Plasma ADMA Predicts Restenosis of Arteriovenous Fistula

Chih-Cheng Wu,*† Szu-Chi Wen,*† Chung-Wei Yang,‡ Shih-Yun Pu,* Kuei-Chin Tsai,* and Jaw-Wen Chen†§

*Department of Medicine and †Hemodialysis Center, Hsinchu General Hospital, Hsinchu, and ‡National Yang-Ming University School of Medicine, †Institute of Pharmacology and Cardiovascular Research Center, National Yang-Ming University School of Medicine, and ‡Department of Medicine, Taipei Veterans General Hospital, Taipei, Taiwan, Republic of China

ABSTRACT

Plasma levels of asymmetrical dimethylarginine (ADMA), an endogenous inhibitor of nitric oxide production, correlate with endothelial dysfunction and the development of cardiovascular events in patients with uremia. It is not known whether endothelial dysfunction contributes to the dysfunction of arteriovenous fistulas (AVFs) in hemodialysis patients. Here, we studied the predictive value of baseline plasma ADMA for symptomatic restenosis of an AVF after percutaneous transluminal angioplasty in dialysis patients. We obtained baseline plasma ADMA levels before percutaneous transluminal angioplasty in 100 consecutive patients with dysfunctional AVFs. Patients were followed up clinically for up to 6 mo after angioplasty for recurrent dysfunction. During the 6 mo after angioplasty, 46 patients experienced recurrent dysfunction of their AVF; of these, follow-up fistulography showed restenosis at the same location in 41, new stenosis at different locations in two, and no significant stenosis in three patients. Up to 60% of the patients with high levels of ADMA (≥0.910 μM) had target lesion restenosis compared with 25% of those with low levels (<0.910 μM; P < 0.001). In multivariate analysis, plasma ADMA independently nearly tripled the risk for recurrent symptomatic stenosis of an AVF after percutaneous transluminal angioplasty (hazard ratio 2.65; 95% confidence interval 1.33 to 5.28). These results suggest a role for ADMA in the progression of symptomatic restenoses of AVFs after percutaneous transluminal angioplasty and call for preventive strategies that target ADMA and/or endothelial dysfunction to decrease the risk for AVF restenosis.


According to recommendations in the National Kidney Foundation Disease Outcomes Quality Initiative (NKF-DOQI) guidelines, creation of native arteriovenous fistulas (AVFs) is preferable to arteriovenous grafts because of their lower morbidity and higher long-term patency.1 Nonetheless, AVFs are also subject eventually to dysfunction and failure, which is usually caused by stenosis in the venous segment of the fistula. Although percutaneous transluminal angioplasty (PTA) is effective in treating these stenotic lesions, its benefit is attenuated by a high restenotic rate within 6 mo.1 Several medical, mechanical, and genetic factors have been identified as being associated with functional or anatomic patency of AVFs2–5; however, the major cause of individual variation in the development of symptomatic restenosis in dysfunctional fistulas remains unknown.

Asymmetrical dimethylarginine (ADMA) is an endogenous inhibitor of nitric oxide (NO) synthase and has been implicated as an important contributor to endothelial dysfunction.6,7 ADMA is not excreted in patients with ESRD, and its concentration

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Correspondence: Dr. Jaw-Wen Chen, Department of Medicine, Taipei Veterans General Hospital, No. 201, Sec.2, Shih-Pai Road, Taipei, Taiwan, Republic of China. Phone: +886-2-2871-2121 ext, 3493; Fax: +886-2-3871-1601; E-mail: jwchen@vghtpe.gov.tw

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in plasma is two to six times higher than in normal control subjects. In patients with ESRD, ADMA is correlated with the development of atherosclerosis and cardiovascular events, which suggests that accumulation of ADMA might be an important cardiovascular risk factor; however, the role of ADMA in the restenosis process after PTA of AVFs is not known. We hypothesized that plasma ADMA levels could be related to future development of symptomatic restenosis after PTA for dysfunctional AVFs. This study was then conducted to investigate prospectively the predictive value of baseline plasma ADMA levels for symptomatic angiographically documented restenosis after PTA in a cohort of patients with dysfunctional AVFs.

