Usefulness of indium-111-oxine-labeled leukocyte scintigraphy in diagnosis of inflammation associated with chronic aortic dissection

Keiko TAKAHASHI,* Mitsumasa OHYANAGI,* Hitoshi NARUSE,* Miho MASAI,* Tadaaki IWASAKI,* Minoru FUKUCHI** and Takashi MIYAMOTO***

*Department of Internal Medicine, Cardiovascular Division, Hyogo College of Medicine
**Department of Nuclear Medicine, Hyogo College of Medicine
***Department of Thoracic and Cardiovascular Surgery, Hyogo College of Medicine

Background: Patients with chronic aortic dissection require monitoring for indications of disease progression. In present study, inflammation adjacent to associated aortic wall was evaluated by indium-111-oxine-labeled leukocyte scintigraphy, since inflammation of the blood vessel wall often associates with progression of chronic aortic dissection. Methods and Results: Fifteen patients with aortic dissection underwent indium-111-oxine-labeled leukocyte scintigraphy. Seven showed positive images at sites corresponding to the actual sites of the dissociated aorta. Four patients with positive images underwent surgery. Histologic examination revealed inflammatory and necrotic changes of the aortic wall. During a mean follow-up period of 2.3 years, progression of aortic dissection was observed in two of the seven patients with positive intimal imaging. Conclusion: Indium-111-oxine-labeled leukocyte scintigraphy may be a useful noninvasive technique to assess the persistent inflammation in patients with chronic aortic dissection.

Key words: aortic dissection, inflammation, leukocyte scintigraphy

INTRODUCTION

In patients with aortic dissection who have survived the acute phase, careful follow-up is required even in the absence of symptoms. The death rate during the acute phase is reportedly high.1,2 However, even in the chronic phase, life-threatening complications such as progression and rupture of the dissected lumen may occur.3 The prognosis of chronic aortic dissection has been reported to depend on the presence of a thrombosed false lumen3 and the level of blood pressure control.4 Cassells et al. reported that in the human carotid artery, higher temperature of the blood vessel walls and more invasive inflammation decrease the stability of the blood vessel wall and increase the likelihood of disease progress.5 Therefore, there is a possible association between persistent inflammation and the morbid progression of aortic dissection. In general, transesophageal echocardiography and magnetic resonance imaging (MRI) are used to follow patients with chronic aortic dissection.6,7 However, these methods are not capable of assessing the inflammation in the chronically dissected aortic wall. To assess inflammation in the blood vessel wall during the chronic phase of aortic dissection in a non-invasive manner, we performed indium-111-oxine-labeled leukocyte scintigraphy (In-WBC) and examined how the findings correlated with disease progression.

METHODS

Patient selection
Fifteen patients with chronic aortic dissection who did not undergo operation during the acute phase were evaluated. At least 2 weeks had passed since the acute onset. There were 4 women and 11 men, ranging in age from 34 to 78 years. According to the DeBakey classification,1 1 dissection was type I, 1 was type II, and 13 were type III. The
patients did not have any symptoms at the time of examination, and were mainly receiving medical treatment for hypertension. Patients with Marfan's syndrome or other aortic diseases were excluded from the study. Written informed consents were obtained from all patients.

**Indium-111-oxine-labeled leukocyte imaging protocol**

Mixed leukocyte labeling was performed using the method of Thakur et al. and autologous leukocytes. After 45 ml of venous blood was collected using a heparinized syringe, and allowed to stand for 60 min, the supernatant was transferred to a sterile test tube and centrifuged at 450 G for 5 min to separate the leukocytes from plasma. After saline was added, the suspension was recentrifuged. The supernatant fluid was disposed and the sedimentated leukocytes were resuspended in previously isolated plasma were used. Next, 37 MBq of indium-111-oxine (Nycomed Amersham Plc, Buckinghamshire, UK) was added to the preparation. After incubating the washed leukocytes for 15 min, additional plasma was added and the suspension centrifuged. After decanting the supernatant fluid, the sedimentated labeled-leukocytes were resuspended in plasma. The degree of leukocyte labeling was calculated as (Cell-associated activity)/(total activity) × 100%.

Approximately 18.5 MBq of labeled leukocytes were infused into the patient, and imaging was carried out 48 hr later using a Starmac 3000XCT gamma camera (General Electric, Milwaukee, WI). Anterior and posterior whole-body views were acquired for 15 min each. A medium energy collimator was utilized. In some of the patients, distinction between the blood pool and the thoracic aortic aneurysm was not possible in the images acquired after 24 hr. In contrast, identification of the structures was possible in the images acquired after 48 hr. The diameter of the aneurysm was measured by CT imaging. Markers of systemic inflammation were analyzed in another blood sample drawn on the day of imaging, and included the leukocyte count, C-reactive protein concentration, and erythrocyte sedimentation rate.

