

Myocarditis

Myocardial Expression of Fas and Recovery of Left Ventricular Function in Patients With Recent-Onset Cardiomyopathy

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OBJECTIVES	This study aimed to evaluate the role of gene expression for predicting myocardial recovery in recent-onset cardiomyopathy.
BACKGROUND	Apoptosis may limit ventricular recovery. We examined the myocardial expression of Fas, Fas ligand (FasL), tumor necrosis factor (TNF)-alpha, and TNF receptor 1 (TNFR1), and myocardial recovery in patients from the multicenter Intervention in Myocarditis and Acute Cardiomyopathy (IMAC) study.
METHODS	Endomyocardial biopsy samples were obtained in 20 patients with recent-onset (<6 months) idiopathic dilated cardiomyopathy (left ventricular ejection fraction [LVEF] \leq 0.40). The LVEF was assessed at baseline and at 6 and 12 months by nuclear scans. Myocardial expression was assessed by ribonuclease (RNase) protection, normalized to a constitutively active gene (glyceraldehydes 3-phosphate dehydrogenase [GAPDH]) and reported as percent GAPDH expression. The change in LVEF at 6 and 12 months was compared by tertiles of expression.
RESULTS	For all patients (14 men, 6 women; age 46.5 ± 10.7 years), the mean LVEF was 0.28 ± 0.05 at baseline and 0.40 ± 0.14 at six months. Patients in the highest tertile of Fas expression had minimal improvement at six months (Δ EF = 0.03 ± 0.05) when compared with the intermediate (Δ EF = 0.10 ± 0.13) and lowest tertiles (Δ EF = 0.21 ± 0.11 , change in LVEF by tertile, $p = 0.006$). A similar relationship was seen with TNFR1 expression (highest tertile, Δ EF = 0.06 ± 0.07 ; lowest tertile, Δ EF = 0.21 ± 0.11 , $p = 0.02$). In contrast with Fas and TNFR1, expression of TNF-alpha and FasL did not predict recovery of LV function. In cardiomyopathy of recent onset, increased expression of Fas and TNFR1 was associated with minimal recovery of LV function. Apoptosis limits myocardial recovery, and represents a potential target for therapeutic intervention. (J Am Coll Cardiol 2005;46:1036-42)
CONCLUSIONS	In cardiomyopathy of recent onset, increased expression of Fas and TNFR1 was associated with minimal recovery of LV function. Apoptosis limits myocardial recovery, and represents a potential target for therapeutic intervention. (J Am Coll Cardiol 2005;46:1036-42)

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For patients presenting with recent-onset dilated cardiomyopathy, clinical outcomes and the degree of myocardial recovery vary widely. Significant improvement in left ven-

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tricular ejection fraction (LVEF) is seen in 30% to 40% of patients (1,2), whereas the remainder of patients are left with persistent left ventricular dysfunction and congestive

heart failure (3). Although cellular inflammation is evident on biopsy in a subset of patients (1), histology on endomyocardial biopsy is generally not predictive of outcome. In fact, there are few reliable predictors of subsequent recovery of ventricular function.

In patients with chronic heart failure, loss of myocytes caused by apoptosis or programmed cell death results in progressive myocardial dysfunction (4,5). Fas is a transmembrane cell surface receptor that plays a critical role in apoptosis (6). When engaged by the signaling peptide Fas ligand (FasL), the receptor initiates a cascade that leads to proteolysis and programmed cell death. Other cytokines are important in this pathway, and the interaction of tumor necrosis factor (TNF)-alpha and its receptor, TNFR1, also leads to apoptosis. Patients with progressive left ventricular dysfunction and chronic heart failure have elevated levels of soluble Fas that correlate with prognosis (7-11). In addition, levels of serum FasL are increased in patients with myocarditis and correlate with severity of heart failure (11).

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Manuscript received March 4, 2005; revised manuscript received April 27, 2005, accepted May 3, 2005.

Abbreviations and Acronyms

ACEI	=	angiotensin-converting enzyme inhibitor
FasL	=	Fas ligand
FLICE	=	Fas-associated death domain-like interleukin-1 β -converting enzyme
GAPDH	=	glyceraldehydes 3-phosphate dehydrogenase
IMAC	=	Intervention in Myocarditis and Acute Cardiomyopathy study
LVEF	=	left ventricular ejection fraction
NYHA	=	New York Heart Association
RNA	=	ribonucleic acid
TNF	=	tumor necrosis factor
TNFR	=	tumor necrosis factor receptor

The impact of myocardial expression of mediators of apoptosis on myocardial recovery has not been previously evaluated in cardiomyopathy of recent onset.

