Soluble Elastin Fragments in Serum Are Elevated in Acute Aortic Dissection

Tadashi Shinohara, Kimihiro Suzuki, Makoto Okada, Masaru Shiigai, Masashi Shimizu, Tadaaki Maehara, Fumitaka Ohsuzu

Objective—We aimed to establish an enzyme-linked immunosorbent assay for measuring soluble elastin fragments (sELAF) in serum and to reveal its usefulness in diagnosing acute aortic dissection (AAD).

Methods and Results—An enzyme-linked immunosorbent assay to measure sELAF in serum was developed by using the newly created double monoclonal antibodies, which recognize the different epitopes of human aortic elastin. Twenty-five AAD patients, 50 patients with acute myocardial infarction (AMI), and 474 healthy individuals were enrolled in the study. The sELAF levels from healthy subjects gradually increased with aging. When the cutoff point for positivity was set at the mean + 3 SD (ie, 3 SD above the mean in healthy subjects at each age), 16 AAD patients (64.0%) were found to be positive, whereas only 1 AMI patient was found to be positive (2.0%). AAD patients with either an open or a partially open pseudolumen were found to be 88.9% positive for sELAF, whereas those with its early closure were 0% positive. The difference in the sELAF levels between AAD patients with and without a thrombotic closure of false lumen was significant (60.3 ± 15.6 versus 135.4 ± 53.2 ng/mL, respectively; P < 0.005).

Conclusions—The sELAF level in serum may be a useful marker for helping in the diagnosis and screening of AAD and may also help to distinguish AAD from AMI. (Arterioscler Thromb Vasc Biol. 2003;23:1839-1844.)

Key Words: matrix protein ■ elastin degradation ■ aortic media ■ intimal tear ■ false lumen

Acute aortic dissection (AAD) is a life-threatening medical emergency of the aorta that is associated with a high mortality. In AAD patients who do not receive immediate treatment, mortality is estimated to be 35% within the initial 24 hours, 50% within 48 hours, and 80% after 2 weeks. Therefore, rapid diagnosis and timely management play an essential role in patient survival.

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The typical manifestation of AAD is an acute onset of severe chest and/or back pain. To make an accurate diagnosis of AAD, it is helpful to use the recently improved noninvasive imaging modalities, such as transthoracic and transesophageal echocardiography, computed tomography (CT), and magnetic resonance imaging (MRI). However, it is still difficult for physicians to decide to use such modalities when the signs and symptoms of the underlying disease are unclear or misleading, especially at the onset. In fact, up to 30% of AAD patients remain undiagnosed until autopsy.

The biochemical methods for diagnosing acute myocardial infarction (AMI) have advanced greatly in the past quarter century; these methods include the use of creatinine phosphokinase (CK), the MB isozyme of CK, cardiac myosin light chain, troponin T, and human heart fatty acid binding protein. Meanwhile, for the diagnosis of AAD, smooth muscle myosin heavy chain and C-reactive protein (CRP) in the serum have both been reported to be such candidates. However, they also have several drawbacks: the former rapidly disappears within the first 12 hours after onset, whereas the levels of the latter, which is a nonspecific marker of inflammation, begin to increase only the day after onset. Therefore, the establishment and clinical availability of another simplified laboratory test(s) to help make a timely and accurate diagnosis of AAD is urgently needed.

Elastin is one of the major structural matrix proteins of the arterial wall. Mature elastin is composed of soluble elastin subunits, which are intermolecularly cross-linked into a fibrous network (desmosine and isodesmosine formation) and thus construct a highly polymerized insoluble protein. The main pathological feature of the aortic media in AAD is a higher grade of elastin degradation. Once an initial tear is formed, the dissection tends to expand to the degraded elastin layers, along with an inflammatory infiltrate, a major source of proteolytic enzymes such as elastases and metalloproteinases, which thus dramatically promote the fragmentation process of the elastin network in the media. As a result, soluble elastin fragments (sELAF) are supposed to be released into the circulating blood. Therefore, sELAF in the...
serum might be a new and potentially useful variable for aid in the diagnosis of AAD. The purposes of the present study are (1) to establish an immunoassay system for measuring sELAF in the serum and (2) to reveal its usefulness in diagnosing AAD.

