Indium 111–monoclonal antimyosin antibody imaging in the diagnosis of acute myocarditis

TSUNEHIRO YASUDA, M.D., IGOR F. PALACIOS, M.D., G. WILLIAM DEC, M.D., JOHN T. FALLON, M.D., PH.D., HERMAN K. GOLD, M.D., ROBERT C. LEINBACH, M.D., H. WILLIAM STRAUSS, M.D., BAN AN KHAW, PH.D., AND EDGAR HABER, M.D.

ABSTRACT A definitive diagnosis of myocarditis requires right ventricular biopsy. Despite its specificity, however, right ventricular biopsy may lack sensitivity due to the focal nature of the disease. Because indium 111–monoclonal antimyosin antibody imaging can be used to detect myocardial necrosis, this procedure was performed on 28 patients clinically suspected of having myocarditis, 25 of whom had left ventricular ejection fractions of less than 45%, and the results were compared with those of right ventricular biopsy performed within 48 hr of the scan. Antimyosin scans were positive in nine patients who had evidence of myocarditis on right ventricular biopsy, and negative in 11 who had no evidence of myocarditis by biopsy. The remaining eight had positive antimyosin scans but showed no evidence of myocarditis on right ventricular biopsy. On the basis of a right ventricular biopsy standard, the sensitivity of this method was 100%, the specificity 58%. We conclude that antimyosin antibody imaging is a reliable screening method for the evaluation of patients suspected of having myocarditis, and that a positive antimyosin scan indicates the need for right ventricular biopsy to establish the histologic diagnosis.

Circulation 76, No. 2, 306–311, 1987.

ALTHOUGH A PRESUMPTIVE diagnosis of myocarditis can be made on the basis of clinical findings, the histologic demonstration of a cell infiltrate associated with necrotic or degenerative myocytes is necessary for a definitive diagnosis.[1] Right ventricular endomyocardial biopsy is a widely used technique, but its sensitivity for myocarditis has not been established. Furthermore, because of the focal nature of the disease, right ventricular endomyocardial biopsy may lack sensitivity because it may fail to sample a sufficient number of myocardial sites.[2]

Radiolabeled Fab of monoclonal antimyosin antibodies bind to cells that have lost the integrity of their plasma membranes; intracellular myosin is exposed to extracellular fluid when the membrane degenerates.[3, 4] Scintigraphic examinations with these antibodies have been used to localize and quantify regions of myocardial necrosis in myocardial infarction.[5–7] Because myocardial necrosis is an obligatory component of myocarditis,[1] the present study was performed to evaluate the applicability of this technique in the diagnosis of myocarditis.

Methods

Patient population. Twenty-eight patients were studied who presented at the Massachusetts General Hospital between 1984 and 1985 with histories and clinical findings suggestive of acute myocarditis (table 1). All patients underwent right and left heart catheterization, right ventricular endomyocardial biopsy, and imaging with monoclonal antimyosin Fab labeled with indium-111 (^{111}In), and all were demonstrated to have normal coronary arteries by selective coronary artery cineangiography.

Three patients had left ventricular ejection fractions greater than 45% (two men, one woman). One patient presented with chest pain and electrocardiographic changes resembling an evolving myocardial infarction; normal coronary arteries and no evidence of spasm were demonstrated during emergency coronary cineangiography. Another presented with pericardial effusion, chest pain, and atrial fibrillation, and one had unexplained bisided congestive heart failure, a normal left ventricular ejection fraction, and hemodynamic findings consistent with constrictive versus restrictive physiology. The pericardium appeared normal on two-dimensional echocardiography and computed body tomography.

Twenty-five patients presented with global left ventricular dysfunction demonstrated by cardiac catheterization or equilibrium-gated blood pool scanning (13 men, 12 women; mean age 50 ± 3 years, range 19 to 77; average left ventricular ejection fraction 27 ± 2%). Of these 25 patients, four (16%) presented with ventricular tachyarrhythmias and one (4%) with chest pain.

From the Division of Nuclear Medicine, Department of Radiology, the Cardiac Unit, Department of Medicine and the Department of Pathology, Massachusetts General Hospital, Boston.

Supported in part by a grant from Centocor, Incorporated.

