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Biochemical response to cryothermal and radiofrequency exposure of the human myocardium at surgical ablation of atrial fibrillation: a randomized controlled trial

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Abstract

Background: Surgical cryothermia and radiofrequency (RF) ablations for atrial fibrillation (AF) seem to result in similar sinus rhythm restoration, but the biochemical consequences of the two methods are unclear. We aimed to compare the biochemical responses to the two ablative methods in concomitant mitral valve surgery (MVS).

Methods: Sixty mitral valve surgery patients with AF were prospectively included. Forty-one patients planned for ablation were randomized to cryothermia ($n = 20$) or radiofrequency ($n = 21$) ablation and 19 served as controls. Markers for myocardial injury, inflammation, cell stress, apoptosis, and heart failure were analyzed pre- and postoperatively at different time points.

Results: Troponin T and creatine kinase isoenzyme MB (CK-MB) peak levels were significantly higher in the cryothermia group compared with the RF group (12,805 [6140–15,700] vs. 2790 [1880–4180] ng/L; $P = 0.002$ and 271 [217–357] vs. 79 [66–93] μg/L; $P < 0.001$, respectively). Both groups had significantly higher levels than the no-ablation group. There were no group differences in C-reactive protein (CRP) and N-terminal pro-B-type natriuretic peptide (NT-proBNP), but there were correlations between pre- and postoperative levels of both CRP ($r_s = 0.41$, $P = 0.001$) and NT-proBNP ($r_s = 0.48$, $P < 0.001$). Protease-activated receptor 1 (PAR-1) and heat shock protein 27 (HSP27) were significantly increased in the cryoablation group.

Conclusions: Cryoablation results in a larger myocardial injury and possibly more elevated apoptotic activity and cell stress compared with the RF technique. The type of ablation device did not have any significant influence on the postoperative inflammatory response nor on the early postoperative levels of NT-proBNP.

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Keywords: Atrial fibrillation, Mitral valve surgery, Concomitant surgical ablation, Myocardial injury, Heart failure, Apoptosis, Cell stress, Cryoablation, Radiofrequency

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Background

Surgical treatment for atrial fibrillation (AF) consists of a well-defined set of atrial lesions in order to electrically isolate trigger areas and block reentry loops. This was first achieved with the cut-and-sew method, which is somewhat more demanding and time-consuming than the newer methods that create scars using thermal devices [1]. The thermal methods are mostly used along with other cardiac surgery to treat AF [2]. The two recommended methods are cryothermal and bipolar radiofrequency (RF) as they have shown similar results in restoring sinus rhythm in cohort studies [3].

The ablation results in three zones; the central zone of the injury, which is in immediate connection to the probe application, a transitional zone that is either undergoing apoptosis or recovering from reversible damage, and the surrounding tissue that is unaffected by ablation [4]. RF energy produces heat at the interface between the probe and the tissue, while cryoablation uses cold injury to create cellular damage. Two of the main differences between RF and cryoablation are the size of the transitional zone and the tissue response to thermal and cryogenic methods. In RF, the transitional zone is narrower because of the steep decrease of the temperature gradient. The tissue response in the transitional zone in cryoablation is the product of vascular injury and ischemia, apoptosis, and immunomodulation [5]. These responses are much less pronounced with RF ablation [4]. A third difference between the methods is the thermal effect in the central zone. In RF, the central zone undergoes coagulative necrosis at temperatures ≥50 °C, which denature most of the intracellular proteins. In cryoablation, fibroblasts and collagen fibers resist freezing injury and the architecture of the tissue, though devitalized, remains as a structure for repair [6].

The effects of these two energy sources in open cardiac surgery on biochemical response are not well known, and the choice of ablation method has so far been based mainly on institutional and personal preferences. Since the mechanisms of tissue damage differ considerably between the two thermal ablation methods, the purpose of this study was to show whether the respective biochemical responses to ablation concomitant with surgery might differ in terms of markers for myocardial injury (Tn-T, CK-MB, ASAT, ALAT) inflammation (CRP, IL-6, IL-18), cell stress and apoptosis (STK4, PAR-1, HSP27, TRAIL R2), and heart failure (NT-proBNP). See Table 1.

Methods

Study design

This was a partly randomized, parallel, single-center study comprising patients with AF scheduled for mitral valve surgery. A group of patients ineligible for AF ablation were included as controls and were not subject to randomization.

Study population

Of 63 consecutive patients, 3 declined participation. Sixty patients with preoperative AF scheduled for mitral

Table 1 Inflammatory, cell stress and apoptosis specific proteins included in the CVD II panel and used in the study

Interleukin-6 (IL-6)	IL-6 is a cytokine involved in the acute phase response to tissue injury or to an inflammatory stimulus. It acts both locally and systemically and it has its peak concentration a few hours after the end of ECC and gradually decreases within the following 24 h [7].
Interleukin-18 (IL-18)	IL-18 has a central role in the inflammatory cascade and it is widely expressed by monocytes/macrophages. High circulating levels have been associated with cardiovascular diseases, ranging from coronary artery disease to heart failure. In particular, a strong association between increased IL-18 levels and atrial fibrillation has been seen [8].
Serine/threonine-protein Kinase 4 (STK4)	STK4, also known as MST1, is a cytoplasmic kinase. It is a critical component of the Hippo pathway and a key regulator of organ size and regeneration. It acts by inducing autophagy [9], apoptosis and inhibition of cell proliferation. Recently it was shown that STK4 plays an important role in regulating stress response and apoptosis in myocardial injury [10].
Protease-activated receptor 1 (PAR-1)	PAR-1 is a G-protein coupled receptor, activated by the coagulation protease thrombin, as well as other proteases. In the heart, it is expressed by cardiomyocytes and cardiac fibroblasts. PAR-1 plays an important role in cardiac remodeling after myocardial infarction. Elevated PAR-1 expression in human heart failure contributes to pathological cardiac remodeling (cardiac fibrosis) and hypertrophy [11].
Heat shock protein 27 (HSP27)	Heat shock proteins are a ubiquitous family of chaperones. They represent the key players within cellular stress responses by stabilizing protein folding of newly synthesized proteins. With a molecular weight of 27 kDa in humans, HSP27 has a dual role, depending on its intracellular or extracellular location [12]. The intracellular overexpression protects cardiac myocytes against ischemic injury. When located in the extracellular spaces HSP27 contributes to the mechanism underlying post ischemic myocardial inflammatory response and cardiac functional injury [13, 14].
TNF-related apoptosis inducing ligand-receptor 2 (TRAIL-R2)	TRAIL-R2 is a cell surface receptor for TNF-related apoptosis- inducing ligand with the capacity to induce apoptotic signaling cascades leading to cell death High TRAIL-R2 levels were recently shown to be a predictor of long term mortality in patients with acute myocardial infarction [15].

