Usefulness of Circulating Caspase-3 p17 and Caspase-1 p20 Peptides and Cardiac Troponin 1 During Cardioplegia to Gauge Myocardial Preservation

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Evidence is accumulating that cardiac apoptosis occurs and contributes to myocyte cell death during myocardial ischemia. Cardioplegia, defined as the temporary cessation of cardiac activity during cardiac surgery, is a clinically controlled condition with myocardial ischemia and reperfusion. Our goal was to determine whether the apoptotic biomarker caspase-3 p17 is elevated in the coronary sinus (CS) during cardioplegia and if any elevations were reflected in the peripheral venous (PV) blood. Levels of the necrotic biomarker cardiac troponin I (cTnI) and the inflammatory marker caspase-1 p20 were also quantified in CS and PV. Blood was drawn before and at the end of cardioplegia in PV and CS and levels of p20, p17, and cTnI were measured. cTnI, p20, and p17 PV levels were significantly elevated compared with the control population before and at the end of cardioplegia. PV levels of all 3 markers increased after cardioplegia. CS levels were higher than PV levels for all 3 markers at both time points. Our data are consistent with the occurrence of cardiac apoptosis and inflammation during cardioplegia, in addition to necrosis. The heart-derived markers contributed to the peripheral levels and suggest that measurement of PV biomarker concentrations can be used to gauge cardiac preservation. **(Am J Cardiol 2019;123:899−904)**

During acute myocardial infarction, ischemic myocardial cell death determines the extent of damage, which is a major determinant of mortality and postinfarction cardiac function.[1,2] A significant portion of cell death that occurs after acute myocardial infarction is secondary to contraction band necrosis caused by both acute ischemia and reperfusion of myocardium,[3,4] and increasing evidence supports the occurrence of cardiac myocyte apoptosis during myocardial ischemia.[5-11] Cardiac apoptosis also occurs in cardioplegia during cardiopulmonary bypass surgery,[12] and is accompanied by elevation of caspase-3 in tissue samples obtained during surgery.[13,14] However, whether caspase-3 can be used as a serum biomarker for apoptosis is not known. Our previous work demonstrated that the p17 fragment of caspase-3 was elevated in the serum of patients during acute myocardial infarcts,[15] consistent with the concept that ischemic infarcts involve both necrosis and apoptosis. Therefore, circulating caspase-3 p17 could provide a noninvasive marker for myocyte apoptosis to gauge cardioprotection during cardiac surgery. Given the importance of inflammation in the pathogenesis of atherosclerosis and vascular disease, we also investigated the levels of the inflammatory biomarker caspase-1 p20 during cardiac surgery, which could be utilized to develop therapies to minimize inflammation and improve outcomes. We investigated whether these biomarkers for necrosis, apoptosis, and inflammation in peripheral venous (PV) serum were elevated in patients undergoing cardioplegia compared with a healthy control population, increased over time during surgery, and had a cardiac (coronary sinus [CS]) origin.

Methods

Recruitment of study patients occurred prospectively at the University of Connecticut Health Center from 1/9/2012 to 9/22/2016. The target population comprises male and female subjects over the age of 18 who underwent cardiopulmonary bypass as part of cardiac surgery. The types of cardiac surgery underwent by study patients include coronary artery bypass grafting (CABG) only (CABG only group; n = 26), and valve or valve plus CABG (valve plus group; n = 22). Exclusion criteria include pregnancy and patients that were unable to provide informed consent. Control subjects were recruited at the University of Connecticut Health Center from 11/20/07 to 05/04/10. The participants were male (67%) and female (33%) subjects over age of 50 who did not undergo any cardiac surgery. Eighty seven percents of the participants are Caucasian. The blood was collected from venous catheterization. The p20 and p17 fragments were quantified by ELISA (Cell Signaling Technology, Danvers, MA).

