Antimyosin Scintigraphy and Immunohistologic Analysis of Endomyocardial Biopsy in Patients With Clinically Suspected Myocarditis—Evidence of Myocardial Cell Damage and Inflammation in the Absence of Histologic Signs of Myocarditis

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Objectives. This study compares the results of antimyosin scintigraphy in patients with clinically suspected myocarditis with histologic and immunohistologic findings in the endomyocardial biopsy.

Background. In patients with clinically suspected myocarditis, antimyosin scintigraphy often demonstrates myocardial cell damage but histologic evaluation of the endomyocardial biopsy often fails to show evidence of myocarditis. Recently developed immunohistologic techniques appear to be more sensitive for the detection of myocardial inflammation than histologic analysis alone. Studies comparing antimyosin scintigraphy and immunohistologic analysis of the endomyocardial biopsy in patients with clinically suspected myocarditis are not yet available.

Methods. Sixty-five patients with clinically suspected myocarditis underwent antimyosin scintigraphy. Antimyosin antibody uptake was correlated with histologic and immunohistologic findings in the endomyocardial biopsy.

Results. Antimyosin scintigraphy showed evidence of myocardial cell damage in 36 (55%) of the 65 patients and was negative in 29 (45%) patients. Histologic analysis of the endomyocardial biopsy revealed myocarditis in nine patients: six had a positive and three had a negative antimyosin scan, respectively. Thirty (83%) of 36 patients with evidence of myocardial cell damage on antimyosin scintigraphy were histologically negative for myocarditis. Immunohistologic analysis showed evidence of myocarditis in 31 (86%) of 36 patients with a positive antimyosin scan and also in 17 (59%) of 29 patients with a normal scan (p < 0.047).

Conclusions. Antimyosin scintigraphy often shows myocyte injury in patients with clinically suspected myocarditis. Histologic analysis of the endomyocardial biopsy alone is often negative, but additional immunohistologic analysis of the endomyocardial biopsy frequently provides evidence of myocardial inflammation in these patients. With immunohistologic analysis as the reference method, antimyosin scintigraphy has a high specificity but a lower sensitivity for the detection of myocarditis.

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In patients with clinically suspected myocarditis, the diagnosis is established by endomyocardial biopsy. Using histologic criteria alone, the diagnostic value of the endomyocardial biopsy has been questioned because of a low yield of diagnostic biopsies in patients with recent onset heart failure of unknown origin (1–3), probably due to sampling error (4,5). Additionally, even when using uniform criteria (6), the histologic analysis of the endomyocardial biopsy is associated with a high interobserver variability (7). Recently, additional immunohistologic methods have been developed using monoclonal antibodies to detect and quantify lymphocytic infiltrates in the myocardium and to analyze the expression of human lymphocyte antigen I and II antigens in the heart (8–12). Using these techniques, the presence of a chronic inflammatory process has been demonstrated in patients with dilated cardiomyopathy (13). Unfortunately, immunohistology is unable to detect myocyte damage, which is an obligatory component of myocarditis (6). Myocyte injury can be studied noninvasively by determining myocardial uptake of indium-111-labeled monoclonal antimyosin antibodies. This method has been successfully used to detect very sensitive areas of myocardial infarction (14) and myocyte injury in cardiac transplant rejection and anthracycline therapy (15–17).

In patients with clinically suspected myocarditis or dilated cardiomyopathy, discrepancies between a high prevalence of positive antimyosin scans and often negative histologic findings in the endomyocardial biopsy have been reported (18–20), indicating a high sensitivity but a low specificity of the antimyosin scintigraphy for the detection of myocarditis. No studies are available comparing results of antimyosin scintigraphy with immunohistologic findings in endomyocardial biopsy specimen.

The present study was performed to analyze the diagnostic value of antimyosin scintigraphy in patients with clinically suspected myocarditis.
suspected myocarditis by comparing the results of antimony scintigraphy with immunohistologic and histologic findings in endomyocardial biopsy specimen.

