Apoptosis in Patients With Acute Myocarditis

Antonio Abbate, MD, PhD[a,*], Gianfranco Sinagra, MD[b], Rossana Bussani, MD[c], Nicholas N. Hoke, BS[a], Marco Merlo, MD[b], Amit Varma, MD[a], Stefano Toldo, MS[a,d], Fadi N. Salloum, PhD[a], Giuseppe G.L. Biondi-Zoccai, MD[e], George W. Vetrovec, MD[a], Filippo Crea, MD[d], Furio Silvestri, MD[c], and Alfonso Baldi, MD[f]

Acute myocarditis is an acute inflammatory syndrome characterized by acute myocardial damage and dysfunction followed by a variable recovery over time with some patients progressing toward severe dilated cardiomyopathy. Cardiomyocyte apoptosis, a key pathologic feature of heart failure, may play a critical role in functional recovery in patients with acute myocarditis. The aim of the study was to investigate whether apoptosis predicts functional recovery in patients with acute myocarditis. Sixteen patients with biopsy-documented acute myocarditis were followed for 1 year with serial transthoracic echocardiography. Functional recovery was defined as 12-month left ventricular ejection fraction >40%. Cardiomyocyte apoptosis, leukocyte infiltration, and cell proliferation was assessed in all samples. A group of cases in which the diagnosis of acute myocarditis was made after death was also selected for comparison, and morphologically normal hearts from patients who died from a noncardiac cause were selected as controls. Six patients (38%) had functional recovery at 12 months, whereas 10 (62%) did not. The 2 groups had similar characteristics except for lower baseline left ventricular ejection fraction in the group with functional recovery. Apoptotic rate was found to be significantly higher in patients with acute myocarditis than in control hearts, and, unexpectedly, patients with functional recovery had significantly higher apoptotic rates than patients without recovery (3.2% vs 0.5%, p = 0.001). None of the patients with apoptotic rates below the median had functional recovery versus 86% of patients with apoptotic rates above the median (p <0.001). In conclusion, higher rates of cardiomyocyte apoptosis in patients with acute myocarditis are associated with functional recovery at 1 year. **© 2009 Elsevier Inc. All rights reserved.** **(Am J Cardiol 2009;104:995–1000)**

Observational studies have shown that patients presenting acutely with severely decompensated heart failure from acute myocarditis often rapidly recover with intense supportive measures, whereas patients that present with more of a "subacute" myocarditis are prone to chronic, virus- or immune-mediated cardiomyocyte injury leading to adverse cardiac remodeling and progression to dilated cardiomyopathy.[1–6] Apoptosis, also called programmed cell death, is an energy-dependent, actively regulated process of cell death characterized by DNA fragmentation, cell shrinkage, and condensation of chromatin. There is growing evidence that apoptosis mediates myocardial damage in acute myocarditis[7–11] and in recent-onset cardiomyopathy.[12] Although it has been established that apoptosis is a fundamental mechanism by which the heart attempts to adapt to physiologic and pathophysiologic demands and stresses, it is also known that apoptosis may lead to adverse cardiac remodeling.[13] We hypothesized that the presence and extent of apoptosis could predict functional recovery in patients with acute myocarditis. To test this hypothesis, we selected 16 patients with biopsy-documented acute myocarditis and followed them over time with serial transthoracic echocardiography. A group of cases in which the diagnosis of acute myocarditis was made after death was also selected for comparison.

Methods

Prospective patients with a clinical syndrome suggestive of acute myocarditis, including those presenting within 3 months of an acute illness in the absence of any evidence of myocardial ischemia but had new-onset impaired global or regional systolic dysfunction, increased cardiac markers such as troponin I and creatine kinase-MB, or sustained/nonsustained ventricular tachycardia or ventricular fibrillation were evaluated for inclusion. Those patients in whom acute myocarditis was diagnosed at pathology according to the Dallas criteria[14] were included in the study, leading to the selection of 16 cases. These patients were classified as an acute presentation if the onset of symptoms was within 1 week of admission or a subacute presentation if symptoms started 1 week to 4 weeks before admission. The study was approved by the institutional review board and all patients gave informed written consent before inclusion.