RESULTS

Patients Characteristics
A total of 111 patients with dysfunctional AVFs were enrolled. After diagnostic fistulography or PTA, 11 patients were excluded: Four because of central vein lesions, five because of arterial lesions, one because of acute thrombosis, and one because of failed PTA. Therefore, the study group consisted of 100 patients (42 men, 58 women, mean age 61 ± 12 yr). There were 69 patients with inflow vein stenosis and 31 patients with outflow vein stenosis. PTA was performed successfully in all patients without major complications.

Baseline Plasma Parameters
In each patient, blood sampling was taken for baseline plasma parameters including ADMA in the morning hours before either hemodialysis or PTA. The time interval between cessation of last dialysis and baseline blood sampling usually varied from 18 to 24 h. Only in three patients (two with restenosis and one without restenosis), the last hemodialysis session before blood sampling was terminated prematurely (<4 h) because of inadequate flow.

Compared with the 30 control subjects with normal AVF function [stenosis(−) 0.83 ± 0.17 μM], the 100 study patients had a significantly higher baseline plasma ADMA level [stenosis(+) 0.92 ± 0.18 μM; P = 0.01; Figure 1, left]. There was no significant difference in age [stenosis(+) versus stenosis(−) 61 ± 12 versus 60 ± 13 yr; P = 0.73], gender (42 versus 33% men; P = 0.47), history of hypertension (51 versus 70%; P = 0.06), history of type 2 diabetes (35 versus 40%; P = 0.68), and fistula age (42 ± 44 versus 57 ± 32 mo; P = 0.07) between these two groups.

Among the 100 study patients, those with a history of previous PTA for AVF stenosis [stenosis(+) 0.97 ± 0.20 μM] had increased baseline ADMA levels as compared with the others without previous PTA [stenosis(−) 0.88 ± 0.15 μM; P = 0.02; Figure 1, right]. The preprocedure percentage stenosis was also higher in the restenosis(+) group. There was no difference in classic risk factors, biochemical profiles, medications, and access blood flow rate before (476 ± 84 versus 464 ± 100 ml/min; P = 0.55) and immediately after PTA (738 ± 83 versus 735 ± 113 ml/min; P = 0.89) between the two groups.

Clinical Follow-up
All patients received 6 mo of clinical follow-up. No patients were lost to follow-up apart from three patients who died of pneumonia, sudden cardiac death, or suicide, respectively.

During follow-up, 46 patients had clinical findings indicative of AVF dysfunction. They received follow-up fistulography: 41 patients had restenosis at the same location, two patients had new stenosis at different locations, and three patients had no significant stenosis. The characteristics of the 41 patients with and the 59 without symptomatic restenosis are shown in Table 1. Patients with symptomatic restenosis had higher baseline ADMA levels and preprocedure percentage stenosis than those without. There were no differences in the pre- and postprocedure access blood flow rate between the two parties of patients.

Preprocedural Plasma ADMA Levels and Symptomatic Restenosis
The patients with symptomatic AVF restenosis were then grouped in tertiles according to their baseline plasma ADMA levels (tertile 1, <0.835 μM; tertile 2, 0.835 to 0.955 μM; tertile 3, >0.955 μM). As shown in Figure 2, there were eight target lesion restenoses in patients of tertile 1, 13 in tertile 2, and 20 in tertile 3, indicating the significant association of baseline ADMA levels with future recurrence of symptomatic AVF stenosis after PTA (P = 0.01, by Kaplan-Meier analysis).

On the basis of receiver operator characteristic analysis, baseline plasma ADMA level of 0.910 μM was found to be the best cutoff value for predicting symptomatic restenosis (Figure 3). Patients were divided into high (>0.910 μM) versus low (<0.910 μM) ADMA groups. The high ADMA group had 60% target lesion restenosis, compared with 25% in the low ADMA group (P < 0.001). There was no significant difference in clas-
sic risk factors, biochemical profiles, and medications between these two groups, except for longer lesion length and higher preprocedure and postprocedure stenosis in the high ADMA group (Table 2). Kaplan-Meier analysis showed that high ADMA was associated with a significant higher restenosis rate than low ADMA (P < 0.001; Figure 4).

Finally, by considering plasma ADMA levels as a continuous variable, the hazard ratio (HR) of the presence of symptomatic restenosis would be increased by 21% when baseline plasma ADMA levels increased by 0.1 μM (HR 1.21; 95% confidence interval 1.04 to 1.40; P = 0.01).

