Scintigraphic quantification of myocardial necrosis in patients after intravenous injection of myosin-specific antibody

BAN AN KHAW, PH.D., HERMAN K. GOLD, M.D., TSUNEIRO YASUDA, M.D., ROBERT C. LEINBACH, M.D., MACHITO KANKE, M.D., John T. FALLON, M.D., PH.D., MARTHA BARLAI-KOVACH, M.S., H. WILLIAM STRAUSS, M.D., FLORENCE SHEEHAN, M.D., AND EDGAR HABER, M.D.

ABSTRACT The Fab fragments of antmyosin antibodies, labeled with \(^{99m}\)Tc, were used in the scintigraphic examination of 30 patients with myocardial infarction. The ability to detect necrosis and determine its extent from the antmyosin scan were compared with the results of quantitative regional wall motion analysis by contrast ventriculography at 10 to 14 days and \(^{99m}\)Tc-pyrophosphate imaging. Antimyosin images recorded by planar and single photon-emission computed tomography (SPECT) delineated areas of myocardial necrosis in 27 of 30 patients (90%) compared with a 91% sensitivity of pyrophosphate in 21 of 23 patients. Infarct size was determined by both antimyosin and pyrophosphate SPECT images. Results by both techniques showed a significant correlation with computer-derived hypokinetic segment length \((r = .79 \text{ for both, } p = .002)\) and peak creatine kinase \((r = .9 \text{ for both, } p < .01)\). Although sensitivity for and correlations with markers of necrosis were similar with both techniques, infarct size by pyrophosphate SPECT was 1.7 times larger than infarct size by antimyosin SPECT \((p < .01)\). Certain zones in the infarct area were differentially labeled; the nature and irreversibility of injury within these zones remains to be clarified.


WE PREVIOUSLY described a method for localizing and quantifying regions of myocardial necrosis based on the binding of radiolabeled myosin-specific antibodies to cells that have lost the integrity of their plasma membranes.\(^1\)\(^2\) Unlike other methods that are dependent on blood flow, concentration of antibody in the center of an infarct is greater than that at the periphery, with antibody concentration inversely proportional to blood flow.\(^3\)\(^5\) The location of radiolabeled antimyosin antibody corresponds exactly to histochemically delineated regions of myocardial infarction.\(^1\)\(^6\) The present study describes our initial experience with this antibody imaging technique for the detection and quantification of acute myocardial infarction in man. We compare this method with that of analysis of peak creatine kinase (CK) and two other currently available techniques for the quantitation of infarct size.\(^7\)\(^9\)

Methods

Patient selection. Thirty patients with suspected acute myocardial infarction were studied. Criteria for selection were precordial chest pain typical of cardiac ischemia of at least 30 min duration, ST elevation of at least 0.1 mV in two or more leads of the electrocardiogram with subsequent evolution of an electrocardiographic infarct pattern, and at least twice normal elevation of CK, with associated elevation of the MB isoenzyme. Patients with prior infarction or left bundle-branch block were excluded. Infarctions were categorized as anterior or inferior on the basis of the leads showing ST elevation and Q wave development. Twenty-seven of 30 patients received streptokinase therapy. Reflow was seen in 25.

Administration of radiolabeled tracer. Antimyosin Fab was administered within 24 hr of admission to the hospital according to the following protocol. Before injection, informed consent was obtained from all subjects. To test for sensitivity to the radiolabeled antibody, 0.1 ml of \(^{99m}\)Tc-labeled antimyosin Fab was administered intradermally. If no wheal and flare were observed in the next 15 to 30 min, the patients were injected intravenously with approximately 500 \(\mu g\) of antimyosin Fab labeled with 15 to 25 mCi of \(^{99m}\)Tc. Ungated images in the anterior and 40 to 50 degree left anterior oblique (LAO) views were recorded at intervals ranging from 6 to 24 hr after injection to define the myocardial location of the tracer and to determine...
the time of maximal target-to-background contrast. All 30 patients underwent planar antimyosin imaging with a high-resolution parallel-hole collimator (pulse height analyzer set at a centerline of 140 keV with a 20% window). Initial images were obtained at bedside with a portable gamma camera (Technicare 420/550, Solon, OH) and at least one more set of anterior and 45 degree LAO images were obtained at times indicated in table 1.

