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Mast cell activation and arterial hypotension during proximal aortic repair requiring hypothermic circulatory arrest

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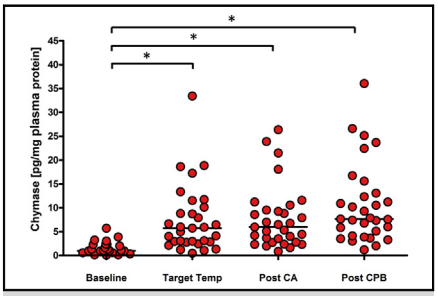
ABSTRACT

Objective: Aortic surgeries requiring hypothermic circulatory arrest evoke systemic inflammatory responses that often manifest as vasoplegia and hypotension. Because mast cells can rapidly release vasoactive and proinflammatory effectors, we investigated their role in intraoperative hypotension.

Methods: We studied 31 patients undergoing proximal aortic repair with hypothermic circulatory arrest between June 2013 and April 2015 at Duke University Medical Center. Plasma samples were obtained at different intraoperative time points to quantify chymase, interleukin-6, interleukin-8, tumor necrosis factor alpha, and white blood cell CD11b expression. Hypotension was defined as the area (minutes × millimeters mercury) below a mean arterial pressure of 55 mm Hg. Biomarker responses and their association with intraoperative hypotension were analyzed by 2-sample *t* test and Wilcoxon rank sum test. Multivariable logistic regression analysis was used to examine the association between clinical variables and elevated chymase levels.

Results: Mast cell-specific chymase increased from a median 0.97 pg/mg (interquartile range [IQR], 0.01-1.84 pg/mg) plasma protein at baseline to 5.74 pg/mg (IQR, 2.91-9.48 pg/mg) plasma protein after instituting cardiopulmonary bypass, 6.16 pg/mg (IQR, 3.60-9.41 pg/mg) plasma protein after completing circulatory arrest, and 7.64 pg/mg (IQR, 4.63-12.71 pg/mg) plasma protein after weaning from cardiopulmonary bypass (each P value $< .0001$ vs baseline). Chymase was the only biomarker associated with hypotension during ($P = .0255$) and after ($P = .0221$) cardiopulmonary bypass. Increased temperatures at circulatory arrest and low presurgical hemoglobin levels were independent predictors of increased chymase responses.

Conclusions: Mast cell activation occurs in cardiac surgery requiring cardiopulmonary bypass and hypothermic circulatory arrest and is associated with intraoperative hypotension. (J Thorac Cardiovasc Surg 2016;■:1-9)



Significant release of mast cell-specific chymase during proximal aortic repair surgery.

Central Message

Mast cell activation during proximal aortic repair with hypothermic circulatory arrest is associated with hemodynamic instability.

Perspective

In patients undergoing proximal aortic repair with hypothermic circulatory arrest, we provide the first evidence that mast cell activation is an important mechanism propagating inflammatory and vasomotor disturbances. Well-tolerated drugs exist to stabilize mast cells and could provide a novel therapeutic opportunity to reduce adverse systemic responses in cardiac surgery.

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Episodes of intraoperative hypotension have been linked to adverse outcomes such as end-organ injury and mortality.[1] In cardiac surgery, complex vasomotor disturbances lead to hypotension through generalized vasoplegia and threaten adequate organ perfusion through endothelial leakage and associated tissue edema (reviewed elsewhere[2]). In fact, up

Scanning this QR code will take you to the supplemental figures, tables and video for this article.

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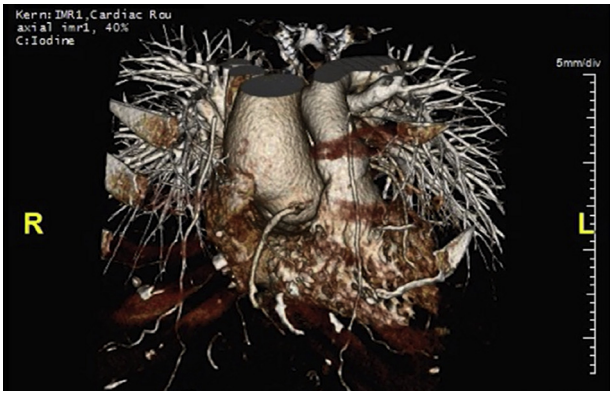
Abbreviations and Acronyms

Δ chymase	= delta baseline chymase
CPB	= cardiopulmonary bypass
HCA	= hypothermic circulatory arrest
IL	= interleukin
I/R	= ischemia/reperfusion
LT	= leukotriene
MAP	= mean arterial pressure
MC	= mast cell
TNFα	= tumor necrosis factor alpha

to 25% of cardiac surgery patients develop a vasoplegic syndrome, a form of distributive shock that is linked to increased length of stay and mortality.[3,4] But even in less dramatic manifestations, prolonged periods spent outside a still poorly defined optimal perfusion range affect postoperative outcomes.[5,6] Currently, the factors that cause such hemodynamic shifts during cardiac surgery are not well understood, impeding substantial advancement of patient care beyond supportive measures.

We have recently shown in a rat model that mast cells (MCs) play an important role in early inflammatory and tissue injury responses following deep hypothermic circulatory arrest.[7] As evidenced by their pivotal role in asthma and anaphylaxis, MCs can rapidly launch local and systemic inflammatory responses through release of potent mediators from preformed stores. These effectors can dramatically change vasomotor function (eg, heparin, histamine, and prostaglandins), endothelial integrity (eg, proteases, prostaglandins, and vascular endothelial growth factor), and can activate further inflammatory components (eg, chemokines and cytokines) (reviewed elsewhere[8]). This identifies MCs as crucial regulators of vascular integrity, tone, and function and as a potent factor in the propagation of systemic inflammation.

Establishing significant MC activation in cardiac surgery constitutes a first step toward establishing a possible causative role of MCs in the development of hemodynamic disturbances and systemic inflammatory activation. Here, we present the first study that specifically addresses the activation pattern of MCs during aortic surgery by measuring intraoperative plasma chymase levels, an MC-specific protease stored in intracellular vesicles and rapidly released upon MC stimulation. Based on our experimental data[7] and the pronounced inflammatory responses regularly observed, we targeted our study to patients undergoing proximal aortic repair necessitating hypothermic circulatory arrest (HCA) (see Videos 1-3 for representative procedure). Furthermore, as a first exploration into the clinical relevance, we characterized the perioperative setting in which MC responses occur and examined their association to intraoperative hypotension.

