similar between the pericardial space and the systemic circulation (Figure 4, *A*). Following surgery, a sharp increase in MMPs was specifically observed in the pericardial space (Figure 4, *A*). MMP-2, -3, -8, and -9 were the predominant MMPs present in both the pericardial space and systemic circulation (Figure 4, *B*). The elevation in MMPs levels in the pericardial space relative to the blood following surgery was driven by increases in MMP-3, MMP-8, and MMP-9 but not MMP-2 (Figure 4, *C*).

TIMPs can dampen MMP activity locally and thus TIMP profiles were measured in the same samples. Total TIMPs levels were consistently elevated in the pericardial

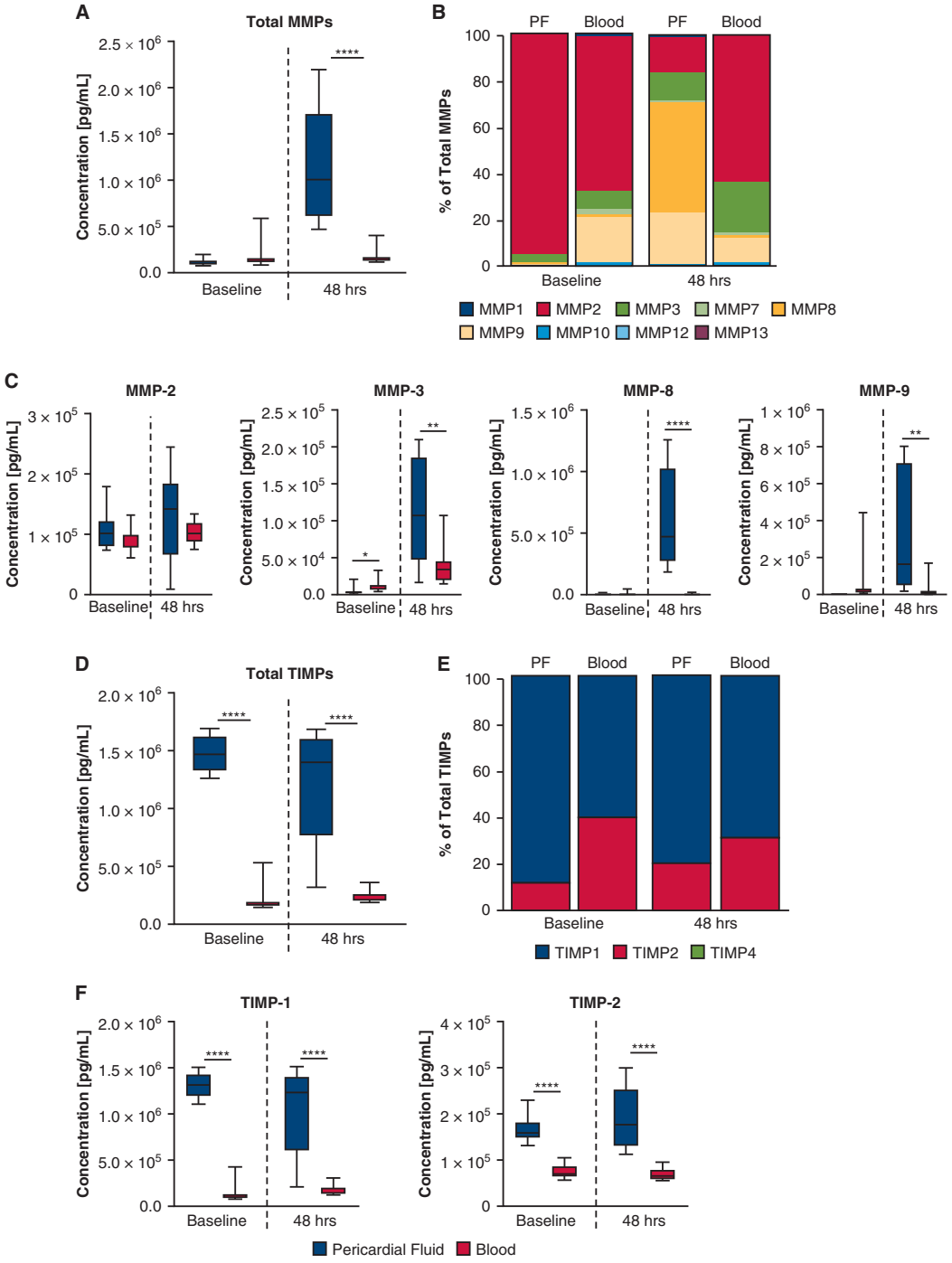

FIGURE 4. The concentrations of matrix metalloproteinases (*MMPs*) and tissue inhibitors of matrix metalloproteinases (*TIMPs*) are both increased in the pericardial space after surgery. Concentrations of MMPs and TIMPs were measured by multiplex analysis and are shown in pg/mL. A, Comparison of total MMPs concentration in pericardial fluid (*PF*) (*blue*) and blood (*red*) at baseline and 48 hours postsurgery, respectively. B, Presentation of each MMP as percentage of total MMPs for baseline (*left*) and 48 hours (*right*) in PF and blood. Shown is the distribution of MMP1, MMP2, MMP3, MMP7, MMP8, MMP9, MMP10, MMP12, and MMP13. C, Representation of MMP-2 (*left*), MMP-3 (*middle left*), MMP-8 (*middle right*), and MMP-9 (*right*) comparisons for PF (*blue*) versus blood (*red*) at baseline and 48 hours. D, Comparison of total TIMPs concentration in PF (*blue*) and blood (*red*) at baseline (*left*) and 48 hours (*right*) postsurgery, respectively. E, Presentation of each TIMP as percentage of total