### Uni- and Multivariate Analysis of Risk Factors for Symptomatic Restenosis

In univariate Cox regression analysis, high ADMA level, high LDL cholesterol level, and high preprocedural percentage diameter stenosis were associated with increased risk for symptomatic restenosis. Then, we performed multivariate Cox regression analysis to identify the independent predictors. As shown in Table 3, only diabetes (HR 1.97; P = 0.04), LDL cholesterol level >130 mg/dl (HR 2.29; P = 0.03), and plasma ADMA level >0.910 μM (HR 2.65; P = 0.005) could independently predict restenosis after PTA.

### DISCUSSION

The findings of our study showed that in addition to type 2 diabetes and elevated LDL cholesterol level, elevated baseline plasma ADMA is an important independent predictor of symptomatic restenosis after PTA for dysfunctional AVFs. In particular, patients with plasma ADMA levels >0.91 μM were identified with a higher restenosis rate. Our findings for the first time indicated the connection between baseline plasma...
ADMA and the consequent development of symptomatic restenosis after PTA on dysfunctional AVFs and suggest the potential pathogenesis role of ADMA and endothelial dysfunction to AVF restenosis.

Although PTA is effective in treating stenotic lesions in dialysis fistulas, its benefit could be attenuated by a high restenosis rate within 6 mo.10,11 The mechanisms of restenosis in arterial systems have been widely investigated; however, direct application of these mechanisms to the venous segment of AVFs may not be valid. At the anatomic level, veins tend to have a less well-defined internal elastic lamina.12 Veins also tend to produce less NO and prostacyclin, which could predispose them to endothelial injury.13 In addition, the presence of uremia could predispose to endothelial dysfunction.14 In contrast to the development of restenosis in arterial systems, there is no correlation between restenosis in AVFs and cardiovascular risk factors or anatomic factors, except for the poorer patency that has been observed in longer lesions.15,16 Genotype polymorphisms of TGF-β1, methylene tetrahydrofolate reductase, and heme oxygenase 1 have been identified as independent predictors for AVF stenosis.3–5 Nonetheless, in a substantial portion of patients, the causes of rapid progression and individual variations in the development of restenosis remain unknown.

Since 1992, there has been considerable interest in the pathophysiologic relevance of ADMA in vascular diseases.17 Its pathogenic role in vascular damage is now supported by a variety of experimental data in animals and in vitro models as well as by observational studies in various disease states.18–21 It is theorized that ADMA is involved in endothelial dysfunction through competition with L-arginine as the substrate for NO synthase, resulting in decreased production of endothelium-dependent NO. The endothelium plays a crucial role in the maintenance of vascular homeostasis, and NO is the most important mediator of this process. The deprivation of NO production has several possible effects, including platelet aggregation and adhesion, leukocyte adhesion, smooth muscle proliferation, and extracellular matrix formation, which may contribute to the progression of atherosclerosis;22,23 however, until now, there has been no direct evidence linking ADMA and NO synthesis in vivo in humans.

The pathogenesis of restenosis after balloon injury is somewhat different from that of atherosclerosis. Restenotic lesions after balloon injury are characterized by neointimal hyperplasia, originating from smooth muscle cell migration, proliferation, and extracellular matrix accumulation.14,24,25 In animal studies, impaired NO bioavailability plays a critical role in the complex process of restenosis.26 Administration of L-arginine may reduce neointimal formation and vascular remodeling after arterial injury by balloon angioplasty, and the beneficial effect is abolished by administration of inhibitors of NO synthase.27 In addition, restoration of NO production by adenovirus-mediated NO synthase gene transfer significantly reduced luminal narrowing after balloon angioplasty in animals.28,29 All of these animal studies suggest that vascular NO deficiency may be related to restenosis after balloon angioplasty. Clinical reports in humans to support this linkage are rare. In one study with patients receiving percutaneous coronary intervention (PCI), plasma ADMA levels independently predicted subsequent cardiovascular events, most of them coming from target lesion restenosis.30 In another study with patients undergoing PCI, intramural delivery of L-arginine caused significant reduction in neointimal volume as measured by intravascular ultrasound 6 mo after PCI.31 Nonetheless, to answer the question of causality with certainty, it will be
necessary to investigate whether restenosis could be attenuated by therapeutic strategies that decrease plasma ADMA levels. Thus, it remains elusive whether this molecule is simply a bystander or a contributor to restenosis.

