**Association Between Thrombotic Microangiopathy and Reduced ADAMTS13 Activity in Malignant Hypertension**

Bert-Jan H. van den Born, Niels V. van der Hoeven, Evelyn Groot, Peter J. Lenting, Joost C.M. Meijers, Marcel Levi, Gert A. van Montfrans

**Abstract**—The thrombotic microangiopathy observed in malignant hypertension is similar to that of thrombotic thrombocytopenic purpura, which is associated with a deficiency of ADAMTS13, a von Willebrand factor (VWF)—cleaving protease that cleaves large prothrombogenic multimers. We hypothesized that ADAMTS13 is deficient in malignant hypertension and that the severity of thrombotic microangiopathy is associated with decreased ADAMTS13 activity. We included 20 patients with malignant and 20 patients with severe hypertension, and 20 matched normotensive individuals served as control subjects. VWF, active VWF, and free hemoglobin were assessed to explore predictors of ADAMTS13 activity. Patients with malignant hypertension had lower ADAMTS13 activity (80% interquartile range: 53% to 130%) compared with control subjects (99% interquartile range: 82% to 129%; P<0.01) but not compared with patients with severe hypertension (P=0.14). ADAMTS13 activity negatively correlated with lactic dehydrogenase levels after logarithmic transformation (r=−0.65; P<0.001) and was associated with platelet count (r=0.34; P=0.04) and the presence of schistocytes (r=−0.37; P=0.02). Apart from the association with thrombotic microangiopathy, ADAMTS13 was inversely associated with creatinine (r=−0.42; P=0.008). Increasing levels of VWF were associated with a decrease in ADAMTS13 activity (r=−0.34; P=0.03). There was no significant association between ADAMTS13 activity and other parameters, including blood pressure. In conclusion, ADAMTS13 is decreased in malignant hypertension and associated with the severity of thrombotic microangiopathy, likely because of the release of VWF after endothelium stimulation. A severe deficiency could not be demonstrated. More studies are needed to identify the role of ADAMTS13 in the thrombotic microangiopathy and ischemic complications of malignant hypertension. *(Hypertension. 2008;51:862-866.)*

**Key Words:** hypertensive crisis ■ thrombotic microangiopathy ■ coagulation ■ kidney failure ■ endothelium

Malignant hypertension is a condition characterized by severe hypertension and acute ischemic complications and frequently complicated by a thrombotic microangiopathy (TMA). TMA is characterized by thrombosis of small vessels, intravascular hemolysis with fragmentation of red blood cells (schistocytes), elevated lactic dehydrogenase (LDH) levels, and consumption of platelets. TMA was observed in 27% of patients with malignant hypertension presenting at the emergency department of our hospital and was associated with renal impairment at presentation but an increased recovery of renal function during follow-up.1 The pathogenesis of TMA in malignant hypertension is incompletely understood. Several mechanisms may lead to the TMA associated with malignant hypertension.

Malignant hypertension bears resemblance to thrombotic thrombocytopenic purpura (TTP). TTP is associated with a congenital or acquired deficiency of ADAMTS13, a zinc-containing metalloprotease that cleaves large von Willebrand factor (VWF) multimers, thereby decreasing their prothrombogenic properties.2,3 Deficiency of ADAMTS13 (activity) leads to the appearance of unusually large prothrombogenic multimers (UL-VWF) in the circulation resulting in thrombocytopenia, intravascular hemolysis, and ischemic microvascular complications because of thrombus formation.4 First, a secondary deficiency of ADAMTS13 activity has been demonstrated in situations where high levels of circulating VWF are present as a result of endothelial stimulation or damage.5,6 This may suggest that, in a state of marked endothelial activation or damage, such as malignant hypertension, a deficiency of ADAMTS13 activity may develop as a result of binding to the A2 domain of the VWF molecule. Second, apart from the association with VWF, high fluid shear rates may result in conformational changes of the VWF molecule and promote binding of ADAMTS13, thereby reducing its activity.7 Recently, an antibody fragment has been developed that allows quantification of this conforma-
tional change. This conformational change of the VWF molecule is known as “active VWF.”8 Finally, the degree of extracellular free hemoglobin (Hb) that may accompany the destruction of red blood cells has been shown to inhibit ADAMTS13 in vitro and may also be responsible for a secondary deficiency of ADAMTS13 in vivo.9

We hypothesized whether ADAMTS13 is deficient in malignant hypertension and associated with TMA severity. In addition, we explored possible mechanisms influencing ADAMTS13 activity by measuring VWF, active VWF, free Hb, and multimer size.