**Methods**

**Patient selection.** Sixty-five patients (42 men, 23 women, mean age 48.5 ± 12.5 years, range 21 to 70) were studied who presented with histories and clinical findings suggestive of myocarditis. All patients underwent right and left heart catheterization, left ventriculography, right ventricular endomyocardial biopsy and antimony scintigraphy with monoclonal antimony Fab labeled with indium-111. Coronary artery disease was excluded by coronary angiography in all patients.

Forty-six patients presented with left ventricular dysfunction (left ventricular ejection fraction <55%) demonstrated by left ventriculography. Eleven patients presented with new onset of anginal symptoms accompanied by electrocardiographic changes (ST-segment depression or negative T waves) that could be interpreted as myocardial ischemia. However, other causes, like pericardial disease or myocardial hypertrophy, could not be excluded solely from the electrocardiogram. Normal coronary arteries and no evidence of coronary spasm were demonstrated by coronary angiography in all of these patients. A small pericardial effusion was present in 3 patients, and 24 h or 48 h after the injection of the radiopharmacon to assess the presence of the antimyosin antibodies in the myocardial wall was classified visually by an experienced observer unaware of the clinical data in the anterior and the lateral projections, respectively, comparing the images taken 30 min and 24 or 48 h after the injection of the radioimmunoassay to assess the presence of the antimyosin antibodies in the myocardial wall. Images were taken with a large field-of-view high resolution camera, using a medium energy all purpose collimator and two 20% windows centered on 247 and 173 keV peaks. Images were collected for 10 min into a 128 × 128 pixel matrix.

**Analysis of endomyocardial biopsy specimen.** Endomyocardial biopsies were taken transvenously via the femoral approach from the right heart side of the interventricular septum. From each patient, several biopsies (at least five) were analyzed. The endomyocardial biopsies were examined histologically according to the Dallas Criteria (6). The histologic sections were analyzed by light microscopy for evidence of myocardial necrosis, interstitial fibrosis and for the presence of lymphocytic infiltrates. As the histologic evaluation of myocardial biopsies is known to be difficult and affected by many problems (21,22), including high interobserver variability (7) and sampling error (5), the biopsy specimens were therefore examined additionally with immunohistologic techniques using various monoclonal antibodies. Antibodies directed against surface antigens of human lymphocytes (CD3, CD4, CD8) were used to detect and quantitate lymphocytic infiltrates in myocardial tissue (8–13). Additionally, using antibodies against the major histocompatibility antigens (MHC), the expression of MHC I and II antigens was analyzed. With these techniques, the diagnostic accuracy in the biopsies could be increased (11) and the interobserver variability could be minimized (12,13).

The biopsy specimens were classified as “acute myocarditis” when histologic sections of endomyocardial biopsies revealed lymphocytic infiltrates in the neighborhood of myocardial necrosis (6). “Borderline myocarditis” was diagnosed when lymphocytic infiltrates were present in histologic sections without myocardial necrosis (6). When the immunohistologic analysis revealed pathologically increased lymphocytic infiltrates (>2.0 lymphocytes/high power field) (23) and an increased expression of MHC I and II antigens, the biopsy was classified as lymphocytic myocarditis. When neither the histologic nor the immunohistologic analysis revealed myocytolysis or lymphocytic infiltrates, the biopsies were classified as no myocarditis.

**Antimyosin scintigraphy.** All antimyosin scintigraphy studies were done within 3 weeks of the time of myocardial biopsy, the interval being mainly dependent on patient’s agreement and organizational factors such as availability of antibody and tracer.

Each patient was given 0.5 mg of R11D10-Fab-DPTA monoclonal antimyosin antibodies labeled with 75 MBq of indium-111 (Myosint, Centocor, The Netherlands) intravenously. Imaging was done in the anterior and lateral projections at 30 min after the injection to view the heart chambers, and 24 h or 48 h after the injection of the radiopharmaceutical to assess the presence of the antimyosin antibodies in the myocardial wall. Images were taken with a large field-of-view high resolution camera, using a medium energy all purpose collimator and two 20% windows centered on 247 and 173 keV peaks. Images were collected for 10 min into a 128 × 128 pixel matrix.