We examined the myocardial expression of Fas and FasL, their relationship to expression of the proinflammatory cytokines TNF-alpha and its receptor TNFR1, and their impact on subsequent myocardial recovery in a series of patients with recent-onset cardiomyopathy.

METHODS

Patients. Endomyocardial biopsy samples were obtained in 20 patients with recent-onset idiopathic dilated cardiomyopathy from the right ventricle using standard techniques. Nineteen patients were recruited from the gene expression substudy of the Intervention in Myocarditis and Acute Cardiomyopathy (IMAC) study (12), and the remaining patient was from the previous pilot study (13). All patients had an LVEF \leq 0.40, were within six months of their onset of cardiac symptoms, and had an extensive evaluation including right heart catheterization and right ventricular biopsy. Baseline assessment of LVEF by radionuclide scan was performed, and LVEF was reassessed at 6 and 12 months after presentation.

Gene expression. Samples were immediately frozen in liquid nitrogen and stored at -80°C until analysis. To measure myocardial gene expression, total ribonucleic acid (RNA) was extracted from frozen tissues by using an acid guanidium thiocyanate-phenol-chloroform method. A commercially available multi-probe RNase protection assay kit (Riboquant, PharMingen, San Diego, California) was used to evaluate transcript levels, with the assay performed according to the manufacturer's protocol. The value of each hybridized probe was quantified by the PhosphoImager using ImageQuant software (Molecular Dynamics, Sunnyvale, California) and normalized to that of a constitutively active gene, glyceraldehydes 3-phosphate dehydrogenase (GAPDH) included in each template set as an internal control (arbitrarily set as = 1). Myocardial levels of mRNA for TNF-alpha, TNF receptor-1 (TNFR1), Fas, FasL, and Fas-associated death domain-like interleukin-1-beta-

converting enzyme (FLICE) were then expressed as percents of GAPDH mRNA level.

Statistical analysis. Statistical analysis was performed using the statistics software SPSS (SPSS Inc., Chicago, Illinois). Results are presented as mean values \pm standard deviation. Patients were grouped into tertiles based on gene expression of Fas, FasL, TNF, and TNFR1. For continuous variables, assessment of significant differences between ordered tertile means was performed by one-way analysis of variance; significance of a linear trend across ordered means is reported. An exact version of the Mantel-Haentzsel trend test was used to compare distributions of all categorical variables by ordered tertiles. Pearson's correlation coefficient was used as a measure of linear association between two variables. Differences were considered significant at a value of $p < 0.05$.

RESULTS

Clinical characteristics. The 20 patients (14 men and 6 women) had a mean age of 46.5 ± 10.7 years and a New York Heart Association (NYHA) functional class I/II/III/IV = 4/9/7/0 (Tables 1 and 2). The LVEF at baseline was 0.28 ± 0.05 , and the left ventricular end diastolic dimension was 6.5 ± 0.9 cm. Their mean pulmonary capillary wedge pressure was 11.9 ± 7.8 mm Hg, cardiac output and cardiac index were 5.3 ± 1.5 l/min and 2.6 ± 0.6 l/min, respectively, pulmonary artery pressure was 21.2 ± 9.1 mm Hg, and mean right atrial pressure was 4.3 ± 3.6 mm Hg. No patient was febrile at the time of entry. Nine of 20 patients (45%) gave a history of a significant febrile illness within the past year (mean duration between febrile illness and study entry, 5.9 ± 3.6 months). All had a normal white blood cell count at time of entry (mean, 6.9 ± 1.5 ; range, 4.9 to $10.0 \times 10^3/\text{mm}^3$). Endomyocardial biopsies were interpreted as negative for myocarditis in 19 of 20 patients, and borderline myocarditis in one individual. Medical therapy at time of presentation and right ventricular biopsy included angiotensin-converting enzyme inhibitors (ACEIs) in 19 (95%) and an angiotensin receptor antagonist in the remaining subject. Beta-adrenergic receptor antagonists were utilized in three patients (15%) at baseline and at 6- (30%) and 12-month follow-up.