Patients and Methods

Study Population

We used a prospective case-control design to compare patients with malignant hypertension, defined according to the World Health Organization criteria as severe hypertension (blood pressure [BP] >120 mm Hg diastolic) with bilateral linear or flame-shaped hemorrhages or “cotton-wool” exudates with or without papilledema, on funduscopic examination, with patients having severe hypertension (BP >120 mm Hg diastolic) without these retinal lesions. Normotensive control subjects were included to differentiate between the ischemic complications corroboration malignant hypertension and the influence of BP. To assess TMA severity, we determined LDH levels, platelet count, and schistocytes at presentation to determine associations between these TMA parameters and ADAMTS13 activity. The primary outcome measure was the difference in ADAMTS13 activity between BP groups. The second outcome measure was the association between ADAMTS13 activity and TMA severity. To explore the mechanisms leading to a deficiency of ADAMTS13, we assessed VWF, active VWF, free Hb, and VWF multimer size and assessed the influence of these parameters on ADAMTS13 activity.

We included 20 patients with malignant hypertension and 20 patients with severe hypertension, presenting at a large teaching hospital serving a multiethnic community in Amsterdam, the Netherlands. Twenty age-, sex-, and ethnicity-matched normotensive persons served as control subjects. Excluded were patients <18 years of age, pregnant women, and patients on dialysis before admission. BP was measured using an aneroid sphygmomanometer in the recumbent position. Retinal examinations were carried out by myriadiasis by an ophthalmologist. Schistocytes were considered present if “several” red cell fragments could be detected on a peripheral blood film. Macroalbuminuria was defined as urinary protein excretion >300 mg/L or 2+ for dipstick proteinuria. Left ventricular hypertrophy was defined according to the Sokolow-Lyon criteria. Additional screening for secondary hypertension consisted of imaging studies of the renal arteries in 25 patients (66%), ≥1 endocrine test in 16 patients (42%), and renal biopsy in 7 patients (18%).

All of the blood samples were taken just before initiating antihypertensive treatment and were immediately analyzed or frozen as plasma at −80°C. The study was approved by the local medical ethics committee. Informed consent was obtained from all of the participants.

Laboratory Procedures

ADAMTS13 activity was determined using the rapid fluorescence resonance energy transfer assay.10 The reference range was 54% to 150% in 60 healthy individuals (SD: 26.5%), the intra-assay variation was 1% to 6% (3% to 6% in the lower range), and the interassay variation was 2% to 5% (3% to 6% in the lower range). VWF antigen was determined by ELISA (Dako). Active VWF was determined by immunosorbent assay using an antibody fragment (AU/VWFa-11) that specifically recognizes the glycoprotein Ib-β binding configuration, as described previously.8 This antibody fragment allows for the detection of active VWF in the plasma of patients characterized by spontaneous VWF-platelet interactions and discriminates active from resting VWF. The ratio of the slope for different plasma samples over the slope for normal pooled plasma was calculated and represents the relative amount of active VWF. In previous experiments, the intra-assay variation was 7.1% and the interassay variation was 13.7%. Free Hb was assessed in plasma by absorption spectrophotometry using a cyanide-free hemoglobin reagent (Cell Dyn 4000, Abbott Diagnostics). Reference values are 5.0 to 28.0 μmol/L. Compared with a standardized serial dilution, linearity was R²=0.996 in the reference range (0 to 1.0 mmol/L) and R²=0.984 in the lower range (0 to 30.0 μmol/L). The coefficient of variation is 10.9% at a range of 17.0 μmol/L (SD: 1.8 μmol/L). False-positive results can be obtained with lipemic or hemolytic plasma and were considered if free Hb concentration was ≥40 μmol/L. The multimeric pattern of VWF was analyzed using 2.5% agarose gel electrophoresis, followed by immunoblotting according to methods described previously.11

