Correlation of Circulating von Willebrand Factor Levels With Cardiovascular Hemodynamics

William F. Penny, MD; Mark Weinstein, PhD; Edwin W. Salzman, MD; and J. Anthony Ware, MD

Background. Valvular heart disease is associated with a decreased platelet circulating time and a thrombotic tendency. The possibility that these events are related to changes in von Willebrand factor (vWF), a multimeric glycoprotein released from endothelial cells and platelets that mediates platelet adhesion to the vascular subendothelium, has not been examined.

Methods and Results. We measured the vWF antigen (vWF:Ag) concentration in 43 patients undergoing cardiac catheterization for the evaluation of mitral (n = 17) or aortic (n = 10) stenosis or nonvalvular heart disease (n = 16). Mean vWF:Ag concentration was significantly higher in patients with mitral stenosis than in those without (212±84 versus 150±79 units/dl, p<0.02); this elevation was associated with a significant elevation of pulmonary vascular resistance (PVR) in the patients with mitral stenosis (186±49 versus 133±81 dynes-sec-cm⁻⁵, p<0.02). The vWF:Ag levels in the entire group of patients (regardless of the presence or type of valvular disease) varied directly with PVR (r=0.72, p<0.0001) and with pulmonary artery pressure (r=0.60, p<0.0001) and inversely with cardiac output (r=0.64, p<0.0001). Changes in PVR, pulmonary artery pressure, or cardiac output could not be correlated with circulating levels of fibrinogen or β-thromboglobulin, which may be released from activated platelets, nor with the endothelial cell product tissue plasminogen activator.

Conclusions. The association of high vWF:Ag levels with increased PVR and decreased cardiac output in patients both with and without mitral stenosis suggests a hemodynamically induced increase in the endothelial release of vWF, which might contribute to a thrombotic tendency in these patients. (Circulation 1991;83:1630–1636)

An increased tendency toward thrombosis and thromboembolism has been recognized in patients with congestive heart failure and valvular heart disease,¹ but the pathogenesis remains unclear. Steele et al² reported shortened platelet survival, which could suggest enhanced platelet adhesion or aggregation followed by removal of altered platelets from the circulation, in patients with mitral stenosis. Decreased fibrinolytic and anticoagulant capacity, as evidenced by decreased antithrombin III and increased α₂-antiplasmin levels, have also been found in patients with mitral stenosis.³ These findings suggest that platelet activation and coagulation abnormalities may add to the mechanical effects of valvular stenosis, left atrial enlargement, and stasis, and thus may promote thromboembolism in these patients.

A large multimeric glycoprotein, von Willebrand factor (vWF), is found in endothelial cells, plasma, and platelet alpha granules and mediates platelet adhesion to the vascular subendothelium. As part of a randomized trial to evaluate the effect of desmopressin acetate on postoperative blood loss,⁴,⁵ we noted an elevation in the circulating levels of vWF antigen (vWF:Ag) in patients with mitral stenosis(1630–1636)
even before the administration of desmopressin acetate. While the role of vWF in the pathophysiology of adult valvular heart disease is not known, a few reports associate other cardiovascular abnormalities with disorders of vWF. An increased blood concentration of vWF has been described in patients with unstable angina and myocardial infarction, and in patients with a history of arterial thromboembolism. The possible importance of the pulmonary circulation was first emphasized by Carvalho et al., who described alterations of vWF in patients with pulmonary hypertension related to acute respiratory failure. Additional investigations found an increase in vWF-ristocetin cofactor activity in patients with primary pulmonary hypertension, and elevations of vWF:Ag concentrations and decreases in the frequency of high-molecular weight (HMW) forms of vWF in patients with pulmonary hypertension, both with and without congenital cardiac defects. Immunocytochemical analysis of pulmonary endothelium from patients with pulmonary hypertension suggested an increased synthesis of vWF, primarily in the pulmonary arteries. In contrast, Gill et al. noted a decreased vWF:Ag level in six of 12 children with congenital atrial or ventricular septal defects or aortic stenosis; the largest vWF multimers were absent in all patients, with normalization of the multimer ratio occurring in four of five patients following corrective surgery.

To investigate the hypothesis that valvular heart disease could be associated with elevated levels of vWF:Ag and thus might contribute to the thrombotic tendency seen in such patients, we measured the concentrations of vWF:Ag and the vWF multimeric distribution in adult patients with various forms of valvular and ischemic heart disease and correlated these findings with hemodynamic measurements at cardiac catheterization.