Sixteen of the 30 patients also underwent single photon-emission computed tomography (SPECT) at, on average, 15 hr after antimyosin injection. In these patients, SPECT preceded planar imaging. The patients were positioned so that the circle of smallest diameter 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 128/128 matrix and stored for subsequent analysis. Blood pressure, heart rate, and the electrocardiogram were observed continuously for 30 min after injection of antimyosin, and then intermittently for 24 hr. Seventeen patients were studied with polyclonal Fab and 13 with monoclonal Fab.

On the third day after admission, 23 of 30 patients received 25 mCi of Tc-labeled stannous pyrophosphate intravenously. Four to six hours later, planar images were obtained in the anterior and LAO views. Immediately before acquisition of the planar images, 12 of these 23 patients underwent SPECT imaging as described above.

**Image analysis.** Planar antimyosin Fab gamma images were interpreted directly from the computer video display by two observers blinded to the clinical data. One observer read the images at two different times greater than 6 months apart. The final analysis was performed by both observers and there was concordance among the three readings (except for the reading of the septal location in the two patients with inferior myocardial infarction). The images were evaluated for the presence or absence of tracer uptake and the zone of involvement was determined as anterior, inferior, or septal. The area of the infarction was estimated from the planar image by manually outlining the zone of tracer uptake and counting the number of picture elements. The size of each element was determined by imaging a measured phantom. For patients with anterior infarction the anterior or LAO view was used, and for patients with inferior infarction the LAO view only was used.

The quantitative assessment of infarct size was obtained from SPECT images. The images were reconstructed with a filtered backprojection algorithm into transverse, sagittal, and coronal projections with a thickness of approximately 1 cm (three pixels). Infarct size was calculated by widely outlining the zone of increased tracer concentration, applying a 60% threshold to the region, counting the number of pixels in the zone of infarction in each slice, multiplying the pixel number by the slice thickness, and then multiplying the results by a calibration factor derived from a three-dimensional phantom.

The planar pyrophosphate scans were similarly evaluated by two observers, blinded to infarct location. Quantitation of specific localization was performed by the method of Jansen et al., which employs a similar selection of regions of interest, data filtration, and a 65% threshold. The SPECT pyrophosphate images were analyzed after application of this threshold by the same technique used with antimyosin.

**Wall motion analysis.** All 30 patients underwent coronary angiography and left ventriculography 10 to 14 days after admission to the hospital. Quantitative left ventricular regional wall motion analysis was performed with the 30 degree right anterior oblique (RAO) contrast ventriculogram. Cineangiographic films were projected and end-diastolic and end-systolic endocardial contours were traced from normal sinus beats by observers blinded to infarct location. Wall motion was measured by the centerline method along 100 chords constructed perpendicular to a centerline drawn midway between the end-systolic and end-diastolic contours, and was normalized for heart size and expressed in units of standard deviations from the mean motion in 64 normal subjects by a method previously described. The chords were numbered clockwise beginning at the anterior aspect of the aortic valve.

A region of interest was defined as the location of the obstructed coronary artery. The location of this region was determined by superimposing the diastolic RAO left ventricular outline (a transparency) on the 30 degree RAO diastolic right and left coronary arteriograms, respectively, and by identifying the numbers of the chords nearest to the proximal and distal ends of

---

**TABLE 1**

Comparison of infarct location by electrocardiography with that determined by planar and SPECT antimyosin imaging, 10 day hypokinetic segmental length, maximum total CK serum levels, and percent CK-MB isoenzyme