[a]VCU Pauley Heart Center, Virginia Commonwealth University, Richmond, Virginia; Institutes of [b]Cardiology and [c]Pathologic Anatomy, University of Trieste, Trieste, [d]Institute of Cardiology, Catholic University, Rome, [e]Institute of Cardiology, University of Turin, Turin, and [f]Department of Biochemistry, Section of Pathology, Second University of Naples, Naples, Italy. Manuscript received April 2, 2009; revised manuscript received and accepted May 13, 2009.

*Corresponding author: Tel: 804-828-0513; fax: 1-360-323-1204.

E-mail address: aabbate@mcvh-vcu.edu (A. Abbate).

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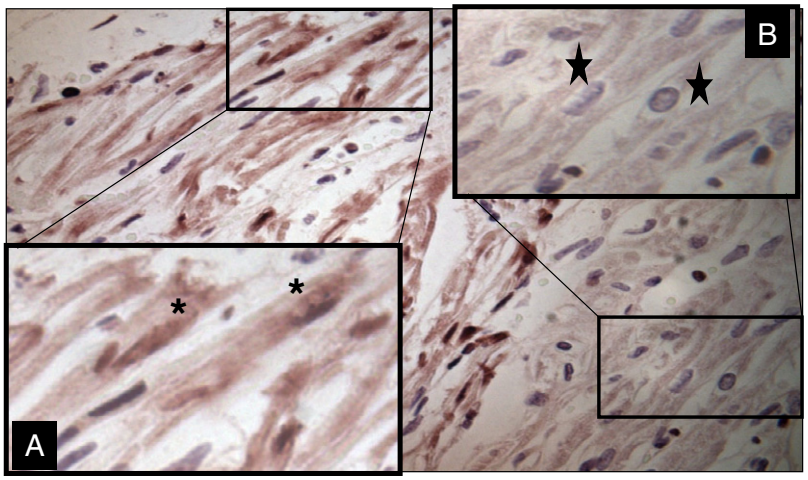

Figure 1. Example of double staining for TUNEL and activated caspase-3. *(A)* TUNEL$^+$ (nuclei) and caspase-3$^+$ (cytoplasm) cardiomyocytes *(*)* and *(B)* TUNEL$^-$ (nuclei) (★) cardiomyocytes, which show only mild staining for active caspase-3 (cytoplasm).

To assess whether severity of the disease at presentation and early mortality was associated with apoptosis, we selected 8 subjects at autopsy with fulminant myocarditis who died shortly after admission and another 8 subjects admitted with progressive heart failure that led to in-hospital death and were found to have acute myocarditis at pathology. Also, to determine whether apoptosis seen in endomyocardial biopsy (EMB) represented a diffuse or spotty process, we compared the results obtained in the EMB samples to those derived from postmortem samples of patients with acute myocarditis who had died and underwent autopsy comparing samples from areas of the myocardium that were frankly necrotic to areas that were apparently normal at standard pathology. A final group of 4 patients who died from noncardiac causes and had normal cardiac function served as control hearts.

Baseline and 12-month follow-up transthoracic echocardiograms were obtained in all cases. Echocardiography was performed by the same operator the day before EMB and each report was formulated according to American Society of Echocardiography guidelines.[15] Left ventricular (LV) end-diastolic and end-systolic diameters were measured with 2-dimensionally guided M-mode echocardiography. In particular, LV ejection fraction (EF) was calculated from apical 4- and 2-chamber views using the modified Simpson formula. The patients were then divided in 2 groups according to functional recovery based on final LVEF, namely $>$40% (recovery) and $\leq$40% (no recovery). As an additional criterion for recovery we used LV end-diastolic volume index at 12-month follow-up, namely $\leq$70 ml/m^2 (recovery) and $>$70 ml/m^2 (no recovery).

Multiple biopsies (median 7, range 4 to 9) were taken from the left and right ventricles in patients with acute myocarditis. Biopsy sample sites were chosen according to findings on transthoracic echocardiogram and were taken with a dedicated bioptome advanced through a 7-Fr guiding catheter to reach the specified regions of interest in the left and right ventricles. Tissue samples were 1 to 3 mm in diameter and were immediately harvested under sterile conditions and fixed in 10% buffered formalin. In the autopsic group, multiple samples from the left and right ventricles were taken including areas frankly necrotic and areas that appeared normal at gross pathology. In the control group, 2 random samples in the left ventricle were taken. Tissue specimens were processed for each sample and morphologic analysis of tissue structure, cellular integrity, and nuclear integrity was performed by light microscopy. Each specimen for each case was assessed for the presence of active myocarditis according to the Dallas criteria.[13] In 8 patients, samples were also immediately frozen using liquid nitrogen and stored at -80°C for the detection of viral genome.