Baseline gene expression and subsequent improvements in LVEF. The mean expression levels of TNF-alpha and TNFR1 (55 kD) were $0.12 \pm 0.04\%$ and $1.87 \pm 0.43\%$ of GAPDH transcript levels. The expression levels for the apoptosis genes were: Fas, $0.22 \pm 0.07\%$; FLICE, $0.15 \pm 0.05\%$; and FasL, $0.008 \pm 0.007\%$. There was a significant correlation between myocardial expression of Fas and TNF-alpha ($r = 0.76$, $p < 0.01$, Fig. 1) and between expression of Fas and TNFR1 ($r = 0.66$, $p < 0.05$).

Overall, there was significant recovery of left ventricular function at the six-month reassessment (baseline LVEF, 0.28 ± 0.05 ; six-month LVEF, 0.40 ± 0.14 ; $p < 0.01$),

Table 1. Baseline Characteristics by Tertiles of Fas Expression

	Total (n = 20)	Low Fas (n = 7)	Intermediate Fas (n = 7)	High Fas (n = 6)	p Value
Age (yrs)	46 ± 11	42 ± 13	46 ± 12	52 ± 3	NS
% Male	70	43	71	100	0.04
NYHA functional class (I/II/III)	4/9/7	0/3/4	1/3/3	3/3/0	0.01
Months of symptoms	2.2 ± 1.6	2.0 ± 1.8	2.4 ± 1.7	2.2 ± 1.5	NS
LVEF, baseline	0.28 ± 0.05	0.27 ± 0.04	0.29 ± 0.07	0.28 ± 0.05	NS
Peak MVO ₂	21.5 ± 4.7	20.5 ± 3.2	21.8 ± 5.7	22.3 ± 5.4	NS
LVEDD (cm)	6.6 ± 0.9	6.2 ± 0.7	6.6 ± 0.9	6.9 ± 1.0	NS
PCWP (mm Hg)	12 ± 8	10 ± 8	13 ± 9	12 ± 6	NS
Cardiac output (l/min)	5.3 ± 1.5	4.8 ± 1.8	5.2 ± 1.2	6.2 ± 1.5	NS
RA (mm Hg)	4 ± 4	4 ± 4	5 ± 4	4 ± 3	NS
ACEI or ARB	100%	100%	100%	100%	NS
Beta-blockers	15%	14%	29%	0%	NS
BP systolic, baseline	116 ± 16	123 ± 24	109 ± 14	118 ± 10	NS
BP systolic, 6 months	120 ± 20	129 ± 26	110 ± 14	122 ± 19	NS
BP systolic, 12 months	117 ± 16	120 ± 12	116 ± 20	114 ± 14	NS
BP diastolic, baseline	74 ± 10	77 ± 10	67 ± 7	79 ± 10	NS
BP diastolic, 6 months	76 ± 13	76 ± 12	71 ± 11	81 ± 17	NS
BP diastolic, 12 months	74 ± 11	70 ± 9	74 ± 14	78 ± 10	NS

ACEI = angiotensin-converting enzyme inhibitor; ARB = angiotensin receptor blocker; BP = blood pressure; LVEDD = left ventricular end-diastolic dimension; LVEF = left ventricular ejection fraction; MVO₂ = maximum oxygen consumption (cc/kg/min); NS = not significant; NYHA = New York Heart Association; PCWP = pulmonary capillary wedge pressure; RA = right atrial pressure.

which was maintained at the 12-month assessment (LVEF at 12 months, 0.40 ± 0.13, compared with baseline, p < 0.01; compared with six months, p = NS; Tables 3 and 4). A significant negative correlation exists between baseline myocardial expression of Fas and the subsequent change in LVEF at 6 months (r = -0.54, p = 0.01) and 12 months (r = -0.50, p = 0.02). In contrast, there was no significant correlation between baseline expression of TNF-alpha and the change in LVEF (6 months: r = -0.37, p = 0.10; 12 months: r = -0.23, p = 0.32).

