Myocardial Apoptosis Predicts Postoperative Course after Aortic Valve Replacement in Patients with Severe Left Ventricular Hypertrophy

Mario Gaudino[1], Amedeo Anselmi[1], Antonio Abbate[3], Leonarda Galiuto[2], Nicola Luciani[1], Franco Glieca[1], Gianfederico Possati[1]

Departments of [1]Cardiac Surgery and [2]Cardiology, Catholic University of the Sacred Heart, Rome, Italy, [3]Division of Internal Medicine, Virginia Commonwealth University, Richmond, VA, USA

Background and aim of the study: **Myocardial apoptosis has been implicated in heart failure and post-infarct remodeling. In some patients with severe aortic stenosis, delayed valvular replacement is associated with a poor in-hospital outcome. The study aim was to evaluate the impact of cardiomyocyte apoptosis on the postoperative course after aortic valve replacement (AVR) for severe aortic stenosis.**
Methods: **During elective AVR, myocardial biopsies were obtained from the left ventricle of 11 patients with severe left ventricular hypertrophy (LVH), and the samples analyzed for apoptosis.**
Results: **The mean apoptotic rate was 10.4 ± 3.7‰ (range: 5-16‰). The apoptotic rate correlated directly with preoperative NYHA functional class, duration of intensive care unit (ICU) stay, number of days of postoperative acute renal insufficiency, and serum level of troponin T at 24 h; the apoptotic rate correlated inversely with cardiac index at 24 h postoperatively. At multivariate analysis, the apoptotic rate and left ventricular mass index were independent predictors of prolonged ICU stay. The apoptotic rate and duration of cardiopulmonary bypass were predictive of the duration of postoperative acute renal insufficiency.**
Conclusion: **The study results showed an association between myocardial apoptosis and postoperative outcome in patients with severe LVH submitted for AVR. Non-invasive correlates of apoptosis may be introduced as a means of identifying patients at a higher operative risk, and may help in the evaluation of asymptomatic patients with severe aortic stenosis. Anti-apoptotic strategies before and during surgery would possibly ameliorate the surgical results.**

The Journal of Heart Valve Disease 2007;16:344-348

Aortic valve stenosis is among the most frequent valve diseases in the Western world (1) for which, to date, surgical valve replacement represents the state of the art and the only curative treatment (2). Although the in-hospital outcome after isolated aortic valve replacement (AVR) has steadily improved in recent years and postoperative mortality is actually estimated at approximately 4% (3), there still exists a particular subset of patients in whom surgical results are poor and in-hospital fatality rate remains unacceptably high (1).

Thus, a method of identifying those patients who are at higher operative risk is required. Both, experimental reports (4) and review articles (5) have suggested that ischemia/reperfusion damage, cardioplegic arrest and cardiopulmonary bypass (CPB) elicit apoptotic myocardial cell death per se. Very few data exist, however, concerning the role of apoptosis during the postoperative period after valve surgery. Based on recent findings which related the role of myocardial apoptosis to the pressure-overloaded left ventricle (6), the study aim was to ascertain whether the apoptotic burden in severe aortic stenosis has a clinical correlate after AVR.

Address for correspondence:
Amedeo Anselmi MD, Divisione di Cardiochirurgia, Policlinico Universitario A. Gemelli, Largo A. Gemelli 8, 00168 Rome, Italy
e-mail: amedeo.anselmi@aliceposta.it

Clinical material and methods

Patient selection and preoperative evaluation

Eleven patients (nine females, two males) scheduled for elective AVR for symptomatic severe stenosis (aortic valve area ≤0.5 cm^2/m^2) at the authors' institution were included in the study, based on the following criteria: Left ventricular hypertrophy (LVH) (end-diastolic wall thickness ≥12 mm); preserved systolic function (left ventricular ejection fraction (LVEF) >50%); absence of aortic insufficiency, moderate to severe valvulopathy at any position other than aortic, coronary artery disease (at angiography), and past

diagnosis of any type of cardiomyopathy.

Preoperatively, each patient was subjected to transthoracic echocardiography in order to determine the cardiac volume and left ventricular mass index (LVMI).

All patients provided their informed consent to participate in the study, the protocol of which was approved by the Ethical Committee of the Catholic University, Rome.

Surgical technique and sample harvesting

All operations were performed via a median sternotomy, with complete CPB and right atrial and ascending aorta cannulation. Anterograde-retrograde multidose blood cardioplegia was employed in all study patients, according to a standardized protocol. A 'free-hand' myocardial biopsy was obtained from the anterolateral free wall of the left ventricle after median sternotomy, and immediately before CPB.