Analysis of the antimyosin scintigraphy was done by visual interpretation and by calculating the heart/lung ratio. The accumulation of the antimyosin antibodies in the myocardial wall was classified visually by an experienced observer unaware of the clinical data in the anterior and the lateral projections, respectively, comparing the images taken 30 min and 24 or 48 h after injection of the antimyosin antibodies, respectively. Antimyosin uptake was assessed semiquantitatively as 0 = no myocardial uptake, 1 = faint/mild uptake, 2 = clear uptake, 3 = intense uptake. The heart/lung ratio was calculated in the anterior view by dividing the average counts per pixel over the myocardium by the average counts per pixel over the lung. A ratio ≤1.6 was considered normal, a ratio >1.6 was considered pathologic. The heart/lung ratio was not determined in the lateral view because exact definition of the lung region often proved to be difficult in this view. Visual analysis, which compared the size of the radioactive heart region 48 h after antibody injection with the size of the blood pool activity.
30 min after injection, was superior to the calculation of the heart/lung ratio in detecting antibody accumulation in the myocardial wall in patients with large left ventricles where high blood pool activity increased the heart/lung ratio even in the absence of antibody accumulation in the myocardial wall. A positive antimyosin scan is demonstrated in Figure 1.

**Statistical analysis.** Agreement between antimyosin scintigraphy and biopsy results was measured using Fisher’s exact test (two-tailed) or the chi-square test. Continuous variables were analyzed with the unpaired t test; p values <0.05 were considered to indicate statistical significance.

**Results**

**Antimyosin scintigraphy in patients with clinically suspected myocarditis.** The results of the antimyosin scintigraphy in the 65 patients are shown in Table 1. The heart/lung ratio in the anterior view ranged from 1.20 to 2.30 (mean 1.69 ± 0.26) and was pathologic in 33 (51%) patients. By visual analysis, antimyosin antibody uptake in the myocardial wall was detectable in 36 (55%) of 65 patients. Eight of 36 patients with visually detectable antimyosin accumulation in the myocardial wall had a normal heart/lung ratio. Three of the eight patients had antimyosin deposition only in inferobasal region of the heart; in five patients the tracer uptake was distributed diffusely over the myocardium. In 5 of 29 patients with no visible accumulation of antimyosin antibodies in the ventricular wall, the heart/lung ratio was >1.6. The left ventricular end diastolic volumes of these patients tended to be larger (437 ± 132 ml) than the left ventricular end diastolic volumes of the patients with a heart/lung ratio <1.6 (252 ± 134 ml) (p = NS). The heart/lung ratio seemed to be increased because of a higher blood pool activity in the ventricles rather than accumulation of antibodies in the ventricular wall. For comparison of scintigraphic results with findings in the endomyocardial biopsies, scans with clear accumulation of antimyosin antibodies in the ventricular wall but a heart/lung ratio ≤1.6 were considered positive; scans without visible antibody accumulation in the ventricular wall but a heart/lung ratio >1.6 were considered negative. Finally, 36 (55%) antimyosin scans were considered positive and 29 (45%) antimyosin scans were considered negative. Four patients presented with elevated creatine kinase levels. Two of these patients had a positive antimyosin scintigraphy; in two patients the antimyosin scintigraphy was normal.

**Antimyosin scintigraphy and results of endomyocardial biopsy.** In patients with a positive antimyosin scan (AMS+), myocarditis was diagnosed (histologically and/or immunohistologically) in 31 of 36 patients (86%); in 5 AMS+ patients (14%) the endomyocardial biopsy was negative for myocarditis (Fig. 2). In patients with a normal antimyosin scan (AMS−), evidence for myocarditis in the endomyocardial biopsy was found histologically and/or immunohistologically in 17 of 29 patients (59%). In 12 AMS-patients (41%), the biopsy did not reveal any sign of myocarditis. In 10 patients, antimyosin scintigraphy and endomyocardial biopsy were performed within 6 weeks of the onset of symptoms. The antimyosin scan was positive in five patients and negative in the remaining five patients. Evidence of myocarditis was detected in the endomyocardial biopsy in all of these patients. The sensitivity of the antimyosin scintigraphy for the detection of myocarditis (histologically and/or immunohistologically) was 66%, the speci-