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Imaging time (hr)</th>
<th>ECG location</th>
<th>AM-planar (pixel No.)</th>
<th>AM-SPECT (g)</th>
<th>HK-2SD (cm)</th>
<th>Peak CK (units)</th>
<th>%MB</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>13.5,20</td>
<td>Ant</td>
<td>77,71</td>
<td>10.3</td>
<td>2970</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>12</td>
<td>Ant</td>
<td>458,48</td>
<td>6.2</td>
<td>1140</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>23</td>
<td>Ant</td>
<td>764,83</td>
<td>13.2</td>
<td>3580</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>11.5</td>
<td>Ant</td>
<td>310,26</td>
<td>2.0</td>
<td>784</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>1.10,12</td>
<td>Ant</td>
<td>405,44</td>
<td>3.9</td>
<td>850</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>2.7,16</td>
<td>Ant</td>
<td>443,26</td>
<td>1.5</td>
<td>571</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>18</td>
<td>Ant</td>
<td>418,64</td>
<td>10.0</td>
<td>2204</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>6,17</td>
<td>Ant</td>
<td>361,49</td>
<td>16.0</td>
<td>2290</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>16</td>
<td>Ant</td>
<td>845,40</td>
<td>10.9</td>
<td>1830</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>13.5</td>
<td>Ant</td>
<td>933,NA</td>
<td>17.8</td>
<td>2344</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>9.18</td>
<td>Ant</td>
<td>977,NA</td>
<td>16.8</td>
<td>7390</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>20</td>
<td>Ant</td>
<td>1230,NA</td>
<td>19.7</td>
<td>1926</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>14.16</td>
<td>Ant</td>
<td>74,NA</td>
<td>5.3</td>
<td>463</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>18</td>
<td>Ant</td>
<td>460,NA</td>
<td>9.8</td>
<td>711</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>13</td>
<td>Ant</td>
<td>577,NA</td>
<td>20.5</td>
<td>2910</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>4.22</td>
<td>Ant</td>
<td>682,NA</td>
<td>16.8</td>
<td>976</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>12.24</td>
<td>Ant</td>
<td>724,NA</td>
<td>19.9</td>
<td>1950</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>11.24</td>
<td>Ant</td>
<td>718,NA</td>
<td>18.5</td>
<td>2310</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>19</td>
<td>Ant</td>
<td>202,9.4</td>
<td>5.8</td>
<td>583</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>16</td>
<td>Inf</td>
<td>0,15</td>
<td>4.9</td>
<td>620</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>5.5</td>
<td>Inf</td>
<td>0,0</td>
<td>211</td>
<td>15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>2.10,20</td>
<td>Inf</td>
<td>0,0</td>
<td>2.7</td>
<td>130</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>16</td>
<td>Inf</td>
<td>107,12</td>
<td>6.0</td>
<td>157</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>15</td>
<td>Inf</td>
<td>274,4</td>
<td>0.0</td>
<td>360</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>6.24</td>
<td>Inf</td>
<td>180,NA</td>
<td>1.7</td>
<td>780</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>26</td>
<td>14.9</td>
<td>Inf</td>
<td>55,33</td>
<td>3.7</td>
<td>1080</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>27</td>
<td>8.5,21</td>
<td>Inf</td>
<td>109,NA</td>
<td>3.2</td>
<td>486</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>28</td>
<td>16</td>
<td>Inf</td>
<td>70,NA</td>
<td>4.2</td>
<td>329</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>29</td>
<td>10.12,16</td>
<td>Inf</td>
<td>0,NA</td>
<td>3.6</td>
<td>274</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>8.20</td>
<td>Inf</td>
<td>157,NA</td>
<td>7.6</td>
<td>819</td>
<td>13</td>
<td></td>
</tr>
</tbody>
</table>

ECG = electrocardiographic; AM = antimyosin Fab; HK = hypokinesia; NA = not available.

*All except patients 5, 6, 16, and 22 underwent initial imaging at the time of injection.

Reperfusion with streptokinase.

Patients injected with Tc-labeled monoclonal antimyosin Fab.

Spontaneous reflow.
the infarct-related artery, defined as the artery undergoing thrombolysis and supplying the area of the myocardium that was acutely dysfunctional. The motion of chords lying within this zone was measured, and hypokinesis was defined as motion that was two or more standard deviations below normal. The length of the hypokinetic segment (in centimeters) was calculated by multiplying the number of contiguous hypokinetic chords by the left ventricular end-diastolic perimeter, corrected for magnification. The presence of a segment of the contour with function depressed below the 2 SD threshold has been previously shown to be correlated with myocardial fibrosis. However, this is not a direct measure of the extent of infarction.