Methods

Study Design and Subjects

Twenty-five patients with AAD (within 48 hours after onset, 12 men and 13 women), 50 patients with AMI (within 48 hours after onset, 32 men and 18 women), 20 patients complaining of chest pain, the causes of which excluded AAD and AMI (within 48 hours after the onset, 8 men and 12 women), 40 hypertensive patients without medication (28 men and 12 women), and 474 healthy individuals (289 men and 185 women) were enrolled in the present study. Serum samples for testing sELAFs were collected soon after admission; in some patients, samples were also collected at specified time intervals thereafter. The samples were stored frozen at -30°C until they were used in testing. The local ethics committee approved the study protocol. Informed consent was obtained from each subject after the nature and purpose of the study were fully explained. For some AAD patients with sudden death soon after admission or diagnosed at autopsy, consent was obtained from each family afterward.

The protocol for the enrolled patients included the documentation of the patient characteristics. Clinical variables included the following: age; sex; time of onset; time of admission; type of dissection according to the Stanford or DeBakey classifications; prehospital events; history of past illnesses; family history; underlying aortic disease, including Marfan syndrome, anulooaortic ectasia, trauma, and syphils; physical examination on admission; complications such as myocardial infarction, cardiac tamponade, aortic rupture, acute renal failure, and cardiogenic shock; electrocardiographic findings; image diagnostic findings, including those from plain chest radiography, echocardiography, CT, MRI, and angiography; and details of medical or surgical therapy and outcomes.

Complete blood count, CRP, and serum chemistry panels including aspartate transaminase, alanine transaminase, lactate dehydrogenase (LDH), total bilirubin, blood urea nitrogen, serum creatinine, CK, and the MB isozyme of CK were measured serially.

Immunostudy of sELAFs in Serum

An enzyme-linked immunosorbent assay (ELISA) system to measure the concentration of sELAFs in serum was developed with the use of monoclonal antibodies (mAbs) newly created according to standard hybridoma technology.24,25 BALB/c mice were immunized with highly purified human aortic soluble elastin (Elastin Product Co) emulsified with Freund's complete adjuvant 3 times at 3-week intervals. After the last booster injection, splenocytes removed from the mouse with the highest serum titration for the antigen were fused with murine SP 2/0 myeloma cells. The hybridoma-producing mAbs reactive with the soluble elastin were screened. Finally, several hybridoma cells were selected and cloned by limiting dilution. A high amount of mAbs was obtained as ascitic fluid of BALB/c mice primed with the stune, and the mAbs were purified by protein A–Sepharose affinity chromatography.

We assessed combinations of mAbs by a double monoclonal antibody sandwich assay. Specificity to recognize human soluble elastin of each mAb was confirmed by Western blot analysis (data not shown). Primary mAbs were absorbed onto 96-well microplates (Nunc A/S), and secondary mAbs were labeled with horseradish peroxidase. The combination of the paired mAbs (designated as HASG-30 and HASG-61-1) with the highest assay sensitivity and specificity was chosen for the following analysis.

Each sample was diluted 10 times with the dilution buffer (PBS containing 1% skim milk and 2 mmol/L EDTA) and then added to the mAb HASG-30 immobilized 96-well microplate and incubated for 1 hour at 37°C. Each well was washed with 20 mmol/L Tris-HCl buffer containing 0.15 mol/L NaCl and 0.1% Tween 20 detergent (TBS-Tween 20) 3 times and then incubated with peroxidase-labeled mAb HASG-61-1 for 1 hour at 37°C. Finally, each well was washed 3 times with TBS-Tween 20 and added to 3,3',5,5'- tetramethylbenzidine substrate solution (Sigma) for 10 minutes at room temperature. The enzymatic reaction was terminated by adding HCl solution. Thereafter, the reaction was measured at 450 nm with a T-max microplate reader (Molecular Device Co).

Statistical Analysis

Results are presented as mean ± SD. Comparisons between means of 2 independent samples were analyzed by using paired or unpaired Student t tests. An analysis of multiple groups was performed with a 1-way ANOVA and the Tukey-Kramer multiple comparisons test. Statistical significance was defined as P < 0.05. Statistical testing was performed with commercially available statistical software (Stat-View version 5.0, SAS Institute Inc).