Tertile analysis. The clinical characteristics by Fas tertile are listed in Table 1. Patients were divided into tertiles on the basis of Fas expression into those with low (Fas

transcript level < 0.22% of GAPDH, n = 7), intermediate (0.22% to 0.26%, n = 7) and high expression (>0.26%, n = 6). Patients with high Fas expression had minimal improvement in LVEF (change in LVEF at six months = 0.03 ± 0.05), those with intermediate expression had a greater increase (0.10 ± 0.13), whereas patients with the lowest expression had the greatest improvement (0.21 ± 0.11; comparison change in LVEF by tertile, p = 0.006; Fig. 2). This difference in LV recovery based on Fas expression at baseline persisted at 12 months (change in LVEF by tertiles low/intermediate/high = 0.22 ± 0.13/0.09 ± 0.13/0.02 ± 0.06, p = 0.004, Fig. 3). Fas expression was inversely associated with NYHA functional class (mean Fas expres-

Table 2. Baseline Characteristics by Tertiles of TNFR1 Expression

	Low TNFR1 (n = 7)	Intermediate TNFR1 (n = 7)	High TNFR1s (n = 6)	p Value
Age (yrs)	43 ± 13	50 ± 3	47 ± 13	NS
% Male	43	71	100	0.04
NYHA functional class (I/II/III)	0/2/5	1/5/1	3/2/1	0.01
Months of symptoms	2.1 ± 1.7	2.7 ± 1.5	1.8 ± 1.7	NS
LVEF, baseline	0.28 ± 0.06	0.29 ± 0.05	0.27 ± 0.06	NS
Peak MVO ₂	20.2 ± 3.1	21.3 ± 6.4	23.2 ± 4.9	NS
LVEDD (cm)	6.2 ± 0.7	6.6 ± 0.9	6.9 ± 1.0	NS
PCWP (mm Hg)	8 ± 5	14 ± 8	14 ± 9	NS
Cardiac output (l/min)	4.2 ± 1.8	6.0 ± 1.6	6.0 ± 1.6	0.03
RA (mm Hg)	3 ± 3	5 ± 4	6 ± 4	NS
ACEI or ARB	100%	100%	100%	NS
Beta-blockers	14%	14%	17%	NS
BP systolic, baseline	116 ± 27	116 ± 10	115 ± 8	NS
BP systolic, 6 months	125 ± 26	116 ± 14	117 ± 21	NS
BP systolic, 12 months	117 ± 13	122 ± 9	111 ± 24	NS
BP diastolic, baseline	73 ± 14	75 ± 9	74 ± 6	NS
BP diastolic, 6 months	74 ± 13	76 ± 11	77 ± 17	NS
BP diastolic, 12 months	71 ± 8	81 ± 11	71 ± 12	NS

TNFR1 = tumor necrosis factor receptor 1; other abbreviations as in Table 1.

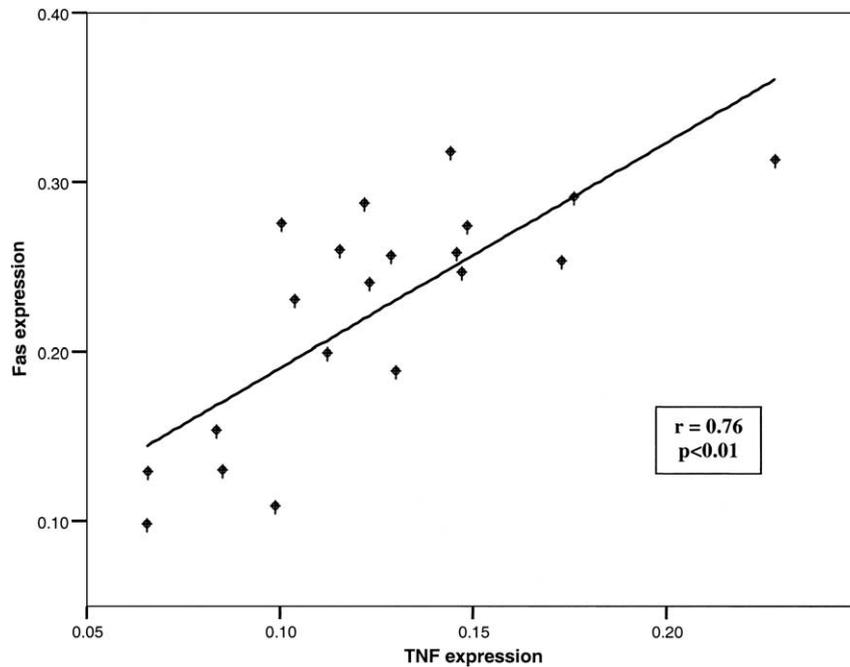


Figure 1. Relationship between baseline myocardial tumor necrosis factor (TNF)-alpha and Fas gene expression, noted as percent glyceraldehydes 3-phosphate dehydrogenase (GAPDH) expression. Correlation of Fas and TNF expression: $r = 0.76$, $p < 0.01$.

sion in NYHA class I/II/III = $0.27 \pm 0.11/0.23 \pm 0.07/0.18 \pm 0.06$, $p = 0.03$). The NYHA functional class reflects functional status at the time of enrollment, and the four class I patients were all class II to III at the time of initial presentation.