TIMPs for baseline (*left*) and 48 hours (*right*) in PF and blood. Shown is the distribution of TIMP1, TIMP2, and TIMP4. F, Representation of TIMP-1 (*left*) and TIMP-2 (*right*) comparisons for PF (*blue*) versus blood (*red*) at baseline and 48 hours. Each comparison shows n = 12. The *lower and upper whiskers* represent the minimum and maximum values of nonoutliers. For statistical analysis unpaired Student *t* test was performed to determine differences between PF and blood at each time point, respectively. *$P \leq .05$. **$P \leq .01$. ****$P \leq .0001$.

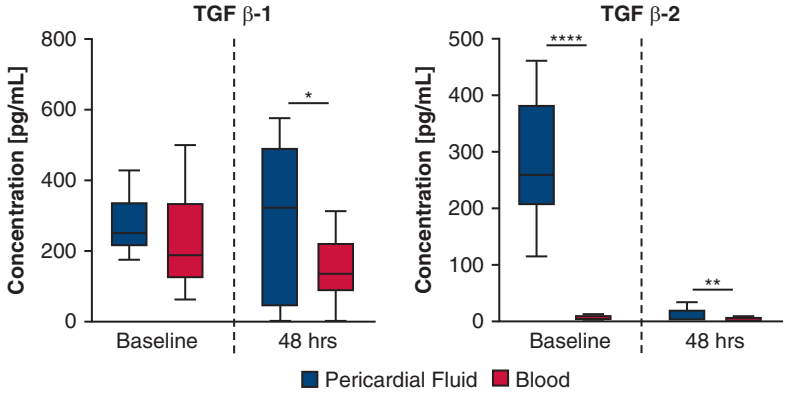

FIGURE 5. The concentrations of transformation growth factor β (*TGFβ*) 1 and TGFβ-2 distinguish between pericardial fluid and blood. Comparison for TGFβ-1 (*left*) and TGFβ-2 (*right*) is shown at baseline and 48 hours postsurgery in pericardial fluid (*blue*) and blood (*red*), respectively. Concentrations are measured by multiplex analysis and shown in picograms per milliliter. Each comparison represents n = 12. The *lower and upper whiskers* represent the minimum and maximum values of nonoutliers. For statistical analysis unpaired Student *t* test was performed to determine differences between PF and blood at each timepoint, respectively. *$P \leq .05$. **$P \leq .01$. ****$P \leq .0001$.

space compared with systemic circulation at both baseline and after surgery (Figure 4, *D*). The relative proportions of the main TIMPs present, TIMP-1 and TIMP-2, were preserved in both the blood and pericardial following surgery (Figure 4, *E*). As such, both TIMP-1 and TIMP-2 levels were significantly elevated in the pericardial fluid compared with the blood before and after surgery (Figure 4, *F*).