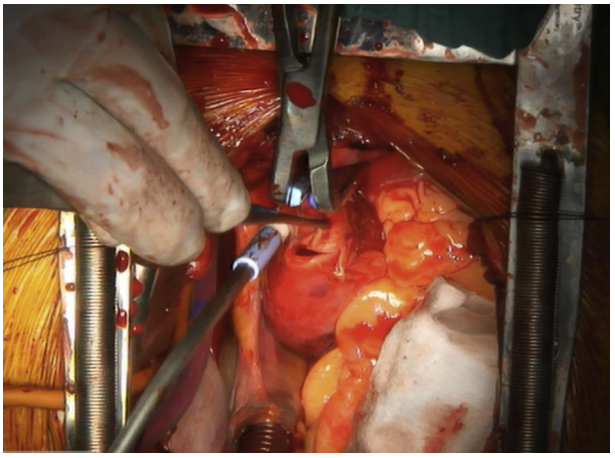


VIDEO 1. An example of proximal aortic repair surgery requiring hypothermic circulatory arrest at the Duke University Medical center. Part 1: Case presentation, cannulations including right axillary cannulation and crossclamp. Video available at: http://www.jtcvs.org.

METHODS

Patient Selection and Data Collection

As part of our ongoing research effort, we conducted a pilot study to define inflammatory responses and cognitive outcomes in patients undergoing proximal aortic arch surgery and requiring HCA between June 2013 and April 2015 at the Duke University Medical Center. The Institutional Review Board for Clinical Investigations at Duke University Medical Center approved this study and informed consent was obtained preoperatively from all patients. We included all patients aged $\geq$ 40 years except those with a history of symptomatic cerebrovascular disease and substantial residual deficit, alcoholism, psychiatric illness, renal failure (creatinine > 2.0 mg/dL), or those with less than a seventh-grade education, or unable to complete neuropsychological testing or cranial magnetic resonance imaging. Patient data were extracted from the electronic health record software (Epic, Verona, Wis) and included patient demographic characteristics, procedure type, preoperative medications, pre- and



VIDEO 2. An example of proximal aortic repair surgery requiring hypothermic circulatory arrest at the Duke University Medical center. Part 2: Moderate hypothermic circulatory arrest with antegrade cerebral perfusion, transverse aortic arch reconstruction (Hemi-Arch), and preparation of the aortic root for reconstruction. Video available at: http://www.jtcvs.org.

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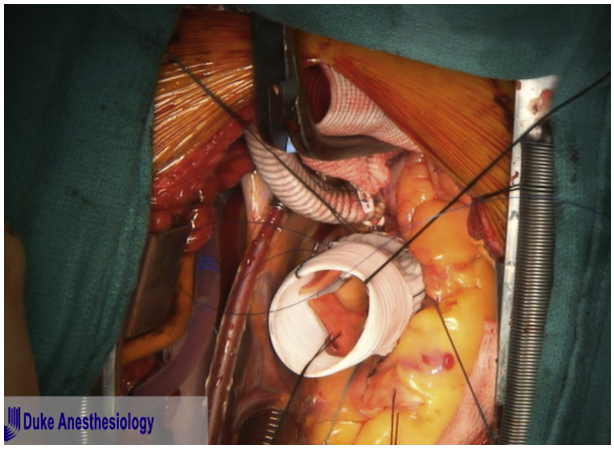

VIDEO 3. An example of proximal aortic repair surgery requiring hypothermic circulatory arrest at the Duke University Medical center. Part 3: T. David-V valve-sparing aortic root replacement with coronary reconstruction and anastomosis of aortic arch graft and aortic root graft. Video available at: http://www.jtcvs.org.

intraoperative drug administration, laboratory values, and timing of intraoperative events. Cumulative doses of vasopressor drugs were normalized to body weight and calculated from the total intraoperative doses of norepinephrine (in micrograms), epinephrine (in micrograms), and vasopressin (in international units). Mean arterial pressures (MAPs) from the left radial arterial line were electronically charted every minute. Values < 30 mm Hg or > 250 mm Hg were excluded as artifactual.

Anesthesia, Surgery, and Cardiopulmonary Bypass

Anesthesia, surgical, and postoperative care were provided according to the standards of care established for proximal aortic arch surgery requiring HCA at Duke University Medical Center. General anesthesia was induced with midazolam and/or propofol, fentanyl, and rocuronium or cisatracurium before tracheal intubation. Anesthesia was maintained using isoflurane in an air-oxygen mixture with an inspiratory oxygen concentration $\geq$ 50% and end-tidal anesthetic gas concentrations were monitored throughout the case. As part of this institution's neuroprotection protocol, all patients received 1000 mg methylprednisolone intravenously in the preoperative area. Surgery was performed by median sternotomy with intraoperative transesophageal echocardiographic and invasive arterial blood pressure monitoring as previously described.[9] Temperature management during circulatory arrest was determined at the surgeon's discretion. Patients undergoing moderate HCA were cooled to 20°C to 26°C as defined by nasopharyngeal temperature, whereas for patients undergoing deep HCA, cooling was performed until electrocerebral inactivity was demonstrated by electroencephalography. Upon completion of cooling, the circulation was halted to allow opening of the aortic arch. Adjunctive antegrade (n = 30) or retrograde cerebral perfusion (n = 1) was used for cerebral protection in all cases. After completion of the hemiarch anastomosis, cardiopulmonary bypass (CPB) was reinstituted. After a 5-minute period of cold reperfusion, patients were carefully rewarmed and additionally required cardiac procedures were completed. Weaning from CPB was performed at a core temperature of 36°C. Antifibrinolytic therapy using aminocaproic acid was routinely administered intraoperatively. Transfusion decisions were made according to Society of Cardiovascular Anesthesiologists/Society of Thoracic Surgeons published guidelines[10] using surgical field observations, chest tube output, activated clotting time, platelet count, fibrinogen level, thrombelastogram data, and prothrombin and partial thromboplastin time tests. Antiplatelet therapy was held preoperatively except for 81 mg aspirin and was restarted on postoperative day 1 unless active bleeding was present.