For TNFR1, patients were divided into low (TNFR1 transcript level $<1.67\%$ of GAPDH, $n = 7$), intermediate (1.67% to 2.0% , $n = 7$), and high expression ($>2.0\%$, $n = 6$). Similar tertile analysis showed that high TNFR1 expression was associated with a limited increase in LVEF and low TNFR1 expression predicted greater improvement in LVEF at 6 and 12 months (change in LVEF by TNFR1 expression tertiles at 6 months, low/intermediate/high = $0.21 \pm 0.11/0.08 \pm 0.13/0.06 \pm 0.07$, $p = 0.02$, Fig. 4; 12 months = $0.22 \pm 0.12/0.08 \pm 0.12/0.04 \pm 0.10$, $p = 0.01$, Fig. 5). Tertile analyses of TNF-alpha, FasL, and FLICE did not predict subsequent changes in LVEF.

Male gender was associated with a higher expression of Fas and TNFR1 ($p = 0.04$). Tertile analysis was repeated in the male subset ($n = 14$). Fas and TNFR1 tertiles remained predictive of LV improvement at 6 months (Fas, $p = 0.02$; TNFR1, $p = 0.08$) and 12 months (Fas, $p = 0.004$;

TNFR1, $p = 0.01$) when evaluated in male patients, suggesting that differences in gender did not underlie apparent differences in the tertiles overall.

Impact of medical therapy on LV recovery. Beta-blocker therapy was used by 3 of 20 (15%) of patients at baseline, 5 (25%) at 6 months, and 6 (30%) at 12 months. The low percentage of patients on beta-blockers was reflective of evolving practice guidelines during the time of enrollment (1996 to 1998). The percentage of patients on beta-blockers by Fas tertile (low, intermediate, high) was not significantly different at baseline (Tables 1 and 2) or at 6 or 12 months. The mean LVEF was significantly higher for patients on beta-blockers at 12-month follow-up, ($n = 6$, LVEF = 0.50 ± 14) when compared with those not on therapy ($n = 14$, LVEF = 0.35 ± 0.10 , $p = 0.01$). Therefore, to ensure that beta-blocker therapy was not acting as a confounder, analysis of the change in EF by Fas tertile was repeated exclusively in patients not treated with beta-blockers during follow-up. Even in this smaller cohort ($n = 14$), high Fas expression remained significantly linked to less improvement in LVEF for both the 6-month ($p = 0.014$) and the 12-month ($p = 0.017$) end points. For the small subset (n

Table 3. Left Ventricular Ejection Fraction by Fas Tertile

	Total (n = 20)	Low Fas (n = 7)	Intermediate Fas (n = 7)	High Fas (n = 6)	p Value
LVEF, baseline	0.28 ± 0.05	0.27 ± 0.04	0.29 ± 0.07	0.28 ± 0.05	NS
LVEF, 6 months	0.40 ± 0.14	0.49 ± 0.13	0.39 ± 0.16	0.32 ± 0.05	0.03
LVEF, 12 months	0.40 ± 0.13	0.49 ± 0.10	0.39 ± 0.14	0.30 ± 0.07	0.006
Change in EF, 6 months	0.12 ± 0.13	0.22 ± 0.12	0.10 ± 0.13	0.03 ± 0.05	0.006
Change in EF, 12 months	0.12 ± 0.13	0.22 ± 0.13	0.09 ± 0.13	0.02 ± 0.06	0.004

Abbreviations as in Table 2.