The Concentration of TGFβ-1 is Twice as High in the Pericardial Space Compared With Blood After Surgery

Given the important role TGFβ has in driving inflammatory processes, we probed the baseline and post-operative samples for concentration of TGFβ-1 and TGFβ-2 (Figure 5). Although a significant difference was not found in between native pericardial and baseline blood concentration of TGFβ-1, after surgery, TGFβ-1 had a significantly higher concentration in the pericardial space compared with blood ($P = .04$). TGFβ-2 was found at a significantly higher concentration in the pericardial space before ($P < .0001$) and after surgery ($P = .001$) when compared with blood.

DISCUSSION

Patients undergoing CABG surgery often experience postoperative complications such as atrial fibrillation, PPS, and postsurgical pericardial adhesions. These complications are associated with increased risk of morbidity, mortality, and can incur a significant economic burden on health care systems. Although not well understood, inflammation has been shown to play an important role in driving these complications. To date, the systemic post-CPB inflammatory response has been extensively studied.[10-20] It is believed that CPB and ischemia–reperfusion injury play an important role in activating systemic inflammatory cascades. However, the inflammatory processes that occur in the pericardial space after surgery have been poorly characterized and there is a paucity of data comparing the local inflammatory response to the systemic inflammation that occurs in these patients. In this study, we provide a comparison between the local and systemic inflammatory processes that are present in patients undergoing cardiac surgery.

To do so, we included 12 patients undergoing CABG surgery, and compared the local post-operative inflammatory response to systemic inflammation and show that the local response is distinct when compared with systemic postsurgical inflammation. Given the present study is a subpopulation analysis of our recent work,[22] the enrolled patients had all presented with an ACS. Although we have previously shown that ACS alters the immune cell composition of the pericardial space,[21] the present study focuses on the postoperative changes that can be consistently analyzed because native PF was removed in entirety during each operation. Seven patients had normal left ventricular ejection fraction and none of them were experiencing heart failure symptoms worse than New York Heart Association functional class II. Future studies should assess whether degree of left ventricle dysfunction; severity of heart failure at time of surgery; and type, duration, and indication of the operation can influence the local versus systemic postoperative inflammatory response.

Furthermore, given this was a pilot clinical study, we collected samples at only 1 time point after surgery. Ongoing work focuses on better delineating how the local and systemic postoperative response evolves over the first 72-hours after CABG surgery by collecting samples at more time points after surgery. Current work by our group is also exploring whether or not age, gender, and comorbidities influence the local postsurgical inflammatory response. As more patients are enrolled, it will be feasible

to also determine medications and severity of comorbidities influences the pericardial inflammatory process after surgery.

The findings of our study may have significant clinical implications. Many complications associated with cardiac surgery, such as POAF, postsurgical pericardial adhesions, and PPS are driven by inflammation.[3-11] Although there is also evidence to suggest that systemic factors and physiological parameters such as heart rate variability can contribute to atrial fibrillation,[8,27-31] a better understanding of the inflammatory processes that take place in the pericardial space after surgery may explain the precise mechanisms that drive POAF, postsurgical pericardial adhesion formation, and PPS. Within the first 48 hours after CABG surgery, compared with blood, the pericardial space is populated by significantly higher concentrations of potent proinflammatory and anti-inflammatory mediators, such as IL-6 and TGFβ, respectively.

Although speculative at this stage, there may be a link between the proinflammatory factors and POAF, for instance, an association may be found between anti-inflammatory and reparative makers and pericardial adhesion formation. Moreover, MMPs and TIMPs, which are critical to tissue and extracellular matrix remodeling, have differential concentrations in the pericardial space versus peripheral blood after surgery. These mediators can also contribute to the