Sample Collection and Analysis

Study blood samples were drawn from the arterial line into ethylenediaminetetraacetic acid tubes following induction of anesthesia (baseline) when reaching the target temperature before HCA (target temp), 5 minutes after HCA (post-HCA), and after terminating CPB (post-CPB) (see Figure 1). Samples were immediately transferred to ice, centrifuged, and plasma was stored at −80°C until analysis. The following enzyme-linked immunosorbent assays were performed: chymase (Antibody Online, Aachen, Germany), interleukin (IL)-6, IL-8, and tumor

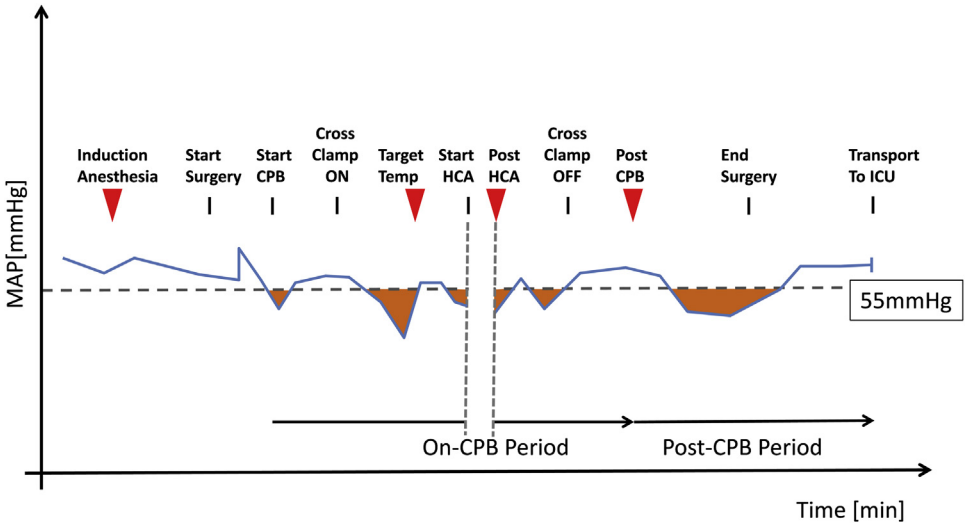

FIGURE 1. Sample draw time-points and hemodynamic quantitation strategy. Relevant time-points are graphed versus time against an idealized mean arterial pressure (MAP) tracing (*blue line*). A cutoff of 55 mm Hg was chosen and the area (in minutes × millimeters mercury) underneath the cutoff was calculated (*orange shaded areas*). Cumulative values were then determined for an on-cardipulmonary bypass (CPB) period (excluding hypothermic circulatory arrest [HCA] period) and a post-CPB period. Red triangles denote blood draw. *ICU*, Intensive care unit.

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necrosis factor alpha (TNFα) (eBioscience, San Diego, Calif). Results were normalized against the protein content of the respective sample, as determined by Bradford assay (BioRad, Hercules, Calif) to account for hemodilution during CPB. In addition, leukocytes were isolated from the buffy coat following the separation of plasma. After hypotonic lysis of residual red blood cells, leukocytes were fixed and stained with allophycocyanin (APC)–coupled anti-CD45 (BD Pharmigen, San Jose, Calif) and fluorescein isothiocyanate (FITC)–coupled anti-CD11b antibody (eBioscience, San Diego, Calif) and the percentage of CD11b-positive cells in the CD45-gated population was established by fluorescence-activated cell sorting. Lactate levels were obtained as part of routine blood gas analyses and the difference between the first measurement after anesthesia induction and the last before transport to the intensive care unit was calculated.

Statistical Analysis

Continuous variables are presented as means $\pm$ standard deviation or medians (interquartile range), and categorical variables as group frequencies and percentages. Measured values of inflammatory markers at target temperature, post-HCA, and post-CPB were compared with baseline values using Wilcoxon signed rank test. Repeated measures analysis using generalized estimating equations was performed to examine the trends over time for the serum measurements of chymase, IL-8, and CD11b but not for IL-6 and TNFα because of missing values at the post-HCA and post-CPB time points.

Intraoperative hypotension was defined as episodes with an MAP below 55 mm Hg. This was quantified as cumulative area (in minutes $\times$ millimeters mercury) between the actual MAP and 55 mm Hg, using a modification of the approach from Levin and colleagues.[3]

Testing for an association between inflammatory marker measurements and hypotension was performed for 2 intraoperative time periods (Figure 1). The on-CPB period extended from initiation of CPB to termination of CPB, excluding the time of circulatory arrest. The post-CPB period was defined as the interval between CPB termination and the last valid MAP reading before transfer to the intensive care unit. The MAP area below 55 mm Hg from these periods was examined against the delta baseline chymase (Δ chymase) value at the target temperature measurement (for on-CPB period) and at the post-CPB measurement (for post-CPB period). Upon examination of the distribution of Δ chymase measurements, we found that they had a non-normal distribution. We attempted several different methods of data transformation, but none of these transformation techniques resulted in a normal distribution or showed a linear association with MAP area below 55 mm Hg. This precluded us from using linear analysis models and nonlinear methods such as smoothing splines did not improve model fit. Therefore, we decided to dichotomize chymase responses as outlined below. Because there is no predefined threshold for chymase, we evaluated each possible Δ chymase dichotomization cutoff for its relationship to MAP area below 55 mm Hg, using either 2 samples t test or Wilcoxon rank sum test. To determine the optimal cutoff, we first defined a candidate region where consistent association signals were observed between Δ chymase values and MAP area below 55 mm Hg. Then, we chose cutoffs that yielded significant differences and a balanced grouping between high and low responders. The chosen cutoffs were close to (for the on-CPB period) or identical to (for the post-CPB period) the median. C-indices, as estimates of prediction accuracy determined by logistic regression for the chosen Δ chymase cutoff by MAP area, were 0.697 and 0.742, respectively. These Δ chymase cutoff values were then used to dichotomize our cohort into high-responders and low-responders (measured responses above or below cutoff value for respective time period).

The same search method was applied to examine the association of IL-6, IL-8, TNFα, and CD11b with MAP area below 55 mm Hg. However, no reliable cutoff could be determined using the above approach and we therefore used the median for these biomarkers.

Univariable and multivariable logistic regression analyses were applied to evaluate the association between perioperative variables and increased chymase responder status as determined at the post-CPB blood draw. We examined preoperative risk factors, including those from the European System for Cardiac Operative Risk Evaluation score,[11] the durations of CPB, HCA, and aortic crossclamp, the temperature during circulatory arrest, and the amount of blood product transfusions. Variables with $P < .05$ from the univariable analyses were tested for collinearity and entered into a multivariable logistic model. Backward stepwise selection was used for the final multivariable logistic model. Odds ratios and corresponding 95% confidence limits are reported in Table 1. All analyses were performed using the SAS version 9.3 (SAS Institute Inc, Cary, NC).

RESULTS

A total of 31 patients (22 men, 9 women) aged 60.1 $\pm$ 12.6 years were recruited to this study. Patient characteristics and details of the procedures performed are outlined in Table 2.