CVD Cardiovascular disease, *ECC* Extra corporeal circulation, *MST* Mammalian sterile 20-like 1

valve surgery were enrolled consecutively between Sept 2013 and October 2015 at Linkoping University Hospital. Patients judged to benefit from AF ablation were randomly assigned, in a 1:1 ratio in blocks of 10, to undergo concomitant AF ablation with either a cryodevice (cryo group, $n = 20$) or RF device (RF group, $n = 21$). Patients with no AF symptoms, and those for whom additional aortic cross clamp time and thereby prolonged ischemia was regarded as too hazardous, underwent mitral valve surgery without AF ablation (no-maze group, $n = 19$). The decision to ablate or not was made before enrollment by a group of experts in arrhythmia, consisting of cardiologists and cardiac surgeons. The study was approved by the Regional Ethical Review Board (2012/371–31) and patients were recruited after written, informed consent by the surgeon the day before surgery. This study is the first part of the registered clinical trial: DOI https://doi.org/10.1186/ISRCTN14454361.

Ablation methods

For cryoablation, the argon powered Cardioblate CryoFlex Surgical Ablation Probe (Medtronic Inc., Minneapolis, MN) was used. The probe was applied for 120 s and the temperature fell rapidly (Joule-Thompson effect) to between − 130 °C and − 150 °C at each ablation line. The left atrial lesions consisted of three lines for the left atrium and three for the right atrial wall and followed the Cox IV pattern [16–18]. All but the intercaval lesions were created during cardioplegic arrest. For RF ablation, the Cardioblate BP2 Irrigated Bipolar Surgical Ablation System (Medtronic Inc., Minneapolis, MN) was used. This system uses irrigation and impedance-based power adjustments to reach tissue temperatures between 50 °C and 80 °C during ablation, according to the manufacturer. Each line was subject to at least three complete ablation periods. During RF ablation, the right and the left pulmonary vein orifices were isolated pairwise and epicardially on bypass [19], but before cardioplegia was given. During cardioplegic arrest, the left atrial ablation was completed according to the Cox IV pattern [20].

Procedures

All patients were operated on by the same surgeon. Myocardial arrest was induced and maintained with combined antegrade and retrograde cold blood cardioplegia. Biatrial lesions were induced, during cardioplegia arrest, in all ablation patients except for one in the cryo group and three in the RF group, who underwent only left-sided ablations. The left atrial appendage was stapled and excised with an Endo GIA Reinforced device (Medtronic Inc., Minneapolis, MN). The surgical procedures and patient characteristics are summarized in Table 1.

Blood sampling and biochemical analyses

Blood was collected preoperatively on the day before surgery (Pre), at the end of surgery (T0), 3 h (T3), 20 h (T20), and 3 days (D3) postoperatively. Troponin T (Tn-T) was analyzed at Pre, T0, T3, T20, and D3. Creatine kinase isoenzyme MB (CK-MB) and aspartate aminotransferase (ASAT) were analyzed at Pre, T0, T3, and T20. C-reactive protein (CRP) and alanine aminotransferase (ALAT) were analyzed at Pre and T20. NT-proBNP was analyzed at Pre, T20, and D3.

CK-MB, Tn-T, and NT-proBNP was analyzed in serum samples using an electrochemiluminescence immunoassay. A particle-enhanced immunoturbidimetric assay was used for CRP measurement in plasma samples preoperatively and postoperatively, and pyridoxal phosphate activation for ASAT and ALAT. The cobas c701 and e602 analyzers (Roche Diagnostics GmbH, Mannheim, Germany) were used. Additional biomarkers were analyzed using the Proseek Multiplex CVD II panel (Olink Proteomics, Uppsala, Sweden), simultaneously measuring 92 proteins. This analysis provides very high sensitivity and specificity as it is performed using a matched pair of antibodies coupled to unique oligonucleotides and measured by quantitative real-time PCR. For this study, blood samples from Pre, T0, T3, and T20 were fractionated and plasma was stored at − 70 °C before being sent to the Clinical Biomarkers National Facility (Science for Life Laboratory, Uppsala) for analysis.

In this survey, interleukins 6 and 18 (IL-6 and IL-18) were selected as markers for inflammation, and serine/threonine-protein kinase 4 (STK4) also known as MST1, proteinase activated receptor 1 (PAR-1), heat shock 27 kDa protein (HSP27), and TNF-related apoptosis-inducing ligand receptor 2 (TRAIL-R2) were selected as markers of cellular stress/apoptosis. Data from the Proseek Multiplex CVD II panel are presented as the differences in normalized protein expression (NPX) between preoperative levels and T0, T3, and T20 (ΔT0, ΔT3, and ΔT20). The log-2 transformed data were linearized before calculating differences from preoperative values. Data from 3.8% of the samples were excluded from analysis due to assay failure or failure to pass quality control.