Preparation of antimyosin Fab. Human cardiac myosin was purified from cadaver myocardium by the method of Katz et al.12 Polyclonal Fab was produced by immunizing rabbits with human cardiac myosin in complete Freund’s adjuvant.3 Rabbit antisera, collected under sterile conditions, was applied to an affinity column of human cardiac myosin covalently bound to Sepharose 4B.3 After washing with sterile and apyrogenic saline, the antibody was eluted with 5M guanidinium HCl and dialyzed against isotonic saline. Fab was prepared by papain digestion13 and separated from Fc by protein A-Sepharose affinity chromatography.14 The resulting preparation was shown to be free of intact antibody and Fc by Ouchterlonory agarose double-diffusion analysis.15 Antimyosin Fab was then covalently coupled to diethylenetriaminepentaacetic acid (DTPA) by the carboxylic anhydride method.5,16–19 The Fab was then dialyzed against sterile, pyrogen-free 0.3M phosphate, pH 8.0, 0.15M NaCl, and samples were submitted for sterility and pyrogen testing (Laberco, NJ). The solution was then frozen in 300 to 500 µl aliquots and, when required, thawed and labeled with 99mTc as described.

Monoclonal antimyosin Fab was prepared by intraperitoneal injection of BALB/c mice with 50 µg of human cardiac myosin in complete Freund’s adjuvant, followed 10 days later by an intravenous injection of 10 µg in 0.3M phosphate-buffered saline, pH 7.0.5 After another 3 days, the spleens of two immunized mice were aseptically removed and the spleen cells were fused with the plasmacytoma line Sp2/0-Ag14, as previously described.5 The cells were then distributed into five, 96 well cell culture plates in hypoxanthine/aminopterin/thymidine medium. After sufficient growth, the supernatants were screened for the presence of antimyosin antibody by a solid-phase radioimmunoassay. Positive hybrids were cloned by the limiting dilution method. A clone designated R11D10, isotyped immunoglobulin (Ig) G2a, was selected and expanded as an ascites tumor in BALB/c mice. Ascites fluid was obtained under aseptic conditions and subsequently processed with use of sterile and pyrogen-free reagents and glassware. The gamma globulin fraction of the ascites was isolated by protein A-Sepharose chromatography as described by Ey et al.14 Sodium dodecyl sulfate polyacrylamide gel electrophoresis showed that the monoclonal antibody was 95% homogeneous. Fab was prepared by papain digestion13 followed by protein A-Sepharose chromatography to remove Fc and undigested antibody. Pyrogenicity and sterility testing and aliquoting and DTPA derivitization were carried out as described above for the polyclonal Fab.

Labeling DTPA-antimyosin Fab with 99mTc. All procedures were carried out in a laminar flow hood under aseptic conditions with the use of sterile reagents, solutions, and glassware. A 5 × 10⁶M excess of fresh crystalline sodium dithionite was added to a solution containing 100 to 150 mCi of 99mTcO₄⁻. The molar concentration of Tc was computed by the method of Lamson et al.20 Reduction was allowed to proceed for exactly 10 min, after which the solution was added dropwise to 300 to 600 µg DTPA-antimyosin-Fab in 500 µl of 0.3M Na phosphate (pH 8.0), 0.15M NaCl, and stirred vigorously in a vortex mixer. After 5 min at room temperature, the reaction mixture was fractionated on a PD-10 column that had been prewashed with 1 to 2 ml of 1% human serum albumin in lactated Ringer’s solution, followed by lactated Ringer’s solution alone. The void volume of this column was collected and applied to a 10 ml Sepharose 4B column that had been first washed with 5M quanidinium HCl, then with 1 to 2 ml of 1% human serum albumin in lactated Ringer’s solution, and finally equilibrated in lactated Ringer’s solution alone. One milliliter fractions were collected and their radioactivity was monitored in an ionization chamber. The fractions representing the peak and descending slopes of radioactivity were subjected to Millipore filtration and used in clinical studies. Typical yields were 15 to 25 mCi of 99mTc-antimyosin Fab.

Statistical analyses were by the linear regression method, and the slopes of the linear regressions were compared by the standard t test.

Results

Skin tests before administration of antimyosin were negative in all patients studied. Arterial pressure, heart rate, and the electrocardiogram showed no changes after administration of antimyosin. Neither early allergic symptoms nor late symptoms suggestive of serum sickness were noted.

Clinical documentation of myocardial infarction. Electrocardiograms localized the infarct to the anterior wall in 19 patients and to the inferior wall in 11. All patients evolved pathologic Q waves. The peak CK (normal 0 to 55 U) was 1434 ± 1477(SD) U (range 130 to 7390), with MB fractions ranging from 7% to 25%.