Clinical follow up

All patients followed a standard anesthesiological protocol. During the stay in the intensive care unit (ICU), all data related to the clinical course were registered in a prospective database. For this purpose, the following definitions were applied:

- Acute renal insufficiency: postoperative serum creatinine level >2 mg/dl or an increase >2 mg/dl in serum creatinine level with respect to the preoperative level.
- ICU stay: the number of days (or part thereof) since admission to the ICU.
- Cardiac index: cardiac output related to body surface area.

Variables tested for association with apoptotic rate included the rate/duration of major and minor complications, duration of ICU and hospital stays, serum markers of myocardial and end-organ damage, and hemodynamic indices of left ventricular performance.

Samples processing

Myocardial biopsies were analyzed for apoptosis as described elsewhere (7). Briefly, samples were stored in buffered paraformaldehyde immediately after harvest, and then embedded in paraffin and sectioned. The sections were stained for in-situ end-labeling for DNA fragmentation (terminal deoxynucleotidyl transferase-mediated dUTP labeling; TUNEL assay) with the Apoptag kit (Oncor, Gaithersburg, Maryland, USA). In order to confirm the myocardial nature of cells, sections were incubated with antibodies against alpha sarcomeric actin; the sections were then stained with antibodies against anti-activated caspase-3 (cleaved caspase-3 (Asp-175) antibody; Cell Signaling Technology; Beverly, Massachusetts, USA). Suitable

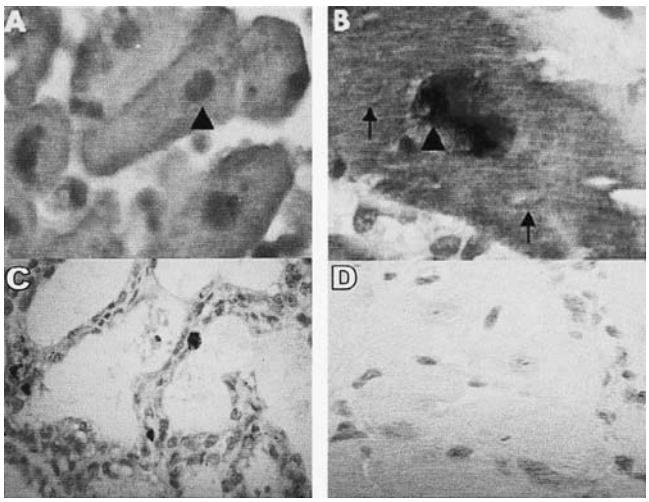

Figure 1: Histology of surgical biopsies. A) Dark brown nuclear staining (arrowhead) indicating TUNEL positivity. Non-apoptotic nuclei appear blue. B) Co-localization of TUNEL (arrowhead) and red cytoplasmic staining for cleaved caspase-3 (arrows). C) Positive control for TUNEL assay. D) Negative control for TUNEL assay.

negative and positive controls were set up, as specified elsewhere (7). Briefly, controls for TUNEL were performed as indicated by the manufacturer (positive control: normal female rodent mammary gland three to five days after weaning of rat pups and staining, omitting active terminal deoxynucleotidyl transferase but including proteinase K digestion). The control for activated caspase-3 was a human lymph node (strong immunoreactivity was evident in the apoptotic-prone germinal center B-lymphocytes and not in the mantle zone) (Fig. 1). The apoptotic rate was defined as the rate of double-positive (TUNEL-positive and cleaved caspase 3-positive) cardiomyocytes per field, and expressed as ‰ of cardiomyocytes.

Statistical analysis

Data were analyzed using an electronic database and SPSS 11.0 for Windows (SPSS, Chicago, IL, USA). Continuous data were presented as mean ± SD. Linear correlation analysis between continuous variables was assessed with Pearson's product-moment (bivariate normal distribution). Following univariate analysis, a multiple linear regression model was built, with the duration of ICU stay as the single continuous response variable. Variables such as patient-specific comorbidities, cross-clamp time and CPB time were included in the final model in order to control for their potential influence on outcomes. The assumptions of linear regression were checked and met, and the model was validated through a common 'jack-knife' procedure. An alpha level of 0.05 was adopted.