Apoptosis was detected by terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick-end labeling (TUNEL) of DNA fragmentation using the peroxidase-based ApopTag kit (Oncor, Gaithersburg, Maryland) according to the supplier's instructions. Several series of TUNEL-stained sections were subsequently costained to assess for activated caspase-3 (cleaved caspase-3 antibody, Cell Signaling Technology, Beverly, Massachusetts). In TUNEL and caspase-3 detection experiments, positive and negative controls were prepared. Cardiomyocytes were defined as apoptotic if colocalization of TUNEL and caspase-3 was evident. TUNEL$^-$/caspase-3$^+$ cells were not considered apoptotic because caspase-3 activation may represent a reversible step. Similarly we chose not to use annexin V because it marks cells with early commitment to apoptosis but it may represent a reversible step. Figure 1 displays an example of double staining for TUNEL and activated caspase-3 showing TUNEL$^+$/caspase-3$^+$ cells and TUNEL$^-$ cells, which show only mild cytoplasmic staining for active caspase-3. Twenty random fields (40$\times$) per section were analyzed and the apoptotic rate was expressed as percent double-positive cardiomyocytes of the total number per field. Subsequently, tissue samples were also assessed for immunohistochemical evidence of leukocyte infiltration and cell proliferation. To determine the degree of leukocyte infiltration, the number of leukocytes in the myocardium was measured as the number of CD45$^+$ cells/mm^2 (using a mouse monoclonal anti-human CD45 antibody, Laboratory Vision, Fremont, California). An anti–Ki-67 antibody (rabbit polyclonal antibody, dilution 1:50, AbCam, Cambridge, Massachusetts) was used to determine the number of pro-

Table 1
Clinical characteristics of six patients with acute myocarditis and functional recovery and ten patients without functional recovery

Demographics	
Age	44 (32–52)
Men	8 (50%)
Co-morbidities and risk factors	
Diabetes mellitus	0 (0%)
Hyperlipidemia	5 (31%)
Hypertension	1 (6%)
Tobacco use	6 (38%)
Presenting symptoms	
Flulike symptoms	5 (31%)
Dyspnea	11 (69%)
Chest pain	0 (0%)
Palpitations	4 (25%)
Syncope	2 (12%)
Rash	0 (0%)
Asthenia	3 (19%)
Clinical syndrome at presentation	
Decompensated heart failure	11 (69%)
Compensated heart failure	1 (16%)
Life-threatening arrhythmias	4 (25%)
New York Heart Association classification	2 (2–2)
Electrocardiographic conduction abnormalities	6 (38%)
ST-segment and T-wave changes	7 (44%)
Physical examination findings	
Heart rate (median)	76 (70–95)
Systolic blood pressure (mm Hg)	110 (100–130)
S_3 gallop	9 (56%)
Rales	3 (19%)
Jugular venous distension	4 (25%)
Lower extremity edema	2 (12%)
Hemodynamic parameters	
EF (%)	29 (25–36)
End-diastolic volume index (ml/m^2)	80 (71–102)
End-systolic volume index (ml/m^2)	55 (50–78)
Cardiac index (L/min × m^2)	2.5 (1.9–3.3)
Laboratory data	
C-reactive protein (mg/L)	10 (3–32)
Erythrocyte sedimentation rate (mm/h)	14 (7–23)
White blood cell count ($\times 10^3$/ml)	11.2 (7.9–13.1)
Creatine kinase (IU/L)	57 (49.90)
Creatine kinase >200 IU/L	2 (12%)
Discharge medications	
ACE inhibitor or ARB	12 (75%)
β blockers	14 (88%)
Digoxin	4 (25%)
Loop diuretics	11 (69%)
Spironolactone	4 (25%)
Statins	1 (6%)
Prednisone and azathioprine	11 (69%)

Values are medians (interquartile ranges) or numbers of subjects (percentages). Consecutive number presented is based on age in each group.

ACE = angiotensin-converting enzyme; ARB = angiotensin receptor blocker.

liferating cells in the myocardium. Percent Ki-67$^+$ cardiomyocyte nuclei were calculated.