Localization of antimyosin. Planar images showed discrete localization of antimyosin in 26 of 30 patients (87%) (table 1). Among those with anterior infarcts, planar images were positive in 19 of 19 patients. In the inferior infarct group, seven of 11 patients (64%) had discrete tracer localization (table 1). In all but two cases, the location of myocardial damage delineated by antimyosin imaging was concordant with the infarct location by electrocardiography. These two patients had inferior infarction, as documented by electrocardiography, septal uptake of antimyosin, and inferoposterobasal hypokinesia (table 1, patients 26 and 28). Although discrete myocardial antimyosin localization was detected as early as 7 hr after intravenous injection, the usual time for visualization was from 12 to 20 hr after injection and 16 to 24 hr after infarction, with optimal visualization occurring at about 18 hr after administration of antimyosin.

Tomographic antimyosin images were obtained in 10 patients with anterior infarction (all positive) and six patients with inferior infarction (four positive). In one patient with inferior infarction planar images were negative while SPECT images were positive. Of the two inferior infarction patients with negative planar and SPECT images (table 1, patients 21 and 22) one
had no angiographic hypokinesis and the other had a 2.7 cm hypokinetic segment length. Therefore, the combined sensitivity of planar and SPECT images with 99mTc-labeled antimyosin was 90% in this group of patients with confirmed myocardial infarction.

Figure 1 shows the LAO planar and sagittal SPECT 99mTc-antimyosin images from patient 1 (with anterior infarction). Uptake is homogeneous within the infarct. The infarct edges are more apparent in the SPECT images.

Comparative sensitivities for detection of infarction by planar pyrophosphate and antimyosin imaging are shown in table 2. Among the 14 patients with anterior infarct, abnormal 99mTc-pyrophosphate planar scans were found in all. Of the nine patients with inferior infarcts, pyrophosphate scans were positive in seven patients.
Isolated. Pyrophosphate images were positive in two of the three patients with negative planar antimyosin scans, and negative in one patient with a positive antimyosin scan. In this group of patients, the sensitivity of pyrophosphate scanning for detection of infarction was 91%.

Twelve of the 16 patients who underwent antimyosin SPECT also underwent pyrophosphate SPECT (table 3). Infarct weight by SPECT delineated by antimyosin averaged 29.2 ± 28 vs 56.5 ± 52 g by pyrophosphate SPECT. This difference is significant at the p = .001 level. Figure 2 shows the correlation between infarct size determined by antimyosin SPECT and that determined by pyrophosphate SPECT (y = 1.697x + 7.04; r = .914, p = .001). Figure 3 shows transverse sagittal and coronal matching pairs of pyrophosphate and antimyosin SPECT images from patient 1 (figure 1). The relationship between infarct weight in grams determined by pyrophosphate SPECT and angiographic hypokinetic segment length in these same 12 patients is shown in figure 4, bottom. The correlation was again linear (y = 8.24x + 7.97; r = .79, p = .002). Results of antimyosin and pyrophosphate SPECT correlated with the length of the hypokinetic segments to a similar degree. However, the slope of the pyrophosphate SPECT relationship was 1.87 times greater than that of the antimyosin SPECT relationship (p = .002). Planar antimyosin and pyrophosphate pixel numbers correlated with peak CK (r = .75 and .76, respectively), but the correlation with SPECT images was closer (r = .96 and .92, respectively).

Discussion
Antibodies permit the highly specific identification of tissues by unique antigens. In patients with myocardial infarction, exposed myosin provides a distinct target for antimyosin antibody. The use of Fab rather than intact IgG reduces unwanted immunogenic reactions and results in a more rapid blood (background) clearance. Antibody fragments can be labeled with radionuclides without a significant loss of antibody activity or specificity.1-6, 16, 17 Among the patients reported here, all of whom had confirmed infarctions, false-negative results were seen only in those with small inferior infarcts. Reasons for the failure to image these lesions might include obscuring of tracer uptake by liver activity, residual blood pool activity, and greater

TABLE 2
Comparison of infarct detection by planar antimyosin and pyrophosphate imaging