Statistical Analysis

A sample size of 20 patients in each group was calculated to allow detection of a difference in ADAMTS13 activity of ≥1 SD between BP groups (26.5%) with a 80% power and α level of 0.05. Baseline variables were described using mean and SD, median and range, or interquartile range for variables with a skewed distribution. Baseline differences were calculated to compare patients with severe and malignant hypertension using an independent samples t test for parametric and Mann-Whitney U test for nonparametric distributions where appropriate. Categorical variables were calculated using χ² with Yates’ correction. We assessed differences in ADAMTS13 activity between BP groups using 1-way ANOVA followed by Tukey’s test for multiple comparisons to assess differences between BP groups. Linear regression analysis was used to assess the association between ADAMTS13 activity and LDH, platelet count, and schistocytes expressing TMA severity and renal insufficiency between those with severe and malignant hypertension. To assess predictors of ADAMTS13, the univariate correlations with ADAMTS13 were tested. The bivariate interactions between predictors of ADAMTS13 were also determined. Finally, cross-correlations were assessed to explore the associations among ADAMTS13 activity, VWF, active VWF, and free Hb. P<0.05 was considered to indicate a statistically significant difference. For statistical analysis, the SPSS software package for Windows was used, version 14.0.

Results

The clinical characteristics of the patients with malignant hypertension, patients with severe hypertension, and normotensive control subjects are shown in Table 1. Two patients with severe hypertension had TMA, as evidenced by a low platelet count, elevated LDH, and fragmented red blood cells in a peripheral blood smear. A secondary cause for the hypertension was established in 5 patients (25%) with malignant hypertension and in 3 patients (15%) with severe hypertension. Secondary causes of hypertension included IgA nephropathy (1 patient), renal artery stenosis because of polyarteritis nodosa (1 patient), bilateral hydropnephrosis (1 patient), a cortisol producing adrenal carcinoma (1 patient) and corticoid therapy (1 patient) in the malignant hypertensive group and IgA nephropathy (2 patients) and systemic lupus erythematous nephritis (1 patient) in the group with severe hypertension.

The individual data for ADAMTS13 activity by BP group are depicted in Figure 1. Patients with malignant hypertension had lower ADAMTS13 activity (80%; interquartile range: 53% to 130%) compared with control subjects (99%; interquartile range: 82% to 129%; P<0.01) but not compared with patients with severe hypertension (P=0.14). The association
between ADAMTS13 activity and log-LDH is depicted in Figure 2. ADAMTS13 activity significantly and negatively correlated with log-LDH ($r = -0.65; P = 0.001$), explaining 42% of its variations. ADAMTS13 activity was also associated with platelet count ($r = 0.34; P = 0.04$) and the presence of schistocytes ($r = -0.37; P = 0.02$). Correcting for LDH, platelet count, or schistocytes removed the association between ADAMTS13 activity and BP groups ($P = 0.10$ for all of the variables). Apart from the association with these TMA parameters, ADAMTS13 was negatively associated with creatinine ($r = -0.42; P = 0.008$).

The only variable that had a significant association with ADAMTS13 activity was VWF; increasing VWF levels were associated with a decrease in ADAMTS13 activity ($r = -0.34; P = 0.03$). Associations between ADAMTS13 and active VWF ($r = -0.22; P = 0.17$), free Hb ($r = 0.12; P = 0.44$), diastolic BP ($r = -0.26; P = 0.11$), and systolic BP ($r = -0.19; P = 0.24$) were not significant. The cross-correlations among ADAMTS13, VWF, active VWF, and free Hb are depicted in Table 2. The multimeric pattern of VWF was analyzed in 8 patients with the lowest ADAMTS13 activity and in a patient with the highest active VWF. However, no difference in multimer size could be demonstrated in these patients. Seven free Hb samples showed abnormal high values ($\geq 40 \mu\text{mol/L}$), 3 of which were macroscopically lipemic. The results were not different after excluding these samples.