**Table 1. Visual Uptake of Antimyosin Antibodies in the Ventricular Wall**

<table>
<thead>
<tr>
<th>Heart/lung ratio</th>
<th>0–1</th>
<th>2–3</th>
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<tbody>
<tr>
<td>≤1.6</td>
<td>24</td>
<td>8</td>
</tr>
<tr>
<td>&gt;1.6</td>
<td>5</td>
<td>28</td>
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Antimyosin scintigraphy in 65 patients with clinically suspected myocarditis. Heart/lung ratio and visual assessment of antimyosin antibody uptake in the ventricular wall. The heart/lung ratio was calculated in the anterior view by dividing the average counts per pixel over the myocardium by the average counts per pixel over the lung. Antimyosin antibody uptake into the ventricular wall was assessed semiquantitatively as 0–1 = no or faint/mild uptake, 2–3 = clear or intense uptake.
myocarditis was found immunohistologically in 5 (14%) of 36 AMS+ patients and in 12 (41%) of 29 AMS− patients. With right ventricular immunohistologic findings as the diagnostic method for the presence of myocarditis, the diagnostic value of the antmyosin scintigraphy had to be calculated as follows: sensitivity, 65%; specificity, 71%; predictive value of an abnormal scan, 86%; predictive value of a normal scan, 41%.

Antimyosin scintigraphy and hemodynamic measurements.
The mean ejection fraction of all patients was 50.1 ± 21.0% (range 21% to 85%). There was no difference in the ejection fraction between patients with a positive (48.4 ± 17.6%) and a negative (52.4 ± 24.7%) antmyosin scan (p = NS) or between patients with myocarditis (51.2 ± 20.2%) and without myocarditis (47.0 ± 23.0%) (p = NS). The ejection fraction of patients with myocarditis and a positive antmyosin scan (50.2 ± 17.7) was higher than the ejection fraction of patients with a positive antmyosin scan and no evidence of myocarditis (35.5 ± 10.3), but this difference did not reach statistical significance (p < 0.082). The ejection fraction of patients with myocarditis and a positive antmyosin scan (50.2 ± 17.7) was not different from the ejection fraction of patients with myocarditis and a negative antmyosin scan (53.5 ± 24.4) (p = NS). The mean end diastolic volume of all patients was 265 ± 122 ml (range 68 to 625 ml). There was also no difference in the end diastolic volume between patients with a positive (252 ± 96 ml) and a negative (283 ± 148 ml) antmyosin scan (p = NS) or between patients with myocarditis (258 ± 112 ml) and without myocarditis (287 ± 122 ml) (p = NS).

Discussion
Previous studies investigated the diagnostic value of antimyosin scintigraphy in patients with myocarditis and dilated cardiomyopathy (18–20,24). They correlated antimyosin scintigraphy with the results of the histologic analysis of the endomyocardial biopsy, classified according to the Dallas Criteria (6), and found a high sensitivity but a low specificity of the antimyosin scintigraphy for the detection of myocarditis with many scintigraphically positive cases in which the endomyocardial biopsy failed to show myocarditis on histologic analysis. In the present study, similar values for sensitivity and specificity of the antimyosin scintigraphy for the detection of myocarditis were found, with histologic analysis of the endomyocardial biopsy as the reference method. However, similar to other studies (18–20), the majority of patients with a positive antmyosin scan were histologically negative for myocarditis. This group of patients had been classified by Obrador et al. (20) as dilated cardiomyopathy with unexplained ongoing myocardial cell damage. Several possible explanations for the discrepancy between antimyosin scintigraphy and endomyocardial biopsy have been discussed, including subendocardial necrosis due to cardiac dilatation, a focal nature of myocarditis, persistent viral infection of the myocardium or sampling error of the biopsies (18,20). The histologic evaluation of myocardial biopsies alone is known to be difficult and affected by many problems (21,22), including high interobserver vari-