Measurements of inflammatory responses are shown in Figure 2 and detailed in Table E1. MC-specific chymase increased significantly from a baseline median of 0.97 pg/mg (interquartile range [IQR], 0.01-1.84 pg/mg) plasma protein, to a median of 5.74 pg/mg (IQR, 2.92-9.48 pg/mg) plasma protein with institution of CPB and cooling. Chymase levels did not increase substantially with reperfusion after HCA (median, 6.16 pg/mg [IQR, 3.60-9.41 pg/mg] plasma protein), but rose slightly to a median of 7.64 pg/mg (IQR, 4.63-12.71 pg/mg) plasma protein after completing CPB ($P < .0001$ vs baseline for all time points). IL-8 showed a similar response, increasing from a median of 0.27 pg/mg (IQR, 0.19-0.40 pg/mg) plasma protein at baseline to a median of 0.46 pg/mg (IQR, 0.35-0.75 pg/mg), 0.65 pg/mg (IQR, 0.35-0.89 pg/mg), and 0.67 pg/mg (IQR, 0.51-1.04 pg/mg) plasma protein at target temperature, post-HCA and post-CPB, respectively ($P < .0001$ vs baseline for all time points). Similarly, we found that neutrophil activation occurred early during surgery, with the percentage of CD11b-positive and CD45-positive cells increasing from a median of 15.7% (IQR, 7.9%-26.6%) at baseline to a median of 20.5% (IQR, 14.4%-30.9%), 27.1% (IQR, 15.6%-40%), and 30.2% (IQR, 20.7%-50.3%) at target temperature, post-HCA, and post-CPB, respectively ($P < .0001$ vs baseline for all time points). In contrast, TNFα and IL-6 rose only at the later time points of surgery; that is, TNFα from a median of 0.19 pg/mg (IQR, 0.11-0.28 pg/mg) plasma protein at baseline to a median of 0.39 pg/mg (IQR, 0.11- 0.49 pg/mg) at post-CPB ($P < .0001$ vs baseline) and IL-6 from a median of 0.08 pg/mg (IQR, 0.03-0.11 pg/mg) plasma protein at baseline to a median of 0.28 pg/mg (IQR, 0.13-1.02 pg/mg) plasma protein post-CPB ($P < .0001$ vs baseline). Repeated measure analysis of chymase, IL-8, and CD11b levels taken at different intraoperative time points significantly increased over time from baseline. (Figure E1).

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TABLE 1. Univariable and multivariable analysis

	Univariable analysis				Multivariable analysis			
Variable	Odds ratio	95% CI LL	95% CI UL	*P* value	Odds ratio	95% CI LL	95% CI UL	*P* value
Gender (M vs F)	0.35	0.06	1.68	.2009				
Age (y)	1.03	0.98	1.10	.2836				
Preoperative calcium blocker	2.31	0.20	53.10	.5142				
Preoperative ACE inhibitor/ARB	4.50	1.03	22.98	.0544				
Preoperative β-blocker	0.39	0.09	1.63	.2045				
ASA (4 vs 3)	0.86	0.20	3.61	.8326				
Chronic renal insufficiency	1.08	0.12	10.07	.9449				
Previous cardiac surgery	2.31	0.20	53.10	.5142				
Preoperative EF (TEE) (>50 vs 31-50)	2.00	0.17	45.99	.5887				
PMH allergy/autoimmune Disease	4.33	0.90	25.54	.0788				
Hemoglobin preoperative (g/dL)	0.32	0.11	0.69	**.0157**	0.32	0.08	0.78	**.0370**
WBC count preoperative (10^9/L)	0.75	0.40	1.33	.3341				
Platelet count preoperative (10^9/L)	1.00	0.99	1.02	.5729				
Preinduction MAP (mm Hg)	0.97	0.92	1.02	.2424				
Additional valve surgery	8.36	1.61	65.89	**.0198**				
CPB time (min)	1.00	0.99	1.02	.6479				
Crossclamp time (min)	1.00	0.98	1.02	.8484				
HCA time (min)	1.06	0.95	1.20	.2998				
NP temp at circulatory arrest (°C)	1.34	1.08	1.75	**.0146**	1.36	1.07	1.89	**.0276**
MAP area on-CPB (mm Hg × min)	1.00	1.00	1.01	.2116				
Postoperative EF (TEE) (>50 vs 31-50)	2.40	0.50	13.79	.2896				
Transfusion RBC (units)	4.71	1.53	24.07	**.0259**				
Transfusion FFP (units)	0.96	0.69	1.31	.7916				
Transfusion platelets (units)	1.45	0.53	4.50	.4842				
Transfusion cryoprecipitate (units)	0.78	0.300	1.94	.5929				
Hemoglobin postoperative (g/dL)	0.97	0.40	2.30	.9381				
WBC count 24 h postoperative (1000/μL)	1.07	0.90	1.30	.4534				
WBC count 48 h postoperative (1000/μL)	1.08	0.93	1.29	.3318				
Baseline chymase (pg/mg plasma protein)	1.66	1.01	3.16	.0745				
Delta-chymase target temp (pg/mg plasma protein)	1.43	1.12	2.05	**.0193**				
Delta-IL-6 target temp (pg/mg plasma protein)	1.60	0.20	11.41	.5989				
Delta-IL-6 post-CPB (pg/mg plasma protein)	1.51	0.70	4.15	.3202				
Delta-IL-8 target-temp (pg/mg plasma protein)	0.97	0.31	3.44	.9616				
Delta-IL-8 post-CPB (pg/mg plasma protein)	0.96	0.65	1.41	.8379				
Delta-TNFα target temp (pg/mg plasma protein)	1.68	0.00	3683.40	.8898				
Delta-TNFα post-CPB (pg/mg plasma protein)	14.04	0.20	2571.36	.2519				
CD11b target temp (fold increase over baseline)	1.19	0.28	5.36	.8169				
CD11b post-CPB (fold increase over baseline)	0.90	0.52	1.39	.6318				

Predictors of increased chymase responses. Variables were examined against increased chymase responder status as measured at the post-CPB blood draw. Hosmer-Lemeshow goodness-of-fit: χ^2, 10; *df*, 2; *P* value = .10. Boldface denotes statistical significance, $P \leq .05$. *95% CI LL*, 95% Confidence interval lower limit; *95% CI UL*, 95% confidence interval upper limit; *ACE*, angiotensin-converting enzyme; *ARB*, angiotensin II receptor blocker; *ASA*, American Society of Anesthesiologists physical status classification system; *EF*, ejection; *TEE*, transesophageal echocardiography; *PMH*, past medical history; *WBC*, white blood cell; *MAP*, mean arterial pressure; *CPB*, cardiopulmonary bypass; *HCA*, hypothermic circulatory arrest; *NP temp*, nasopharyngeal temperature; *RBC*, red blood cell; *FFP*, fresh-frozen plasma; *Delta-*, delta baseline; *IL*, interleukin; *TNFα*, tumor necrosis factor alpha.