**Methods**

**Patients**

A nonconsecutive group of 37 adult patients was chosen prospectively from those undergoing cardiac catheterization for the evaluation of cardiac valvular abnormalities, cardiomyopathy, or coronary artery disease. Blood specimens were obtained by antecubital phlebotomy following informed verbal consent in all patients. In a subgroup of seven arbitrarily selected subjects, blood was also obtained from the pulmonary artery and either the left ventricle or a femoral artery by simultaneous draw through catheters. No patient had undergone a surgical procedure within the 72 hours prior to blood sampling. A complete list of current medications was obtained in all patients. Cardiac catheterization was performed within 24 hours after blood sampling in 35 of the 37 patients and 5 and 7 days following blood collection in the other two patients. Two patients were subsequently excluded from analysis: one who had been extubated from a mechanical ventilator immediately prior to catheterization, and one who had inadequate hemodynamic tracings. An additional eight patients whose vWF:Ag concentration was measured as part of a separate study and had subsequently undergone cardiac catheterization within 7–30 days were included in the study and analyzed retrospectively.

Thus, data from a total of 43 patients were analyzed. Seventeen patients had mitral stenosis and 10 had aortic stenosis. Of the 16 patients who did not have mitral or aortic stenosis, nine were catheterized for the evaluation of coronary artery disease, one for dilated cardiomyopathy, four for severe mitral regurgitation, and two for aortic regurgitation.

**Hemodynamic Measurements**

Patients underwent right- and left-heart catheterization, with pressures expressed as millimeters mercury and cardiac output calculated by the Fick method. Pulmonary vascular resistance (PVR) was calculated as (mean pulmonary artery pressure—mean pulmonary capillary wedge pressure)/cardiac output and expressed as dynes per second per cm². Valve areas were calculated using the method of Gorlin and Gorlin.

Patients were classified as having mitral stenosis if there was a demonstrable diastolic gradient across the mitral valve at catheterization and either a history of acute rheumatic fever or evidence of rheumatic mitral valve deformity on an echocardiogram; in one patient the gradient was less than 3 mm Hg and the valve area could not be accurately quantified. Patients were classified as having aortic stenosis if a peak-to-peak systolic gradient across the aortic valve of at least 10 mm Hg was identified at catheterization in the presence of echocardiographic evidence of aortic valve leaflet deformity. Patients with mitral or aortic stenosis were so classified regardless of the presence of coronary artery disease.

**von Willebrand Factor Determination**

Peripheral venous blood obtained by phlebotomy was collected into tubes containing ethylenediamine tetraacetic acid (EDTA) with aprotinin, immediately placed on ice, and then spun at 1,500g for 10 minutes at 4°C. Platelet-poor plasma was decanted and spun again, divided into aliquots, and frozen at −70°C. Blood specimens obtained from the pulmonary and systemic arterial catheters were collected and prepared similarly. The vWF:Ag concentration was measured using an enzyme-linked immunosorbent assay (ELISA). Immulon II microtiter plates (Dynatech Laboratories, Inc., Chantilly, Va.) were coated with a 1:1,000 dilution of rabbit anti-human vWF antibody (Accurate Chemical and Scientific Corp., Westbury, N.Y.) in 0.1 M NaHCO₃. After washing once with 0.01% fatty acid–free bovine serum albumin (BSA) (Sigma Chemical Co., St. Louis), in 0.056 M Na₂HPO₄, 0.011 M KH₂PO₄, and 0.15 M NaCl (phosphate buffered saline [PBS]), and once with water, the wells were blocked with 0.5% BSA and 0.5% dry milk in PBS. The plates were then washed three
times with 0.1% BSA in PBS and incubated for at least 2 hours at 37°C with the test plasma diluted 1/10 to 1/320 in PBS. The plates were then washed three times in PBS, incubated for 1.5 hours at 37°C with 100 µl of a 1/1,000 dilution of horseradish peroxidase–labeled rabbit anti-human factor VIII–related antibody (Accurate), and again washed three times with PBS. Plate wells were treated with 100 µl o-phenylenediamine (5.5 mg in 10 ml 0.1 M sodium citrate, pH 5.0) for 3.5 minutes, the reaction was stopped with 100 µl of 1 M H2SO4, and the optical absorbance of the wells was read at 490 nm. The values are expressed in units per deciliter and are normalized to the values obtained in normal plasma (100 units/dl).

Quantification of vWF multimers was performed as described previously.4 Briefly, samples of plasma, adjusted to a vWF concentration of 15 units/dl, were applied to a 1% agarose–sodium dodecyl sulfate gel. Following electrophoresis, the gel was overlaid with [125I]-anti-vWF antibody, and vWF multimers were detected by autoradiography. Densitometric analysis of the autoradiograph was used to quantify the relative amount of HMW vWF multimers, defined as those vWF forms with a molecular mass greater than about 8×10^6 d, the size of multimers at the optical density maximum of an autoradiograph of vWF from pooled normal plasma.4 The percent of HMW multimers present in an individual plasma sample was divided by the percent found in pooled normal plasma to obtain the normalized ratio of HMW multimers. The mean±SD ratio in a healthy age-matched control group is 0.93±0.17.4

β-Thromboglobulin Assay

The β-thromboglobulin (β-TG) concentration was measured in plasma samples from blood collected into EDTA, aprotinin, and 1 µM prostaglandin E1, using a two-syringe technique and discarding the initial 2 ml blood drawn. Plasma was prepared as above, and the β-TG concentration was measured using a standard commercial radioimmunoassay kit (Amer sham Corp., Arlington Heights, Ill.).