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Figure 3. Antimyosin scintigraphy and separate analysis of histologic and immunohistologic findings in the endomyocardial biopsy in 65 patients with clinically suspected myocarditis. AMS = antimyosin scintigraphy. + = positive antimyosin scan with clear antimyosin antibody uptake in the ventricular wall; − = negative antimyosin scan with no clear antimyosin antibody uptake in the ventricular wall. Histo = results of histologic analysis of the endomyocardial biopsy; acute MC = acute myocarditis; bord MC = borderline myocarditis; no MC = no myocarditis. ImHisto = results of immunohistologic analysis of the endomyocardial biopsy; lymph MC = lymphocytic myocarditis; no MC = no myocarditis.

Patients

AMS | Histo | ImHisto
---|---|---
+ | acute MC | lymph MC
| 36 | 1 | 1
| 5 | bord MC | no MC
| 29 | 8 | 39
| 56 | 17

| | no MC |
---|---|
| 30 |

Sensitivity, 65%; specificity, 71%; predictive value of an abnormal scan, 86%; predictive value of a normal scan, 41%.
ability (7) and sampling error (5). Therefore, in the present study the biopsy specimens were analyzed additionally with immunohistologic techniques (8–11). Using these methods, Kühl et al. (23) found evidence of ongoing myocardial inflammation in 48% of patients presenting with a clinical picture of dilated cardiomyopathy and no evidence of myocarditis on histologic analysis. However, it has to be pointed out that the pathophysiologic reason responsible for the myocardial inflammation, whether it is viral, ischemic, toxic or other, cannot be detected by immunohistologic analysis of the endomyocardial biopsy. To identify the pathophysiologic agent, other techniques such as in situ hybridization (25) or the polymerase chain reaction (26) for the detection of viral genome in the myocardium have to be employed.

**Antimyosin scintigraphy and immunohistologic analysis of the endomyocardial biopsy.** The present study provides the first report that compares antimyosin scintigraphy with immunohistologic analysis of the endomyocardial biopsy in patients with clinically suspected myocarditis. The prevalence of a positive antimyosin scan was similar to the results of previously reported studies (18–20). Histologic analysis of the endomyocardial biopsy specimen alone could establish the diagnosis of myocarditis only in a small number of these patients. However, additional immunohistologic evaluation revealed evidence of myocarditis in most of the patients with a positive antimyosin scan. With immunohistologic analysis of the endomyocardial biopsy as the reference method, the antimyosin scintigraphy has a high specificity and a lower sensitivity for the detection of myocarditis. The positive predictive value of antimyosin scintigraphy is high; the negative predictive value is low for the detection of myocarditis with immunohistologic analysis as the reference method.

The present studies underscore the low diagnostic sensitivity of the histologic analysis of endomyocardial biopsy both for the detection of myocyte damage and myocardial inflammation, respectively. Therefore, the diagnosis of myocarditis should not be based on histologic analysis of the endomyocardial biopsy alone. The presence of myocarditis should also be considered if immunohistologic analysis of the endomyocardial biopsy shows lymphocytic infiltrates and an activated immunologic process, and if evidence of ongoing myocardial damage can be demonstrated, either by antimyosin scintigraphy or probably by measurement of cardiac troponin T (27).

**Discrepancies between antimyosin scintigraphy and endomyocardial biopsy.** A positive antimyosin scan was also found in 5 of 36 patients who had negative biopsy findings in both histologic and immunohistologic analysis, respectively. Visual analysis of the antimyosin scans showed that three of these patients had antimyosin antibody accumulation only in the dorsal or lateral parts of the left ventricle. Apparently, myocardial damage was not evenly distributed over the entire heart in these patients but rather a more focal process was present only in some heart regions. As the endomyocardial biopsy is taken from the interventricular septum, it is likely that the diagnosis might be missed in patients with focal myocarditis only in other regions of the heart. In two patients, the biopsy was negative histologically and immunohistologically despite a uniform distribution of staining over the left ventricle. In these patients, the diagnosis of myocarditis possibly may be missed because of a sampling error of the endomyocardial biopsy, probably due to the “patchy” nature of the myocardial inflammation on a microscopical scale. The ejection fraction of patients with a positive antimyosin scan and no evidence of myocarditis was somewhat lower than the ejection fraction of patients with myocarditis and a positive antimyosin scan so that other pathogenetic mechanisms like subendocardial ischemia may also be responsible for the presence of myocardial cell damage and a positive antimyosin scan in the absence of myocardial inflammation.