**Radionuclide angiography protocol**

Radionuclide (RN) angiography was performed using technetium-99m-labeled human serum albumin to determine the location of large blood vessels and to differentiate the vertebral, aortic, and cardiac pool activity for each indium-111-oxine labeled leukocyte image.

**Histopathologic characteristics**

Four subjects who had DeBakey type I or II dissections underwent surgery and resected specimens were examined histopathologically. The walls of the excised aortic aneurysms were examined microscopically after staining with hematoxylin and eosin to determine the extent of inflammation. The extent of tissue inflammation was graded faint, moderate, or severe.

**Table 1** Clinical characteristics of patients with aortic dissection

<table>
<thead>
<tr>
<th>Patients</th>
<th>Age</th>
<th>Gender</th>
<th>Days</th>
<th>DeBakey</th>
<th>Image</th>
<th>Thrombosed</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>77</td>
<td>M</td>
<td>29</td>
<td>III</td>
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<td>-</td>
</tr>
<tr>
<td>2</td>
<td>63</td>
<td>M</td>
<td>30</td>
<td>III</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
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<td>F</td>
<td>36</td>
<td>III</td>
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</tr>
<tr>
<td>4</td>
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<td>29</td>
<td>III</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>72</td>
<td>M</td>
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<td>M</td>
<td>33</td>
<td>II</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>77</td>
<td>M</td>
<td>24</td>
<td>III</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>55</td>
<td>F</td>
<td>18</td>
<td>III</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>57</td>
<td>M</td>
<td>23</td>
<td>I</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>66</td>
<td>M</td>
<td>30</td>
<td>III</td>
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</tr>
<tr>
<td>11</td>
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<td>M</td>
<td>29</td>
<td>III</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>12</td>
<td>70</td>
<td>F</td>
<td>31</td>
<td>III</td>
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<td>+</td>
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<tr>
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<td>68</td>
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<td>30</td>
<td>III</td>
<td>-</td>
<td>+</td>
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<td>III</td>
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<td>+</td>
</tr>
<tr>
<td>15</td>
<td>72</td>
<td>M</td>
<td>32</td>
<td>III</td>
<td>-</td>
<td>-</td>
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</tbody>
</table>

M/F, male/female; Days, interval from clinical onset to imaging; DeBakey, DeBakey type; Image +/-, positive/negative with indium-111-oxine labeled leukocyte scintigraphy; Thrombosed +/-, opened/closed of false lumen with computed tomography

**Table 2** Indium-111-labeled leukocyte imaging and inflammation reaction

<table>
<thead>
<tr>
<th>n</th>
<th>WBC (µl)</th>
<th>CRP (mg/dl)</th>
<th>ESR (mm/1 hr)</th>
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<tr>
<td>positive imaging</td>
<td>7</td>
<td>6614 ± 1401</td>
<td>0.56 ± 0.32</td>
</tr>
<tr>
<td>negative imaging</td>
<td>8</td>
<td>6725 ± 656</td>
<td>0.59 ± 0.43</td>
</tr>
</tbody>
</table>

WBC, white blood cell; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; positive imaging vs. negative imaging, ns.

**Patient follow-up**

All subjects were followed periodically with either CT scanning or transesophageal echocardiography to assess the condition of the disassociated lumen. CT scanning was also performed at the same time as with In-WBC studies, and the accumulation of In-WBC as well as the condition of the dissected lumen were assessed.

**Data analysis**

Accumulation of indium-111-oxine-labeled leukocytes was determined visually from scintigraphs and graded as either being negative or positive. The mean (± SD) peripheral blood leukocyte count, C-reactive protein concentration and erythrocyte sedimentation rate were determined, for patients with positive or negative scintigrams. A t-test was used for statistical analysis. A value of p < 0.05 was considered statistically significant.