Table 4. Left Ventricular Ejection Fraction by TNFR1 Tertile

	Low TNFR1 (n = 7)	Intermediate TNFR1 (n = 7)	High TNFR1 (n = 6)	p Value
LVEF, baseline	0.29 ± 0.06	0.29 ± 0.05	0.27 ± 0.06	NS
LVEF, 6 months	0.50 ± 0.11	0.37 ± 0.15	0.33 ± 0.09	0.02
LVEF, 12 months	0.51 ± 0.08	0.37 ± 0.13	0.31 ± 0.09	0.003
Change in EF, 6 months	0.21 ± 0.11	0.08 ± 0.13	0.06 ± 0.07	0.02
Change in EF, 12 months	0.22 ± 0.12	0.08 ± 0.12	0.04 ± 0.10	0.01

Abbreviations as in Table 2.

= 6) on beta-blockers, the improvement in LVEF seemed to be greater for patients on beta-blockers from the lowest Fas tertile (n = 3, increase in LVEF = 0.30 ± 0.10) than for those in higher tertiles of Fas expression (n = 3, change in EF = 0.12 ± 0.21); however, this was not significant given the limited number of patients.

All patients were on ACEI therapy at study entry. The ACEI dose was increased in 8 of 20 patients (40%) during follow-up. The number of patients with an increase in ACEI therapy was similar in all Fas tertiles (43%, 43%, 33%, p = 0.92), and was not associated with increased LVEF (ACE increased, n = 8; LVEF = 0.40 ± 0.16; not increased, LVEF = 0.40 ± 0.11; p = 0.92). Although statistical power was limited in the smaller subsets, tertiles of Fas expression seemed predictive of subsequent LV recovery in both groups for the 6-month (ACE not increased, p = 0.05; ACE increased, p = 0.10) and 12-month end points (ACE not increased, p = 0.08; ACE increased, p = 0.02).

DISCUSSION

In this study of patients with recent-onset cardiomyopathy, patients with lower expression of the receptors that initiate

the apoptosis pathway, Fas and TNFR1, had significantly more LV recovery during subsequent follow-up, with mean improvements in LVEF of more than 20 EF units at 6 and 12 months. In contrast, patients with the highest Fas and TNFR1 expression had minimal improvements of LVEF evident during the course of the study. This analysis suggests that activation of apoptotic pathways may limit myocardial recovery in this disorder, and that the levels of Fas and TNFR1 expression may be useful clinical predictors of the potential for subsequent LV recovery.

In the IMAC study of acute cardiomyopathy and myocarditis, biopsy status, hemodynamic assessment, and metabolic stress testing all failed to predict subsequent improvements in LVEF (12). In cardiomyopathy of recent onset, the percentage of patients with biopsy-proven myocarditis in published series is variable, with as few as 10% showing inflammatory infiltrates (14). Evidence of cellular inflammation on biopsy does not seem to predict clinical outcome (1). In fact, patients with fulminant myocarditis seem to have a significant chance of complete recovery (15). For patients with recent-onset cardiomyopathy, the utility of endomyocardial biopsy remains uncertain and the procedure is not routinely performed. However, the findings of the current investigation suggest that the incorporation of gene

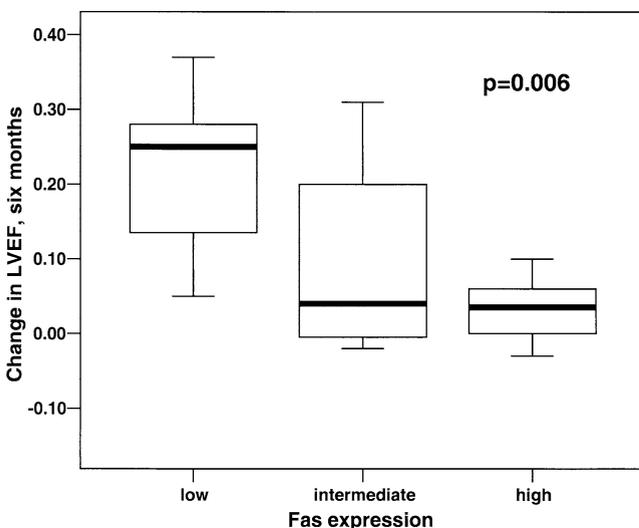


Figure 2. Box plot of distribution change in left ventricular ejection fraction (LVEF) at six months by tertiles of baseline Fas expression. **Solid black lines** = median; **rectangles** = quartile range; **whiskers** = total range. Higher Fas expression is associated with less improvement in LVEF at six months, p = 0.006.