Statistical methods

Sample-size calculation was based on the difference in markers for myocardial injury in pilot patients with and without concomitant cryoablation. The number of patients needed in each group to detect such a large difference was 3. We included 60 patients in the study to ensure at least 17 patients each in the cryoablation and RF ablation groups, enough to detect an effect size of 1. Continuous variables are presented as medians [25th to

75th percentile] and categorical variables as counts (percentages). For comparisons of the three groups, the Kruskal-Wallis test was used, taking into account multiple comparisons of mean ranks for all groups' P values, and post-hoc comparisons of mean ranks of all pairs of groups when the Kruskal-Wallis test showed significance. The Spearman rank test was used for correlations between two variables. Multivariable linear regression models were used to find the factors influencing myocardial injury. The Tn-T and CK-MB at 3 h postoperatively and ASAT at 20 h postoperatively were chosen as dependent factors and log-10 transformed to align with the assumption of Gaussian distribution. Study groups, age, gender, cross clamp time (CCT), coronary artery disease, and the extent of ablation were tested as predictors. The models were fit using an enter method. Multicollinearity was found between the study groups and the extent of ablation. Therefore, the extent of ablation was excluded from the models. For comparison of the markers of myocardial injury between the three study groups over time-repeated measurements, analysis of variance was used. A P value < 0.05 was considered statistically significant. Statistical analyses were conducted with Statistica 10.0 (StatSoft Inc., Tulsa, OK) and SPSS 22.0 (SPSS, Inc., Chicago, IL).

Results

There were no significant differences between the cryo and the RF groups with regard to preoperative, operative, and postoperative characteristics (Table 2). Patients in the No-maze group were older compared with the patients in the intervention groups, but did not differ markedly in other preoperative comorbidities.

Markers of myocardial injury and their thermal stability

The highest levels of Tn-T, CK-MB, and ASAT were measured 3 h after surgery (Table 3). There were, however, significant differences in Tn-T and CK-MB between all three groups, with the cryo group having the highest levels. There was no significant difference in ASAT between the cryo and the RF groups but both had higher levels than the no-maze group (Fig. 1). Correcting for the patients with only left atrial ablation lines did not influence the median levels of the markers for myocardial injury or the differences between groups. The AF ablation and method explained a large proportion of variance in the peak levels of markers for myocardial injury. Other explanatory variables were CCT, absence of coronary artery disease, and age (Table 4).

We conducted in vitro experiments to evaluate if locally increased temperatures during RF procedures might create a detection bias because of denaturation. To analyze thermal effects on TnT, CK-MB, ASAT, and ALAT detection, plasma samples from 10 patients were heated to 37 °C, 56 °C, 65 °C, and 80 °C for 3 min and the results were compared with a non-heated sample (Fig. 2). Detection of Tn-T and ASAT was unaffected by temperature while ALAT detection was partially affected at 65 °C (Fig. 2). CK-MB levels were temperature dependent and decreased dramatically even at 56 °C, suggesting that the lower levels measured during RF are most probably due to protein denaturation.

Multivariable linear regression models showing the factors with significant influence on myocardial injury. The Tn-T and CK-MB at 3 h postoperatively and ASAT at 20 h postoperatively were chosen as dependent factors and log10 transformed to align with the assumption of Gaussian distribution. The models were fit by an enter method. The extent of ablation was excluded from the models due to multicollinearity with the study groups. 95%CI: 95% confidence interval, RF: radio frequency, Tn-T: troponin T, CK-MB: creatine kinase isoenzyme MB, ASAT: aspartate aminotransferase, T3: 3 h postoperatively, T20: 20 h postoperatively.

Markers of inflammation (CRP, IL-6, and IL-18)

There were no significant differences in postoperative CRP levels between the three groups (Table 2). Both preoperative CRP and CCT correlated with CRP 20 h postoperatively ($r_s = 0.41$, $P = 0.001$ and $r_s = 0.22$, $P = 0.01$, respectively) in the whole study population. No significant differences between the three groups of patients regarding the pro-inflammatory cytokines IL-6 and IL-18 were found at any time point (Table 3).

Markers of cell stress and apoptosis (PAR-1, HSP27, STK4, TRAIL R2)

PAR-1 elevation from preoperation to 3 h after surgery (ΔT3) was significantly higher in the cryo compared with the RF group ($P = 0.002$) (Table 3, Fig. 3). ΔT3 HSP27 levels also differed significantly between the three study groups ($P = 0.007$) and in pairwise comparison, increased levels were found in the cryo compared with the RF group ($P = 0.002$) and compared with the no-maze group ($P = 0.001$), but there was no difference between RF and No-maze patients (Table 3, Fig. 4). At ΔT3, the decrease in STK4 had a tendency to be more pronounced in the RF group compared with the cryo group, although not statistically significant ($P = 0.06$). TRAIL-R2 did not show any significant differences between the study groups.

Marker of heart failure (NT-proBNP)

There were no significant differences in postoperative NT-proBNP levels between the three groups (Table 3). Preoperative NT-proBNP correlated with NT-proBNP at 20 h and 3 days postoperatively ($r_s = 0.48$, $P < 0.001$ and $r_s = 0.58$, $P < 0.001$, respectively) but there were no