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>ECG location</th>
<th>AM (pixel No.)</th>
<th>PYP (pixel No.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A</td>
<td>Ant</td>
<td>777</td>
<td>572</td>
</tr>
<tr>
<td>2A</td>
<td>Ant</td>
<td>458</td>
<td>588</td>
</tr>
<tr>
<td>3A</td>
<td>Ant</td>
<td>764</td>
<td>739</td>
</tr>
<tr>
<td>4A</td>
<td>Ant</td>
<td>310</td>
<td>598</td>
</tr>
<tr>
<td>5A</td>
<td>Ant</td>
<td>405</td>
<td>442</td>
</tr>
<tr>
<td>7A</td>
<td>Ant</td>
<td>418</td>
<td>510</td>
</tr>
<tr>
<td>8A</td>
<td>Ant</td>
<td>361</td>
<td>545</td>
</tr>
<tr>
<td>10</td>
<td>Ant</td>
<td>933</td>
<td>1019</td>
</tr>
<tr>
<td>12A</td>
<td>Ant</td>
<td>1230</td>
<td>1299</td>
</tr>
<tr>
<td>13</td>
<td>Ant</td>
<td>74</td>
<td>280</td>
</tr>
<tr>
<td>14A</td>
<td>Ant</td>
<td>460</td>
<td>392</td>
</tr>
<tr>
<td>15A</td>
<td>Ant</td>
<td>577</td>
<td>1103</td>
</tr>
<tr>
<td>18A</td>
<td>Ant</td>
<td>718</td>
<td>887</td>
</tr>
<tr>
<td>19A</td>
<td>Ant</td>
<td>202</td>
<td>415</td>
</tr>
<tr>
<td>20A</td>
<td>Inf</td>
<td>0</td>
<td>146</td>
</tr>
<tr>
<td>21A</td>
<td>Inf</td>
<td>0</td>
<td>253</td>
</tr>
<tr>
<td>22B</td>
<td>Inf</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>23A</td>
<td>Inf</td>
<td>107</td>
<td>158</td>
</tr>
<tr>
<td>24A</td>
<td>Inf</td>
<td>274</td>
<td>185</td>
</tr>
<tr>
<td>25A</td>
<td>Inf</td>
<td>180</td>
<td>0</td>
</tr>
<tr>
<td>26A</td>
<td>Inf</td>
<td>55</td>
<td>249</td>
</tr>
<tr>
<td>27</td>
<td>Inf</td>
<td>134</td>
<td>209</td>
</tr>
<tr>
<td>28</td>
<td>Inf</td>
<td>70</td>
<td>70</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>369 ± 334</td>
<td>463 ± 355</td>
<td></td>
</tr>
</tbody>
</table>

ECG = electrocardiographic; AM = antimyosin; PYP = pyrophosphate.
AReperfusion with streptokinase.
BSpontaneous reflow.

(78%). Pyrophosphate images were positive in two of the three patients with negative planar antimyosin scans, and negative in one patient with a positive antimyosin scan. In this group of patients, the sensitivity of pyrophosphate scanning for detection of infarction was 91%.

Twelve of the 16 patients who underwent antimyosin SPECT also underwent pyrophosphate SPECT (table 3). Infarct weight by SPECT delineated by antimyosin averaged 29.2 ± 28 vs 56.5 ± 52 g by pyrophosphate SPECT. This difference is significant at the p = .001 level. Figure 2 shows the correlation between infarct size determined by antimyosin SPECT and that determined by pyrophosphate SPECT (y = 1.697x + 7.04; r = .914, p = .001). Figure 3 shows transverse sagittal and coronal matching pairs of pyrophosphate and antimyosin SPECT images from patient 1 (figure 1). The relationship between infarct weight in grams determined by pyrophosphate SPECT and angiographic hypokinetic segment length (in 12 patients) is shown in figure 4, top. Linear regression analysis demonstrated a linear correlation (y = 4.41x + 3.19; r = .79, p = .002). The relationship between infarct weight in grams determined by pyrophosphate SPECT and angiographic hypokinetic segment length in these same 12 patients is shown in figure 4, bottom. The correlation was again linear (y = 8.24x + 7.97; r = .79, p = .002). Results of antimyosin and pyrophosphate SPECT correlated with the length of the hypokinetic segments to a similar degree. However, the slope of the pyrophosphate SPECT relationship was 1.87 times greater than that of the antimyosin SPECT relationship (p = .002). Planar antimyosin and pyrophosphate pixel numbers correlated with peak CK (r = .75 and .76, respectively), but the correlation with SPECT images was closer (r = .96 and .92, respectively).