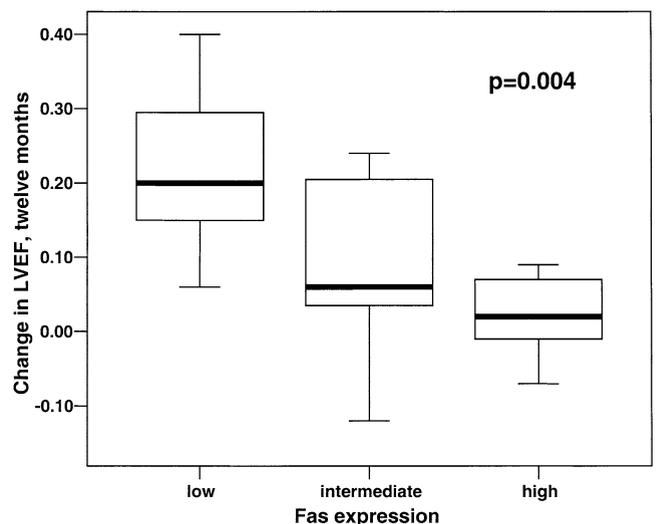


Figure 3. Change in left ventricular ejection fraction (LVEF) at 12 months by tertiles of baseline Fas expression. Higher Fas expression is associated with less improvement in LVEF at 12 months, p = 0.004.

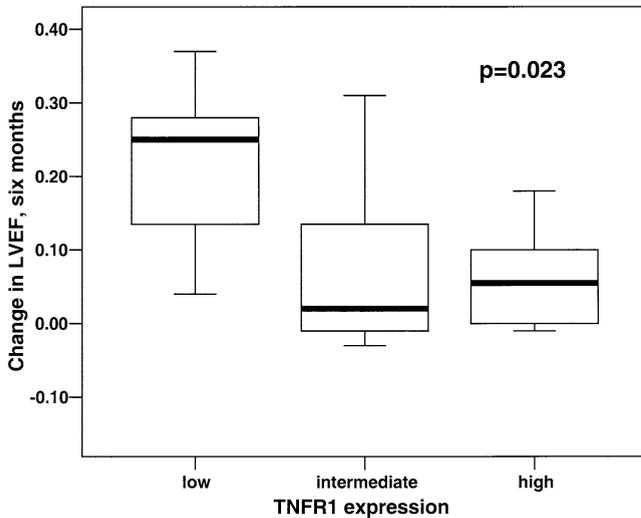


Figure 4. Change in left ventricular ejection fraction (LVEF) at 12 months by tertiles of baseline tumor necrosis factor receptor 1 (TNFR1) expression. Higher TNFR1 expression is associated with less improvement in LVEF at six months, $p = 0.023$.

expression profiles may significantly improve the predictive value of myocardial assessment.

The Fas/FasL system is involved in the pathogenesis of autoimmune myocarditis (16) and peripartum cardiomyopathy (17). Elevated levels of soluble Fas and FasL have been shown in patients with myocarditis (10) and chronic congestive heart failure (6-9), and have been found to predict clinical outcomes (11). The TNFR1 receptor, when activated by TNF-alpha, induces several cellular processes, including apoptosis (18). Patients with increased expression of Fas and TNFR1 may be showing a more profound response to myocardial injury, resulting in increased cell death and diminished potential for recovery. Alternatively, these patients may have undergone biopsy at a more advanced stage of disease progression, in which increase in programmed cell death is evident. Whether increased expression represents a distinct response to injury or a simply a different stage of the inflammatory/apoptotic response cannot be determined by the current study.

Male gender was associated with higher Fas and TNFR1 expression. The murine model of autoimmune disease shows significant gender differences in the expression of genes associated with programmed cell death (19). Testosterone stimulates apoptosis in vitro in renal tubular cells, and may contribute to the more rapid progression of diabetic nephropathy in men (20). In a rat ischemia-reperfusion model, reduction of androgens through surgical castration or hormonal therapy decreases pro-inflammatory and pro-apoptotic gene expression and facilitates myocardial recovery (21). The tendency of androgens to stimulate cytokine and pro-apoptotic gene expression would theoretically limit myocardial recovery in men relative to women and may underlie the male predominance in idiopathic dilated cardiomyopathy (22).