**RESULTS**

1) **Indium-111-oxine-labeled leukocyte imaging**

Seven of the 15 patients showed accumulation at the site
Fig. 1 Registered indium-111-oxine leukocyte (In-WBC) imaging: Positive accumulation in the descending aorta with chronic aortic dissection. (left: anterior view, right: posterior view)

Fig. 2 Registered indium-111-oxine leukocyte (In-WBC) imaging: Negative accumulation in the descending aorta with chronic aortic dissection. (left: anterior view, right: posterior view)

Fig. 3 Registered computed tomography (CT): Upper, In-WBC accumulated in the descending aorta. The false lumen is not thrombosed. Lower, negative accumulation with chronic aortic dissection. The false lumen is thrombosed.

of the aortic dissection (Table 1). The accumulation areas in the positive patients almost corresponded to the entry site of the aortic dissection. The blood tests showed that the leukocyte count and CRP were within the normal ranges, but the erythrocyte sedimentation rate was augmented in all cases. However, there were no significant differences between the positive group and the negative group (Table 2, Figs. 1, 2, and 3). In six patients within the positive group, CT scanning did not indicate the presence of false lumen thrombosis. Nonetheless, CT scanning revealed false lumen thrombosis in all patients in the negative group (Table 1).

2) Radionuclide angiography
The RN angiographic images did not show any blood pooling in the accumulation areas of the In-WBC studies, allowing differentiation between the In-WBC accumulation areas and the normal blood pooling in the vessels (Fig. 4).

3) Histopathologic characteristics
Histopathologic examination clearly revealed inflammatory and necrotic changes in the aortic wall (Table 3, Fig. 5). Currently, all subjects of the negative group are receiving medical treatment and no histopathologic evaluation has been carried out.
Table 3  Scintigraphical imaging and histopathological findings

<table>
<thead>
<tr>
<th>Patients</th>
<th>Age</th>
<th>Gender</th>
<th>Days</th>
<th>Debakey</th>
<th>Image</th>
<th>Adhesion</th>
<th>Histopathological findings</th>
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<tbody>
<tr>
<td>1.</td>
<td>77</td>
<td>M</td>
<td>29</td>
<td>III</td>
<td>+</td>
<td>+/-</td>
<td>+++</td>
</tr>
<tr>
<td>3.</td>
<td>67</td>
<td>F</td>
<td>36</td>
<td>III</td>
<td>+</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>6.</td>
<td>36</td>
<td>M</td>
<td>33</td>
<td>II</td>
<td>+</td>
<td>+/-</td>
<td>++</td>
</tr>
<tr>
<td>8.</td>
<td>55</td>
<td>F</td>
<td>18</td>
<td>III</td>
<td>-</td>
<td>+</td>
<td>+/-</td>
</tr>
<tr>
<td>9.</td>
<td>57</td>
<td>M</td>
<td>23</td>
<td>I</td>
<td>-</td>
<td>+/-</td>
<td>+</td>
</tr>
</tbody>
</table>

M/F, male/female; Days, interval from clinical onset to imaging; Debakey, Debakey type; +/-, positive/negative imaging of indium-111-oxine labeled leukocyte; Adhesion (+/-, +, +++), adhesion of peri-dissecting wall of aorta (faint, moderate, severe); Histopathological findings (+/-, +, ++, +++), the degree of inflammation of aortic wall (almost none, faint, moderate, severe)

Fig. 4 Registered radionuclide angiographic imaging (case of Fig. 1, lateral view); did not show any blood pooling in the accumulation areas of the In-WBC studies.

4) Patient follow-up

During a mean follow-up period of 2 to 3 years (mean period 29 months), progression of the aortic dissection occurred in two subjects in the positive group who were diagnosed as DeBakey type III at the time of onset. One of these patients had an enlarged false lumen and is deliberately being treated, while the other required an emergency surgery as the dissection progressed to DeBakey type I.

DISCUSSION

In the present study, we evaluated the association between persistent inflammation and the progression of aortic dissection using scintigraphy. Many studies have demonstrated that scintigraphy is a useful diagnostic tool for the evaluation of patients with inflammatory diseases. Gallium (Ga) scintigraphy is widely used in the evaluation of fever of unknown origin and infectious diseases. A new method to evaluate inflammatory activity in chronic aortic dissection is necessary.

Fig. 5 Histopathologic findings. Infiltration of inflammatory cells in the region of In-WBC accumulation in chronic aortic dissection.