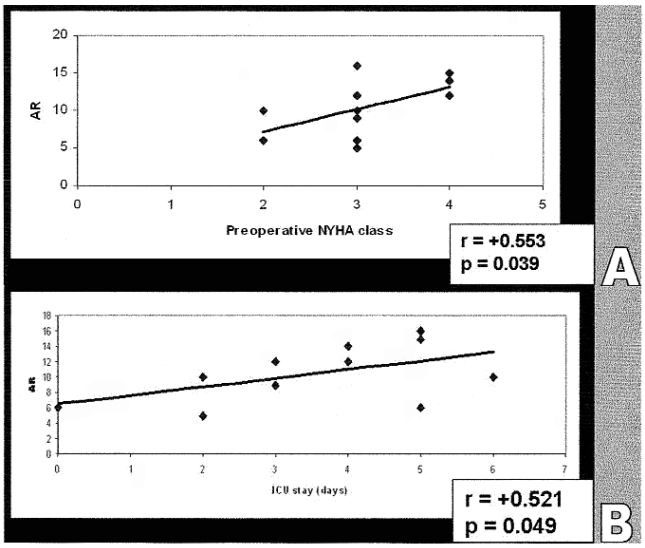

Figure 2: A) Correlation between apoptotic rate (AR) and preoperative NYHA functional class. B) Correlation between apoptotic rate (AR) and duration of intensive care unit (ICU) stay.

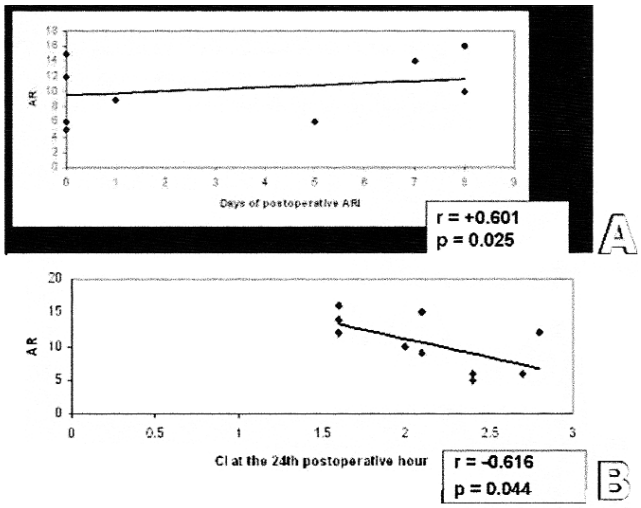

Figure 3: A) Correlation between apoptotic rate (AR) and duration of postoperative acute renal insufficiency (ARI). B) Correlation between apoptotic rate (AR) and cardiac index (CI) at 24 h postoperatively.

Results

The baseline features of the study population and intraoperative details are listed in Table I. All patients had concentric LVH and normal left ventricular end-diastolic and end-systolic volumes. The mean aortic valve area corrected for body surface was 0.3 mm^2/m^2 (range: 0.2 to 0.4 mm^2/m^2). Four patients with concomitant arterial hypertension received treatment with angiotensin-converting enzyme inhibitors, and three also with β-blockers. The mean apoptotic rate in the samples was 10.4 ± 3.7‰ (range: 5 to 16‰).

Only one postoperative death occurred due to multi-organ failure. This patient displayed the highest apoptotic rate (16‰) of the study population at myocardial biopsy. The patient displaying the lowest apoptotic rate in the study group had received an angiotensin converting-enzyme inhibitor (enalapril 10 mg/day) preoperatively for the treatment of hypertension.

Table I: Preoperative demographics and intraoperative data.

Parameter	Value
Age (years)*	72.2 ± 8.2
Gender ratio (M:F)	2:9
NYHA class III/IV (n)	9
LVMI (g/m^2)*	160.9 ± 18.8
LVEF (%)*	63 ± 5.9
EDV (ml/m^2)*	88.4 ± 18.6
Hypertension (n)	4
CPB time (min)*	65 ± 13.1

*Values are mean ± SD.
CPB: Cardiopulmonary bypass; EDV: End-diastolic volume; LVEF: Left ventricular ejection fraction; LVMI: Left ventricular mass index.