Results

Patient Characteristics

A total of 25 AAD patients (12 men and 13 women) were enrolled in the present study (Table 1). Their ages (mean ± SD) were 64.5 ± 15.4 years for the men and 65.1 ± 12.7 years for the women (P = 0.92). The lag time from the onset to admission was 11.5 ± 14.9 hours. There were 17 patients classified as Stanford type A and 8 patients classified as Stanford type B. In terms of the DeBakey classification, 16 patients were type I; 1 patient was type II; and 8 patients were type III. All patients complained of chest and/or back pain.

Four patients (patient Nos. 6, 8, 15, and 16 in Table 1) exhibited complications involving neurological disorders after onset. The diseases associated with AAD included hypertension in 23 patients, Marfan syndrome in 2 patients, and anulooaortic ectasia in 7 patients. Twenty-three patients were diagnosed as having AAD according to the findings of enhanced CT and echocardiography. Angiography was performed in 3 patients, and MRI was performed in 5 patients.

Fifteen AAD cases were negative (<0.6 mg/mL) for CRP on admission (Table 1). Eleven patients and 15 patients showed almost normal ECG and chest radiographic findings on admission, respectively. Circulatory shock, as defined by a systolic blood pressure of <80 mm Hg and a urine output of <20 mL/h, was found in 6 patients (Nos. 4, 6, 8, 10, 14, and 16). Renal dysfunction, as defined by serum creatinine levels of >1.5 mg/dL, was found in 5 patients (Nos. 1, 3, 14, 23, and 25).

Among 17 patients with Stanford type A dissection, 10 received surgical repair (Table 1). The reasons for treatment with medication alone in 3 type A patients were as follows: 2 patients (Nos. 3 and 4) did not want to undergo surgery, and 1 patient (No. 11) was too aged.

Analytical Performance of ELISA

The concentration of sELAF was determined with the use of a calibration curve for the ELISA system and standard highly purified human aortic soluble elastin (from 0.69 to 500 ng/mL). Three sera, serially diluted from 1/2 up to 1/128 in the dilution buffer, exhibited a linear recovery pattern (data not shown). In experiments to determine analytic recovery with the use of 2 reconstructed serum samples, the percentages of recovery were 101.1% and 100.0%, respectively. The intra-assay and interassay coefficients of variation ranged.
from 3.0% to 4.2% and 1.6% to 8.4%, respectively. Freeze-and-thaw treatment (from 1 to 8 times) little affected the measured values of sELAF (coefficient of variation 3.2%, n/H11005/H11001/10).

Clinical Measurement of sELAF in Serum by ELISA

Figure 1 shows sELAF levels in 474 healthy control subjects (mean age 40.6 years, range 13 to 89 years). In men (mean age 40.9 years, range 18 to 82 years) and women (mean age 40.3 years, range 13 to 89 years), the levels gradually increased with age. There was no significant difference between the levels in men and women.

The sELAF levels in patients with AAD and in those with AMI at admission are shown in Figure 2. In the 25 patients with AAD, the sELAF level was 114.7/H11006/H1100556.9 ng/mL, whereas in patients with AMI, it was 56.1/H11006/H1100514.9 ng/mL (Table 2). In the 20 patients with chest pain without AAD or AMI and in the 40 hypertensive patients without medication, the levels were 47.3/H11006/H1100513.5 and 47.7/H11006/H1100522.3 ng/mL, respectively. When the cutoff point for positivity was set at the mean + 3 SD (ie, 3 SD above the mean in healthy subjects at each age), 16 patients with AAD (64.0%) were shown to be positive (Figure 2). The specificity at the cutoff point was 99.8% (473 of 474). The predictive values of a positive or negative test in the population were 94.1% (16 of 17) or 98.1% (473 of 482), respectively.

<table>
<thead>
<tr>
<th>TABLE 1. Characteristics of the 25 AAD Patients</th>
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HT indicates hypertension; HL, hyperlipidemia; DM, diabetes mellitus; AAE, annuloaortic ectasia; Marfan, Marfan syndrome; Asc-R, ascending aorta replacement; pAr-R, proximal arch replacement; tAr-R, total arch replacement.

Delayed indicates 2 months or later after the onset.
respectively. When the cutoff point was set at the mean +2 SD (positivity 68.0%, specificity 96.6%), the predictive value of a positive or negative test was 51.5% or 98.3%, respectively. Therefore, we used the cutoff point set at the mean +3 SD at each age in the following analysis. Accordingly, the cutoff level at 30 years was 68.3 ng/mL; at 40 years, 75.6 ng/mL; at 50 years, 84.1 ng/mL; at 60 years, 112.6 ng/mL; and at 70 years, 116.4 ng/mL. Under these conditions, 1 patient with AMI (2.0%), 1 patient with hypertension (2.5%), and none of patients with chest pain without AAD or AMI (0%) exhibited positivity (Table 2).