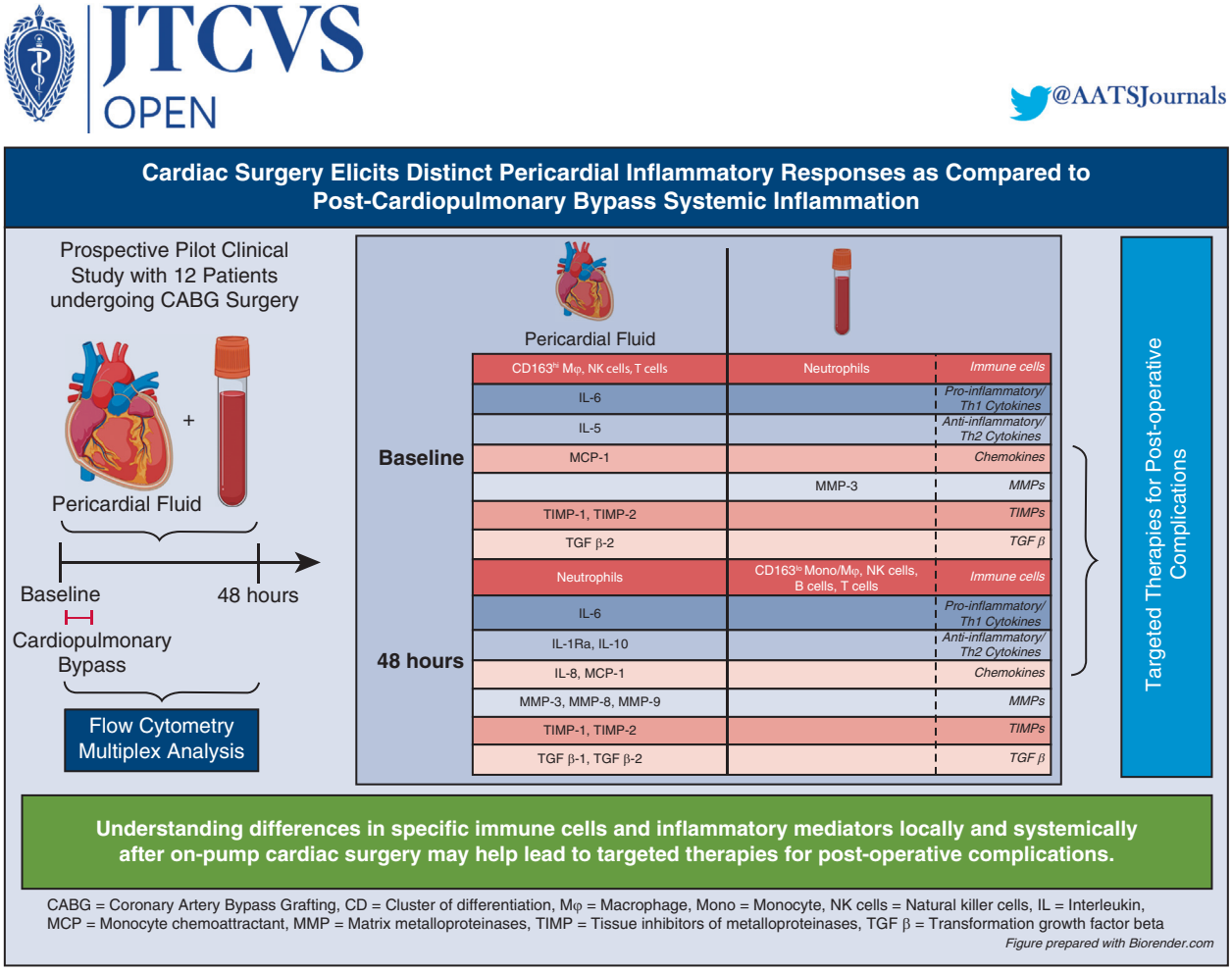

FIGURE 6. Graphical summary of the study. Twelve patients undergoing coronary artery bypass graft (*CABG*) surgery were enrolled in the study. Native pericardial fluid and baseline (preoperative) venous blood were collected. Upon the conclusion of the surgery, a drain was placed in the pericardial space to allow for collecting postoperative pericardial fluid. In addition to retrieving post-operative pericardial effluent at 48-hours postcardiopulmonary bypass, venous blood was obtained. All samples were analyzed for immune cells and inflammatory mediators, including cytokines, matrix metalloproteinases, tissue inhibitors of metalloproteinases, and growth factors. The local postoperative inflammatory profile is distinct from the systemic postcardiopulmonary bypass inflammatory response. Such a distinct response may have important clinical implications, where certain local pericardial factors can contribute to post-operative atrial fibrillation, postsurgical pericardial adhesion formation, and postpericardiotomy syndrome. *CD*, Cluster of differentiation; *CD163^{hi} Mϕ*, CD163^{hi} macrophages; *IL*, interleukin; *MCP-1*, monocyte chemoattractant-1; *TIMP*, tissue inhibitors of matrix metalloproteinases; *TGFβ*, transformational growth factor β; *IL-1Ra*, interleukin 1 receptor antagonist; *MMP*, matrix metalloproteinases.

local remodeling phenomena that takes place postoperatively. Despite having an abundant neutrophil presence in both the pericardial space and blood after surgery, the local mediator environment differed considerably. This likely reflects different activation statuses for these immune cells present in the pericardial space and the contribution of other cell types (eg, mesothelial cells and fibroblasts) in establishing the local milieu.

Furthermore, we demonstrate that, although there are significant differences in specific immune cells and inflammatory mediators when comparing between blood and the pericardial space after surgery, not all factors are significantly elevated in the pericardial space postsurgery. This is clinically relevant because specific markers can be targeted, as opposed to using broad anti-inflammatory agents. We have recently shown that therapeutic agents can be delivered to the pericardial space to direct immunomodulatory activities (Vasanthan and colleagues, unpublished work). Therefore, it is feasible to target the mediators that are present in the pericardial space after surgery to reduce or mitigate inflammatory responses. Moreover, because we currently lack robust preclinical models for POAF, postsurgical pericardial adhesions, and PPS, it will be important to design and complete large animal studies that can assess whether or not postoperative pericardial contents contribute to these complications.