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Blood was collected from catheters located in the CS and PV. These catheters were part of normal cardiopulmonary bypass procedures and sample collections were approved by the Institutional Review Board. Blood was collected before initiation of cardioplegia and after cardioplegia had ceased (Time 1 and Time 3, respectively). Time 1 samples were collected before the application of cross clamp and initiation of bypass, while Time 3 samples were collected after bypass was ended and normal physiologic systemic circulation was restored. Blood samples were collected from the CS during cardioplegia (named Time 2) in addition to Time 1 and 3. Time 2 samples were collected within 1 hour of initiation of cardioplegia. PV samples were collected at Time 1 and Time 3 when the CS and PV circulations were reconnected. The total amount of blood required was no more than 3 tablespoons (≤45 ml).

The p17 fragment of caspase-3 protein was measured by a solid-phase sandwich ELISA (Cell Signaling Technology, Danvers, MA) as previously described. Similarly, the p20 fragment of caspase-1 was quantified by ELISA. cTnI protein was determined by the Access AccuTnI assay (Beckman Coulter, Brea, CA) and levels were measured using a Beckman DXI 800 (Beckman Coulter, Brea, CA).

Chart review of the enrolled patients was performed to obtain age, gender, ethnicity, race, surgery details, medical history, smoking history, physical exam findings, current medications, electrocardiograms, laboratory data, echocardiogram results, cardiac catheterization data, and hospitalization outcomes.

The data were statistically analyzed using computer software MATLAB R2017b and GraphPad Prism 5. All the biomarker data were visually inspected and tested for normality using Kolmogorov-Smirnov test to determine appropriate statistical approaches. Most of the biomarker data did not satisfy the normality assumptions and nonparametric methods were used. All the variables were presented as median with interquartile range (IQR) or percentage. Differences between cTnI levels of surgery patients and normal cutoff value (0.01 ng/ml) were tested using one-sample Wilcoxon rank sum test. Two-samples Wilcoxon rank sum tests were used to determine whether median biomarkers (p17 and p20) of the surgery patients were different from the healthy patient population. Comparisons of biomarker levels over time (at Time 1, 2, and 3) in the CS were accomplished with the use of Friedman tests followed by post hoc analyses. The Wilcoxon matched pair signed rank test compared biomarker levels between Time 1 and Time 3 in the PV samples and was used to test the difference between CS and PV levels in the same subjects. To evaluate the correlation between biomarkers, Spearman correlation coefficients were calculated. The Wilcoxon rank sum tests (not matched) were used to determine whether biomarkers were different in CABG only versus valve plus surgeries and in patients versus healthy subjects. Significance level was defined as $p < 0.05$.

Cardioplegia utilized a platform composed of "Master-follower" whole blood microcardioplegia delivered by Terumo System 1 heart-lung machine and CP-50 disposable cardioplegia circuit (Terumo Cardiovascular Ann Arbor Michigan). Cardioplegia solution contains 200 ml isotonic sodium chloride, 4.06 mEq/ml magnesium sulfate, and 2 mEq/ml potassium chloride (total potassium 100 mEq) with initiation by a 12 mg bolus of adenosine. There were slight differences in cardioplegia protocol by 2 surgeons. However, in general, initial cardioplegia was given warm or cold to induce myocardial arrest followed by cold dose to reach a septal temperature of 10°C. Doses were delivered repeatedly with frequency determined by cross-clamp time or ECG findings using both antegrade and retrograde approaches. A terminal dose was sometimes given just before cross-clamp removal.

Results

Between September 2012 and September 2016, 48 patients were included in this study. Table 1 presents the baseline demographic and clinical characteristics of the study population. Participants enrolled in the study were mostly elderly (70% > 65 years old), male (75%), Caucasian (52%), and overweight/obese (71% with a BMI ≥ 25).