Address for correspondence: Editor's Office, Cardiac Unit, Jackson 13, Massachusetts General Hospital, Boston, MA 02114.

Received Oct. 7, 1986; revision accepted May 7, 1987.

TABLE 1
Characteristics of 28 patients suspected of having myocarditis

Patient No.	Age (yr)	Sex	Clinical score	Presentation	Biopsy results	AMAb scan	Initial LVEF	Six month follow-up LVEF
1	53	F	3	CP	Myoc	+	56	70
2	63	M	3	CP, AF, eff	Myoc	+	66	
3	44	M	0	CHF	Myoc	+	63	58
4	57	M	1	CHF	Myoc	+	15	55[B]
5[A]	46	F	3	CHF	Myoc	+	36	24
6	44	F	2	CHF	Myoc	+	34	55[B]
7	64	F	0	CHF	Myoc	+	27	44[B]
8	47	F	0	VT, VF	Myoc	+	20	30[B]
9	52	F	2	CHF	Myoc	+	37	34
10	25	M	0	VT, VF	NSC	+	20	18
11	29	M	3	CHF	Normal	+	20	65[B]
12	25	M	3	CP	Normal	+	44	54[B]
13	51	M	2	CHF	Normal	+	22	73[B]
14	46	F	2	CHF	Normal	+	23	40[B]
15	53	F	0	CHF	Normal	+	12	10
16	58	M	1	CHF	NSC	+	18	19
17	70	M	1	CHF	NSC	+	44	30
18[A]	47	M	3	CHF	NSC	−	17	19
19	71	F	1	CHF	NSC	−	45	56[B]
20[A]	19	M	0	VT, VF	NSC	−	29	28
21	66	F	1	VT, VF	NSC	−	30	27
22	77	M	2	CHF	Normal	−	36	26
23	51	F	0	CHF	NSC	−	21	45[B]
24	43	F	1	CHF	NSC	−	10	
25	61	M	1	CHF	NSC	−	21	21
26	65	F	0	CHF	NSC	−	24	
27[A]	36	M	0	CHF	NSC	−	15	17
28	46	M	1	CHF	NSC	−	29	70[B]

AF = atrial fibrillation; AMAb = antimyosin antibody; CHF = congestive heart failure; CP = chest pain; eff = pericardial effusion; LVEF = left ventricular ejection fraction; Myoc = myocarditis; NSC = nonspecific change; VT = ventricular tachycardia; VF = ventricular fibrillation.

[A]Previous bout of biopsy-proven myocarditis.

[B]Patients with abnormal left ventricular ejection fractions who showed an improvement of 10% or more.

The remaining 20 (80%) presented with acute onset of heart failure. Heart failure was present for less than 1 year in three of the 20 in this group, and for less than 6 months in 17. Four of the 20 had had a previous episode of biopsy-proven myocarditis, and presented with another episode of heart failure and symptoms suggestive of relapse.

Transvenous right ventricular endomyocardial biopsy and pathologic evaluation. Patients underwent right ventricular endomyocardial biopsy through the right jugular vein as previously described.[2, 8, 9] Multiple biopsy specimens (usually six or seven, each measuring 1 to 3 mm in diameter) were obtained with a Caves-Schultz-Stanford bioptome. Three to four samples were immediately fixed by immersion in buffered 10% formalin for histologic study by light microscopy. One sample was placed in 2.5% buffered glutaraldehyde for electron microscopy, and one was snap-frozen for immunofluorescence studies. Paraffin sections were stained with hematoxylin and eosin, Masson trichrome, and Congo red. The frozen sample was sectioned and stained with hemotoxylin and eosin, and with toluidine blue. Sections of 1 μm were cut from each of the specimens embedded in plastic and stained with toluidine blue.

Biopsy specimens were analyzed without knowledge of the scan results. Specimens were divided into three categories on the basis of light microscopic studies: specimens defined as showing myocarditis contained an inflammatory infiltrate adjacent to necrotic or degenerative myocytes, with or without interstitial fibrosis[1] (figure 1); specimens showing myocyte hypertrophy, interstitial fibrosis and/or replacement fibrosis but no evidence of myocarditis were placed in the nonspecific change category; normal specimens showed no abnormalities.