The search for viral genome was completed in 8 patients. Standard techniques for total RNA and DNA isolation and polymerase chain reaction were used with the addition of specific primers for enterovirus species, adenovirus species, Epstein-Barr virus, parvovirus B19, herpes simplex viruses 1 and 2.

Statistical analysis was performed with SPSS 10.1 for Windows (SPSS, Inc., Chicago, Illinois). Quantitative results are expressed as median (interquartile range) because of potential deviations from assumptions of normality. Uncorrected chi-square test or Fisher's exact test were used for categorical variables, when appropriate. Continuous variables were analyzed with Mann-Whitney U or Kruskal-Wallis test, when appropriate. Spearman correlation test was used to correlate 2 continuous variables. Analysis for variance test for repeated measurements was used to compare changes over time with post hoc comparisons between the 2 groups. Two-tailed statistical significance was at the 0.05 level. Bonferroni correction was used to control type I error when comparisons between multiple groups were made.

Results

Clinical characteristics of patients with acute myocarditis are presented in Tables 1 and 2. Six patients (38%) showed functional recovery in LVEF >40% at follow-up, whereas 10 (62%) did not (Table 1). Baseline LVEF was significantly lower in patients with recovery versus those without recovery ($p = 0.031$). Creatine kinase levels at admission were higher in patients with recovery versus those without recovery; however, only 3 patients had increased levels above the upper limit of normal. We found no other differences in clinical parameters including presenting symptoms, physical examination signs, cardiac dimensions at echocardiography, or therapy (including immunosuppressive therapy, which consisted of prednisone 1 mg/kg/day for 2 weeks and a taper over 6 months and azathioprine 1 to 2 mg/kg/day for 6 months), comparing patients with and without functional recovery (Table 1). All patients with functional recovery were alive at 24 months, whereas 3 of the 10 patients without recovery had died.

Apoptotic rate was significantly higher in the EMB samples of patients with acute myocarditis versus control hearts (1.1%, 0.4 to 2.2, vs 0.01%, 0.01 to 0.01, $p < 0.001$; Figure 2). Unexpectedly, patients with functional recovery had a significantly higher apoptotic rate than patients without recovery (3.2%, 1.1 to 8.0, vs 0.5%, 0.3 to 1.0, $p = 0.001$; Figure 2). Five of the 6 patients with functional recovery (LVEF >40%) had also LV end-diastolic volume index <70 ml/m^2. Similarly patients with follow-up LV end-diastolic volume index ≤70 ml/m^2 had significantly higher apoptotic rate versus those with LV end-diastolic volume index >70 ml/m^2 (1.8%, 1.2 to 8.0, vs 0.4%, 0.3 to 1.0, $p = 0.019$). Six of the 7 patients (87%) with an apoptotic rate above the median showed functional recovery compared to 0 of the 9 patients (0%) with an apoptotic rate below the median ($p < 0.001$; Figure 2). No association between pattern of clinical presentation (acute vs subacute) and apoptotic rates was found. Apoptotic rate also correlated with LVEF at follow-up, with higher apoptotic rates being associated with greater LVEF ($R = +0.54$, $p = 0.030$) but not with LVEF at baseline. Furthermore, serial changes in LVEF were significantly more favorable in patients with apoptotic rates above the median (1.1%) compared to those with apoptotic rates below the median ($p = 0.025$; Figure 3).

Table 2
Clinical characteristics of six patients with acute myocarditis and functional recovery and ten patients without functional recovery

	Age (years)/Sex	Time From Symptom Onset (wks)	Flulike Syndrome	Decompensated Heart Failure	Life-Threatening Arrhythmias	Immunosuppressive Therapy*	ACE Inhibitors	Beta Blockers	Spironolactone
Functional recovery									
1	35/F	1–4	0	0	+	+	+	+	0
2	47/F	>4	0	+	0	+	+	+	+
3	48/F	>4	+	+	0	0	+	+	+
4	51/M	1–4	0	+	0	+	+	+	0
5	51/M	1–4	+	0	+	0	0	+	0
6	72/M	1–4	0	+	0	+	+	+	0
No functional recovery									
1	20/F	>4	0	+	0	+	+	0	0
2	21/F	<1	0	+	0	0	+	+	0
3	22/M	<1	+	+	0	+	+	+	0
4	32/F	>4	0	+	0	+	+	+	+
5	32/M	>4	0	0	+	+	0	0	0
6	33/F	>4	+	+	0	+	+	+	0
7	42/M	>4	0	+	0	+	+	+	0
8	55/M	1–4	0	+	0	+	+	+	+
9	56/M	<1	0	0	0	0	0	+	0
10	68/M	<1	+	0	+	0	0	+	0

Consecutive number presented is based on age in each group.
* Immunosuppressive therapy consisted of prednisone and azathioprine.
Abbreviation as in Table 1.