We next examined whether chymase responses were associated with increased intraoperative hypotension. As shown in Figure 3, chymase high-responders displayed a significantly increased MAP area below 55 mm Hg during CPB, when compared with low-responders (median, 188 minutes × mm Hg [IQR, 140-392 minutes × mm Hg] vs median, 123 minutes × mm Hg [IQR, 76-212 minutes × mm Hg]; $P = .0255$). Similarly, hypotensive episodes were significantly more pronounced after terminating CPB in chymase high-responders, compared with low-responders (median, 22.2 minutes × mm Hg [IQR, 1.1-88.6 minutes × mm Hg] vs 0.4 minutes × mm Hg [IQR, 0-5.1 minutes × mm Hg]; $P = .0221$).

In addition, chymase high-responders displayed a trend toward increased cumulative use of vasopressor drugs ($P = .07$) as well as increased intraoperative lactate production ($P = .121$) (Figure E2).

Subsequently, we also examined the association between, IL-6, IL-8, TNF, and CD11b and intraoperative hypotension and found that there was no statistically significant

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TABLE 2. Patient demographic characteristics and intervention details

Patient variable	Result
Age (y)	61.23 ± 12.4
Male	22 (71)
Female	9 (29)
ASA I	0
ASA II	0
ASA III	18 (58)
ASA IV	13 (41)
Preoperative ACE inhibitor/ARB	13 (42)
Preoperative calcium blocker	3 (10)
Preoperative β-blocker	14 (45)
Preoperative heparin	0
Preoperative hemoglobin (g/dL)	13.96 ± 1.53
EuroSCORE variables	
Chronic pulmonary disease	4 (13)
Extracardiac arteriopathy	31 (100)
Neurological dysfunction	0
Serum creatinine > 200 mmol/L	4 (13)
Poor mobility	1 (3)
Preoperative EF (TEE) > 50	20 (65)
Preoperative EF (TEE) 31-50	11 (36)
Preoperative EF (TEE) < 30	0
Recent myocardial infarction	1 (3)
Pulmonary hypertension	3 (10)
Previous cardiac surgery	3 (10)
Diabetic on insulin	0
Logistic EuroSCORE (%)	18.9 ± 12
Surgical variables	
Ascending aorta only	9 (29)
Ascending aorta + CABG	2 (6)
Ascending aorta + valve	20 (65)
Antegrade cerebral perfusion	30 (97)
Retrograde cerebral perfusion	1 (3)
CPB time (min)	169.5 ± 45.4
Crossclamp time (min)	118.8 ± 35.7
Circulatory arrest time (min)	13 (11-16)
NP temp at circulatory arrest [C°]	22.4 ± 4.0
Postoperative EF (TEE) > 50	21 (68)
Postoperative EF (TEE) 31-50	10 (32)
Postoperative EF (TEE) < 30	0
FFP transfusion (units)	4 (0-4)
RBC transfusion (units)	0 (0-1)
Platelet transfusion (units)	2 (1.5-2)
Cryoprecipitate transfusion (units)	0 (0-1)
Postoperative hemoglobin (g/dL)	10.1 ± 0.84

Values are expressed as mean ± standard deviation, median (interquartile range), or n (%). *ASA*, American Society of Anesthesiologists physical status classification system; *ACE*, angiotensin-enzyme; *ARB*, angiotensin II receptor blocker; *EuroSCORE*, European System for Cardiac Operative Risk Evaluation score; *EF*, ejection fraction; *TEE*, transesophageal echocardiography; *CABG*, coronary artery bypass grafting; *CPB*, cardiopulmonary bypass; *NP temp*, nasopharyngeal temperature; *FFP*, fresh-frozen plasma; *RBC*, red blood cell.

association between any of these markers and hypotension during on-CPB or post-CPB periods (Table E2).

We next aimed at better defining the conditions related to increased chymase responses. In univariable analysis, several preoperative and intraoperative variables were significantly associated with increased Δ chymase levels at the end of CPB (Table 1). In multivariable analysis, preoperative hemoglobin, and nasopharyngeal temperature at circulatory arrest emerged as independent predictors of increased chymase responses. As such, for every gram per deciliter increase of preoperative hemoglobin, the odds for an increased chymase response was 0.32 and for every degree Celsius increase at circulatory arrest result was 1.36 times the original odds.

DISCUSSION

This study of 31 patients undergoing proximal aortic repair with CPB and HCA demonstrates, for the first time, substantial intraoperative release of the MC-specific degranulation product, chymase. Importantly, MC responses, but not other inflammatory markers, such as IL-6, IL-8, TNFα, or CD11b expression in white blood cells, were associated with significant intraoperative hypotension. Given the profound inflammatory effects of MC products in asthma and anaphylaxis, this work identified MCs as potentially crucial effectors of inflammatory and tissue-injurious signaling in cardiac surgery. Designed as a hypothesis-forming study, our data strongly encourage future research to determine whether modulation of MC activity can be used to improve outcomes in this setting. Well-established drugs that block MC degranulation exist and have been successfully used in animal models.[7]

Systemic inflammation following cardiac surgery is a multifactorial process, and may have profound adverse effects on injured and normal tissue. Despite compelling evidence for relevant MC activation in animal models of trauma,[12] hemorrhagic shock,[13] ischemia/reperfusion (I/R) injury,[14] but also rat HCA[7] and porcine extracorporeal membrane oxygenation,[15] corresponding human studies have been lacking. MCs are a ubiquitously present population of potent inflammatory surveillance cells that rapidly respond to inflammatory triggers and tissue stress signals with release of preformed and de novo synthesized proinflammatory, vasoactive, and tissue-disruptive effectors (reviewed elsewhere[8]). However, previous attempts to quantify MC activation during adult or pediatric cardiac surgery have yielded incoherent results.[16-18] Fayaz and colleagues[16] observed rapid histamine release upon initiation of CPB, but dismissed MCs as the source because of concurrently decreasing MC-tryptase levels. Based on our own unpublished observations, we think that tryptase binding to heparin may interfere with detection in the setting of CPB. Instead, in our hands chymase constituted a robust marker of MC activation during cardiac surgery. These chymase measurements showed that in contrast to the rat model,[7] MC responses in patients were vigorous