Samples for p20 (n = 66) and p17 (n = 56) were obtained from PV blood of a healthy control population and compared with PV biomarker levels of cardiac surgery patients

Table 1
Baseline characteristics of the study patient cohort

Variable	Value (Mean ± Standard Deviation)	Number (n = 48)
Age (years)	69 ± 10	48 (100 %)
Men		36 (75 %)
Women		12 (25 %)
White		25 (52 %)
Hispanic		1 (2 %)
Asian		2 (4 %)
N/A		20 (42 %)
Body mass index (Kg/m^2)		
18.5 ≤ body mass index <25	22.8 ± 1.2	8 (17 %)
25 ≤ body mass index <29.9	27.1 ± 1.4	18 (38 %)
Body mass index ≥30	37.4 ± 7.4	16 (33 %)
N/A		6 (13 %)
Diastolic blood pressure (mm Hg)	66 ± 10.6	45 (94 %)
Systolic blood pressure (mm Hg)	126.6 ± 17.4	45 (94 %)
Coronary arterial disease*		34 (71 %)
Hypertension		36 (75 %)
Peripheral vascular disease		15 (31 %)
Diabetes mellitus		16 (33 %)
Atrial fibrillation		12 (25 %)
Dyslipidemia†		31 (64 %)
Stroke		4 (8 %)
Chronic kidney disease		4 (8 %)
Smoker		
Never		10 (21 %)
Former		17 (35 %)
Current		11 (23 %)
N/A		10 (21 %)
Prior coronary artery bypass grafting (CABG) only		0 (0 %)
Prior valve replacement + CABG		0 (0 %)
Prior percutaneous coronary intervention (PCI) or PCI Stent		15 (31 %)

* Coronary artery disease was defined as atherosclerotic vascular disease as determined by coronary angiography or positive stress testing.

† Hyperlipidemia was defined as a total cholesterol >200 mg/dl and/or low-density lipoprotein (LDL) cholesterol fraction >130 mg/dl.

(Figures 1 to 3). At Time 1 and 3, that is, before and at the end of cardioplegia, cTnI was elevated in the PV, indicating some degree of cardiac ischemia or injury (Figure 1). Of interest, PV levels of p20 (Figure 2) and p17 (Figure 3) were also higher in cardiac surgery patients than in healthy individuals.

We sought to determine whether cTnI, p17, and p20 levels increase after patients underwent cardioplegia. The levels of cTnI (ng/ml) in the CS and PV increased with time (Figure 4). Median (IQR) cTnI levels in CS across Time 1, 2, and 3 were 0.12 (0.06 to 0.41), 0.25 (0.17 to 0.37), and 0.49 (0.25 to 0.78), respectively, where values at Time 2 or 3 were significantly elevated compared with Time 1. In PV, median (IQR) cTnI was 0.11 (0.06 to 0.23) at Time 1 and was significantly increased at Time 3 (0.35 [0.21 to 0.95]; median [IQR]).

The CS p20 caspase-1 level (pg/ml) also rose over time (Figure 5). The median (IQR) CS p20 level rose from 118.16 (83.30 to 188.01) at Time 1 to 141.94 (100.67 to 330.71) at Time 2 ($p = 0.05$ Time 2 vs Time 1), and then to 151. 91 (116.99 to 211.94) at Time 3. In the PV, the median levels of p20 showed significant increases over time (Time 1 vs Time 3, $p = 0.01$). Median (IQR) PV p20 levels increased from 114.93 (72.31 to 143.66) at Time 1, to 137.11 (110.88 to 171.51) at Time 3.

Similarly, the p17 of caspase-3 (ng/ml) increased with time during cardioplegia in both CS and PV (Figure 6). The median (IQR) levels of p17 (ng/ml) increased from 6.85 (3.26 to 15.28) at Time 1, to 9.14 (5.03 to 25.10) at Time 2 (Time 2 vs Time 1, $p = 0.028$), and then to 10.90 (5.02 to 21.25) at Time 3 (Time 3 vs Time 1, $p = 0.09$). In the PV, p17 levels increased with time of cardioplegia. Median (IQR) levels of p17 (ng/ml) increased from 7.71