### Table 1. Patient Characteristics by BP Category

<table>
<thead>
<tr>
<th>Patient Characteristics</th>
<th>Normotensive Control Subjects (n=20)</th>
<th>Severe Hypertension (n=20)</th>
<th>Malignant Hypertension (n=20)</th>
<th>$P$ (MHT vs SHT)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean±SD, y</td>
<td>43±9</td>
<td>46±15</td>
<td>42±11</td>
<td>0.34</td>
</tr>
<tr>
<td>Male/female</td>
<td>11/9</td>
<td>11/9</td>
<td>15/5</td>
<td>0.32</td>
</tr>
<tr>
<td>Black, n (%)</td>
<td>10 (50)</td>
<td>12 (60)</td>
<td>10 (50)</td>
<td>0.75</td>
</tr>
<tr>
<td>Systolic BP, mean±SD, mm Hg</td>
<td>125±11</td>
<td>226±20</td>
<td>229±23</td>
<td>0.74</td>
</tr>
<tr>
<td>Diastolic BP, mean±SD, mm Hg</td>
<td>80±9</td>
<td>133±14</td>
<td>150±14</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Current smoker, n (%)</td>
<td>2 (10)</td>
<td>5 (25)</td>
<td>5 (25)</td>
<td>1.00</td>
</tr>
<tr>
<td>Diabetes, n (%)</td>
<td>0</td>
<td>2 (10)</td>
<td>1 (5)</td>
<td>1.00</td>
</tr>
<tr>
<td>Previous hypertension, n (%)</td>
<td>14 (70)</td>
<td>7 (35)</td>
<td>1.06</td>
<td>0.06</td>
</tr>
<tr>
<td>On active treatment, n (%)</td>
<td>4 (20)</td>
<td>0</td>
<td>0.11</td>
<td></td>
</tr>
<tr>
<td>Serum creatinine, median (range), $\mu\text{mol/L}$</td>
<td>92 (72 to 142)</td>
<td>149 (74 to 1545)</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>Macroalbuminuria, n (%)*</td>
<td>5 (28)</td>
<td>17 (85)</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>Left ventricular hypertrophy, n (%)</td>
<td>8 (40)</td>
<td>17 (85)</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>Hemoglobin, median (range), mmol/L</td>
<td>9.3 (7.3 to 10.9)</td>
<td>8.6 (4.0 to 10.3)</td>
<td>0.17</td>
<td></td>
</tr>
<tr>
<td>Platelets, median (range), $\times10^9/\text{L}$†</td>
<td>234 (102 to 363)</td>
<td>246 (45 to 345)</td>
<td>0.76</td>
<td></td>
</tr>
<tr>
<td>Lactic dehydrogenase, median (range), U/L‡</td>
<td>226 (126 to 471)</td>
<td>325 (161 to 1467)</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>Schistocytes, n (%)</td>
<td>2 (10)</td>
<td>6 (30)</td>
<td>0.24</td>
<td></td>
</tr>
</tbody>
</table>

$P$ values are calculated for differences between patients with severe and malignant hypertension. MHT indicates malignant hypertension; SHT, severe hypertension.

*Data are missing for 2 in the group with severe hypertension.
†Data are missing for 1 patient in the group with malignant hypertension.
‡Data are missing for 2 patients in the severe hypertension group.

---

**Figure 1.** Plasma levels of ADAMTS13 activity in the 3 BP groups. The solid line represents median values. $P < 0.01$ malignant hypertension vs controls; $P = 0.14$ vs severe hypertension.