In our study, inflammatory markers such as plasma high-sensitivity C-reactive protein (hs-CRP) levels were not related to the development of restenosis after PTA. This differs from the results of previous studies of arterial angioplasty, because most of them have shown an association between elevated plasma hs-CRP levels and adverse clinical outcomes. In our study, inflammatory markers such as plasma high-sensitivity C-reactive protein (hs-CRP) levels were not related to the development of restenosis after PTA. This differs from the results of previous studies of arterial angioplasty, because most of them have shown an association between elevated plasma hs-CRP levels and adverse clinical outcomes. There are several possible reasons for this discrepancy. Although CRP is an independent predictor of adverse clinical outcomes after PCI, lack of angiographic follow-up has prevented the determination of whether the adverse outcome was due to restenosis of the target lesion or progression of atherosclerosis. The majority of arterial vascular disease is characterized by atherosclerosis with various inflammatory components; however, in the venous segment of AVFs, both primary and restenotic lesions are characterized by neointimal hyperplasia, which differs from characteristic atherosclerotic lesions.24,25 The results of our study further suggest that inflammation may play a less dominant role in the pathogenesis of venous restenosis. Hyperhomocysteinemia has also been proposed as a novel risk factor for atherosclerosis in the general population.33 None-theless, its role in patients with ESRD remains controversial.34 Previous studies investigating the association between homocysteine and restenosis or clinical outcomes after PCI also showed the conflicting results. In our study, there was no association between plasma homocysteine levels and symptomatic restenosis after PTA. Our findings concerning the associations among inflammation, homocysteine, and restenosis after PTA should then be confirmed in a larger cohort. Plasma

### Table 2. Patient characteristics and the events at follow-up according to baseline plasma ADMA level

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>ADMA &gt;0.91 μM</th>
<th>ADMA &lt;0.91 μM</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr; mean ± SD)</td>
<td>62 ± 12</td>
<td>61 ± 13</td>
<td>0.520</td>
</tr>
<tr>
<td>Gender (men/women)</td>
<td>22/23</td>
<td>20/35</td>
<td>0.230</td>
</tr>
<tr>
<td>Risk factors (n [%])</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>hypertension</td>
<td>24 (53)</td>
<td>27 (49)</td>
<td>0.690</td>
</tr>
<tr>
<td>diabetes</td>
<td>17 (38)</td>
<td>18 (33)</td>
<td>0.400</td>
</tr>
<tr>
<td>current smoker</td>
<td>3 (7)</td>
<td>5 (9)</td>
<td>1.000</td>
</tr>
<tr>
<td>Plasma biochemical data (mean ± SD)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>110 ± 32</td>
<td>104 ± 30</td>
<td>0.340</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>50 ± 13</td>
<td>53 ± 20</td>
<td>0.310</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>170 ± 87</td>
<td>158 ± 95</td>
<td>0.510</td>
</tr>
<tr>
<td>calcium (mg/dl)</td>
<td>9.95 ± 0.97</td>
<td>9.90 ± 0.91</td>
<td>0.800</td>
</tr>
<tr>
<td>phosphate (mg/dl)</td>
<td>4.52 ± 1.59</td>
<td>4.36 ± 1.58</td>
<td>0.620</td>
</tr>
<tr>
<td>albumin (mg/dl)</td>
<td>3.58 ± 0.38</td>
<td>3.63 ± 0.47</td>
<td>0.560</td>
</tr>
<tr>
<td>creatinine (mg/dl)*</td>
<td>10.30 ± 2.50</td>
<td>10.50 ± 1.90</td>
<td>0.750</td>
</tr>
<tr>
<td>Kt/V</td>
<td>1.69 ± 0.38</td>
<td>1.79 ± 0.44</td>
<td>0.240</td>
</tr>
<tr>
<td>Medications (n [%])</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>antiplatelet agents</td>
<td>16 (36)</td>
<td>17 (31)</td>
<td>0.530</td>
</tr>
<tr>
<td>nitrates</td>
<td>5 (11)</td>
<td>7 (13)</td>
<td>0.990</td>
</tr>
<tr>
<td>β blockers</td>
<td>5 (12)</td>
<td>12 (22)</td>
<td>0.280</td>
</tr>
<tr>
<td>calcium antagonists</td>
<td>9 (20)</td>
<td>12 (22)</td>
<td>0.990</td>
</tr>
<tr>
<td>ACEI/ARB</td>
<td>4 (9)</td>
<td>8 (15)</td>
<td>0.540</td>
</tr>
<tr>
<td>lipid-lowering agents</td>
<td>4 (9)</td>
<td>10 (18)</td>
<td>0.260</td>
</tr>
<tr>
<td>Lesion characteristics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>fistula age (mo; mean ± SD)</td>
<td>38 ± 50</td>
<td>44 ± 37</td>
<td>0.490</td>
</tr>
<tr>
<td>upper arm lesion (n [%])</td>
<td>15 (33)</td>
<td>16 (29)</td>
<td>0.660</td>
</tr>
<tr>
<td>reference diameter (mm; mean ± SD)b</td>
<td>6.52 ± 1.00</td>
<td>6.46 ± 0.95</td>
<td>0.770</td>
</tr>
<tr>
<td>lesion length (cm; mean ± SD)</td>
<td>2.16 ± 0.60</td>
<td>1.78 ± 0.76</td>
<td>0.008</td>
</tr>
<tr>
<td>preprocedure stenosis (%; mean ± SD)</td>
<td>76.30 ± 10.00</td>
<td>69.80 ± 11.80</td>
<td>0.004</td>
</tr>
<tr>
<td>postprocedure stenosis (%; mean ± SD)</td>
<td>12.30 ± 11.00</td>
<td>7.90 ± 9.60</td>
<td>0.030</td>
</tr>
<tr>
<td>Plasma biomarkers (mean ± SD)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>hs-CRP (mg/dl)</td>
<td>0.78 ± 0.89</td>
<td>0.80 ± 1.03</td>
<td>0.690</td>
</tr>
<tr>
<td>homocysteine (μM)</td>
<td>20.90 ± 5.60</td>
<td>19.70 ± 8.10</td>
<td>0.830</td>
</tr>
<tr>
<td>ADMA (μM)</td>
<td>1.06 ± 0.15</td>
<td>0.80 ± 0.09</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Events at follow-up (n [%])</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>target lesion restenosis</td>
<td>27 (60)</td>
<td>14 (25)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>access re-intervention</td>
<td>27 (60)</td>
<td>16 (29)</td>
<td>0.002</td>
</tr>
</tbody>
</table>