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\text{Technetium-99m-hexamethylpropylene amine oxime (HMPAO) labeled-leukocyte scintigraphy is also used in the assessment of infectious diseases and in the field of pediatrics.}^{10} \text{ In-WBC scintigraphy has the advantage of being able to identify persistent inflammatory activity.}^{11} \text{ Although the labeling procedure is complex, In-WBC scintigraphy also can be applied to the detection of blood vessel inflammation.}^{11} \text{ The sensitivity for the identification of inflammatory lesions with In-WBC is reported to be 84 to 95\%.}^{11,12} \text{ Therefore In-WBC scintigraphy was used as a tool to detect persistent inflammation in patients with chronic aortic dissection. Indium-111-oxine-labeled leukocytes usually accumulate in the liver, spleen, bone marrow, and great vessels in 18 to 24 h.}^{11} \text{ This accumulation decays physiologically in 24 to 48 h. Thus, it is technically difficult to identify pathologic accumulations of indium-111-oxine-labeled leukocytes in the spine, aorta, and heart after 24 h. For this reason, imaged are taken at 48 h after administering indium-111-oxine-labeled leukocytes.}

In this study, seven subjects (47%) showed accumulations of In-WBC. These positive images indicate persistent inflammation caused by aortic dissection. It has been reported that complications such as aortic rupture are
caused by continuous enlargement of the false lumen. Complete occlusion of the false lumen with blockage of blood flow therefore reduces the incidence of aortic rupture.\textsuperscript{13,14} Patients with a thrombosed false lumen may have a reduced risk of the progression of aortic dissection. In this study, CT scanning revealed a thrombosed false lumen in six (75\%) of eight subjects with negative scintigraphy findings, but six subjects (66\%) with positive scintigraphy findings did not show thrombosed false lumens. It has been reported that 24\% of patients receiving medical treatment developed ruptures adjacent to the intimal entrance.\textsuperscript{15} In the present study, the positive images represented accumulation in areas adjacent to the intimal areas of aortic dissection. Nakashima reported that type III dissection is associated with the presence of severe atherosclerosis.\textsuperscript{16} Rupture of an atherosclerotic lesion may play a role in the initiation of small proportion of dissecting aneurysms.\textsuperscript{16} We demonstrated an association between the degree of local inflammation and potency of the false lumen. Larson and Williams reported that intimal plaques do not develop with inflammation in type I and type II aortic dissection, but do develop with inflammation in type III thoracic aortic dissection.\textsuperscript{4} This may be why so many positive images were observed in the present study: 87\% of subjects had a type III dissection. However, given the association between potency of the false lumen and thrombosis, we conclude that In-WBC scintigraphy is an effective, noninvasive technique for demonstrating the existence of local inflammation secondary to chronic aortic dissection.

We found a clear association between inflammation of the aortic dissection and peripheral blood findings. Some reports have suggested that it is difficult to assess the extent of inflammation from blood tests when the inflammation is localized in vivo.\textsuperscript{17} Our study confirms the difficulty of assessing inflammation by blood tests alone. Suzuki et al. have suggested that measurement of myosin heavy chain can be a tool for the diagnosis of aortic dissection.\textsuperscript{18} In another case report, the use of chemical mediators was suggested.\textsuperscript{19} Nonetheless, these parameters fail to correlate with the prognosis of acute aortic dissection. We previously reported that accumulation of In-WBC in aortic aneurysms indicated severe or moderate infiltration of inflammatory cells in the aneurysm wall.\textsuperscript{20} Our present results (Table 3) show that In-WBC imaging might be able to predict progression of chronic disease. Consequently, In-WBC scintigraphy is an effective follow-up tool.

Aortic dissection is associated with several risk factors,\textsuperscript{21} including Marfan’s syndrome.\textsuperscript{4} Some studies have shown that rupture of the aorta occurs in 70\% of chronic aortic dissection patients with medical follow-up.\textsuperscript{15} One case report described a patient who underwent reoperation for aortic rupture 15 years after emergency surgery for aortic dissection.\textsuperscript{2} One sign that indicates aortic rupture is evidence of blood outside the blood vessels, which can be identified by transesophageal echocardiography.\textsuperscript{1} However, no method is currently available to identify aortic rupture before the occurrence of blood effusion. In the present study, we demonstrated an association between inflammation and progression of the dissection. Also, In-WBC scintigraphy identified local inflammation is undetectable by examination of the peripheral blood. Monitoring once every 3 to 6 months is ideal for observing changes in the diameter of the aorta in patients with aortic disease.\textsuperscript{13,22} Even during the chronic phase of aortic dissection, assessing the aortic wall is important. A statistical discussion is perhaps not valid with only 15 cases. Although there are only 15 cases in our study, we report the results because a clear trend was found after comparing the patients’ outcomes with the pathology tissues. Our results suggest that In-WBC scintigraphy is a useful method for monitoring patients with chronic dissection of the aortic wall.

CONCLUSION

This study was performed to detect local inflammation of the aortic wall using In-WBC scintigraphy in patients with chronic aortic dissection. We were able to detect inflammation that was not recognizable using blood tests, and suggest that In-WBC scintigraphy is a useful method for following patients with this disease.

REFERENCES