Immunohistologic analysis of endomyocardial biopsy also found evidence of myocardial inflammation in 17 of 29 patients with a normal antimyosin scan. Seven of these patients showed a prominent blood pool activity even 48 h after the injection of the antimyosin antibodies; the heart/lung ratio was elevated in five of these seven patients. It cannot be ruled out that high blood pool activity precluded the detection of antibody deposition in the ventricular wall (24). It is also possible that the extent of myocardial cell damage in these patients was too low to be detected by antimyosin scintigraphy. However, it has to be pointed out that the presence of lymphocytic infiltrates in the myocardium does not necessarily imply lymphocyte-mediated myocyte necrosis. Another explanation would be that different time frames exist for the presence of myocardial necrosis (leading to a positive antimyosin scan) and myocardial inflammation (leading to the detection of lymphocytic infiltrates in the endomyocardial biopsy) in the course of the disease “myocarditis,” thus explaining the observed differences between the two diagnostic methods.

On the other hand, different pathogenetic mechanisms for the presence of myocardial damage and myocardial inflammation might be responsible for the discrepancies between antimyosin scintigraphy and endomyocardial biopsy.

**Possible pathogenetic mechanisms for myocardial damage and inflammation in patients with myocarditis.** Using in situ hybridization or the polymerase chain reaction, viral RNA has been detected in the myocardium of patients with myocarditis (25,26,28). In these patients the viral infection is discussed as the pathogenetic mechanism of myocardial inflammation. It cannot be ruled out that the presence of viral structures itself may cause migration of inflammatory cells into the myocardium without actual myocyte damage. In this case, lymphocytic infiltrates would be found in the endomyocardial biopsy but the antimyosin scan would be negative.

Other studies detected autoantibodies against various cardiac antigens in patients with myocarditis (29–33), including autoantibodies against cardiac myosin (34). Antimyosin autoantibodies might possibly interfere with the accumulation of the radioactively labeled antimyosin antibodies used for scintigraphy. The pathogenetic role of the autoantibodies against cardiac antigens in the course of myocarditis is not yet clear. One possible explanation would be “molecular mimicry,” Schwimmbeck et al. (35) identified regions of high homology...
between Coxsackie B3 virus and cardiac myosin. Using synthetic peptides identical to these regions, an immunologic cross-reactivity between Coxsackie B3 virus peptides and cardiac myosin peptides was demonstrated. In contrast, the antimyosin antibodies produced in Coxsackie B3 virus-induced myocarditis in A/J mice did not crossreact with the virus (36).

Recently, evidence of apoptosis (or “programmed” cell death) was detected in the myocardium of patients with chronic heart failure due to dilated cardiomyopathy (37). In contrast to necrosis (or “accidental” cell death), apoptosis is a tightly regulated process driven by a genetic program (38). It cannot be ruled out that apoptosis occurred also in patients in the present study and that apoptosis does not necessarily lead to exposition of intracellular myosin to the extracellular space so that the antimyosin scan might be negative in these cases.

Conclusions. The present study shows that myocardial damage can often be detected in patients with clinically suspected myocarditis by use of antimyosin scintigraphy. Histologic analysis of the endomyocardial biopsy alone is often negative, but additional immunohistologic analysis of the endomyocardial biopsy frequently provides evidence of myocardial inflammation in these patients. With immunohistologic analysis as the reference, antimyosin scintigraphy has a high specificity but a lower sensitivity for the detection of myocarditis.

References