The number of Ki-67$^+$ cardiomyocytes was low (0.1%, 0.0 to 1.0) and not associated with apoptotic rate. The number of infiltrating CD45$^+$ cells was 92/mm^2 (53 to 150) and also did not correlate with apoptotic rate. Parvovirus B19 genome was found in 3 of the 8 cases (38%) and adenovirus genome was detected in 1 case only (12%); no viruses were detected in 4 cases (50%). Apoptotic rate, Ki-67$^+$ rate, and CD45$^+$ infiltrate were similar in cases with and without viral genome presence (data not shown).

We found that apoptotic rate in the autopsy samples (2.0%, 0.6 to 8.6, $p = 0.91$) was not statistically different compared to apoptotic rate in EMB samples. We found no significant difference in apoptotic rates in hearts of patients who died from fulminant myocarditis (3.8%, 0.2 to 8.0) versus those with heart failure (2.0%, 1.5 to 9.0, $p = 0.86$). We also found no differences comparing left to right ventricular samples (data not shown). In many cases, patchy areas of overt necrosis in the left or right ventricle were evident at autopsy. Analyzing separately the samples with and without necrosis, we found that there was no difference in apoptotic rates between affected and unaffected regions, suggesting that in acute myocarditis, apoptosis, unlike necrosis, is a diffuse rather than a patchy process (Figure 4).

Discussion

This study shows for the first time that a higher rate of apoptosis is associated with functional recovery in patients with acute myocarditis at 1-year follow-up. Although previous studies have described the presence of apoptosis in acute myocarditis, the exact role of apoptosis in this setting has remained unclear. Experimental animal models of acute viral myocarditis had suggested a role of apoptosis in promoting cardiac damage and a role in the progression of cardiac dilatation and ventricular dysfunction.[16–18]

In the present study we tried to clarify the correlation between apoptosis and heart failure in acute myocarditis by longitudinally studying patients with serial echocardiography. Unexpectedly we found higher rates of apoptosis in patients who functionally recovered over 1 year, thus suggesting a possible protective role of apoptosis in acute myocarditis. Acute myocarditis may be caused by many different pathogens including viral, bacterial, rickettsial, mycotic, or parasitic organisms, yet in any 1 patient, the true pathogen often remains unknown. Viruses are the most common cause of acute myocarditis.[4] Although the mechanisms and immunologic basis of this association remains to be clarified, 1 potential explanation for this finding is that programmed cell death, in the setting of viral- or immune-mediated inflammation, may be a host defense mechanism.[7,18,19] Apoptosis in this setting might ameliorate any further damage to surrounding myocardial tissue and prevent fibrosis of the region. For example, in murine models of viral myocarditis, viral persistence has been shown to sustain myocardial inflammation and was associated with poor outcomes.[20] In such a model, apoptosis may facilitate clearance of pathogenic factors without promoting an immune response, thus allowing for surrounding myocytes to recover.[21,22]

In acute myocarditis, apoptosis may also play a role in eliminating damaged cells and this may be translated into late functional recovery.[23] Although we found no association between apoptosis and presence of a viral genome, we acknowledge the small number of cases and the single time