Table 2 Pre-, per- and postoperative data of the three study groups

Variables	Cryothermia ($n = 20$)	RF ($n = 21$)	p Value* Cryo vs. RF	No-maze ($n = 19$)	p Value* No-maze vs. Cryo	p Value* No-maze vs. RF	p Value**
Preoperative data							
Female	5 (25%)	12 (57%)		7 (37%)			0.11
Age (years)	71 [65–74]	72 [66–74]	1.0	77 [73–78]	0.02	0.006	0.004
COPD	4 (20%)	4 (19%)		2 (10.5%)			0.69
NYHA III or IV	17 (85%)	14 (67%)		15 (79%)			0.37
Congestive heart failure	12 (60%)	13 (62%)		16 (84%)			0.20
Euroscore II	5.1 [3.2–6.7]	5.4 [4.3–7.0]		4.7 [3.2–7.4]			0.88
Body mass index (kg/m^2)	25 [24–28]	25 [24–29]		26 [23–29]			0.80
Non paroxysmal AF	15 (75%)	15 (71%)		13 (68%)			0.90
LVEF < 50%	5 (26%)	6 (29%)		5 (26%)			0.97
Left atrium area (cm^2)	35 [30–40]	32 [30–36]		32 [25–40]			0.35
TAPSE (mm)	19 [17–22]	16 [16–21]		15 [13–20]			0.17
Right atrium area (cm^2)	28 [26–31]	25 [22–30]		28 [21–31]			0.29
Pulmonary artery pressure (mmHg)	38 [30–50]	43 [32–50]		50 [35–60]			0.10
Operative data							
Surgery time (min)	253 [235–306]	253 [225–286]		220 [195–250]			0.06
Extracorporeal circulation (min)	158 [147–205]	163 [148–193]	1.00	131 [116–178]	0.03	0.03	0.01
Cross clamp time (min)	106 [101–129]	95 [86–120]	0.31	85 [77–100]	0.005	0.36	0.007
Mitral valve replacement	2 (10%)	5 (24%)		5 (26%)			0.39
Tricuspid valve surgery	15 (75%)	15 (71%)		12 (63%)			0.72
Coronary surgery	5 (25%)	6 (29%)		7 (37%)			0.72
Aortic valve replacement	2 (10%)	3 (14%)		2 (11%)			0.90
Aortic surgery	1 (5%)	0		1 (5%)			0.58
Left atrium appendage resection	13 (65%)	20 (95%)	0.29	6 (32%)	0.22	0.002	< 0.001
Inotropic drugs at weaning	18 (90%)	14 (67%)	0.60	8 (42%)	0.03	0.55	0.007
LVAD at weaning	1 (5%)	0		0			0.37
Postoperative outcome							
Time on ventilator (h)	2.6 [1.8–6.9]	4.3 [3.4–11.7]		7.3 [3.5–10.8]			0.08
Sinus rhythm at discharge	13 (65%)	11 (52%)	1.00	1 (5%)	0.004	0.03	< 0.001
30-day mortality	1 (5%)	0		0			0.37
Stroke, permanent	0	1 (5%)		0			0.40
Dialysis	1 (5%)	1 (5%)		2 (11%)			0.72

Data are presented as the median [25th to 75th percentile] or count (percentage). *Post-hoc comparisons of mean ranks of pairs of groups. **Multiple comparisons of mean ranks for all groups, Kruskal-Wallis test. *RF* Radio frequency, *COPD* Chronic obstructive pulmonary disease, *NYHA* New York Heart Association class, *AF* Atrial fibrillation, *LVEF* Left ventricular ejection fraction, *TAPSE* Tricuspid annular plane systolic excursion, *LVAD* Left ventricular assist device

significant correlations between CCT and postoperative NT-proBNP levels ($r_s = 0.11$, $P = 0.4$ and $r_s = -0.14$, $P = 0.3$, respectively).

Discussion

To our knowledge, this is the first randomized prospective study in cardiac surgery comparing the biochemical responses to the two most common methods used to perform ablation concomitant with surgery. We were able to show significantly higher levels of myocardial injury biomarkers, and more elevated apoptotic activity and cell stress, in cryoablation compared with the RF technique.

Myocardial injury

The high levels of CK-MB and Tn-T after both surgical ablative procedures reflect the device-induced atrial myocardial injury. The concentrations of both markers in patients that underwent cryoablation had greater increases at T0, T3, and T20 compared with those who