Ventriculography and clinical evaluation. The initial left ventriculogram was recorded by the radionuclide technique in 24 patients and by the contrast technique in four. All patients were interviewed and examined by one of the investigators before cardiac catheterization. From a review of the clinical history and laboratory data, as previously reported,[2] patients were scored according to the number (0 to 3) of clinical features at the onset of illness that were suggestive of myocarditis; a febrile, virus-like illness just before the development of cardiac symptoms; pericarditis; or laboratory abnormalities (elevation of the serum creatine kinase level, erythrocyte sedimentation rate, or white cell count).

All patients with heart failure due to dilated cardiomyopathy received digoxin and diuretics; in addition, 11 patients received

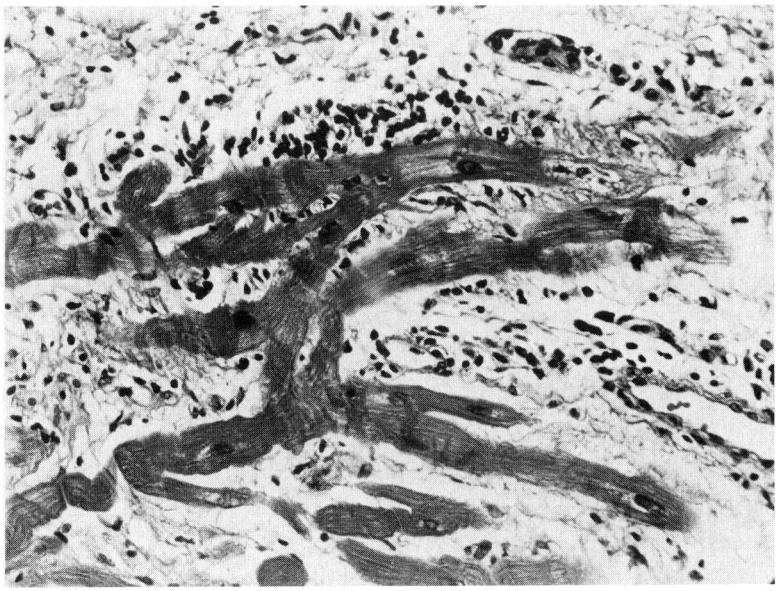

FIGURE 1. Photomicrograph of hemotoxylin and eosin–stained section of right ventricular endomyocardium from patient No. 5 (table 1) showing lymphocytic myocarditis. There is a prominent interstitial infiltrate of mononuclear inflammatory cells and focal degeneration and necrosis of individual myocytes (original magnification ×313).

afterload-reducing agents (captopril, 10; prazosin, one) at the time of initial evaluation of ventricular function. Although the medications were continued with minor adjustment throughout the study, there were no patients in whom afterload-reducing therapy was initiated during the course of the follow-up period. Furthermore, none of the patients who were categorized as having improved ventricular function at the time of follow-up evaluation were receiving afterload-reducing agents. Only one patient (table 1, No. 6.), who presented in cardiogenic shock, received intravenous inotropic therapy with norepinephrine. None of the patients received oral or intravenous phosphodiesterase inhibitors.

Follow-up left ventriculograms were obtained at six months by the radionuclide technique. Ventricular function was considered improved if all three of the following criteria were present: (1) an increase in left ventricular ejection fraction of 10% or more, (2) a decrease in the cardiothoracic ratio of the chest film, and (3) a decrease in symptomatic heart failure by one or more New York Heart Association classes.

Indium 111–monoclonal antimyosin Fab cardiac imaging. We used monoclonal antibody R11D10 (directed against the heavy chain of cardiac myosin) coupled to DTPA[7] for radiolabeling with ^{111}In (kits from Centocor, Malvern, PA). After informed consent was obtained, the radiolabeled, pyrogen-free conjugate was administered to all patients within 24 to 48 hr of myocardial biopsy. To test for hypersensitivity, 0.05 ml of the radioactive agent was administered intradermally. If no wheal or flare was observed within 15 min, 500 μm of antimyosin Fab labeled with 1.8 mCi (66.6 MBq) of ^{111}In was administered intravenously. Ungated planar and single photon–emission computed tomography (SPECT) images were obtained at 24 and 48 hr after administration of the radioactive agents, as previously described.[7] Ungated images were recorded in the anterior and 40 to 50 degree left anterior oblique views with the use of a medium-energy collimator with the pulse height analyzers set at centerlines of 173 and 247 keV (20% window in each). For SPECT imaging, patients were positioned so that the smallest diameter circle could be inscribed by the detector of a rotating gamma camera (Technicare Omega-500/560 AP, Solon, OH). A series of 120 images was collected at 3 degree increments for 20 sec each into a 64/64 matrix and was stored for subsequent analysis. The SPECT images were reconstructed with a filtered backprojection algorithm into transverse, sagittal, and coronal projections with a thickness of approximately 1 cm (3 pixels).