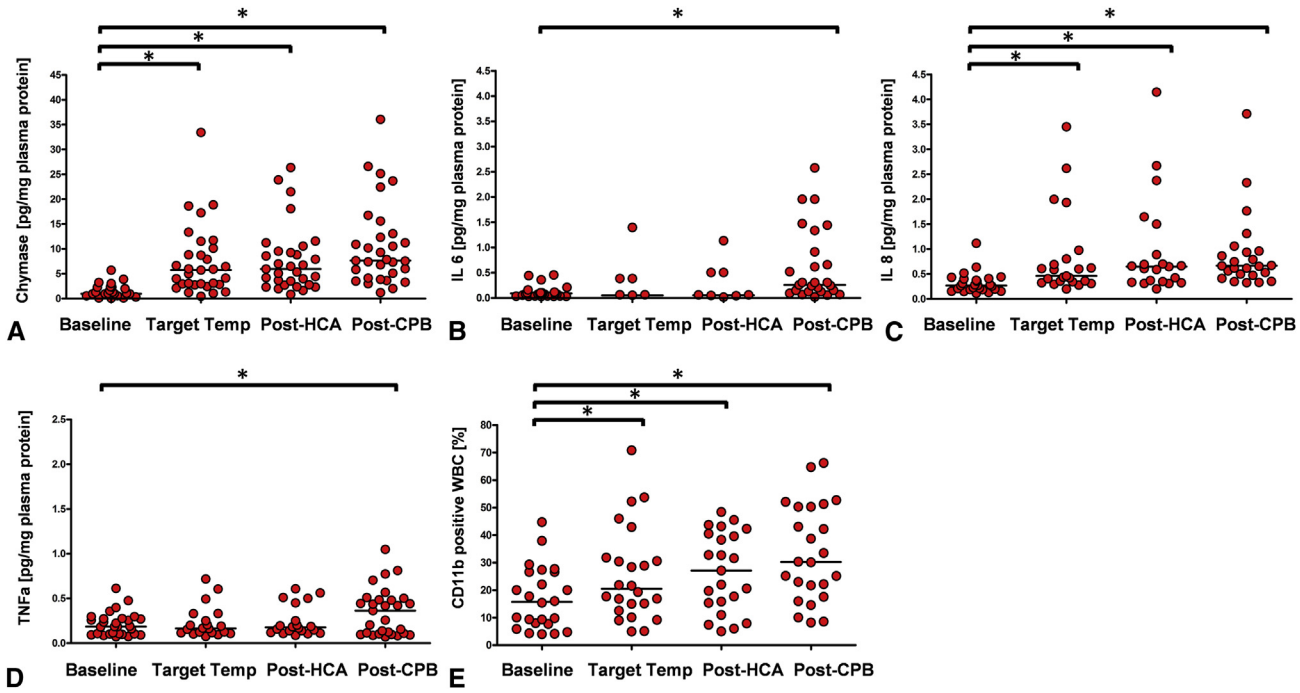

FIGURE 2. Intraoperative responses of inflammatory markers. A, Mast cell-specific chymase. B, Interleukin-6. C, Interleukin-8. D, Tumor necrosis factor alpha. E, The percentage of CD11b-positive white blood cells were determined at baseline, when reaching target temperature on cardiopulmonary bypass (Target Temp), after completion of hypothermic circulatory arrest (Post-HCA), and after completion of cardiopulmonary bypass (Post-CPB). *Increase versus baseline $P \leq .05$ by 2-sample t test.

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after implementation of CPB and cooling, but marginal after HCA. Increased age and comorbidities may make patients (vs young, healthy rats) more sensitive to the inflammatory and circulatory challenges of extracorporeal perfusion. We are currently examining the implications of these findings by examining MC activation in coronary artery bypass grafting procedures. Furthermore, it is noteworthy that MC responses occurred in patients receiving 1000 mg methylprednisolone as part of a neuroprotection protocol. Chymase, but also a significant portion of early cytokine release, stems from degranulation of preformed stores,[15] a process that appears unaffected by steroids.[19]

As the first report on MC activation in a cardiac surgical setting, the present study offers important insights into a possible role for MCs in perioperative responses. Most importantly, this work demonstrated an association between amplified MC responses and intraoperative hypotension. Circulatory instability is often the first clinical

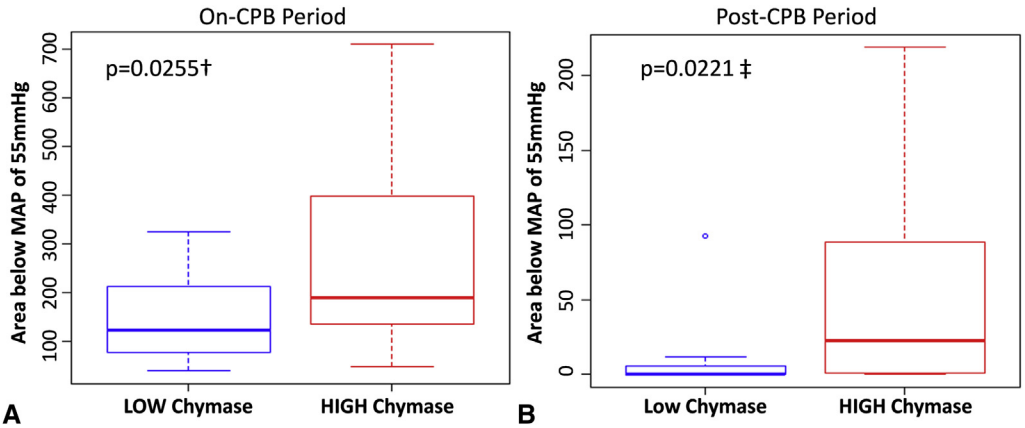

FIGURE 3. Association of plasma chymase responses and intraoperative hypotension. Delta-baseline chymase responses were dichotomized according to the cutoffs chosen for the entire cohort, using chymase levels. A, Target-temperature blood draw for the on-cardiopulmonary bypass (CPB) period. B, At the post-CPB blood draw for the post-CPB period. Hypotensive episodes were quantified by calculating the cumulative area of mean arterial pressure (MAP) below a cutoff of 55 mm Hg (in minutes × millimeters mercury). †Two-sample t test. ‡Wilcoxon rank sum test.

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manifestation of inflammatory events and presents as a predominantly distributive hypotension with inadequate responses to volume expansion. In cardiac surgery, 10% to 20% of patients develop vasoplegic syndrome, which substantially influences immediate clinical care and postoperative outcomes.[3,4] Although this study was not designed to support conclusions on whether MC responses are a cause or effect of hypotension, available data support the notion that MC activation occurs early and promotes inflammation and vasomotor disturbances. Our previous work in a rat model of HCA has shown that pretreatment with the MC stabilizer cromolyn sodium reduced local and systemic inflammation.[7] In addition, MC products have a critical effect on vascular functions. Due to their predominant perivascular distribution, MC mediators may almost immediately influence vascular structures and trigger endothelial barrier breakdown, plasma leakage, and edema (vascular endothelial growth factor, TNFα, the eicosanoids leukotriene [LT] C_4, LTD_4, and LTE_4, and the proteases tryptase and chymase) and cause vasomotor disturbances (histamine and chymase).[8]

CPB and HCA constitute an extremely complex context and multiple events such as I/R,[14] complement activation,[20] and cytokine release[21] may stimulate MCs. Here, our uni- and multivariable analyses provide additional evidence of the clinical setting in which MC activation occurs.