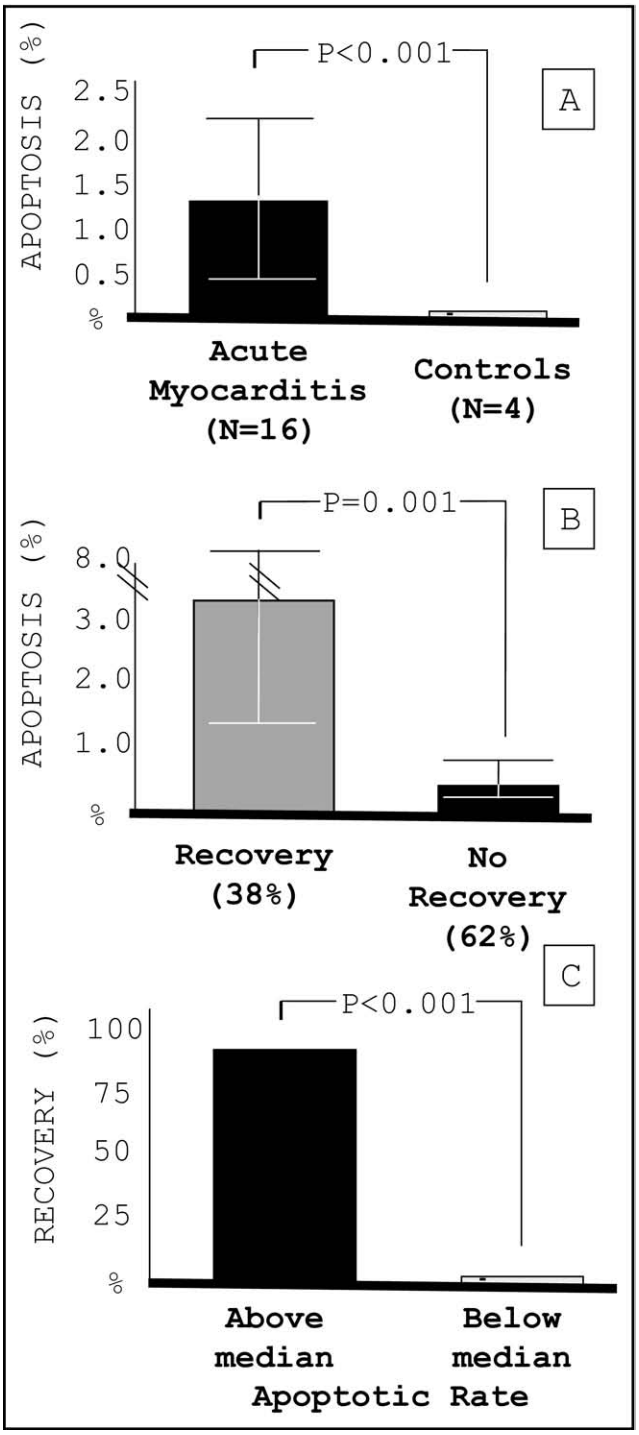

Figure 2. *(A)* Higher rates of apoptotic cardiomyocytes in endomyocardial biopsy specimens of patients with acute myocarditis versus patients who died from noncardiac causes. *(B)* Patients with functional recovery at 1 year (LVEF >40%) had a significantly higher apoptotic rate than patients without recovery (3.2% vs 0.5%, p = 0.001). *(C)* Six of the 7 patients (87%) with an apoptotic rate above the median showed functional recovery versus 0 of 9 patients (0%) with an apoptotic rate below the median (p <0.001).

point of biopsy as limitations of the study. It is indeed possible that the apoptotic rate measured, given the unclear duration of the apoptotic process in the heart, does not

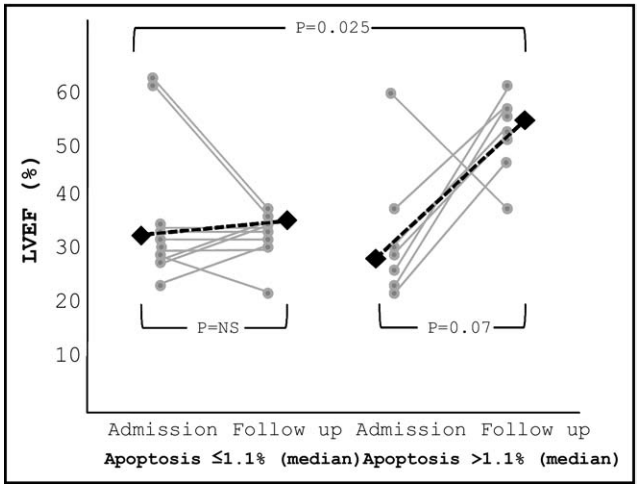

Figure 3. Individual changes in LVEF from baseline to 12 months according to apoptotic rates below and above the median.