*aPredialysis creatinine level.

*bAdjacent segment of normal veins upstream of the stenosis.
ADMA levels have been shown to be associated with a variety of cardiovascular risk factors\(^{17}\); however, in our study, plasma ADMA was not correlated with most of these traditional risk factors. Most of the cardiovascular risk factors were unable to explain adequately the rapid progression and individual variation in the development of symptomatic restenosis in AVFs, except for a mildly increased risk in patients with diabetes and/or high baseline plasma LDL cholesterol levels. In contrast, plasma ADMA was the most predictive of symptomatic restenosis. One possible explanation is that ADMA may mediate the effect of many risk factors on the NO synthase pathway and, in consequence, represents the summative effect of various risk factors on endothelial function.

There are several potential mechanisms for ADMA retention.\(^{8}\) First, dietary factors are associated with plasma ADMA levels, such as high-carbohydrate or high-fat meals.\(^{35,36}\) In addition, approximately 80% of ADMA is degraded by dimethylarginine dimethylaminohydrolase, which is impaired by oxidative stress. Various risk factors could attenuate dimethylaminohydrolase activity \(in\) \(vivo\) and \(in\) \(vitro\), permitting elevation of ADMA.\(^{8}\) Finally, accumulation as a result of impaired renal clearance might be the most important source of increased plasma ADMA level in patients with uremia. Although ADMA can be removed during hemodialysis, plasma levels are still much higher than in healthy control subjects.\(^{8,37}\) The accumulation of plasma ADMA in patients with ESRD might explain why ADMA is a more significant determinant of restenosis than traditional risk factors in this situation.

The plasma concentrations of ADMA in our patients had a very narrow concentration distribution and were very similar to those reported in Japanese hemodialysis patients.\(^{38}\) The ADMA concentrations in the patients of this study were approximately two-fold that reported previously in Taiwanese Chinese nondialysis patients with either cardiac syndrome X or coronary artery disease\(^{30,39}\); however, some other studies have reported much higher ADMA levels in hemodialysis patients. Multiple factors, including ethnicity, may have profound impacts on the difference in plasma ADMA levels.\(^{8}\) Although the plasma ADMA levels in our patients were much lower than that required for physiologic or pathologic effects, the intracellular ADMA level in endothelial cells may be much higher.\(^{10,41}\) Nonetheless, the wide variability in ADMA levels may make a reference value hard to define and hence preclude its application as a common prognostic marker for hemodialysis patients.