Planar and SPECT antimyosin images were interpreted directly from the computer video display by at least three observers who had no knowledge of the biopsy results. We classified the results of radiolabeled antimyosin cardiac imaging as positive or negative. A scan was classified as positive when focal or diffuse uptake of the tracer was present in the planar image and in at least two of the three tomographic reconstructions (figure 2). A scan was negative when no tracer uptake was demonstrated in either planar or tomographic images (figure 3), or when faint uptake was present in the planar but was not confirmed in the SPECT images.

Results

Antimyosin Fab was administered without untoward reaction in all subjects. The clinical scores and histologic, ventriculographic, and antimyosin imaging results are listed in table 1.

Endomyocardial biopsy findings. Right ventricular biopsies were diagnostic for myocarditis in nine patients (32%), showed nonspecific changes in 13 (47%), and were normal in six (21%).

Results of antimyosin imaging. Table 2 shows the correlation between results of right ventricular biopsy and antimyosin imaging. Results of ^{111}In-antimyosin imaging were positive in 17 patients (61%) and negative in 11 (39%). In all 17 patients with positive antimyosin scans, tracer uptake was heterogeneous within the left ventricle. All patients with biopsy-proven myocarditis had positive antimyosin scans. In addition, eight patients with no evidence of myocarditis on biopsy had positive antimyosin scans. Of these eight patients, the biopsy samples of five were classified as normal and three showed nonspecific change. The changes consisted of varying degrees of interstitial and/or replacement fibrosis and myocyte hypertrophy, but no evi-

dence of inflammation. A negative antimyosin scan was obtained in 11 patients; none of them had evidence of myocarditis by biopsy, although most of them (10 patients) showed nonspecific change.

Follow-up. All six patients (left ventricular ejection fraction <45%) with biopsy-proven myocarditis and dilated cardiomyopathy received immunosuppressive therapy: four a combination of azathioprine and prednisone, one prednisone alone, and one cyclosporine and prednisone. Improvement occurred in four of these six patients (67%). No deaths have occurred in this group of patients with biopsy-proven myocarditis.

Spontaneous improvement occurred in four of the eight patients with positive antimyosin scans and biopsy specimens showing no evidence of myocarditis (50%). Each of the four patients who showed improve-