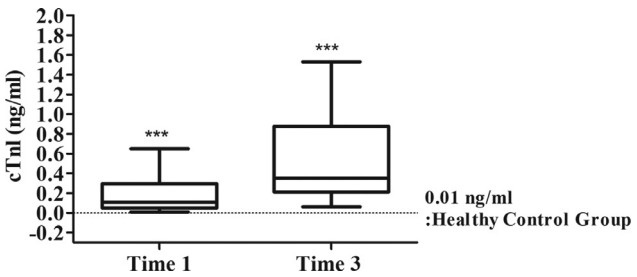

Figure 1. Comparison of PV cTnI levels (ng/ml) in cardiac surgery patients at Time 1 ($n = 48$, 0.11 [0.05 to 0.28]; median [IQR]) and at Time 3 ($n = 36$, 0.35 [0.21 to 0.86]; median [IQR]). Each box and whisker plot indicates the median, IQR, and range of surgery patient PV cTnI. Cardiac surgery patients had elevated PV cTnI levels compared with the healthy control group at both time points. Not all blood draws were feasible or were nonhemolyzed at each time point. ***p <0.001.

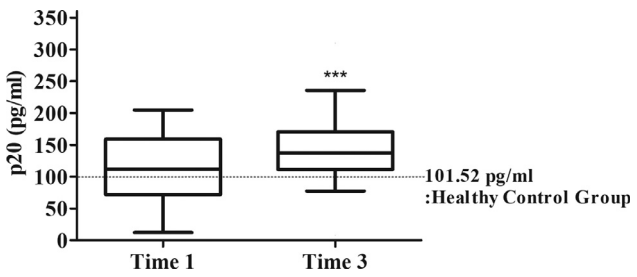

Figure 2. Comparison of PV caspase-1 p20 levels (pg/ml) in cardiac surgery patients at Time 1 ($n = 44$, 111.71 [72.12 to 157.28]; median [IQR]), and at Time 3 ($n = 28$, 136.96 [112.31 to 168.83]; median [IQR])) versus a healthy control group ($n = 56$, 101.52 [79.97 to 131.94]; median [IQR]) before and at the end of cardioplegia (Time 1 and Time 3, respectively). Each box and whisker plot indicates the median, IQR, and range of surgery patient PV p20. Cardiac surgery patients had significantly elevated PV p20 levels compared with the healthy control group at the end of cardioplegia ($p < 0.001$). The smaller sample size here was due to insufficient plasma volume for assaying the p20 levels. ***p <0.001.

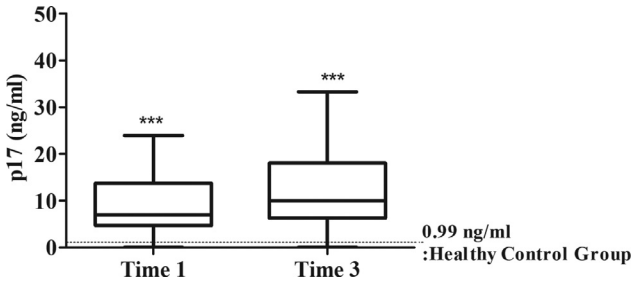

Figure 3. Comparison of PV caspase-3 p17 levels (ng/ml) in cardiac surgery patients at Time 1 ($n = 48$, 6.98 [4.51 to 13.79]; median [IQR])), and at Time 3 ($n = 36$, 11.05 [6.26 to 18.05]; median [IQR]) versus a healthy control group ($n = 66$, 0.998 [0 to 5.44]; median [IQR]) before and at the end of cardioplegia (Time 1 and Time 3, respectively). Each box and whisker plot indicates the median, IQR, and range. Cardiac surgery patients had significantly elevated PV p17 levels compared with the healthy control group at both surgery time points. ***p <0.001.

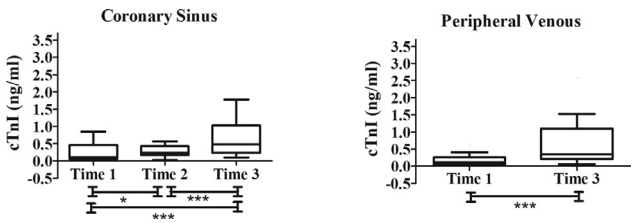

Figure 4. cTnI levels (ng/ml) in blood from the CS ($n = 30$), and PV ($n = 36$) were determined at Time 1, 2, or 3. cTnI levels increased significantly from Time 1 to Time 2 ($p = 0.036$) as well as from Time 2 to Time 3 ($p < 0.001$) in CS. Levels of cTnI also increased from Time 1 to Time 3 ($p < 0.001$) in PV. The sample size reflected available amount of plasma at all 3 time points that permitted assaying cTnI across time. ***p <0.001; *$0.01 \leq p < 0.05$.