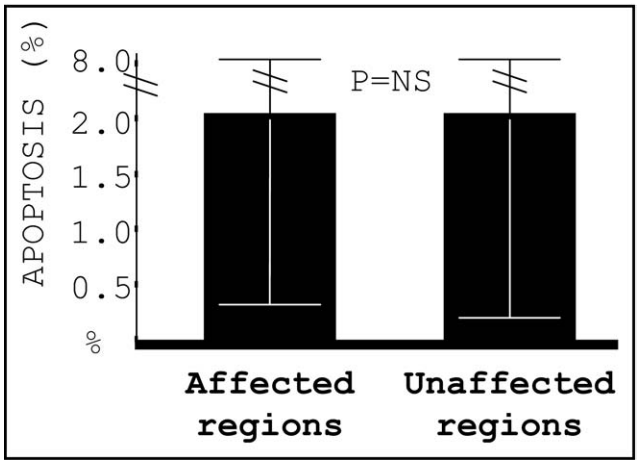

Figure 4. There was no difference in apoptotic rate between affected and unaffected regions of the myocardium, suggesting that apoptosis in acute myocarditis is a diffuse process (p = NS).

represent the true apoptotic burden. Moreover, the specificity of apoptotic markers has been questioned. Although we believe that double staining for DNA fragmentation and for active caspase-3 identifies apoptosis, we cannot exclude that a percentage of these cells is not truly apoptotic because we did not perform electron microscopy, which is the most specific technique. One possibility is that DNA fragments in the nuclei may represent staining for a highly replicating viral genome in the cell, which would ultimately lead to cell death.[24,25] Use of echocardiography alone is a limitation and magnetic resonance would have been preferable. Further, time to symptom onset may not truly reflect disease duration.