As such, increased chymase responses were associated with higher target temperatures at circulatory arrest. A number of recent works[22,23] have highlighted advantages of performing circulatory arrest procedures at higher temperatures. However, the consequences on I/R injury and systemic inflammatory responses are not yet fully defined.[9,24] In fact, conflicting results have been reported on the temperature-dependency of cytokine release in cardiac cases.[25,26] Consistent with a previous report,[13] our data suggest that MC degranulation is blunted at low temperatures, but, as with Rasmussen and colleagues,[26] other inflammatory markers were not affected (data not included). Our previous work in a rat model of HCA revealed substantial intestinal I/R injury.[7] Therefore, although ante- or retrograde cerebral perfusion is thought to confer some degree of brain protection, a concern remains as to I/R injury of abdominal organs. Increased MC activation at higher HCA temperatures may reflect suboptimal ischemic protection at these sites, but further studies are needed to define resultant injury patterns better.

We also observed that low preoperative hemoglobin levels were independent predictors of above-median MC responses. Preoperative anemia reduces oxygen delivery and thus increases the risk of end-organ injury during cardiac surgery.[27] It is possible that both anemia and HCA temperature influence MC activation and related perioperative inflammatory responses through their role in I/R injury. On the other hand, red blood cell transfusions were associated with increased chymase responses in univariable, but not in multivariable, analysis. There is ample evidence that red blood cell transfusion may directly contribute to adverse clinical outcomes in cardiac surgery.[28,29] Although the mechanisms by which transfusions elicit injurious responses remain unknown, there appears to be a distinct inflammatory component.[30] Apart from anaphylactic transfusion reactions, MC activation has been reported due to immunoglobulin E oligomers in donor plasma[31]; however, future studies will have to determine if MCs are also involved in mediating a more general inflammatory response to transfusion.

Study Limitations

Our small-scale study was designed as an exploratory study to identify the presence of MC activation in our cohort and to characterize the clinical setting in which MC activation occurs. To overcome some of the challenges of our limited cohort while still attempting to explore the clinical relevance of MC activation, we analyzed intraoperative hypotension because a dense dataset of MAP measurements was available through electronic charting. It must be noted that we do not routinely use pulmonary artery catheters in these cases. Although independent evaluation by a board-certified echocardiographer pre- and postprocedure did not reveal a significant deterioration of cardiac function, we thus could not calculate vascular resistance as a measure of vasoplegia.[4] Instead, we defined hypotension as an MAP below 55 mm Hg, which according to various reports from cardiac and noncardiac surgery settings showed a strong association between the time spent below this threshold and perioperative adverse outcomes.[1,32,33]

In addition, we found that the distribution of biomarker measurements did not allow analysis using linear models and we therefore resorted to dichotomizing chymase levels into high and low responders. Although we strongly believe that the robust association between hypotension and elevated chymase levels supports the clinical relevance of perioperative MC activation as a concept, this approach may limit the generalizability of our study findings.

Lastly, data on clinical risk factors, medication use, and laboratory values were retrospectively retrieved from electronic medical records. Thus, the effects of certain risk factors and medications may have been biased. In addition, specimen volume was too limited in some patients to measure all of the inflammatory markers.

CONCLUSIONS

We demonstrated, for the first time, significant MC activation during proximal aortic repair surgery requiring circulatory arrest. Multivariable logistic regression analysis indicated that increased circulatory arrest temperatures and low preoperative hemoglobin contribute to such MC

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activation. Importantly, MC activation was significantly associated with relevant intraoperative hypotension.

Conflicts of Interest Statement

Authors have nothing to disclose with regard to commercial support.

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Key Words: cardiac surgical procedures, mast cell, blood pressure, inflammation mediators

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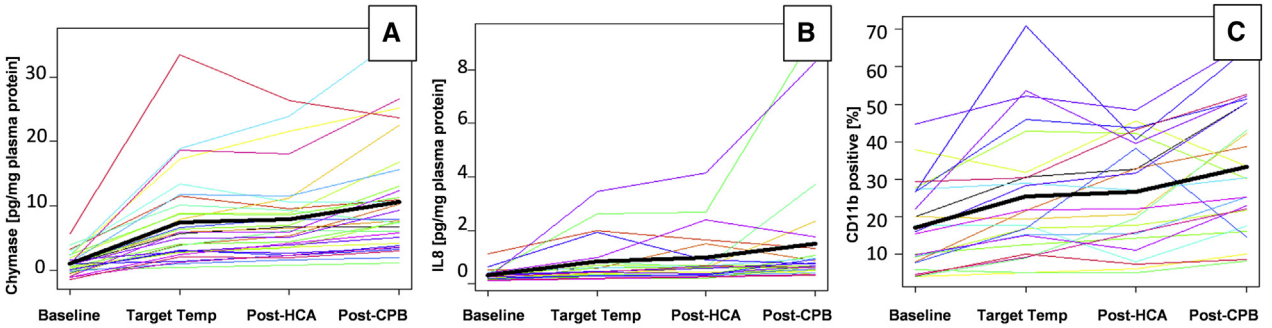

Outcome	Variable	Estimate	Std.err	Wald	Pr(>|W|)
Chymase	**TIme**	25.37	5.62	20.4	**0.0000062**
	Time^2	-3.81	1.09	12.2	**0.00049**
IL8	**Time**	2.5134	1.2124	4.3	**0.038**
	Time^2	-1.1376	0.5839	3.8	0.051
	Time^3	0.1703	0.0878	3.76	0.052
CD11b	**Time**	68.43	31.19	4.81	**0.028**
	Time^2	-28.8	14.42	3.99	**0.046**
	Time^3	3.92	2.01	3.79	0.051

FIGURE E1. Repeated measures analysis to examine the trends over time for measurements of A, Chymase, B, Interleukin-8 (IL8), and C, CD11b. The decision to transform the time scale was based on the curve of mean measurement over time as indicated by the black line in each panel. Estimate denotes the coefficient of time and denotes the slope of the median. *HCA*, Hypothermic circulatory arrest; *CPB*, cardiopulmonary bypass; *Wald*, Wald-type rank test; *Pr(>|W|)*, *P* value.