Some limitations should be considered in the interpretation of our study results. First, our patients were followed up mainly for the clinical symptoms and signs suggesting fistula dysfunction. Only fistulas that had clinical evidence of dysfunction after the initial angioplasty were studied angiographically for restenosis; therefore, we cannot exclude the possible presence of silent restenosis in some fistulas that were not restudied. We did, however, identify a particular group of patients with so-called “symptomatic AVF restenosis” after PTA. They could be easily recognized during routine hemodialysis. Second, substantial ethnic variations in plasma ADMA levels have been reported, and this variation might hamper the application of our cutoff value to other populations. Third, our cohort was relatively small, and the number of events during the follow-up period was limited. Fourth, in this study, only preprocedure ADMA levels were measured, which did not necessarily represent the levels in the whole follow-up period. It is not known whether ADMA levels may change with time, and such changes, if there were any, may contribute to the development of fistula dysfunction in these patients; however, baseline ADMA level was shown to be elevated in patients with a history of fistula stenosis as compared with those without and also elevated in patients with symptomatic restenosis of fistula later at PTA than in those without. It is likely that the processes occurring just before, at the time of, or shortly after PTA could be important to long-term prognosis. Fifth, for the large number of samples and for standardization of analysis, ELISA was used in our study for ADMA determination, rather than the more precise mass spectrometric detection with liquid chromatography. We cannot completely exclude the possibility that ELISA may be detecting other factors that could be responsible for the observed difference. Finally, although we did show that ADMA could be critical to the development of symptomatic restenosis after PTA, there is still no evidence that modifying ADMA levels will reduce the rate of restenosis.

The most important clinical implication of this study is that elevated plasma ADMA and consequent endothelial dysfunction are a possible pathogenic mechanism for the rapid progression of symptomatic restenosis in AVFs. On the basis of this putative mechanism, methods of modifying ADMA levels or improving endothelial dysfunction, such as L-arginine, statins, and blockade of the renin-angiotensin system,\(^{39}\) could be investigated as ways of preventing recurrent AVF dysfunction. Recently, blockade of the renin-angiotensin system with angiotensin-converting enzyme inhibitors was retrospectively shown to prolong primary patency of vascular access.\(^{42}\)
Accordingly, future studies for the causal role of ADMA and endothelial function are warranted to determine whether modulation of ADMA or endothelial function could be a potential strategy to prevent AVF dysfunction and particularly restenosis after PTA in hemodialysis patients.

### CONCISE METHODS

#### Study Participants

From January 2006 to July 2007, a series of patients who had dysfunctional native AVFs and were referred to our institute for PTA were prospectively enrolled into this study. Patients were referred from our hemodialysis center and two nearby hemodialysis centers on the basis of the clinical symptoms and signs suggesting fistula dysfunction (decreased thrill, increased pulsatility, development of collateral veins, limb swelling, and prolonged bleeding from puncture sites) with one or more of the following criteria: Reduction of flow rate of >25% from baseline access flow, total access blood flow rate of <500 ml/min by ultrasound dilution method (Transonic Flow-QC; Transonic Systems, Ithaca, NY), and increased venous pressure during dialysis (dynamic venous pressure exceeded threshold levels for three consecutive times). The participant patients had to have received regular dialysis treatment for at least 6 mo without clinical evidence of acute or chronic inflammation, recent myocardial infarction or unstable angina, or circulatory congestion. According to the same criteria, another 30 patients with patent native AVF and no history of fistula dysfunction and stenosis were also evaluated. They served as the control subjects for ADMA analysis.