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1. D'Ambrosio A, Patti G, Manzoli A, Sinagra G, Di Lenarda A, Silvestri F, Di Sciascio G. The fate of acute myocarditis between spontaneous improvement and evolution to dilated cardiomyopathy: a review. *Heart* 2001;85:499–504.
2. Dec GW Jr, Palacios IF, Fallon JT, Aretz HT, Mills J, Lee DC, Johnson RA. Active myocarditis in the spectrum of acute dilated cardiomyopathies: clinical features, histologic correlates and clinical outcome. *N Engl J Med* 1985;312:885–890.
3. Maron BJ, Towbin JA, Thiene G, Antzelevitch C, Corrado D, Arnett D, Moss AJ, Seidman CE, Young JB. Contemporary definitions and classification of the cardiomyopathies: an American Heart Association scientific statement from the Council on Clinical Cardiology, Heart Failure and Transplantation Committee; quality of care and outcomes research and functional genomics and translational biology interdisciplinary working groups and Council on Epidemiology and Prevention. *Circulation* 2006;113:1807–1816.
4. Kawai C. From myocarditis to cardiomyopathy: mechanisms of cell death; learning from the past for the future. *Circulation* 1999;99: 1091–1100.
5. McCarthy RE III, Boehmer JP, Hruban RH, Hutchins GM, Kasper EK, Hare JM, Baughman KL. Long-term outcome of fulminant myocarditis as compare with acute (non-fulminant) myocarditis. *N Engl J Med* 2000;342:690–695.
6. Liu PP, Mason JW. Advances in the understanding of myocarditis. *Circulation* 2001;104:1076–1082.
7. Saraste A, Arola A, Vuorinen T, Kytö V, Kallajoki M, Pulkki K, Voipio-Pulkki LM, Hyypiä T. Cardiomyocyte apoptosis in experimental coxsackievirus B3 myocarditis. *Cardiovasc Pathol* 2003;12: 255–262.
8. Kytö V, Saraste A, Saukko P, Henn V, Pulkki K, Vuorinen T, Voipio-Pulkki LM. Apoptotic cardiomyocyte death in fatal myocarditis. *Am J Cardiol* 2004;94:746–750.
9. Zorc-Pleskovič R, Alibegović A, Zorc M, Milutinović A, Radovanović N, Petrovič D. Apoptosis of cardiomyocytes in myocarditis. *Folia Biol* 2006;52:6–9.
10. Alter P, Jobmann M, Meyer E, Pankuweit S, Masich B. Apoptosis in myocarditis and dilated cardiomyopathy: does enterovirus genome persistence protect from apoptosis? An endomyocardial biopsy study. *Cardiovasc Pathol* 2001;10:229–234.
11. Fuse K, Kodama M, Okura Y, Ito M, Hirono S, Kato K, Hanawa H, Aizawa Y. Predictors of disease course in patients with acute myocarditis. *Circulation* 2000;102:2829–2835.
12. Sheppard R, Bedi M, Kubota T, Semigran MJ, Dec W, Holubkov R, Feldman AM, Rosenblum WD, McTiernan C, McNamara DM, for the IMAC Investigators. Myocardial expression of Fas and recovery of left ventricular function in patients with recent-onset cardiomyopathy. *J Am Coll Cardiol* 2005;46:1036–1042.
13. Kang PM, Izumo S. Apoptosis and heart failure: a critical review of the literature. *Circulation* 2000;86:1107–1112.
14. Aretz HT, Billingham ME, Edwards WD, Factor SM, Fallon JT, Fenoglio JJ Jr, Olsen EG, Schoen FJ. Myocarditis: a histopathologic definition and classification. *Am J Cardiovasc Pathol* 1987;1:3–14.
15. Schiller NB, Shah PM, Crawford M, DeMaria A, Devereux R, Feigenbaum H, Gutgesell H, Reichek N, Sahn D, Schnittger I. Recommendations for quantitation of the left ventricle by two-dimensional echocardiography. American Society of Echocardiography committee on standards, subcommittee on quantitation of two-dimensional echocardiograms. *J Am Soc Echocardiogr* 1989;2:358–367.
16. Joo CH, Hong HN, Kim EO, Im JO, Yoon SY, Ye JS, Moon MS, Kim D, Lee H, Kim YK. Coxsackievirus B3 induces apoptosis in the early phase of murine myocarditis: a comparative analysis of cardiovirulent and noncardiovirulent strains. *Intervirology* 2003;46:135–140.
17. Kytö V, Saraste A, Fohlman J, Ilbäck NG, Harvala H, Vuorinen T, Hyypiä T. Cardiomyocyte apoptosis after antiviral WIN 54954 treatment in murine coxsackievirus B3 myocarditis. *Scand Cardiovasc J* 2002;12:187–192.
18. Kandolf R, Klingel K, Zell R, Selinka HC, Raab U, Schneider-Brachert W, Bültmann B. Molecular pathogenesis of enterovirus-induced myocarditis: virus persistence and chronic inflammation. *Intervirology* 1993;35:140–151.
19. Gebhard JR, Perry CM, Harkins S, Lane T, Mena I, Asensio VC, Campbell IL, Whitton JL. Coxsackievirus B3-induced myocarditis: perforin exacerbates disease, but plays no detectable role in virus clearance. *Am J Pathol* 1998;153:417–428.
20. Klingel K, Stephan S, Sauter M, Zell R, McManus BM, Bültmann B, Kandolf R. Pathogenesis of murine enterovirus myocarditis: virus dissemination and immune cell targets. *J Virol* 1996;70:8888–8895.
21. Kindermann I, Kindermann M, Kandolf R, Klingel K, Bültmann B, Müller T, Lindinger A, Böhm M. Predictors of outcome in patients with suspected myocarditis. *Circulation* 2008;118:639–648.
22. Frustaci A, Chimenti C, Pieroni M, Salvatori L, Morgante E, Sale P, Ferretti E, Petrangeli E, Gulino A, Russo MA. Cell death, proliferation and repair in human myocarditis responding to immunosuppressive therapy. *Mod Pathol* 2006;19:755–765.
23. Abbate A, Biondi-Zoccai GGL, Baldi A. Pathophysiological role of myocardial apoptosis in post-infarction left ventricular remodeling. *J Cell Physiol* 2002;93:145–153.
24. Taddeo B, Nickoloff BJ, Foreman KE. Caspase inhibitor blocks human immunodeficiency virus 1-induced T-cell death without enhancement of HIV-1 replication and dimethyl sulfoxide increases HIV-1 replication without influencing T-cell survival. *Arch Pathol Lab Med* 2000;124:240–245.
25. Duval R, Delebasseé S, Cardot PJ, Bosgiraud C. Visna-virus induced cytopathic effect in vitro in caused by apoptosis. *Arch Virol* 2002;147: 943–959.