**Association of sELAF Levels to Reference Parameters**

AAD patients with either an open or a partially open pseudolumen showed 88.9% positivity for sELAF (Table 2). The positive or negative predictive values were 94.1% (16 of 17) or 99.6% (473 of 475), respectively. No significance was found between a DeBakey type I and III dissection for the sELAF levels in AAD patients with an open or a partially open pseudolumen (142.7 ± 58.5 ng/mL for type I [n = 12] versus 125.5 ± 44.6 ng/mL for type III [n = 5], P = 0.57).

In contrast, all patients with early thrombogenic closure of dissecting lumen showed 0% positivity. The difference in sELAF levels between the patients with open AAD and those with closed AAD was significant (135.4 ± 53.2 versus 60.3 ± 15.6 ng/mL, respectively; P < 0.005; Table 2). There was no significant difference in age between AAD patients with or without thrombogenic occlusion of false lumen (64.3 ± 11.4 versus 64.3 ± 14.7 years, respectively; P = 0.99), nor was there a significant difference in the lag time from the onset to collecting samples between them (13.5 ± 17.1 versus 10.9 ± 14.6 hours, respectively; P = 0.72).

Other laboratory parameters, such as leukocyte count, hematocrit, CRP, aspartate transaminase, alanine transaminase, LDH, total bilirubin, blood urea nitrogen, creatinine levels, and CK or MB fraction did not show any significant association with sELAF levels.

**Time Courses of sELAF Levels**

The time courses of sELAF levels in 5 patients with AAD (patient Nos. 1, 11, 14, 15, and 19) are shown in Figure 3. Each sELAF level in patient Nos. 1 and 11 was markedly elevated soon after the onset, and subsequently, the level was sustained for >84 hours. In patient Nos. 14 and 15 (DeBakey type I), who received surgical repair (ascending and proximal aortic arch replacement), the sELAF level dramatically increased just after surgery (asterisks in Figure 3), and thereafter, it remained at a high range. The sELAF level in patient No. 19 (aged 64 years) was elevated at first (cutoff value at that age 112.6 ng/mL) and gradually decreased to less than the cutoff value (daggers in Figure 3).

**Discussion**

In the present study, we developed an ELISA system for measuring sELAF in the serum that was reliable and reproducible. Using this system, we demonstrated that (1) 64% of AAD patients (88.9% of those with either an open or a partially open pseudolumen and 0% with a closed pseudolumen) within 48 hours after the onset showed an increase in the sELAF levels in serum, and (2) only 2% of the AMI patients were positive.

Mature elastin is a very stable protein with a biological half-life of 70 years. Elastic architecture and its contents in the aortic media are essential components contributing to the strength and stability of the aorta. Elastin is synthesized and deposited in early childhood, and no further significant synthesis occurs in adult life. With aging, the structure of...
the aortic wall is altered: it dilates and becomes thicker with fragmentation and/or the calcification of elastin fibers. In accordance with such pathological developments, the sELAF levels in sera from healthy subjects gradually increase as the subjects progress from their teens to old age (Figure 1).

In the normal aorta, elastin shows a framework-like continuous structure consisting of elastin laminae and interlaminal fibers that interconnect the laminae. In contrast, the interlaminar elastic fibers become irregular in arrangement and shape and decrease in number in AAD patients.19,20 This disarrangement and decrease of interlaminar elastic fibers may result in medial instability and weakness against various forces, especially against the force that dissects the media. The disruption of the aortic media by blood entering through an intimal tear on the endothelium causes extensive damage to the aortic media.21–23 In accordance with such pathological developments, the sELAF levels in sera from healthy subjects gradually increase as the subjects progress from their teens to old age (Figure 1).

A gradual decrease in the local elastin concentration along the thoracic aorta down to the abdominal segments has been reported.18,22,27 In contrast, Cattell et al28 have demonstrated that the distribution of elastin along the thoracic aorta from the valve to the diaphragm shows no such gradient in amount or concentration. In fact, there was no significant difference between the DeBakey type I and III dissection for sELAF levels in AAD patients with either an open or a partially open pseudolumen.