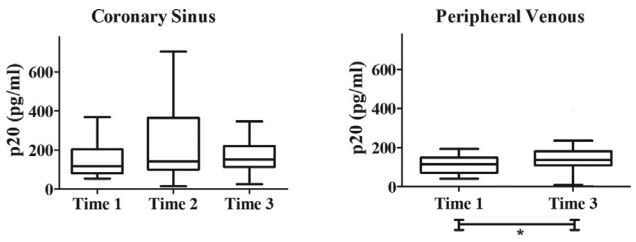

Figure 5. Caspase-1 p20 levels (pg/ml) in blood from CS ($n = 23$) and PV ($n = 25$) were determined at Time 1, 2, or 3. Caspase-1 p20 levels in the CS had an increasing trend over time. PV p20 level was significantly elevated at Time 3 compared with Time 1 ($p = 0.01$). Samples were matched for available amount of plasma at all 3 time points that permitted assaying p20 across time. *$0.01 \leq p < 0.05$.

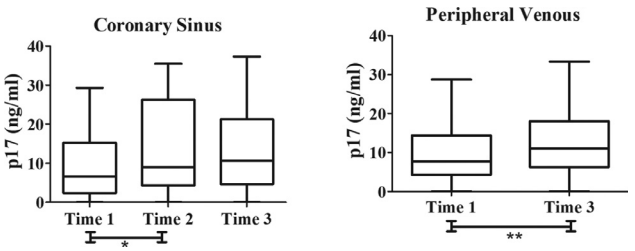

Figure 6. Caspase-3 p17 levels (ng/ml) in blood from CS (n = 30) and PV (n = 36) were determined at Time 1, 2, or 3. In CS, there was a trend for a higher level at Time 3 versus Time 1 and a significantly elevated level was observed at Time 2 versus Time 1 (p = 0.028). In PV, p17 caspase-3 levels were significantly elevated at Time 3 compared with Time 1 (p = 0.001). Samples were matched for available amount of plasma at all 3 time points that permitted assaying p17 across time. **0.001 ≤ p<0.01; *0.01 ≤ p <0.05.

(4.58 to 14.22) at Time 1, to 11.66 (6.25 to 18.05) at Time 3 (Time 3 vs Time 1, p = 0.001).

To determine whether biomarker levels in CS are elevated compared with levels in PV, we made paired comparisons of biomarkers between CS and PV before and after cardioplegia (Table 2). cTnI level in CS was significantly elevated compared with the level in PV at Time 3. Compared with PV p20, the CS p20 was significantly elevated before cardioplegia at Time 1 and after cardioplegia at Time 3. For the CS p17, its level was also elevated compared with p17 in PV before and after cardioplegia.

To ascertain the relationship between the traditional cardiac marker of cell injury cTnI and the apoptotic cell death marker caspase-3 p17 in the CS, we calculated correlations between them at each time point of cardioplegia. As shown in Table 3, in the CS, p17 was positively correlated with cTnI at Time 1 before cardioplegia, indicating the occurrence of both necrotic and apoptotic cell death at that time. In the CS, the inflammatory marker caspase-1 p20 was positively correlated with cTnI during cardioplegia.

To study whether the type of surgery is associated with a different profile of biomarkers, we compared the 3 markers at each time point in those who underwent CABG only versus those with valve or valve plus CABG (valve plus group). There was no difference in the cross-clamp duration between the 2 groups of patients (p = 0.3; Supplementary Figure 1). The CS cTnI levels at the end of cardioplegia were higher in the valve plus group than in the CABG only group (Table 4). We observed a similar difference in CS p20 levels for the valve plus versus CABG only comparison. No differences were observed for p17. These data indicate a greater degree of injury and inflammation during the valve operation.