Tissue Plasminogen Activator Assay

The concentration of tissue plasminogen activator (tPA) in plasma was measured in an aliquot obtained from the same sample of plasma collected for the analysis of vWF. The tPA concentration was quantified using a commercially available ELISA kit, IMUBIND-5, according to the manufacturer’s instructions (American Diagnostica, Inc., Greenwich, Conn.).

Plasma Fibrinogen Assay

Fibrinogen concentrations were determined in plasma samples collected for vWF:Ag and tPA quantification. The same ELISA procedure used to assess vWF:Ag concentration (see above) was employed to measure the fibrinogen concentration, with the following modifications: Immulon-II 96-well plates (Dynatech) were coated with rabbit anti-human fibrinogen antibody (Accurate), plasma samples were diluted 1/2,000 to 1/64,000 with PBS, and horseradish peroxidase–labeled goat anti-human fibrinogen antibody (Cappell, Durham, N.C.) was used to detect bound fibrinogen.

Data Analysis

Values are expressed as mean±SD. Sets of continuous data were analyzed by univariate linear regression and expressed as a correlation coefficient (r) and a probability value. Groups were compared by Student’s two-tailed, unpaired t test, except for the comparisons of vWF:Ag level in pulmonary artery and systemic artery, in which a paired t test was used. In some cases data were not available for all 43 study patients, due to limited clinical data (hemoglobin concentration, platelet count, blood type, oxygen saturation, use of aspirin), insufficient plasma sample (vWF multimer distribution), inclusion of a parameter after initiation of the main study (β-TG, fibrinogen, and tPA antigen concentrations), or the arbitrary selection of patients (pulmonary versus systemic vWF:Ag level).

Results

Analysis of the hemodynamic parameters obtained at cardiac catheterization revealed a strong direct correlation between vWF:Ag level and PVR (r=0.72, p<0.0001) for the entire group of 43 patients studied (Figure 1). The vWF:Ag concentration could be correlated with PVR in patients with either mitral or aortic stenosis as well as in those with other forms of
cardiac disease, as revealed by inspection of Figure 1. The vWF:Ag level was higher (212±84 units/dl) in the patients with mitral stenosis than in the patients without mitral stenosis (150±79 units/dl, p<0.02; Figure 2). This elevation of vWF:Ag levels in patients with mitral stenosis corresponded to an elevation of PVR, which was significantly higher (186±49 dynes-sec-cm⁻⁵) in this group than in the patients without mitral stenosis (133±81 dynes-sec-cm⁻⁵, p<0.02). The relation between vWF:Ag concentration and PVR was similar in the 35 patients undergoing cardiac catheterization within 24 hours after vWF determination and in the 10 patients catheterized 5–30 days following vWF measurement (data not shown). The vWF:Ag concentration was also directly related to the mean pulmonary artery pressure (r=0.60, p=0.0001) and inversely related to the cardiac output (r=0.64, p<0.0001; Figure 3); both correlations were less strong than that for PVR. Systemic vascular resistance and pulmonary capillary wedge pressure were also associated with an increased vWF:Ag level (Table 1). No significant relation was found between vWF:Ag level and mitral or aortic valve area or mean valve gradient, mean systemic arterial pressure, patient age, hemoglobin concentration, or platelet count. Thus, the finding that circulating levels of vWF:Ag were higher in patients with mitral stenosis can be attributed to an effect on PVR and cardiac output, rather than to the mechanical severity of the valvular lesion.

The possibility that variations in inspired oxygen concentration, which can alter PVR, caused these changes in the vWF:Ag concentration was considered. Only three of the 35 patients studied prospectively were administered supplemental oxygen (1–4 l by nasal cannula) during catheterization, and no correlation of vWF:Ag concentration with PO₂ or hemoglobin oxygen saturation at catheterization was

![Figure 2](image-url)

**Figure 2.** Comparison of mean±SD von Willebrand factor antigen concentration (vWF:Ag) and mean±SD pulmonary vascular resistance (PVR) in patients with mitral stenosis (filled bars, n=17) and those with other cardiac disorders (shaded bars, n=26). The y axis is the same for both vWF:Ag and PVR.