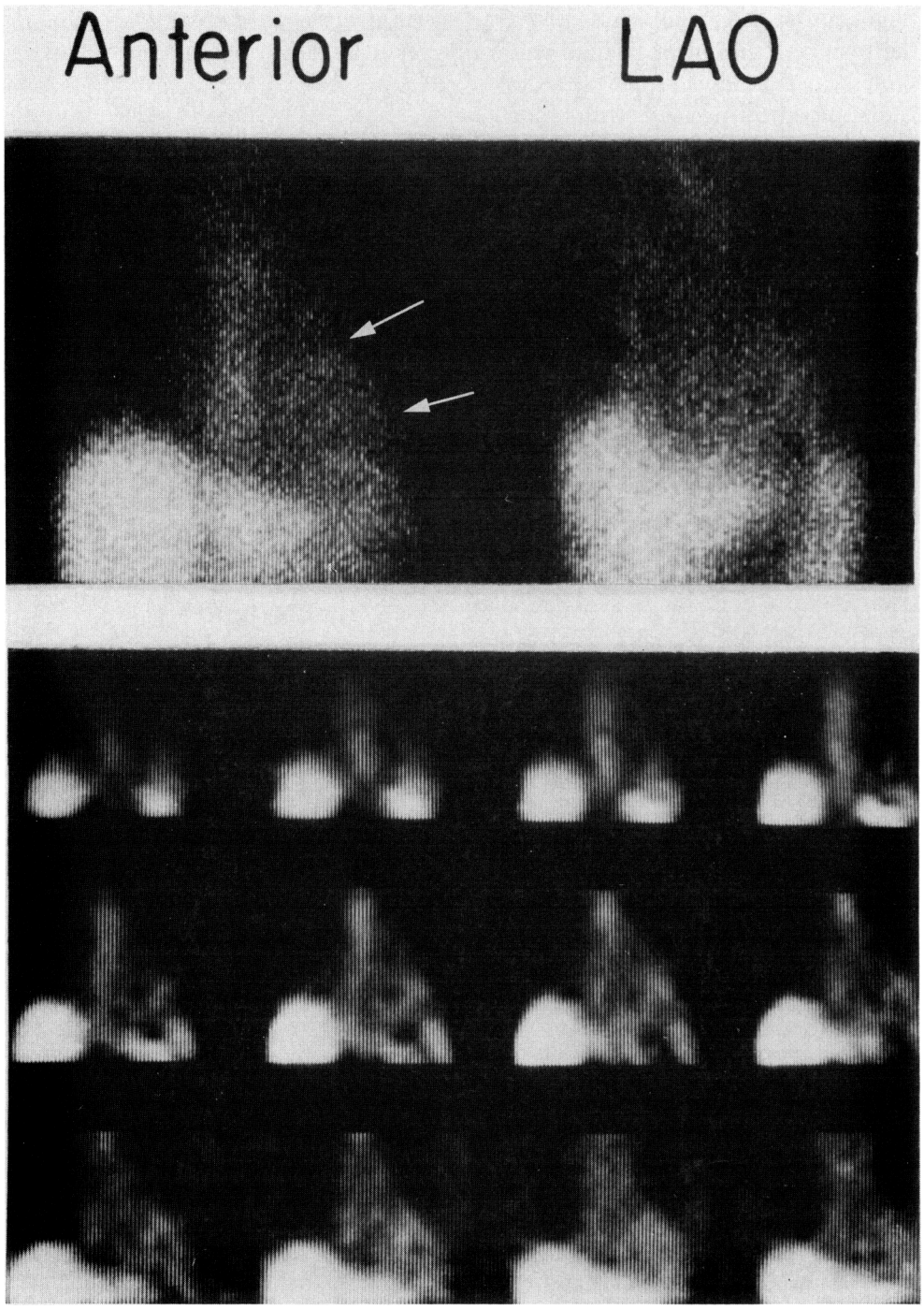

FIGURE 2. A positive antimyosin image. Diffuse uptake in the cardiac region (arrow) is seen in the planar images (*top*) and in the coronal tomographic reconstruction (*bottom*). LAO = left anterior oblique projection.

ment had a clinical score equal to or greater than 2. No deaths have occurred in this group. Finally, improvement also occurred in three of the 11 patients (27%) with negative antimyosin scans.

Discussion

It would be highly desirable to have a noninvasive screening test for myocarditis that could identify those patients who should undergo endomyocardial biopsy.[2] One noninvasive method, the gallium-67 scan, is helpful because an inflammatory cell infiltrate is a histopathologic feature of this disease.[10, 11] Antimyosin scanning provides an opportunity for monitoring the other obligatory abnormality associated with myocarditis, myocardial necrosis.[1] Experimental and clinical studies previously demonstrated that antimyosin Fab was specific only for necrotic myocytes in which intracellular myosin was accessible to extracellular fluid.[3, 4] In this study, a positive antimyosin scan was present in all patients with biopsy-proven myocarditis. Of equal importance, all patients who had a negative antimyosin scan also had a negative biopsy result.

