Pericardial Fluid Chymase Activity Four Hours Post Cardiac Surgery is Related to Intensive Care Unit and Total Hospital Length of Stay

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Introduction

•Operative trauma, reperfusion injury, and other insults from cardiac surgery lead to a robust cardiac inflammatory response and have a significant impact on post-surgical outcomes and hospital utilization.
•Chymase activity and inflammatory cytokines are elevated in pericardial fluid (PCF) as early as 4 hours post surgery and are many-fold higher than in blood
•The post-surgical pericardial environment may provide more insight into risk of adverse postoperative outcomes.

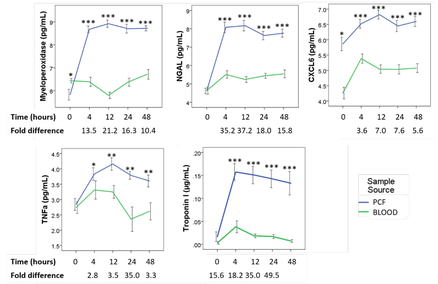

Figure 1. Proinflammatory proteins and troponin are increased in PCF versus blood starting at 4 hours post cardiac surgery. *Adapted from Butts et al. (2017) Circulation. 136(23): 2284-2286.*

Purpose

To examine associations between key biomarkers of cardiac injury and inflammation in the PCF 4 hours after cardiac surgery with intensive care unit (ICU) and total hospital length of stay.

Methods

Patient Population and Sample Collection

•PCF was collected at the time of surgery and from the pericardial drains at 4, 12, and hours after surgery in adult patients (N=30) undergoing cardiac surgery.

Measures

•PCF biomarkers were measured using a Luminex bead-based multiplex assay using fluorescent bead technology (Milliplex kit, Millipore Corp.).
•Troponin-1 and brain natriuretic peptide (BNP) are measures of cardiac injury, TNF-α and chymase are products of mast cells, and myeloperoxidase and neutrophil gelatinase-associated lipocalin (NGAL) are products of neutrophils.
•Radiolabeled (^{125}I) angiotensin-1-12 [Ang-(1-12)] was used as a substrate for the determination of chymase activity, defined as fmoles of Ang II product formed from ^{125}I-Ang-(1–12) substrate per ml per minute (fmol Ang II formation/ml/min).

Primary Outcome: ICU length of stay and Total hospital length of stay

•All patients were directly admitted to the intensive care unit (ICU) from the operating room. Time of arrival in the ICU was considered time zero.

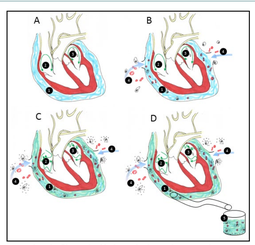

Figure 2. A. (1) Pericardial fluid (blue) bathes the normal heart including the (2) right and (3) left atria. Green arrows in atria represent conduction. B. Surgery disrupts the pericardium and blood and inflammatory cells enter the pericardial space (4). C. (1) Pericardial fluid composition now includes inflammatory cells, cytokines, and highly oxidative proteins, causing atrial fibrillation (green arrows in 2,3) or other adverse outcomes. D. Drainage of pericardial fluid allows for collection of pericardial fluid (5).

Results

Table 1. Demographic and Clinical Characteristics

N=30	Mean ± SD N	Range %
Age (years)	62.63 ± 1.9	38 – 78
BMI (kg/m^2)	28.67 ± 1.2	19 – 51
Female	7	23%
Black	5	17%
Surgery		
CABG	20	67%
Valve Repair	5	17%
CABG + Valve	5	17%
Cardiopulmonary Bypass	17	57%
Pre-operative LVEF (%)	50 ± 11.4	15 – 55