Correlations

The apoptotic rate correlated positively with preoperative NYHA functional class (r = +0.553, p = 0.039; Fig. 2). As described previously, a significant positive correlation was identified between the LVMI and overall ICU stay (r = +0.704, p = 0.16). Most interestingly, the apoptotic rate was found to correlate positively with the number of days of ICU stay (r = +0.521, p = 0.049; Fig. 3). There was a significant correlation between the apoptotic rate and the duration of postoperative acute renal insufficiency (r = +0.601, p = 0.025; Fig. 4). A significant inverse correlation was also identified between the apoptotic rate and the cardiac index at 24 h postoperatively (r = -0.616, p = 0.044; Fig. 3B). Additionally, a strong positive correlation was observed between apoptotic rate and serum troponin T levels at 24 h postoperatively (r = +0.752, p = 0.008; Fig. 4).

Table II: Predictors of prolonged Intensive Care Unit stay (multiple linear regression).

Variable	β-coefficient	95% CI	p-value
AR	0.578	0.502-1.381	0.023
LVMI	0.691	0.003-0.009	0.039
Serum TnT*	0.665	0.665-2.201	0.004

*At 24th postoperative hour.
AR: Apoptotic rate; CI: Confidence interval; LVMI: Left ventricular mass index; TnT: Troponin T.

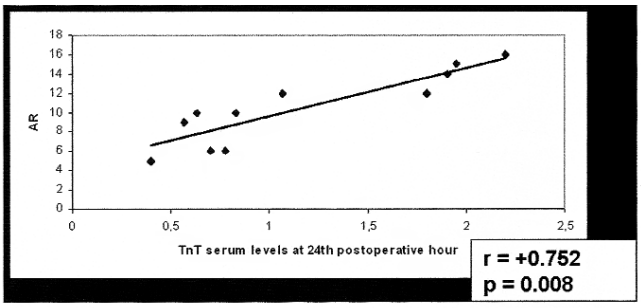

Figure 4: Correlation between apoptotic rate (AR) and troponin T (TnT) serum levels at 24 h postoperatively.

Multiple linear regression

Significant independent predictors of prolonged ICU stay are detailed in Table II. Both, higher LVMI and apoptotic rate were associated with a delayed discharge from the ICU. Subsequently, a second model was built with the number of days of acute renal insufficiency as continuous response variable. The apoptotic rate and CPB time were also identified as significant independent predictors (see Table III).

Discussion

The findings of the present study indicate that a higher apoptotic burden in the human heart is associated with a worse postoperative clinical outcome after AVR in patients with severe LVH. Patients with an elevated apoptotic rate are particularly likely to experience postoperative renal complications, this most likely being linked to a worse cardiac function and less-efficient systemic perfusion compared to individuals with a lower apoptotic rate. In fact, the apoptotic rate correlated inversely with cardiac index at 24 h postoperatively. It is also known that kidney malfunction is among the most sensitive indices of poor organ perfusion during the postoperative period (8). These data were inconclusive however, and the correlation identified in the present study suggest that additional, larger studies must be conducted. In addition, the present protocol was able to detect only end-stage apoptotic cardiomyocytes, while caspase 3-mediated destruction of myofibrillar proteins in the absence of a complete apoptotic process may be responsible for the functional deterioration of cardiomyocytes. Tailored investigations will be needed to clarify this issue.

The present study was undertaken to prove the impact of myocardial apoptosis on the clinical outcome of patients, and then to substantiate future investigations regarding the introduction of non-invasive correlates of apoptosis in the evaluation of these patients. In the present series, a higher apoptotic rate predicted a prolonged ICU stay, which was an obvious surrogate marker of complicated course and difficult myocardial recovery. Although the association between apoptotic rate and postoperative complications such as myocardial infarction or respiratory insufficiency did not reach statistical significance, the troponin T serum level at the 24th postoperative hour increased with increasing apoptotic rate in the surgical biopsy. Rather than being linked by a direct cause-effect relationship, these variables most likely share the same determinant - that is, an increased degree of tissue ischemia related to hypertrophy and surgical stress. In the present study, co-staining for muscle actin was performed, thereby confirming the cardiomyocyte nature of cells undergoing apoptosis and excluding false-positive results (apoptosis of infiltrating cells from a hematopoietic lineage).

Table III: Predictors of duration of postoperative acute renal insufficiency (multiple linear regression).

Variable	β-coefficient	95% CI	p-value
AR	0.552	0.059-1.981	0.046
Duration of CPB	0.548	0.873-2.077	0.045

AR: Apoptotic rate; CI: Confidence interval; CPB: Cardiopulmonary bypass.