Plasma levels of TNF-alpha are elevated in patients with

new-onset cardiomyopathy and correlate with myocardial expression (19). The current study shows a strong correlation between myocardial expression of Fas and TNF-alpha consistent with the role of TNF in initiating the apoptotic pathway. Despite this correlation, TNF-alpha and FasL expression were not predictive of recovery of left ventricular function, suggesting that receptor expression is more reflective of myocardial processes that lead to apoptosis than the circulating ligands. Additionally, the role of TNF-alpha in acute cardiomyopathy is complex; TNF-alpha plays a role in cell survival and protection through induction of the transcription factor nuclear factor kappa-beta (18). The overall impact of TNF expression on LV recovery may therefore represent a synthesis of these two competing roles: protection from viral injury and facilitation of apoptosis.

In acute viral myocarditis, cytokine expression and apoptosis may be beneficial and facilitate viral clearing. Although the current cohort is defined by the recent onset of symptoms, they seem to be beyond the stage of an acute viral trigger. No patient was febrile at the time of endomyocardial biopsy, all had normal white cell counts, and only one had histologic evidence of lymphocytic myocarditis. The apparent negative impact of cytokine and apoptotic myocardial expression in this cohort may reflect a later post-viral stage of this disorder, and the current study cannot address the potential protective role in acute viral myocarditis.

There are several limitations to this study. Given the limited myocardial tissue available, protein levels of Fas and TNFR1 were not assessed. In addition, immunohistochemistry quantifying apoptotic nuclei was not performed. Soluble Fas and FasL were not assessed in the serum, and this study cannot address the predictive value of these more accessible peripheral markers of apoptosis. We have previ-

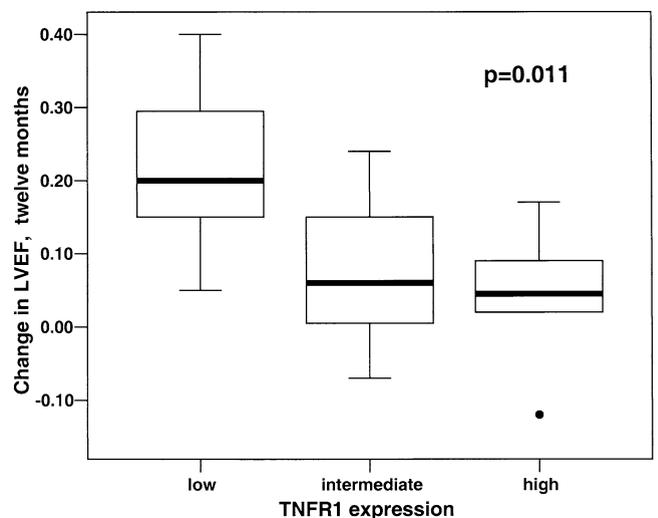


Figure 5. Change in left ventricular ejection fraction (LVEF) at 12 months by tertiles of baseline Fas expression. **Solid black lines** = median; **rectangles** = quartile range; **whiskers** = total range excluding outlier; **filled circle** = outlier. Higher Fas expression is associated with less improvement in LVEF at 12 months, $p = 0.011$. TNFR1 = tumor necrosis factor receptor 1.

ously shown that circulating TNF correlates with myocardial expression (23). In addition, as with routine histology, biopsy studies of gene expression may be subject to sampling error because of regional variations. However, previous studies of myocardial gene expression in the right ventricle septum showed significant correlation with expression in the left ventricular free wall (24). Finally, patients in this study were part of a larger randomized trial of immune globulin therapy, and we cannot rule out that an interaction with the randomized therapy may have impacted the results. The absence of any impact of treatment of immune globulin on subsequent LVEF in the larger trial diminishes this possibility (12).

This study is the first to suggest that the gene expression profile from an endomyocardial biopsy predicts subsequent LV recovery for patients with recent-onset cardiomyopathy. Activation of apoptotic pathways, as delineated by higher Fas and TNFR1 expression, denotes a subset of patients unlikely to recover LV function, whereas patients with minimal Fas and TNFR1 expression experienced a dramatic increase in LVEF during subsequent follow-up. Whether patients with higher Fas and TNFR1 expression were showing a differential response to injury or were captured at a later phase of their illness remains a question for further study. In contrast to the role of the ligands TNF- α and FasL, high expression of the receptors Fas and TNFR1 seems to limit LV recovery and may represent targets for therapeutic intervention.

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