Table 2. STS-PROM, Pericardial Fluid Markers, and Length of Stay

N=30	Mean ± SD	Min – Max
STS-PROM	7.51 ± 3.6	3.2 – 28.3
Troponin-1 (µg/ml)	0.15 ± 0.08	0.06 – 0.24
BNP (pg/ml)	259.84 ± 132.1	93.4 – 468.2
Chymase activity (fmol/ml/min)	8.76 ± 2.5	2.4 – 21.6
TNF-α (pg/ml)	6.15 ± 1.1	1.4 – 16.9
Myeloperoxidase (pg/mL)	550.61 ± 137.7	55.2 – 2226.2
Interleukin-8 (pg/mL)	66.74 ± 29.0	1.7 – 427.2
NGAL (pg/mL)	119.81 ± 17.9	36.6 – 553.0
CXCL6 (pg/mL)	8.67 ± 1.3	2.8 – 20.4
ICU length of stay (days)	2.17 ± 3.8	1 – 21
Total length of stay (days)	6.41 ± 1.3	3 – 40

4-hour PCF chymase activity plus STS-PROM provides the best fit model for prediction of both total hospital and ICU length of stay

Table 3. Zero Truncated Poisson Regression Relating Pericardial Fluid Markers and STS-PROM Score to Hospital Length of Stay

Model	Coefficient	SE	p-value	AIC/AICC/BIC
Univariate Analysis				
STS-PROM	0.093	0.009	<.0001	156.6/157.7/159.4
Chymase (fmol/ml/min)	0.127	0.014	<.0001	117.2/117.7/119.8
CXCL6 (pg/ml)	0.009	0.0001	<.0001	179.2/179.7/181.9
TNF-α (pg/ml)	0.0026	.0015	.0026	200.1/200.7/202.6
Multivariate Analysis				
STS-PROM + Chymase (fmol/ml/min)	0.051 0.068	0.021 0.033	.0132 .04	72.7/74.7/75.0
STS-PROM + BNP (pg/ml)	0.06 0.002	0.02 0.0007	.0001 .01	88.9/90.4/92.1
STS-PROM + CXCL6 (pg/ml)	0.080 0.006	0.009 0.0002	<.0001 .0016	110.1/111.1/114.0
STS-PROM + TNF-α (pg/ml)	0.092 0.001	0.009 0.002	<.0001 .05	108.7/109.9/112.2

STS-PROM – Society of Thoracic Surgeons Predicted Risk of Morbidity and Mortality

Table 4. Zero Truncated Poisson Regression Relating Pericardial Fluid Markers and STS-PROM Score to Intensive Care Unit Length of Stay

Model	Coefficient	SE	p-value	AIC/AICC/BIC
Univariate Analysis				
STS-PROM	0.175	0.020	<.0001	71.3/71.8/74.0
Chymase (fmol/ml/min)	0.241	0.028	<.0001	46.7/47.2/49.3
CXCL6 (pg/ml)	0.002	0.0002	<.0001	105.9/106.4/108.5
Multivariate Analysis				
STS-PROM + Chymase (fmol/ml/min)	0.009 0.271	0.067 0.130	0.89 0.04	30.2/32.2/32.5
STS-PROM + CXCL6 (pg/ml)	0.139 0.001	0.022 0.0005	<.0001 .03	44.64/45.7/48.5

***Statistical analysis*:** For length of stay analysis, zero-truncated Poisson regression models the effect of a predictor variable. Truncation at zero accounts for the reality that no post-cardiac patient spends zero days in the ICU and hospital. The variable coefficients in this model represent the difference in the logs of the expected outcome variable per unit change in the predictor variables. Clinically, a coefficient found to be significantly different from zero means an increase (if positive) or decrease (if negative) in expected length of stay for every unit increase in the predictor variable controlling for other variables in the model.

Proposed mechanism: the increase in chymase activity at 4 hours corresponds with an increase in angiotensin II

- Chymase is a serine protease that converts angiotensin I to angiotensin II at a greater rate than angiotensin converting enzyme (ACE).
- Considering this role of chymase in tissue activation of angiotensin II, we looked at chymase activity and angiotensin II in PCF over time as a possible mechanism of post-operative myocardial remodeling.
- Chymase may play both a direct and indirect (via angiotensin II) role in post-operative cardiac remodeling.