Table 3 Biochemical markers

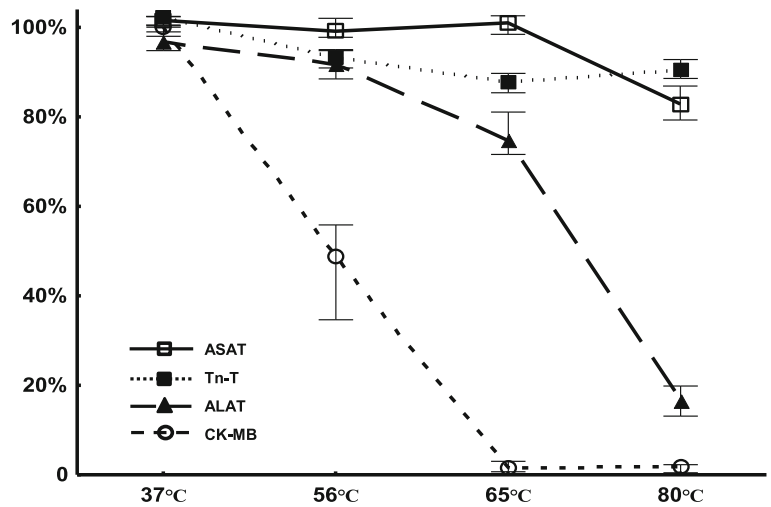
Variables	Cryothermia ($n = 20$)	RF ($n = 21$)	p Value* Cryo vs. RF	No-maze ($n = 19$)	p Value* No-maze vs. Cryo	p Value* No-maze vs. RF	p Value**
Tn-T (ng/L)							
Preoperative	12.5 [8.5–17]	12 [9–16]		15 [11–17]			0.53
T0	8145 [5095–14,440]	2140 [1900–3290]	0.002	709 [517–786]	< 0.001	0.002	< 0.001
T3	12,805 [6140–15,700]	2790 [1880–4180]	0.002	999 [683–1420]	< 0.001	0.002	< 0.001
T20	3400 [2785–4900]	1980 [1640–2530]	0.01	798 [565–929]	< 0.001	0.001	< 0.001
Postoperative day 3	2095 [1645–3275]	1200 [1030–1670]	0.005	576 [437–754]	< 0.001	0.01	< 0.001
CK-MB (μg/L)							
Preoperative	2 [1.5–2.5]	2 [1–3]		2 [2–3]			0.99
T0	269 [243–352]	68 [62–81]	< 0.001	47 [34–59]	< 0.001	0.06	< 0.001
T3	271 [217–357]	79 [66–93]	< 0.001	48 [32–71]	< 0.001	0.09	< 0.001
T20	107 [69–132]	53 [42–57]	0.005	29 [18–43]	< 0.001	0.02	< 0.001
ASAT (μg/L)							
Preoperative	0.5 [0.4–0.5]	0.5 [0.4–0.5]		0.5 [0.4–0.6]			0.58
T0	2.5 [1.8–3.9]	2.4 [1.8–3.4]	1.0	1.2 [0.9–1.5]	< 0.001	0.001	< 0.001
T3	3.3 [2.5–5.9]	2.9 [2–4.1]	0.55	1.3 [1.1–1.7]	< 0.001	< 0.001	< 0.001
T20	3.3 [2.5–3.8]	2.2 [2–2.6]	0.006	1.3 [1.0–1.6]	< 0.001	0.01	< 0.001
ALAT (μg/L)							
Preoperative	0.4 [0.3–0.5]	0.4 [0.4–0.5]		0.4 [0.3–0.5]			0.64
T20	0.5 [0.4–0.7]	0.5 [0.4–0.6]		0.5 [0.3–0.6]			0.16
CRP (mg/L)							
Preoperative	0.9 [0.4–1.9]	1.7 [0.7–3.9]	0.40	2.8 [1.5–7.2]	0.01	0.38	0.01
T20	77 [63–86]	75 [52–104]		77 [60–87]			0.89
IL-6 (NPX)							
ΔT0-Preoperative	300 [221–510]	185 [121–365]		193 [137–386]			0.23
ΔT3-Preoperative	223 [159–333]	141 [106–279]		206 [112–317]			0.20
ΔT20-Preoperative	150 [82–332]	120 [85–193]		222 [135–433]			0.09
IL-18 (NPX)							
ΔT0-Preoperative	−38 [−65 - -19]	−37 [−64–6]		−50 [−60 - -23]			0.87
ΔT3-Preoperative	−5 [−34–9]	−38 [−45 - -3]		−10 [−29 - -3]			0.32
ΔT20-Preoperative	−11 [−33 - -1]	−12 [− 34–7]		−28 [− 64–3]			0.47
STK4 (MST1) (NPX)							
ΔT0-Preoperative	−4.0 [−7.1 - -0.1]	−2.6 [− 12.0 - -1.0]		−5.0 [−8.6 - − 3.1]			0.5
ΔT3-Preoperative	-3.1 [−5.9 - -0.7]	−6.9 [−10.2 - -2.9]		−5.3 [− 7.0 - -1.7]			0.06
ΔT20-Preoperative	−6.6 [−8.5 - -2.4]	− 6.1 [−11.2 - -3.1]		− 8.1 [−10.5 - -3.8]			0.6
PAR-1 (NPX)							
ΔT0-Preoperative	−5.0 [−10.3–17.0]	2.2 [−15.6–16.2]		−7.3 [−18.6–5.6]			0.3
ΔT3-Preoperative	7.8 [0.1–17.6]	−9.8 [−19.3–0.1]	0.002	−5.0 [−14.6–4.8]	0.08	0.9	0.003
ΔT20-Preoperative	−8.1 [− 18.8–8.0]	−17.0 [−21.8–4.0]		− 15.7 [−26.8 - -9.4]			0.3
HSP27 (NPX)							
ΔT0-Preoperative	259 [98–310]	151 [−35–330]	0.6	106 [− 133–174]	0.03	0.6	0.04
ΔT3-Preoperative	196 [129–278]	42 [− 181–156]	0.02	−26 [−115–98]	0.001	1.0	0.007
ΔT20-Preoperative	−40 [− 204–81]	−87 [− 301–80]		− 138 [− 271 - -23]			0.3

Table 3 Biochemical markers *(Continued)*

Variables	Cryothermia ($n = 20$)	RF ($n = 21$)	p Value* Cryo vs. RF	No-maze ($n = 19$)	p Value* No-maze vs. Cryo	p Value* No-maze vs. RF	p Value**
TRAIL-R2 (NPX)							
ΔT0-Preoperative	6.6 [2.7–13.7]	8.7 [7.0–21]		4.2 [2.5–11.3]			0.2
ΔT3-Preoperative	27 [21–38]	23 [17–31]		20 [17–37]			0.1
ΔT20-Preoperative	25 [19–33]	25 [15–40]		25 [16–40]			0.9
NT-proBNP (ng/L)							
Preoperative	1250 [730–1945]	1380 [950–1910]		1620 [1220–3120]			0.65
T20	5290 [3295–10,615]	5470 [3060–9250]		3730 [2750–5810]			0.48
Postoperative day 3	6105 [4300–9185]	6000 [3990–11,200]		5430 [3660–14,000]			0.99

Data are presented as the median [25th to 75th percentile] or count (%). *Post-hoc comparisons of mean ranks of pairs of groups. **Multiple comparisons of mean ranks for all groups, Kruskal-Wallis test. *Cryo* Cryoablation, *RF* Radio frequency, *Tn-T* Troponin T, *T0* End of the operation, *T3* 3 h postoperatively, *T20* 20 h postoperatively, *CK-MB* Creatine kinase isoenzyme MB, *ASAT* Aspartate aminotransferase, *ALAT* Alanine aminotransferase, *CRP* C-reactive protein, *IL-6* Interleukin-6, *NPX* Normalized Protein Expression, *IL-18* Interleukin-18, *STK4* Serine/threonine-protein Kinase 4, *PAR-1* Proteas-activated receptor 1, *HSP27* Heat shock protein 27, *TRAIL R2* TNF-related apoptosis inducing ligand receptor 2, *NT-proBNP* N-terminal pro-B-type natriuretic peptide

underwent RF ablation and with the control group. This initial postoperative biomarker leakage is a confounding factor in the early detection of possible ventricular damage due to ischemia. In perioperative ischemic myocardial injury, CK-MB and Tn-T peaks are seen respectively 20 h postoperatively and at 40 h [21]. We could show that, with ablation, CK-MB and Tn-T leakage have their peaks immediately after the procedure and 3 h later, respectively. We could therefore deduce in line with catheter ablation studies [22–25] that marked elevations of TnT and CK-MB measured early postoperatively indicate direct myocyte damage rather than ischemia. Other factors with weak but statistically significant associations with markers for myocardial injury, beside ablation and ablation method, were CCT, age, and the absence of coronary artery disease. An important aspect to consider upon RF ablation is the heat-induced denaturation of the protein creatine kinase. The true myocardial tissue