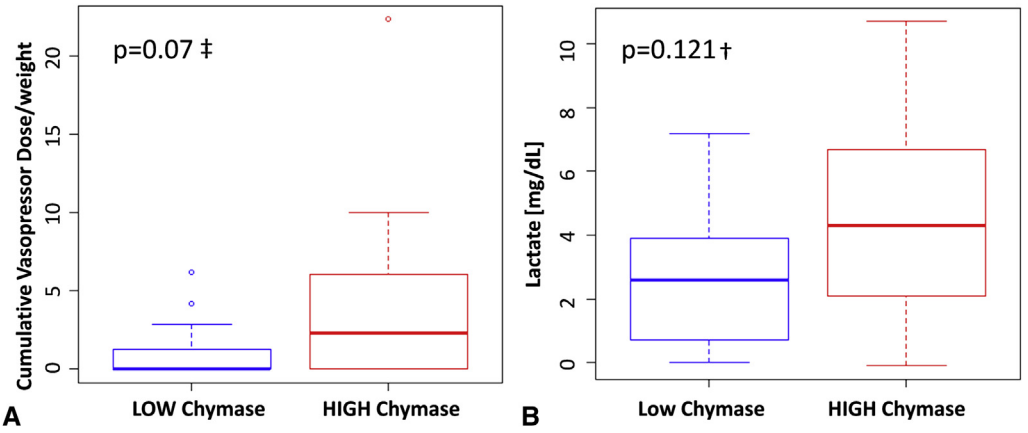

FIGURE E2. Indicators of relevant hypotension are increased in above-median chymase responders. A, Cumulative dose of vasopressors was calculated by adding total intraoperative doses of epinephrine (in micrograms), vasopressin (in international units), and norepinephrine (in micrograms) and adjusted for each patient's body weight. B, Lactate levels are represented as difference between last and first intraoperative lactate level (milligrams per deciliter). †Two-sample *t* test. ‡Wilcoxon rank sum test.

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TABLE E1. Intraoperative measurements of inflammatory markers. Measurements were determined at baseline, when reaching target temperature on cardiopulmonary bypass (CPB) (target temp), after completion of hypothermic circulatory arrest (post-HCA), and after completion of cardiopulmonary bypass (post-CPB) and are expressed for chymase, interleukin-6 (IL-6), interleukin-8 (IL-8) and tumor necrosis factor alpha (TNFα) in pictograms per milligram plasma protein and for CD11b in % CD11b positive white blood cells. Missing measurements of inflammatory markers in some cases were due to sample volume limitations. Estimates are calculated from the mean of the paired difference of a measurement and the respective baseline

					Compared with baseline			
Variable	Time Point	n	Median	Interquartile range	Estimate	95% CI LL	95% CI UL	Wilcoxon signed rank test
Chymase	Baseline	31	0.97	0.01 to 1.84				
	Target temp	31	5.74	2.92 to 9.48	6.48	4.31	8.64	**<.0001**
	Post-HCA	31	6.15	3.60 to 9.41	6.89	4.84	8.93	**<.0001**
	Post-CPB	31	7.64	0.63 to 12.71	9.66	6.79	12.53	**<.0001**
IL-6	Baseline	28	0.08	0.03 to 0.11				
	Target temp	18	−0.08	−0.10 to 0.04	0.10	−0.18	0.39	.2287
	Post-HCA	18	−0.06	−0.10 to 0.05	0.07	−0.14	0.28	.3692
	Post-CPB	28	0.28	0.13 to 1.02	0.60	0.20	1.01	**<.0001**
IL-8	Baseline	26	0.27	0.19 to 0.40				
	Target temp	23	0.46	0.35 to 0.75	0.49	0.15	0.83	**<.0001**
	Post-HCA	21	0.65	0.35 to 0.89	0.62	0.18	1.06	**<.0001**
	Post-CPB	26	0.67	0.51 to 1.04	1.19	0.27	2.10	**<.0001**
TNFα	Baseline	30	0.19	0.11 to 0.28				
	Target temp	20	0.17	0.12 to 0.27	0.05	−0.01	0.11	**.0245**
	Post-HCA	20	0.18	0.13 to 0.30	0.07	0.01	0.13	**.0002**
	Post-CPB	30	0.39	0.11 to 0.50	0.15	0.08	0.22	**<.0001**
CD11b	Baseline	24	15.7	7.9 to 26.6				
	Target temp	24	20.5	14.4 to 30.9	8.42	3.82	13	**<.0001**
	Post-HCA	23	27.1	15.62 to 40.0	9.38	5.42	13.3	**<.0001**
	Post-CPB	24	30.2	20.7 to 50.3	16.21	10.73	21.7	**<.0001**

Boldface denotes statistical significance, $P \leq .05$. *95% CI LL*, 95% Confidence interval lower level; *95% CI UL*, 95% confidence interval upper level; *HCA*, hypothermic circulatory arrest; *CPB*, cardiopulmonary bypass; *IL*, interleukin; *TNFα*, tumor necrosis factor alpha.

TABLE E2. Association of inflammatory markers with intraoperative patient hemodynamic parameters for the on-cardiopulmonary bypass (CPB) and post-CPB period. Delta-baseline responses were dichotomized according to the median for the whole cohort using the respective measurement at the target-temperature blood-draw for the on-CPB period and at the post-CPB blood draw for the post-CPB period. Hemodynamic parameters were quantified by calculation of the area above the mean arterial pressure curve and below a cutoff of 55 mm Hg

		Below or equal to median			Above median			
Marker	Intraoperative period	N	Median	Interquartile range	N	Median	Interquartile range	*P* value
Chymase	On-CPB	16	107.1	70.58-193.3	15	193.4	165.7-411.8	**.0067**†
	Post-CPB	16	0.3631	0-5.07	15	22.24	1.141-88.63	**.0221**‡
Interleukin-6	On-CPB	8	184.5	105.9-291.5	8	249.6	103.4-364.6	.6689†
	Post-CPB	13	0.07	0-7.24	13	2.29	1.131-22.24	.1905‡
Interleukin-8	On-CPB	11	174.4	102-268	11	188.7	129.4-244.5	.7969‡
	Post-CPB	13	1.56	0-22.24	12	4.01	0.8483-17.75	.9559‡
Tumor necrosis factor alpha	On-CPB	10	218.4	179.6-361.6	10	135.1	61-331	.4490‡
	Post-CPB	15	1.6	0.04-9.6	15	7.2	0-71.8	.6127‡
CD11b	On-CPB	12	168.7	121.5-187.5	12	145.3	53.06-249.7	.9323‡
	Post-CPB	12	3.27	0-57.37	12	3.43	0-11.9	.7022‡

Boldface denotes statistical significance, $P \leq .05$. †Two-sample *t* test. ‡Wilcoxon rank sum test.

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000 Mast cell activation and arterial hypotension during proximal aortic repair requiring hypothermic circulatory arrest

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Mast cell activation during proximal aortic repair with hypothermic circulatory arrest is associated with hemodynamic instability.