Discussion
Antibodies permit the highly specific identification of tissues by unique antigens. In patients with myocardial infarction, exposed myosin provides a distinct target for antimyosin antibody. The use of Fab rather than intact IgG reduces unwanted immunogenic reactions and results in a more rapid blood (background) clearance. Antibody fragments can be labeled with radionuclides without a significant loss of antibody activity or specificity.1-6, 16, 17 Among the patients reported here, all of whom had confirmed infarctions, false-negative results were seen only in those with small inferior infarcts. Reasons for the failure to image these lesions might include obscuring of tracer uptake by liver activity, residual blood pool activity, and greater

TABLE 3
Comparison of infarct sizes by antimyosin and pyrophosphate SPECT with hypokinetic myocardial segmental length at 10 to 14 days

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>ECG location</th>
<th>AM-tomo (g)</th>
<th>HK-2SD (cm)</th>
<th>PYP-tomo (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A</td>
<td>Ant</td>
<td>71.0</td>
<td>10.3</td>
<td>139.8</td>
</tr>
<tr>
<td>2A</td>
<td>Ant</td>
<td>48.0</td>
<td>6.2</td>
<td>69.0</td>
</tr>
<tr>
<td>3A</td>
<td>Ant</td>
<td>83.0</td>
<td>13.2</td>
<td>130.6</td>
</tr>
<tr>
<td>4A</td>
<td>Ant</td>
<td>26.0</td>
<td>2.0</td>
<td>63.7</td>
</tr>
<tr>
<td>8A</td>
<td>Ant</td>
<td>49.0</td>
<td>16.0</td>
<td>131.4</td>
</tr>
<tr>
<td>19A</td>
<td>Ant</td>
<td>9.4</td>
<td>5.8</td>
<td>18.1</td>
</tr>
<tr>
<td>20A</td>
<td>Inf</td>
<td>15.0</td>
<td>4.9</td>
<td>6.6</td>
</tr>
<tr>
<td>21A</td>
<td>Inf</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>22B</td>
<td>Inf</td>
<td>0</td>
<td>2.7</td>
<td>0</td>
</tr>
<tr>
<td>23A</td>
<td>Inf</td>
<td>12.0</td>
<td>6.0</td>
<td>24.0</td>
</tr>
<tr>
<td>24A</td>
<td>Inf</td>
<td>4.0</td>
<td>0.0</td>
<td>48.2</td>
</tr>
<tr>
<td>26A</td>
<td>Inf</td>
<td>33.0</td>
<td>3.7</td>
<td>47.8</td>
</tr>
</tbody>
</table>

ECG = electrocardiographic; AM-TOMO = antimyosin infarct size from SPECT; PYP-TOMO = pyrophosphate infarct size from SPECT; HK = hypokinesis.
AReperfusion with streptokinase.
BSpontaneous reflow.
times larger than antomyosin images. Although the length of the hypokinetic segment determined 10 to 14 days after infarction does not provide an unequivocal measurement of myocardial necrosis, it is the best independent criterion available in the living patient.

Some possible explanations for this difference in image size are that (1) the methods of pixel counting and threshold setting were not comparable, (2) the timing of the scans favored pyrophosphate uptake, (3) unrecognized previous myocardial necrosis may have contributed to the overall pyrophosphate image, and (4) one of the two scanning techniques either does not label infarcted tissue or, conversely, labels noninfarcted tissue. The methods of pixel counting were similar for both antomyosin and pyrophosphate SPECT. Regions of interest were outlined in a comparable fashion, the smoothing program was not different, and the mathematics of three-dimensional reconstruction were the same. The target-to-background ratios were on average higher with pyrophosphate. Thresholds for edge detection were 60% with antomyosin and 65% with pyrophosphate. The lower threshold setting, combined with the lower target-to-background ratio of $^{99m}$Tc-antomyosin, would be expected to increase the infarct areas measured with the antomyosin technique relative to those measured with pyrophosphate. The pyrophosphate threshold is that described by Jansen et al.,10 and the 60% antomyosin threshold was determined in animal studies in our laboratories.