	37°C	56°C	65°C	80°C
ASAT	102 [101-102]	99 [98-102]	101 [98-103]	83 [79-87]
Tn-T	102 [100-102]	93 [91-95]	88 [85-90]	90 [89-93]
ALAT	102 [100-104]	97 [93-100]	79 [75-85]	17 [14-21]
CK-MB	100 [98-100]	49 [35-56]	2 [1-3]	2 [0-2]

Fig. 1 The levels of Troponin T (Tn-T), creatine kinase isoenzyme MB (CK-MB), and aspartate aminotransferase (ASAT) preoperatively (Pre), at the end of surgery (T0), and 3 h (T3) and 20 h (T20) postoperatively displayed as median and 25th to 75th percentile. The figures for the mean values, the 25th to 75th percentiles, and the *P* values for comparison of the three groups are given in Table 3. The changes within the three groups over time are significant for all three markers for myocardial injury ($P < 0.001$)

Table 4 Multivariable linear regression models to find factors influencing myocardial injury

Variables	Standardized coefficient β	95% CI	*p* Value
Dependent variable: log10 Tn-T (T3) Adjusted R^2 = 0.79			
Patient groups (No-maze = 1, RF = 2, Cryo = 3)	0.85	0.71 to 0.98	< 0.001
Coronary artery disease (no = 0, yes = 1)	−0.18	−0.32 to −0.05	0.007
Age (years)	0.15	0.02 to 0.28	0.02
Cross clamp time (min)	0.15	0.01 to 0.29	0.04
Dependent variable: log10 CK-MB (T3) Adjusted R^2 = 0.70			
Patient groups (No-maze = 1, RF = 2, Cryo = 3)	0.84	0.70 to 0.98	< 0.001
Dependent variable: log10 ASAT (T20) Adjusted R^2 = 0.64			
Patient groups (No-maze = 1, RF = 2, Cryo = 3)	0.66	0.49 to 0.83	< 0.001
Cross clamp time (min)	0.26	0.08 to 0.44	0.005
Coronary artery disease (no = 0, yes = 1)	−0.20	−0.37 to − 0.03	0.02

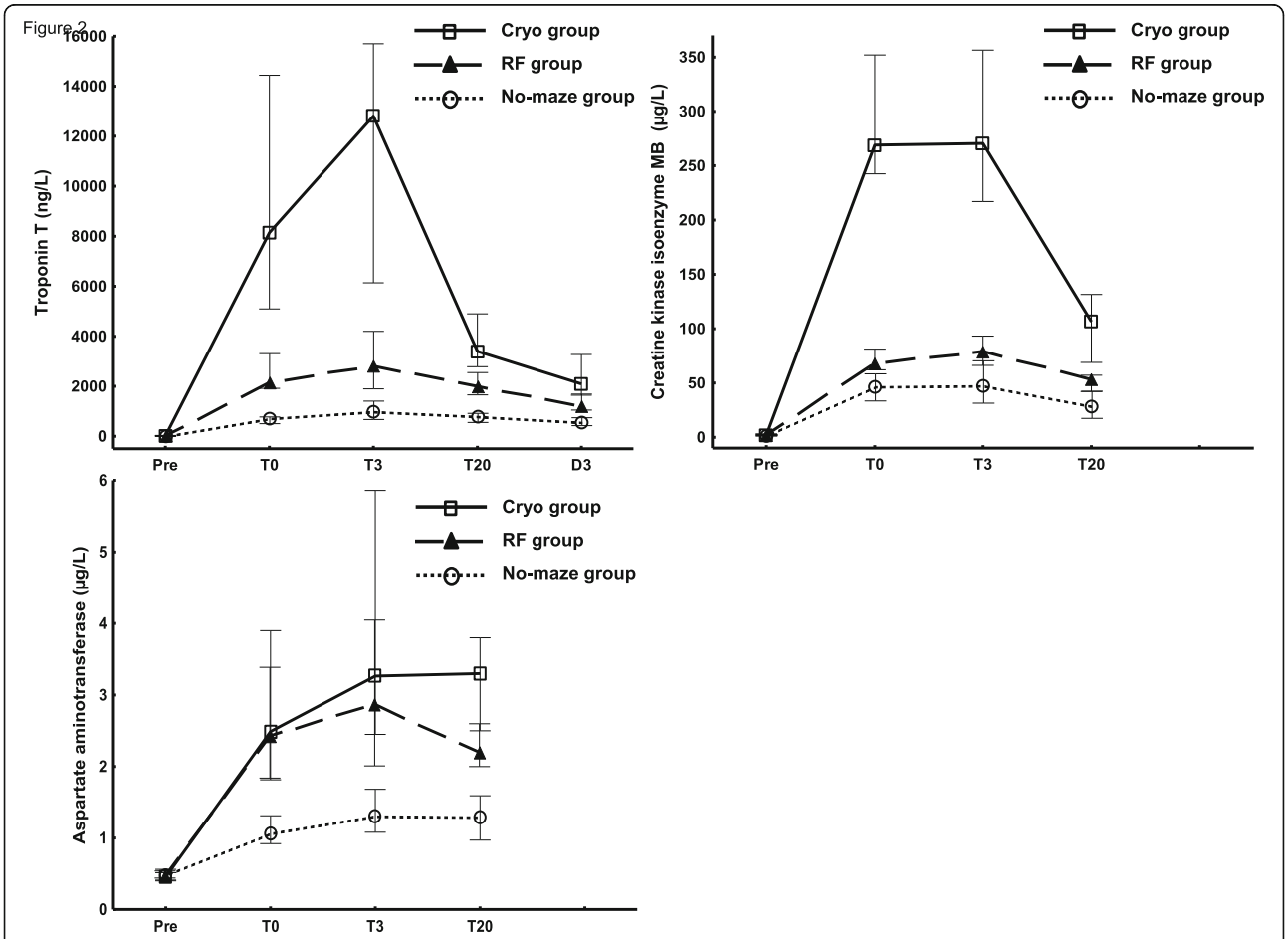


Fig. 2 The detected fractions of aspartate aminotransferase (ASAT), troponin T (Tn-T), alanine aminotransferase (ALAT), and creatine kinase isoenzyme MB (CK-MB) after heating to 37 °C, 56 °C, 65 °C, and 80 °C for 3 min expressed as percentages of the values from non-heated samples (median [25th to 75th percentile]) in 10 patients