The aim of the present study was to disclose the interplays between the apoptotic load in the left ventricle with severe aortic stenosis and the earliest clinical features of patients after valve replacement. Higher apoptotic rates have been already correlated with unfavorable clinical evolution and a worse prognosis in ischemic heart disease (9). The present results, in terms of valvular heart disease, indicate at least that this topic is worthy of further investigation, which will likely identify a direct cause-effect link between apoptosis and a worsening clinical outcome. Moreover, determination of the myocardial expression of survivin may also provide additional useful data for this purpose.

Study implications

Since the results of the present study suggest that myocardial apoptosis influences clinical outcome after AVR in patients with severe LVH, it should be evaluated as a therapeutic target in the surgical management of patients with severe aortic stenosis. In this regard, anti-apoptotic strategies (i.e., supplementation of the cardioplegic solution with the mitochondria-dependent apoptotic pathway inhibitor NIM811, or with caspase inhibitor IDN6734) may possibly improve surgical outcome when applied intraoperatively and/or preoperatively. In addition, given the pathogenetic role of apoptosis in pressure overload, and as

the rate of apoptotic myocyte loss in compensated hypertrophy is non-linear and may vary between patients, non-invasive correlates of apoptosis might help in the identification of asymptomatic patients who should be referred for early surgery, thus lowering the operative risk.

Study limitations

Clearly, the main limitation of the present study was the limited sample size. Nonetheless, the finding of a strong statistical association, despite small patient numbers, suggests that these correlations would likely be confirmed in larger populations. In-vivo myocardial biopsies clearly would provide minimal pathological material, which may also suffer from regional differences in the degree of ischemia. As yet, no evidence exists to support the idea that regional ischemic variations may deeply affect the local apoptotic load. It has been proven that the global mechanical stretching and tissue derangements that are widespread in the hypertrophic ventricle are causative of apoptosis (10-12). The impossibility of determining the in-vivo duration of the apoptotic process makes it difficult to evaluate its real contribution in accelerating progression through failure. However, co-staining for selective DNA fragmentation and cleaved caspase-3 marks the final and irreversible steps of apoptosis, is highly specific, and virtually eliminates false-positive results.

References

1. Carabello BA. Clinical practice. Aortic stenosis. N Engl J Med 2002;346:677-682
2. Schwarz F, Baumann P, Manthey J, et al. The effect of aortic valve replacement on survival. Circulation 1982;66:1105-1110
3. Astor BC, Kaczmarek RG, Hefflin B, Randolph Daley W. Mortality after aortic valve replacement: Results from a nationally representative database. Ann Thorac Surg 2000;70:1939-1945
4. Ramlawi B, Feng J, Mieno S, et al. Indices of apoptosis activation after blood cardioplegia and cardiopulmonary bypass. Circulation 2006;114(suppl.I):I257-I263
5. Anselmi A, Abbate A, Girola F, et al. Myocardial ischemia, stunning, inflammation and apoptosis during cardiac surgery: A review of evidence. Eur J Cardiothorac Surg 2004;25:304-311
6. Galiuto L, Lotrionte M, Crea F, et al. Impaired coronary and myocardial flow in severe aortic stenosis is associated with increased apoptosis: A transthoracic Doppler and myocardial contrast echocardiography study. Heart 2006;92:208-212
7. Abbate A, Biondi-Zoccai GGL, Bussani R, et al. Increased myocardial apoptosis in patients with unfavorable left ventricular remodeling and early asymptomatic post-infarction heart failure. J Am Coll Cardiol 2003;41:753-760
8. Wijeysundera DN, Karkouti K, Beattie WS, Rao V, Ivanov J. Improving the identification of patients at risk of postoperative renal failure after cardiac surgery. Anesthesiology 2006;104:65-72
9. Abbate A, Biondi-Zoccai G, Bussani R, et al. High-risk clinical features predict increased post-infarction myocardial apoptosis and the benefits of as a result of an open infarct-related artery. Eur J Clin Invest 2003;33:662-668
10. Cheng W, Li B, Kajstura J, et al. Stretch-induced programmed myocyte cell death. J Clin Invest 1995;96:2247-2259
11. Liu JJ, Peng L, Bradley CJ, Zulli A, Shen J, Buxton BF. Increased apoptosis in the heart of genetic hypertension, associated with increased fibroblasts. Cardiovasc Res 2000;45:729-735
12. Diez J, Panizo A, Hernandez M, et al. Cardiomyocyte apoptosis and cardiac angiotensin-converting enzyme in spontaneously hypertensive rats. Hypertension 1997;30:1029-1034