Discussion

In the present study, we aimed to determine whether apoptosis occurs in addition to necrosis during ischemia/reperfusion in patients undergoing cardioplegia as part of cardiac surgery. We found that cardioplegia is associated with cTnI elevation in the CS, with a concomitant elevation in the PV. The apoptotic cell death marker caspase-3 p17 was also elevated in the CS compared with healthy subjects, with a similar concomitant elevation in the PV, confirming the presence of apoptotic and necrotic myocardial cell death during cardioplegia and cardiopulmonary bypass surgery.

To investigate the anatomical origin of the biomarkers, comparisons between biomarker levels in the CS and PV blood were performed. The CS levels of cTnI were higher than PV levels both before and after cardioplegia, indicating that the necrotic biomarker predominantly originates from cardiac tissues. A similar significant CS elevation was found for both caspase-1 p20 and caspase-3 p17. Although it is possible that apoptosis and inflammation in noncardiac tissues contributed to the PV levels of caspase-1 p20 and caspase-3 p17, the elevated levels in the CS suggest a cardiac origin. Just like cTnI, it is likely that the elevated PV levels of caspase-1 and -3 are a reflection of the status of cardiac myocytes. Thus, measurement of these apoptotic and inflammatory markers in venous blood may offer a window into these biological processes in the heart.

Both cell death markers increased progressively after the induction and occurrence of cardioplegia. Increased duration of ischemia is well known to result in greater myocardial injury. Thus, it is not surprising that the cardiac levels of both cTnI and caspase-3 p17 increased after cardioplegia.

Table 2
Comparison of coronary sinus (CS) and peripheral vein (PV) biomarker levels cardiac troponin I (cTnI), caspase-1 p20, and caspase-3 p17

Median	Before Cardioplegia				After Cardioplegia			
	CS	PV	p Value	RPD	CS	PV	p Value	RPD (%)
cTnI	0.10	0.11	**0.02***	**7%***	0.57	0.33	**<0.001*****	**30%*****
p20	124.00	111.71	**0.002****	**20%***	163.4	139.9	**0.04***	**6%***
p17	9.19	6.99	**0.04***	**8%***	12.12	11.06	0.05	**3%***

cTnI (ng/ml) at Time 1 (n = 48), at Time 3 (n = 34).
p20 (pg/ml) at Time 1 (n = 42), at Time 3 (n = 28).
p17 (ng/ml) at Time 1 (n = 48), at Time 3 (n = 34).
RPD is relative percentage difference between CS and PV, representing % above the PV level.
Bold values and p values indicate significance. Asterisks indicate significance level.
*** p <0.001.
** 0.001 ≤ p <0.01.
* 0.01 ≤ p <0.05. The sample size reflects availability of the blood samples at the times indicated. There were fewer available CS and PV samples that matched to the same subjects at time point 3.

Table 3
Correlation analysis between cardiac troponin I (cTnI), caspase-1 p20, and caspase-3 p17 in the coronary sinus (CS)

Spearman Correlation (ρ)	Time 1	Time 2	Time 3
cTnI (ng/ml) & p20 (pg/ml)	0.29	**0.39***	0.05
cTnI (ng/ml) & p17 (ng/ml)	**0.42***	0.06	−0.26
p20 (pg/ml) & p17 (ng/ml)	0.20	0.17	0.33

Values are Spearman's rank correlation coefficients (ρ) of CS biomarkers.
cTnI & p20 at Time 1 (n = 45), Time 2 (n = 32), and Time 3 (n = 29).
cTnI & p17 at Time 1 (n = 48), Time 2 (n = 38), and Time 3 (n = 36).
p20 & p17 at Time 1 (n = 45), Time 2 (n = 32), and Time 3 (n = 29).
Bold values indicate significance. Asterisks indicate the level of statistical significance.
* $0.01 \leq p < 0.05$. The sample size reflects availability of the blood samples at the times indicated. The sample from the same subject was analyzed for correlation at each time point for the pair of indicated biomarkers.