Discriminating AAD from AMI is still a common clinical dilemma, and the differential diagnosis is critical, because the management and prognoses for each are quite different. Misdiagnosis of AAD as AMI frequently results in catastrophic hemorrhage or an exacerbation of AAD, especially when thrombolytic drugs are inappropriately administered.29–31

Makita et al11 evaluated the consequences of CRP levels for conservative management in AAD patients. Nevertheless, the CRP levels were rarely elevated, especially within several hours after onset (see Table 1 from Makita et al11), indicating that this parameter may not be useful as an early diagnosis of AAD. Other nonspecific markers for AAD, such as leukocyte count and LDH, are also rarely elevated at onset. The present study demonstrated that the sELAF level in the serum from 1 patient (No. 14 in Table 1) was already elevated at only 0.7 hours after the onset of AAD. When a patient complaining of chest and/or back pain visits any clinical facility, including a small clinic or an emergency room in a large hospital, the measurement of this novel parameter should thus be equally useful for the screening of AAD. If the sELAF levels are elevated, even if the signs and symptoms of AAD are unclear or misleading, physicians can easily decide to use modalities such as echocardiography, CT, or MRI.

In AAD patients with an open or a partially open false lumen, the sELAF level was positive in ≈90% (Figure 2 and Table 2). In contrast, the sELAF levels were not elevated in the AAD subgroup with a closed false lumen. The precise time course of elimination of sELAF is unknown at present. Nevertheless, the sELAF levels are significantly elevated for 48 hours after the onset (patients Nos. 1, 11, 14, and 15 in Figure 3). This might occur because of the continuous release from the AAD lesion into the circulation. If the false lumen is totally packed by thrombus formation, then the sELAF levels should rapidly return to the basal ranges (Figure 2 and Table 2). This might be a limitation when applying this parameter in the diagnosis of AAD; thus, even if this parameter shows negativity in a

The table below shows the percentage of patients positive for sELAF among patients groups.

**Table 2. Percentage of Patients Positive for sELAF Among Patients Group**

<table>
<thead>
<tr>
<th>AAD</th>
<th>Open AAD</th>
<th>Closed AAD</th>
<th>AMI</th>
<th>Chest Pain Without AAD or AMI</th>
<th>HT</th>
</tr>
</thead>
<tbody>
<tr>
<td>n (men)</td>
<td>18 (8)</td>
<td>7 (4)</td>
<td>50 (32)</td>
<td>20 (8)</td>
<td>40 (28)</td>
</tr>
<tr>
<td>Age, y</td>
<td>63.6±14.7</td>
<td>68.8±10.2</td>
<td>60.6±12.4</td>
<td>60.7±8.0</td>
<td>58.9±9.8</td>
</tr>
<tr>
<td>sELAF, ng/mL</td>
<td>135.4±53.2†</td>
<td>60.3±15.6</td>
<td>56.1±14.9</td>
<td>47.3±13.5</td>
<td>47.7±22.3</td>
</tr>
<tr>
<td>% Positive‡ (n)</td>
<td>88.9‡ (16)</td>
<td>0 (0)</td>
<td>2.0 (1)</td>
<td>0 (0)</td>
<td>2.5 (1)</td>
</tr>
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</table>

Results are given as mean±SD. Open AAD indicates AAD with either an open or a partially open pseudolumen; Closed AAD, AAD with a closed pseudolumen by thrombus formation. Chest Pain without AAD or AMI included angina pectoris, musculoskeletal diseases, gastroenterological diseases, or cardiac neurosis.

*P<0.005 vs Closed AAD. †P<0.001 vs the rest of the groups.
‡mean±3SD of healthy controls at each age.

**Figure 3.** Time course of sELAF levels in sera from 5 AAD patients (Nos. 1, 11, 14, 15, and 19). *Sample was collected just after a surgical repair. †sELAF levels are less than the cutoff value (cutoff value at the patient’s age is 112.6 ng/mL).
patient with chest and/or back pain, AAD (especially with a closed pseudolumen) cannot be ruled out.

Another limitation of the present study is that the data were obtained retrospectively. Therefore, a large prospective study should be performed to confirm the diagnostic efficacy and accuracy of this novel assay.

Although it still takes 3 hours to measure the sELAF level in serum, new efforts are now being made to shorten the measurement time of the immunoassay system.

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References


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