The timing of pyrophosphate scans was chosen because high-quality pyrophosphate images can be obtained 2 to 3 days after infarction in both reperfused and nonreperfused hearts. Antomyosin images were obtained 16 to 24 hr after the onset of infarction. Injections were made after resolution of acute injury and, in most cases, after restoration of coronary flow. The residence time of antomyosin in the blood exceeds that of pyrophosphate: the antomyosin blood pool has a half-life of 4 to 6 hr. Therefore, exposure of the necrotic myocardium to circulating antomyosin continued for at least 8 hr. This long residence time combined with the fact that 25 of these patients had reflow suggests that myocardial exposure to antomyosin tracer was adequate. No patients showed evidence of infarct extension between day 1 and day 3. Thus, we do not believe that antomyosin scans at 2 to 3 days would have resulted in larger images.

Because admission criteria for this study excluded patients with prior myocardial infarction, there is no reason for chronic pyrophosphate uptake to have been a factor.22

We therefore must consider whether $^{99m}$Tc-pyro-
phosphate and $^{99m}$Tc-antimyosin do in fact reliably label only necrotic myocardium. Studies by Willerson and his colleagues $^{10,23-28}$ in patients and animals have indicated the specificity of $^{99m}$Tc-pyrophosphate for irreversibly injured myocardial cells. This group also found pyrophosphate uptake in patients with unstable angina but no enzymatic or electrocardiographic evidence of infarction. They attributed pyrophosphate uptake in these patients to “limited myocardial necrosis,” $^{28}$

Bianco et al. $^{30}$ and Gerber et al. $^{31}$ showed localization of $^{99m}$Tc-pyrophosphate in myocardium that had been determined to be uninfarcted by histochemical staining. Siemers et al. $^{32}$ showed that pyrophosphate uptake overestimated pathologic infarct size in the dog, and Zaret et al. $^{33}$ reported $^{99m}$Tc-pyrophosphate uptake in cells that did not show CK depletion. In animal studies in our laboratories, infarcted and reperfused dogs were simultaneously injected intravenously with $^{111}$In-labeled antimyosin and $^{99m}$Tc-pyrophosphate. Five hours after injection, myocardial slices were imaged. The antimyosin image size agreed with histochemical infarct size (13.9 g vs 14.2 g, respectively, $n = 9$), whereas pyrophosphate image size was larger (20 vs 13.9 g, respectively, $n = 9$). $^{34}$ No work to date has shown antimyosin uptake by reversibly injured cells.

Here we demonstrate that intravenous Fab fragments of polyclonal and monoclonal myosin-specific antibody can label infarcted myocardium, with a target-to-background ratio suitable for clinical gamma scanning. No attempt was made to evaluate this technique as a method for detecting myocardial infarction.

FIGURE 3. Tomographically reconstructed matched transverse, sagittal, and coronal images of $^{99m}$Tc-pyrophosphate uptake (top) and $^{99m}$Tc-antimyosin uptake (bottom) in the patient with acute anterior myocardial infarction shown in figure 1. The vertical lines in the transverse sections show areas through which the sagittal sections were reconstructed, and the horizontal lines show areas through which the coronal sections were reconstructed. The image size is larger with pyrophosphate in all reconstructed views.

FIGURE 4. Top, Infarct weight in grams estimated by antimyosin SPECT is shown on the vertical axis, with hypokinetic segment length on the horizontal axis. Bottom, A similar plot with results of pyrophosphate (PYP) SPECT on the vertical axis. Correlation coefficients for both sets of results compared with segment length are similar, but the slope for pyrophosphate is approximately double the slope for antimyosin.
in the general population; all patients in this study had well-documented necrosis. Rather, we evaluated this scanning technique as an improved method of infarct sizing. In this group of predominantly reperfused patients, our findings indicate that infarct size calculated by antimyosin scanning is smaller than the corresponding calculations from pyrophosphate scans. The reason for this difference is not yet known, but may relate to pyrophosphate uptake in border zones that may or may not be irreversibly injured. Further studies are needed with both techniques to determine the nature and fate of myocardium that has been labeled by pyrophosphate but does not show affinity for antimyosin.

References
4. Khaw BA, Beller GA, Haber E: Experimental myocardial infarct imaging following intravenous administration of iodine-131 labeled antibody (Fab')2 fragments specific for cardiac myosin. Circulation 57: 743, 1978
Scintigraphic quantification of myocardial necrosis in patients after intravenous injection of myosin-specific antibody.

Circulation. 1986;74:501-508
doi: 10.1161/01.CIR.74.3.501
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1986 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/74/3/501

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation is online at:
http://circ.ahajournals.org//subscriptions/