Table 4
Coronary sinus (CS) median levels of biomarkers cardiac troponin I (cTnI), caspase-1 p20, and caspase-3 p17 in coronary artery bypass grafting (CABG) only versus valve plus CABG patients (valve plus)

Surgery Type	cTnI (ng/ml)			p20 (pg/ml)			p17 (ng/ml)		
	Time 1	Time 2	Time 3	Time 1	Time 2	Time 3	Time 1	Time 2	Time 3
CABG only	**0.13***	0.24	**0.46***	118.67	134.38	**133.12***	8.81	8.86	11.23
Valve plus	**0.07***	0.31	**0.97***	136.93	265.98	**221.14***	7.95	4.79	10.9

cTnI CABG only at Time 1 (n = 26), Time 2 (n = 26), and Time 3 (n = 23).
cTnI valve plus at Time 1 (n = 22), Time 2 (n = 12), and Time 3 (n = 13).
p20 CABG only at Time 1 (n = 23), Time 2 (n = 23), and Time 3 (n = 18).
p20 valve plus at Time 1 (n = 22), Time 2 (n = 9), and Time 3 (n = 11).
p17 CABG only at Time 1 (n = 26), Time 2 (n = 26), and Time 3 (n = 23).
p17 valve plus at Time 1 (n = 22), Time 2 (n = 12), and Time 3 (n = 13).
Bold values indicate significant differences between groups. Asterisk indicates level of statistical significance.
* $0.01 \leq p < 0.05$.

The progressive increase of both cell death markers in the heart was paralleled by increases in the peripheral circulation. Our data support that cardiac apoptosis occurs during cardioplegia in patients who underwent cardiopulmonary bypass. These data raise the prospect that CS caspase-3 p17 can be developed as a biomarker to gauge the effectiveness of heart protection from apoptosis by cardioplegia. Likewise, cTnI levels in the CS can also be used to determine the degree of cardioprotection against necrosis in cardioplegia. Together, both markers offer promise to indicate the extent of heart protection.

Our current study showed that caspase-1 p20 is elevated in CS and PV during cardioplegia, compared with the healthy control population, and increases with longer duration of cardioplegia. Similar to caspase-3 p17 and cTnI, p20 was increased in the CS compared with PV, consistent with a cardiac origin of the biomarker. It is interesting that caspase-1 p20 is positively associated with cTnI during cardioplegia in the heart, and our data are consistent with the concept that inflammation occurs and may play a role in cardiac injury during cardioplegia, an idea deserving future investigation. Cardiac-derived cTnI and caspase-3 p17 did not show any correlation. As the molecular mechanism and process of necrotic cell death differs from those of apoptotic cell death,[16] this finding is not surprising and may point to different initiating factors.

Our current study has several limitations. It was not always possible to obtain nonhemolyzed blood from the CS at all 3 time points, leaving some subjects with only 2 of the 3 blood samples. This has limited the number of samples matched across time in the same subjects. Nevertheless, we were able to obtain sufficient matched samples to allow analysis. For caspase-1 p20 and caspase-3 p17, tissues other than heart could have contributed to the PV levels. Thus, measuring PV levels of these 2 markers may only partially reflect the occurrence of cardiac inflammation or apoptosis during cardiopulmonary bypass. There is no method to definitively identify caspases of myocardial origin in venous blood. Finally, factors other than cardioplegia, such as general anesthesia and underlying cardiac pathologies, likely also contributed to cardiac inflammation and cell death during the bypass. Biomarker levels before cardioplegia were elevated compared with healthy controls, further supporting that underlying cardiac disease likely contributes to elevations in these biomarkers.

In conclusion, our study showed the ability to quantify the extent of cardiac apoptosis during cardioplegia via circulating p17. Increased cardiac inflammation in cardioplegia was also present and the inflammatory marker caspase-1 p20 correlated with cTnI levels in the heart. Our data suggest that circulating levels of these 3 biomarkers, as measured in both CS and PV blood, offer an indication of the effectiveness of cardioprotection during cardiac bypass surgery. Targeting apoptosis and inflammation may enhance cardioprotection in surgery, providing an avenue for novel therapeutics that improve cardiac outcomes.

Disclosures

The authors have no relevant financial disclosures or conflicts of interest related to this work.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.1016/j.amjcard.2018.12.019.

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