#### Study Protocol

Patients who were eligible for this study were scheduled for diagnostic fistulography (Advantx; GE Healthcare, Buc Cedex, France) and PTA when indicated on a midweek nondialysis day. Data on baseline characteristics and blood samples were collected on the same morning as fistulography. Blood samples were drawn after a 12-h overnight fast.
and withdrawal of medications and before diagnostic or intervention procedures. Cigarette smoking and consumption of beverages containing alcohol or caffeine were also avoided for at least 12 h. Diagnostic fistulography and PTA were performed using standard procedures. After diagnostic fistulography or PTA, patients with insignificant stenosis (<50% diameter stenosis), thrombosed fistulas, arterial side stenosis, or central vein lesions or those who failed to achieve clinical and anatomic success were excluded. Diagnostic fistulography and angiograms of PTA procedures were independently reviewed by another expert angiographer, who was unaware of the patients’ clinical and analytic data. The degree of stenosis was evaluated by two orthogonal planes, and the greatest degree of stenosis was used for subsequent anatomic measurements. Anatomic measurements were made with use of a calibrated reference maker or computer-assisted edge detection software within the angiographic imaging system (Digital DLX; GE Healthcare). The reference vessel was defined as an adjacent segment of normal vein located upstream from the target lesion. The degree of stenosis was reported as the maximum diameter reduction compared with the reference vessel diameter.

After the procedure, aspirin was prescribed for 3 d. Medications for underlying cardiovascular disease or chronic kidney disease were continued. The study was based on the Declaration of Helsinki (edition 6, revised 2000). Informed consent was obtained from all study participants, and this study was approved by the institutional research board of our hospital.

Laboratory Methods
For each patient, a 20-ml blood sample was drawn in the morning. Blood sampling was done after 30 min of quiet rest in a semirecumbent position. The blood samples were centrifuged at 3000 rpm for 10 min at 4°C immediately after collection. The plasma samples were then stored at −80°C until use.

Plasma biochemical parameters including LDL cholesterol, HDL cholesterol, triglycerides, calcium, phosphate, and albumin were analyzed. Plasma hs-CRP levels were measured with a commercially available kit (Dade Behring, Marburg, Germany). The upper normal value of hs-CRP is 0.5 mg/dl in our laboratory. Plasma levels of homocysteine were measured by enzyme immunoassay (Axis Homocysteine EIA; Axis-Shield AS, Oslo, Norway), which has been shown to correlate well with HPLC with a correlation coefficient of 0.94. Plasma levels of ADMA were determined with commercially available ELISA kits (DLD Diagnostika, Hamburg, Germany). The correlation coefficient between liquid chromatography–mass spectrometry ADMA and ELISA ADMA is 0.98. The recovery rate for ADMA was >90%, and the within-assay and between-assay variation coefficients were not more than 7 and 8%, respectively.

Follow-up and Definitions
After the PTA procedure, all of the participants of this study were prospectively followed for 6 mo under the same protocol at respective hemodialysis centers. Follow-up surveillance included physical examination and dynamic venous pressure monitoring at each hemodialysis session, and transonic examination of access blood flow rate immediately, then monthly after intervention. The referring nephrologists were blind to the participants’ ADMA levels. When abnormal clinical or hemodynamic parameters that fulfilled the original referring criteria were detected, patients were referred for repeat fistulography and PTA as appropriate.

Procedural success was defined as the combination of anatomic and clinical success. Anatomic success was defined as <30% residual stenosis. Clinical success was defined as an improvement from baseline in clinical or hemodynamic parameters (e.g., blood flow, venous pressure) that were the initial indicators of access dysfunction. Restenosis was defined as ≥50% diameter reduction at the target lesion within the first 6 mo after PTA. These definitions were in accordance with the guidelines of the Society of Interventional Radiology.45

Statistical Analysis
All data are presented as means ± SD and percentages. We compared categorical data using the χ2 test with Yates correction and Fisher exact test as appropriate. We compared continuous variables using unpaired t test for normally distributed data and Mann-Whitney U tests for non-normally distributed data. To establish a cutoff point between low and high levels, we related percentiles of ADMA values and the rate of restenosis-free survival via a receiver operator characteristic curve. We calculated survival curves of proportions without restenosis by the Kaplan-Meier method and compared them by the log-rank test. We performed univariate and multivariate Cox proportional hazards regression analysis to determine independent predictors of target lesion restenosis for all patients studied. All of the variables with P < 0.2 by univariate analysis were included in the multivariate model. HRs and 95% confidence intervals were calculated. P < 0.05 was considered to be statistically significant. We used the STATISTICA 7.0 software package (StatSoft, Tulsa, OK) for statistical analysis.

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DISCLOSURES
None.

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