Of particular interest are those eight patients who had no evidence of myocarditis on biopsy but who had positive scans. Could normal myocardial biopsies be the result of focal disease with consequent sampling

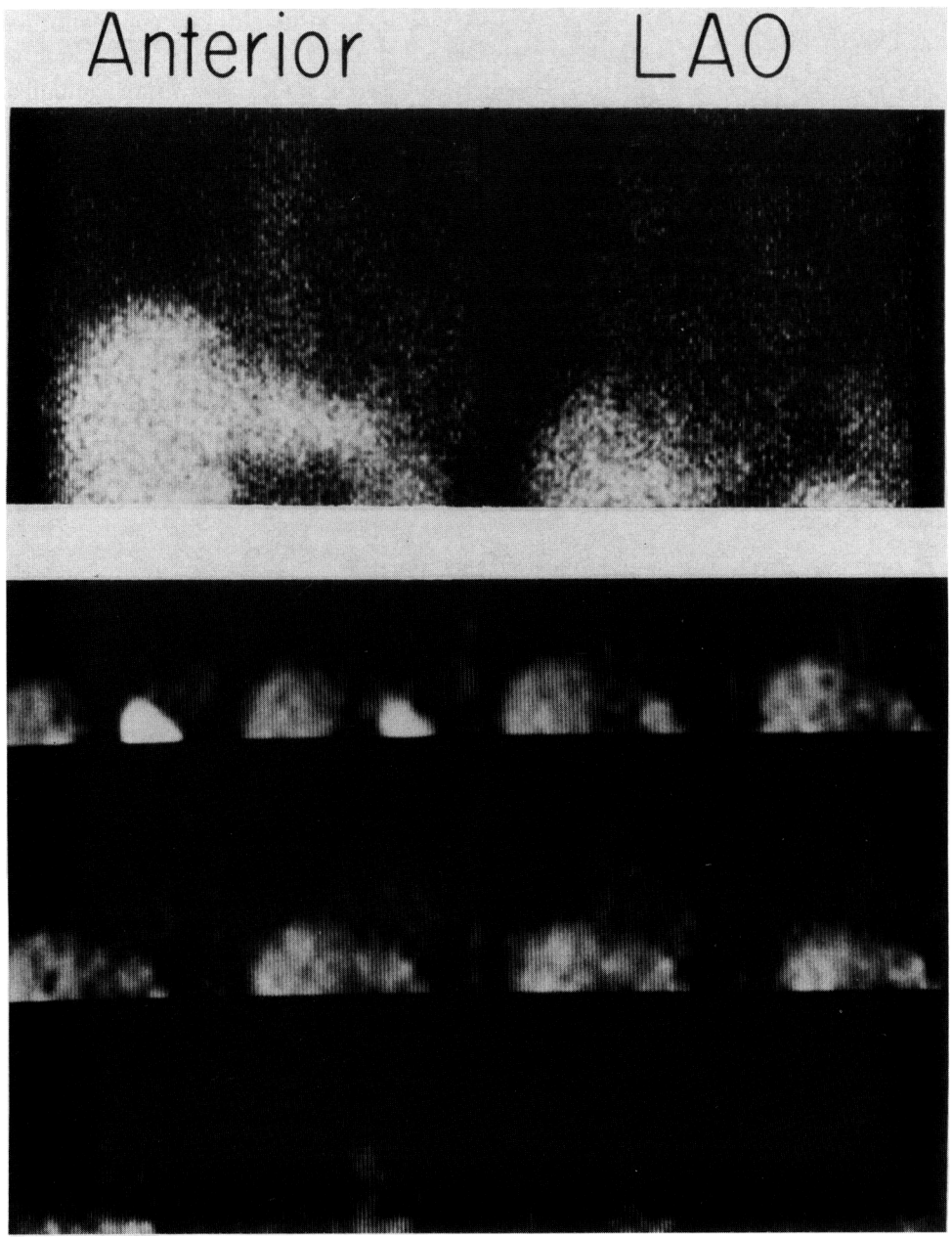

FIGURE 3. A negative antimyosin image. No tracer uptake is seen in either the planar images (*top*) or the coronal tomographic reconstruction (*bottom*). LAO = left anterior oblique projection.

TABLE 2
Comparison of results of right ventricular biopsy and of antimyosin imaging in 28 patients with histories suggestive of myocarditis

Antimyosin scan result	Right ventricular biopsy result	
	Positive	Negative
Positive	9	8
Negative	0	11

The probability that either the two columns or the two rows of this table were drawn from the same population is .004 by the Fisher test.

error? Spontaneous improvement is a feature of acute myocarditis.[2, 12] A spontaneous substantial improvement in ejection fraction occurred in four of these eight patients. The improvement in left ventricular ejection fraction at the time of follow-up radionuclide ventriculography must have been independent of loading changes due to medical therapy, because none of these improved patients required therapy for heart failure at that time. Although these findings suggest that an underlying diagnosis of myocarditis may have been missed because of a sampling error or a failure to identify early myocyte necrosis by histologic criteria,[1] a definitive characterization of this group will require longer term follow-up.