Although our study provides insights into the local versus systemic postsurgical inflammatory responses, there are a few limitations. First, we have a small sample size, and we were not able to ascertain whether or not a link exists between particular pericardial inflammatory mediators and clinical outcomes such as POAF. Second, to have a more feasible pilot study, we performed our comparative analysis at only 1 time point. Finally, because this study focuses on reporting the early cellular and molecular changes that occur in the pericardial space compared with the systemic response, intermediate-, and long-term clinical outcomes have not been collected.

CONCLUSIONS

In this study, we show that the local inflammatory response is different when compared with postcardiopulmonary systemic inflammation, which could have potential clinical implications and possibly therapeutic targets for atrial fibrillation, PPS, and postsurgical pericardial adhesions (Figure 6). Future work should determine whether or not targeting immune mediators that are present in the pericardial space after cardiac surgery can reduce the incidence of atrial fibrillation, PPS, and adhesion formation.

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: cardiac surgery, inflammation, cardiopulmonary bypass, pericardial space

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		
Ghost^{TM} Dye Red710 Viability Dye	TONBO Bioscience	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^{TM} 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		
FlowJo^{TM}10	Becton Dickinson & Company	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

Cat#., Catalog number.

TABLE E2. Immune cell gating strategies

Immune cell population	Gating strategy
B cells	$CD3^{-}$ $CD64^{-}$ $CD16^{-}$ SSC^{lo} $CD56^{-}$ $CD45^{+}$ $CD19^{+}$
$CD163^{hi}$ Macrophages	$CD64^{+}$ $CD14^{+}$ $CD16^{+}$ $CD163^{hi}$
$CD163^{lo}$ monocytes/macrophages	$CD64^{+}$ $CD14^{+}$ $CD16^{-}$ $CD163^{lo}$
Classical dendritic cells type 2	$CD64^{+}$ $CD1c^{+}$ $CD14^{-}$
Inflammatory dendritic cells	$CD64^{+}$ $CD1c^{+}$ $CD14^{+}$
Natural killer cells	$CD3^{-}$ $CD64^{-}$ $CD16^{-}$ SSC^{lo} $CD56^{+}$
Neutrophils	$CD3^{-}$ $CD64^{-}$ $CD16^{+}$ SSC^{hi}
T cells	$CD3^{+}$

CD, Cluster of differentiation.