**Figure 2.** Correlation between ADAMTS13 activity and log (LDH). The solid line represents the regression line with the 95% CI ($R^2=0.42; P<0.001$).
ADAMTS13 activity in Malignant Hypertension

Table 2. Cross-Correlations Among ADAMTS13 Activity, VWF, Active VWF, and Free Hb in Patients With Malignant and Severe Hypertension

<table>
<thead>
<tr>
<th>Factor</th>
<th>ADAMTS13</th>
<th>VWF</th>
<th>Active VWF</th>
<th>Free Hb</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADAMTS13</td>
<td>1.00</td>
<td>−0.34</td>
<td>−0.22 (P=0.03)</td>
<td>0.12 (P=0.44)</td>
</tr>
<tr>
<td>VWF</td>
<td>1.00</td>
<td>1.00</td>
<td>0.63 (P&lt;0.001)</td>
<td>−0.02 (P=0.91)</td>
</tr>
<tr>
<td>Active VWF</td>
<td>1.00</td>
<td>1.00</td>
<td>−0.10 (P=0.51)</td>
<td>1.00</td>
</tr>
<tr>
<td>Free Hb</td>
<td>1.00</td>
<td>1.00</td>
<td>0.22 (P=0.03)</td>
<td>0.10 (P=0.44)</td>
</tr>
</tbody>
</table>

Values represent regression coefficients with P values.

Discussion

We have shown that ADAMTS13 activity is decreased in patients with malignant hypertension compared with normotensive control subjects and that it is associated with TMA severity. Correction for any of the TMA parameters removed the association between ADAMTS13 and BP groups. The presence of TMA in some patients who had severe hypertension but lacked retinal abnormalities may explain why the difference in ADAMTS13 activity between patients with malignant and severe hypertension was not significant. This indicates that the ischemic retinal changes corroborating malignant hypertension may not fully discriminate patients with TMA from patients without TMA. A severe deficiency of ADAMTS13, such as that observed in TTP, could not be demonstrated. Therefore, measurement of ADAMTS13 activity may help to discriminate between TTP and malignant hypertension.

To explore the mechanisms leading to a decrease in ADAMTS13 activity, we assessed several parameters that are implicated in decreasing ADAMTS13 or that inhibit its activity. Of these parameters, only VWF showed a significant association with ADAMTS13. Increased VWF levels were associated with a decreased activity of ADAMTS13. Although a severe ADAMTS13 deficiency was not demonstrated in our study, recent experiments suggest that the balance between ADAMTS13 and VWF may be more important than the concentrations of these parameters alone. It has been established that deficiency of ADAMTS13 alone is not sufficient to develop TMA. Mice that lack ADAMTS13 also require stimulation with epinephrine or collagen and appropriate flow conditions to develop TMA. In humans, patients with a clinical diagnosis of TTP syndrome often have additional disorders (eg, infections) that stimulate the endothelium and may provoke TTP relapses. The endothelial stimulus that initiates the chain of events that lead to TMA may involve VWF. In healthy volunteers, an acute increase in VWF elicited by administration of either desmopressin or endotoxin has been shown to provoke a concomitant decrease in ADAMTS13 activity. This is also associated with the appearance of UL-VWF multimers despite relatively normal ADAMTS13 activity. A (severe) secondary deficiency has also been established in patients with severe sepsis and diffuse intravascular coagulation. The presence of UL-VWF multimers, which were determined in those with an ADAMTS13 activity <20%, was not associated with ADAMTS13 activity in that range. Because VWF levels were not reported, an association between ADAMTS13 activity and VWF remains to be established in these patients. In conclusion, the interaction between VWF and ADAMTS13 appears to be a 2-way association: release of large amounts of VWF reduces ADAMTS13 activity, and vice versa, a lower ADAMTS13 activity may lead to the expression of large prothrombogenic VWF multimers, thereby stimulating platelet binding, thrombus formation, and the development of tissue ischemia and necrosis. In our study, we could not demonstrate the presence of UL-VWF multimers in patients with the lowest ADAMTS13 activity; however, a relative predominance of larger VWF multimers cannot be excluded, because our technique was inappropriate to detect more subtle increases in VWF multimer size. The association between ADAMTS13 activity and renal insufficiency, as observed in our study and in the study of patients with severe sepsis, may support the concept that even small deficiencies of ADAMTS13 may be relevant in conditions of marked endothelial activation.