Results more difficult to explain are those of three of the 11 patients with negative biopsies and negative scans who also showed improvement in their ejection fractions. It may be possible that during the course of myocarditis both necrosis and inflammation may have resolved at the time the patient was studied, yet improvement of ventricular function lagged behind the resolution of tissue injury. Another possibility is that there are other forms of transient depression of ventricular function that are still not characterized.

Thus, antimyosin scintigraphy appears to be a reliable screening method for the evaluation of patients suspected of having myocarditis. A positive antimyosin scan indicates the need for endomyocardial biopsy to establish the histologic diagnosis. If the present results are confirmed in studies of a larger number of patients, it may be possible to avoid biopsy in patients who present with a negative scan.

We thank Martha Barlai-Kovach for her technical assistance, Brenda White for her assistance in preparing the manuscript, and Thomas McVarish for his editorial assistance.

References

1. Aretz HT, Billingham ME, Edwards WD, Factor SM, Fallon JT, Fenoglio JJ, Olsen EGJ, Schoen FJ: Myocarditis: a histopathologic definition and classification. Am J Cardiovasc Pathol **1:** 3, 1986
2. Dec GW Jr, Palacios IF, Fallon JT, Aretz HT, Mills J, Lee DC-S, Johnson RA: Active myocarditis in the spectrum of acute dilated cardiomyopathies: Clinical features, histologic correlates, and clinical outcome. N Engl J Med **312:** 885, 1985
3. Khaw BA, Fallon JT, Beller GA, Haber E: Specificity of localization of myosin-specific antibody fragments in experimental myocardial infarction: Histologic, histochemical, autoradiographic and scintigraphic studies. Circulation **60;** 1527, 1979
4. Khaw BA, Beller GA, Haber E, Smith TW: Localization of cardiac myosin-specific antibody in myocardial infarction. J Clin Invest **58:** 439, 1976
5. Khaw BA, Gold HK, Leinbach RC, Fallon JT, Strauss HW, Pohost GM, Haber E: Early imaging of experimental myocardial infarction by intracoronary administration of ^{131}I-labeled anticardiac myosin $(Fab')_2$ fragments. Circulation **58:** 1137, 1978
6. Khaw BA, Fallon JT, Strauss HW, Haber E: Myocardial infarct imaging of antibodies to canine cardiac myosin with Indium-111-diethylenetriamine pentaacetic acid. Science **209:** 295, 1980
7. Khaw BA, Gold HK, Yasuda T, Leinbach RC, Kanke M, Fallon JT, Barlai-Kovach M, Strauss HW, Sheehan F, Haber E: Scintigraphic quantification of myocardial necrosis in patients after intravenous injection of myosin-specific antibody. Circulation **74:** 501, 1986
8. Mason JW: Techniques for right and left ventricular endomyocardial biopsy. Am J Cardiol **41:** 887, 1978
9. Parrillo JE, Aretz HT, Palacios IF, Fallon JT, Block PC: The results of transvenous endomyocardial biopsy can frequently be used to diagnose myocardial diseases in patients with idiopathic heart failure: endomyocardial biopsies in 100 consecutive patients revealed a substantial incidence of myocarditis. Circulation **69:** 93, 1984
10. O'Connell JB, Robinson JA, Henkin RE, Gunnar RM: Immunosuppressive therapy in patients with congestive cardiomyopathy and myocardial uptake of gallium-67. Circulation **64:** 780, 1981
11. O'Connell JB, Henkin RE, Robinson JA, Subramanian R, Scanlon PJ, Gunnar RM: Gallium-67 imaging in patients with dilated cardiomyopathy and biopsy-proven myocarditis. Circulation **70:** 58, 1984
12. Johnson RA, Palacios IF: Dilated cardiomyopathies of the adult. N Engl J Med **307:** 1051, 1982