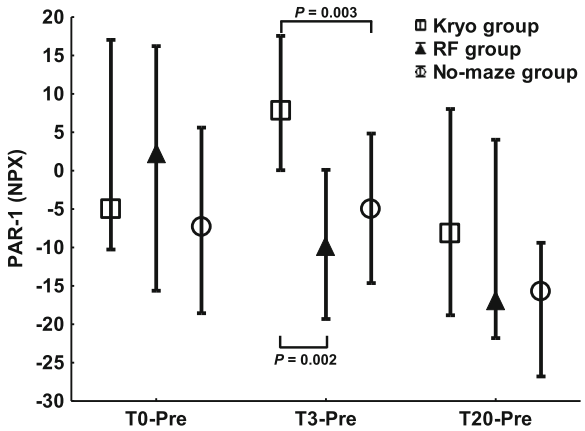


Fig. 3 Protease-activated receptor 1 (PAR-1) changes from preoperatively (Preop) to the end of surgery (T0), and 3 h (T3) and 20 h (T20) postoperatively displayed as median and 25th to 75th percentile in the three study groups. *P* values indicate the significance level at post hoc comparisons of mean ranks of pairs of groups when multiple comparisons of mean ranks for all groups, Kruskal-Wallis test showed significance. NPX: Normalized Protein Expression

damage caused by heating might be underestimated when measured by CK-MB [26]. The central zone of the RF lesion reaches temperatures of > 65 °C and, according to our in vitro experiment and to Haines et al. [27], the immunoassay method used to analyze CK-MB failed to detect heat-denatured CK-MB. This heat-dependent detection bias may explain the comparably modest differences in CK-MB between the RF and No-maze groups, but more importantly it may lead to a falsely large difference in CK-MB between RF and cryoablation [28]. Tn-T

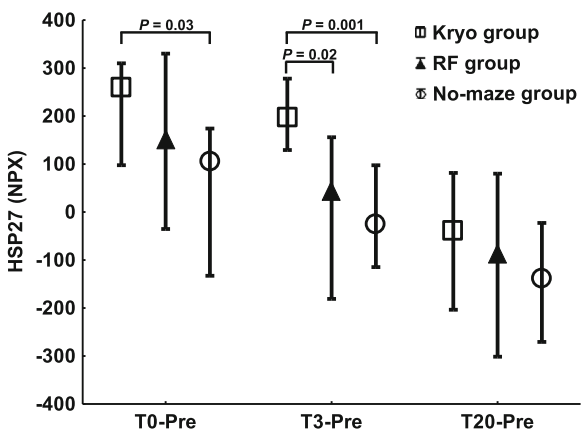


Fig. 4 Heat shock protein 27 (HSP27) changes from preoperatively (Preop) to the end of surgery (T0), and 3 h (T3) and 20 h (T20) postoperatively displayed as median and 25th to 75th percentile in the three study groups. *P* values indicate the significance level at post hoc comparisons of mean ranks of pairs of groups when multiple comparisons of mean ranks for all groups, Kruskal-Wallis test showed significance. NPX: Normalized Protein Expression

analysis was not as sensitive to thermal denaturation and therefore better reflects the differences in myocardial damage between the three study groups. ASAT was stable at temperatures above 65 °C and no significant difference was found between the cryo and RF groups. One explanation for this might be that ASAT is less myocardium-specific compared with CK-MB and Tn-T. This study was not powered to detect differences between cryo- and RF ablation in rhythm outcome. Thus, the larger myocardial injury indicated by the higher levels of cardiac biomarkers at cryoablation did not translate into a significantly better rhythm outcome at discharge compared with RF ablation. Larger randomized studies are warranted to explore possible differences in success rate between the two methods.

Inflammation

There is increasing evidence that AF is associated with inflammation, and active recruitment of macrophages across the atrial endocardium has been demonstrated in AF patients [29]. When inflammatory active cells are present in the atrial wall, the RF and cryoablation methods may result in different amounts of released bioactive inflammatory agents and thereby different systemic inflammatory responses.

Following ablation, tissue repair is initiated by an immediate infiltration of inflammatory cells (lymphocytes and macrophages) in response to the release of cyto- and chemokines from damaged cells. We know from ablation of tumors that cryoablation induces a notably higher postablative immunogenicity in which proinflammatory cytokines are released in higher quantities compared with other ablative modalities [30, 31]. In catheter ablation studies, cryoablation and RF resulted in comparable increases in inflammatory response markers [23, 32].

In our study, the addition of the ablation therapy did not affect the early inflammatory response. Neither CRP, nor IL-6, nor IL-18 differed significantly in levels between the three groups. The inflammatory response to surgical trauma and the extracorporeal circulation probably overrode the possible inflammatory activation caused by ablation. However, we found a significant correlation between CRP preoperatively and CRP 20 h after surgery. Hence, a subclinical, preoperative inflammatory activity might predispose patients to a higher postoperative inflammatory response.

Cell stress and apoptosis

The cornerstone of direct cell injury from freezing is ice crystal formation, which removes water from the cells and leads to the necrosis of the central part of the frozen volume while the peripheral zone shows apoptosis that is thought to arise from mitochondrial damage due to increased bcl-2-associated X protein (BAX) protein expression and caspase activation [6]. Cells that are not immediately killed by direct cryoablation-induced injury

may subsequently die by apoptosis. Apoptotic cell death after cryoablation has therefore been shown in the transitional zone where exposure to temperatures that are not immediately lethal results in irrecoverable cellular injury without immediate cell death [33].