In addition to the association between ADAMTS13 and VWF, we also examined the possibility that high fluid shear rates may be responsible for the relative expression of a more active VWF. High fluid shear rates promote binding of platelets at the A1 domain of the von Willebrand subunit, changing the VWF molecule to the “active” glycoprotein Ib-α binding configuration. This conformational change stimulates cleavage by ADAMTS13 as a result of exposure of the adjacent A2 domain, resulting in a decrease in multimer size. In our study, a more active VWF was positively associated with VWF, showing that with increasing VWF the amount of circulating VWF was also more active. However, we found no association between active VWF and ADAMTS13 activity. In addition, a decrease in multimer size could not be demonstrated in 8 patients with the lowest ADAMTS13 activity or highest active VWF level. These findings suggest that conformational changes in the VWF factor molecule are not a principal determinant of ADAMTS13 activity in malignant hypertension. Finally, because a previous study suggested that free Hb, which is released during hemolysis, may in itself affect ADAMTS13 activity, we also examined the association between free Hb and ADAMTS13 activity. However, no association between free Hb and ADAMTS13 activity could be demonstrated, suggesting that the decrease in ADAMTS13 activity cannot be explained by an inhibitory effect of free Hb.

There are some limitations to our study. First, the association between ADAMTS13 and TMA severity and between ADAMTS13 and renal dysfunction does not prove causality.

It is conceivable that the high arterial pressures associated with severe and malignant hypertension directly result in TMA. The degree of VWF release as a result of the high
arterial pressures and subsequent reductions in ADAMTS13 activity may, therefore, be just a marker for the degree of endothelial damage rather than being causally related to the TMA and renal insufficiency of malignant hypertension. Although there was a lack of association between ADAMTS13 and either systolic or diastolic BP, the acceleration rather than the absolute BP level may be a more important determinant of endothelial damage.

Second, underlying diseases and ABO blood group may have influenced the measurements performed in this study. Inflammatory conditions, such as polyarteritis nodosa and lupus erythematosus, are associated with endothelial activation and may stimulate VWF release and lower ADAMTS13 activity. In contrast, blood group O is generally associated with lower VWF levels and somewhat higher ADAMTS13 activity. The frequency of the inflammatory conditions underlying malignant and severe hypertension were equally distributed between groups and accounted for only a small proportion of the total study population. When these secondary causes were removed, no significant changes occurred in the association between ADAMTS13 activity and BP groups or in the association between ADAMTS13 and VWF. Blood groups were not assessed in this study and may, therefore, have accounted for some of the differences in ADAMTS13 activity between BP groups. However, we estimate that the effect of blood group on ADAMTS13 activity is small and consider it unlikely that blood group would have altered the association between VWF and ADAMTS13 activity.

Perspectives
We have shown that, in patients with malignant hypertension, ADAMTS13 is deficient and associated with the severity of TMA, likely because of the release of large amounts of VWF as a result of endothelium stimulation. The fact that some patients with severe hypertension but no retinopathy also had evidence of TMA may suggest that, in patients with hypertensive crises, a retinal examination can not sufficiently discriminate patients with TMA from those without TMA. A severe deficiency of ADAMTS13, such as that observed in TTP, could not be demonstrated, indicating that assessment of ADAMTS13 activity may help to discriminate between these conditions. The close correlation between ADAMTS13 activity and renal insufficiency in patients with malignant hypertension may suggest that treatment directed at increasing ADAMTS13 activity may also beneficially affect renal function. Further studies are needed to clarify the mechanisms involved in the TMA of malignant hypertension and to examine whether administration of ADAMTS13, or agents that stimulate its function, leads to amelioration of the ischemic renal lesions observed in this condition.

Disclosures
None.

References
Association Between Thrombotic Microangiopathy and Reduced ADAMTS13 Activity in Malignant Hypertension

Bert-Jan H. van den Born, Niels V. van der Hoeven, Evelyn Groot, Peter J. Lenting, Joost C.M. Meijers, Marcel Levi and Gert A. van Montfrans

Hypertension. 2008;51:862-866; originally published online March 10, 2008; doi: 10.1161/HYPERTENSIONAHA.107.103127

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2008 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/51/4/862

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension 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 Hypertension is online at:
http://hyper.ahajournals.org/subscriptions/