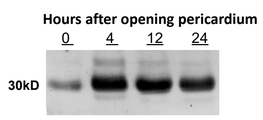

Figure 3. Western blot of representative sample of chymase in pericardial fluid upon opening of the pericardium (time 0) to 4, 12, and 24 hours after surgery.

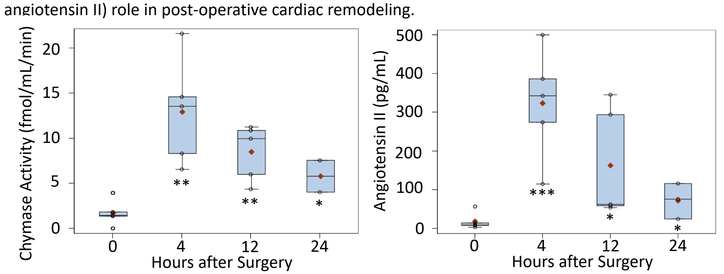

Figure 4. Chymase activity and Angiotensin II peptide levels in pericardial fluid (N=5) upon opening of the pericardium (time 0) to 4, 12, and 24 hour time points after cardiopulmonary bypass. Chymase activity is expressed as chymase-mediated Ang-(1-12) metabolism. *p<.05; **p<.01; ***p<.001 vs time 0.

Conclusions

•This exploratory study found that mast cell and neutrophil inflammatory markers in PCF 4 hours after cardiac surgery were related to both ICU and total hospital length of stay.
•Chymase activity had the best fit relationship with length of stay, suggesting 4-hour chymase activity may improve prediction of patient ICU utilization after cardiac surgery.
•Considering the powerful direct and indirect effects of chymase activity and other inflammatory mediators on the myocardium after acute injury, post-surgical assessment of the fluid bathing the heart may serve as a valuable tool for better precision in determination of individual outcomes.
• A more accurate prediction of post-operative outcomes would benefit individual patients for better management of care and for organizations to optimize resource utilization.

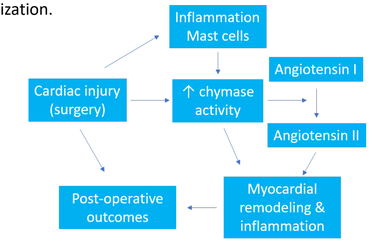

Figure 5. Chymase plays a multifunctional role in acute tissue injury and chronic myocardial remodeling and is the is the primary enzyme responsible for myocardial formation of angiotensin II, a powerful mediator of cardiac remodeling and inflammation.

Limitations

•A larger study to determine cutoff values for chymase activity and relationship to postoperative outcomes such as postoperative atrial fibrillation is needed.
•A larger sample size would allow controlling for multiple testing, which may lead to more precise predictions of outcomes.
•This study was not powered to include baseline predictors such as medications (statins, steroids), demographic factors (age, race, gender), or comorbidities.
•Other enzymes may also be important to measure, such as cathepsin G and neutrophil elastase (from neutrophil degranulation).

Future Directions

•The current findings suggest chymase and other mediators of inflammation in PCF may provide better insight into outcomes after cardiac surgery.
•Postoperative pericardial drainage may be a valuable tool for predicting outcomes after cardiac surgery.
•Further research into mechanisms of post-operative myocardial injury and remodeling may provide improved guidance in treatment
•We have found chymotryptic activity in both mast cells and cardiomyoctes of patients undergoing cardiac surgery (Figure 6)
•However, further insight into the source of chymase in pericardial fluid (e.g. direct release from mast cells, exosomes) is needed

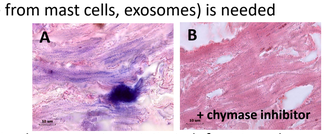

Figure 6. *In situ* chymotryptic activity in left atrium documented by intense blue staining in mast cell and within cardiomyocyte in longitudinal section **(A)** and prevented by pretreatment of tissue with chymase inhibitor (**B**).

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