We found interesting differences in PAR-1 and HSP27. PAR-1 was significantly decreased in the RF group. A possible explanation for this difference could be the small and more distinct RF ablation lines compared with the larger lesions seen with the cryoprobe. Tissue repair is initiated by an immediate infiltration of inflammatory cells and in the following weeks the necrotic tissue is slowly removed and replaced by a fibrous, collagenous scar [6]. Being involved in the fibrotic process that could lead to cardiac fibrosis, PAR-1 might have a key role in pathologic cardiac remodeling of myocytes after ablative atrial injuries. The late consequences of the size of the atrial injury are still unknown.

At no postoperative time points did STK4 and TRAIL-R2 differ significantly between the groups. TRAIL initiates intracellular signaling cascade resulting in the apoptotic cell death after binding to its receptors TRAIL-R1 and TRAIL-R2 [34]. In recent years TRAIL-R2 has been shown to have a unique selectivity for triggering apoptosis in tumor cell while leaving normal tissues intact [35]. Its expression is high in cancer cell lines and increased in viral-induced apoptosis [36], but it is not abundant in most normal human cells [37]. TRAIL-R2 seems therefore to have a minor role in myocyte/myocardium apoptosis compared to apoptosis in other tissues. This could be a possible explanation for not seeing a significant difference between the cryothermally induced apoptosis from the RF one. The mechanism of apoptosis in the transitional areas might only partly be TRAIL-R2 mediated.

HPS27 has a dual role depending on its intracellular or extracellular location. Intracellular HSP27 protects against tissue injury (anti-apoptotic property) allowing the cells to survive potentially lethal conditions such as acute coronary syndromes and reperfusion after ischemic clamping during heart by-pass surgery. Increased expression of HSP27 has been shown to provide cardioprotection against hypoxic injury [14]. Extracellular HSP27 is involved in various immunological processes, being a strong immune modulator, and its abundance in serum is a biomarker for ischemic events [12].

In our study, HSP27 elevation at T3 was significantly higher in the cryo compared with the RF and No-maze groups. This could be the result of the rapidly stimulated HSP27 expression and release by cardiomyocytes in response to the larger cryogenic injury. It might also be the effect of the activated transitional and necrotic central zones which, after the thawing phase, release intracellular HSP27 that is still biologically active after cryogenic injury. Heating of the cellular tissue should result in coagulated and, thereby, biologically inactivated proteins. In contrast, the cryoablation device freezes the myocardium, leading to destruction of the cell membranes, but the intra- and extracellular proteins released to the circulation maintain their bioactivity [4].

Postoperative heart failure

The possible contribution of cryoablation to postoperative heart failure has previously been discussed [38]. The use of inotropes at weaning from extracorporeal circulation was significantly higher in the cryoablation patients compared with the non-ablated patients, but the addition of ablation to mitral valve surgery and the type of energy source did not seem to influence the early postoperative levels of NT-proBNP.

Limitations

The study group size was limited and clinical conclusions cannot, therefore, be based upon the biochemical findings. Larger studies are needed in the future to analyze clinical outcomes in different ablation methods. Only the two ablation groups were randomized. Comparisons between the No-maze group and the two ablation groups should be interpreted cautiously, even though we corrected for some of the differences in the analyses. We think it would have been of interest to analyze more apoptosis-specific proteins than the ones included in the CVD II panel and to follow the cardiac biomarkers up to 4 days postoperatively. The systemic biochemical response to the surgical trauma and to the extracorporeal circulation might obscure the effect of the ablation on the myocardial tissue in this clinical setting. Significant differences between the groups are, however, still relevant.

Conclusions

Our findings indicate increased myocardial injury when surgical ablation for AF is carried out with a cryogenic compared with an RF device. We also showed that the heat-induced denaturation of creatine kinase caused a detection bias that might lead to underestimation of this injury in case of RF. The addition of ablation to mitral valve surgery and the type of ablation device do not have any significant influence on the postoperative inflammatory response nor on the early postoperative NT-proBNP levels.

Higher HSP27 and PAR-1 levels could support the hypothesis that freezing damages tissue by a different intrinsic mechanism than that of RF, in addition to creating a locally wider area of damage to the atrial wall. These results might confirm the importance of apoptosis

as a mechanism of cell death after cryosurgery especially in the periphery of the previously frozen tissue.

The physiologic significance and clinical implications of the more extensive atrial cellular damage caused by cryoablation are still uncertain and should be investigated in further studies.

Abbreviations

ALAT: Alanine aminotransferase; ASAT: Aspartate aminotransferase; CK-MB: Creatine kinase isoenzyme MB; CRP: C-reactive protein; HSP27: Hheat shock protein 27; IL-6: Interleukin − 6; IL-18: Interleukin-18MVSMitral valve surgery; NPX: Normalized protein expression; NT-proBNP: N-terminal pro-B-type natriuretic peptide; PAR-1: Protease-activated receptor 1; RF: Radio frequency; STK4: Serine/threonine-protein kinase 4; Tn-T: Troponin T; TRAIL-R2: TNF-related apoptosis inducing ligand- receptor 2; T0: End of the operation; T3: Three hours postoperatively; T20: Twenty hours postoperatively

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Authors' contributions

All the authors have read the journal's policy on conflicts of interest and they have no conflicts of interest to disclose. All authors have read and understand the journal's authorship agreement and have reviewed this manuscript. G.B., M.Å.A., and F. V Conceived and designed the study. Patient inclusion and data collection: G.B. and F.V. Blood sample handling and laboratory analyses: H.E., G.B., and F.V. Statistical analyses and data interpretation: All authors. Contributed to the writing of the manuscript: All authors. Agree with the manuscript's results and conclusions: All authors. ICMJE criteria for authorship read and met: All authors.

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Availability of data and materials

The complete datasets generated and analyzed during the current study includes clinical data that are not publicly available due to restrictions by Swedish law. They are available from the corresponding author on reasonable request provided that professional secrecy applies.

Ethics approval and consent to participate

The study was approved by the Regional Ethical Review Board (2012/371–31) and patients were recruited after written, informed consent by the surgeon the day before surgery.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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