![Figure 3](image-url)

**Table 1.** Correlation of von Willebrand Factor Antigen Concentration With Hemodynamic and Clinical Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>n</th>
<th>r</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pulmonary vascular resistance (dynes-sec-cm⁻⁵)</td>
<td>43</td>
<td>0.72</td>
<td>0.0001</td>
</tr>
<tr>
<td>Cardiac output (l/min)</td>
<td>43</td>
<td>0.64</td>
<td>0.0001</td>
</tr>
<tr>
<td>Mean pulmonary artery pressure (mm Hg)</td>
<td>43</td>
<td>0.60</td>
<td>0.0001</td>
</tr>
<tr>
<td>Systemic vascular resistance (dynes-sec-cm⁻⁵)</td>
<td>42</td>
<td>0.59</td>
<td>0.0001</td>
</tr>
<tr>
<td>Pulmonary capillary wedge pressure (mm Hg)</td>
<td>43</td>
<td>0.49</td>
<td>0.001</td>
</tr>
<tr>
<td>Valve area* (mm)</td>
<td>26</td>
<td>0.29</td>
<td>NS</td>
</tr>
<tr>
<td>Valve gradient, mean* (mm Hg)</td>
<td>27</td>
<td>0.09</td>
<td>NS</td>
</tr>
<tr>
<td>Mean systemic arterial pressure (mm Hg)</td>
<td>42</td>
<td>0.14</td>
<td>NS</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>43</td>
<td>0.31</td>
<td>0.04</td>
</tr>
<tr>
<td>Sex</td>
<td>43</td>
<td>...</td>
<td>NS</td>
</tr>
<tr>
<td>Oxygen saturation (%)</td>
<td>28</td>
<td>0.29</td>
<td>NS</td>
</tr>
<tr>
<td>Platelet count (mm⁻³)</td>
<td>29</td>
<td>0.05</td>
<td>NS</td>
</tr>
</tbody>
</table>

*Patients with mitral or aortic stenosis only. NS, not significant.
found. We also considered the potential influence of blood type, which has been shown to influence vWF activity, on these findings. Fifteen of the patients were blood type A, four were B, two were AB, and 15 were O. No significant difference in vWF:Ag level was noted among these groups. Thus, neither blood type nor oxygen saturation could explain the correlation between PVR and vWF:Ag levels.

Next, we examined the possibility that alterations in hemodynamics were associated with changes in the ratio of HMW vWF multimeric forms, as well as the vWF:Ag level. No significant relation could be demonstrated between vWF multimer distribution and PVR; as shown in Figure 4, there was no correlation seen for the entire group tested (n=37) nor for the subgroups of patients with mitral or aortic stenosis. The mean±SD HMW multimer ratio for the group (0.79±0.15), however, tended to be lower than that seen in a healthy control group (0.93±0.17). The HMW multimer ratio did not correlate with the vWF:Ag level in either the entire group or in a separate analysis of the patients with mitral stenosis (data not shown).

To determine whether the concentrations of endothelial release products other than vWF were elevated in patients with altered PVR, plasma levels of tPA antigen were measured in 33 consecutive patients. Despite the similarities in sample collection and preparation, no significant relation was found between the tPA level and the vWF:Ag level (r=0.30, p=0.09) or between the tPA level and PVR (r=0.13, p=NS).

A potential source of vWF in addition to the endothelium is the platelet alpha granule, the contents of which are released upon stimulation. To study the contribution of platelet activity to the elevated concentrations of vWF:Ag, levels of β-TG, an index of alpha granule release by activated platelets, were measured in 11 consecutive patients. Plasma β-TG concentration ranged from 8 to 180 ng/ml in this group, but no correlation could be demonstrated between the β-TG level and the vWF:Ag concentration or PVR. Patients who had ingested aspirin within 1 week before vWF:Ag determination had a mean±SD vWF:Ag level of 148±60 units/dl compared with 167±87 units/dl in the patients who had not received aspirin (p=NS). Therefore, platelet activation could not be correlated with PVR, and platelet secretion did not appear to contribute to the vWF:Ag levels measured. Plasma fibrinogen levels were also measured and found not to correlate with PVR, cardiac output, or the vWF:Ag level; thus, no generalized increase in plasma protein content could be detected as a consequence of the altered hemodynamics.

In an effort to investigate the possibility that vWF was selectively released from the pulmonary vascular endothelium, producing a gradient of vWF:Ag concentration across the pulmonary vascular bed, vWF:Ag levels were compared in blood collected from the pulmonary artery and from the femoral artery or left ventricle via a cardiac catheter in seven patients. No significant difference could be detected between pulmonary arterial (190±60 units/dl) and systemic arterial (170±55 units/dl) vWF:Ag concentrations, and thus the pulmonary circulation could not be directly implicated as the source of the vWF:Ag measured.

Discussion

Previous studies from our laboratory have found that circulating levels of vWF:Ag are elevated in patients scheduled to undergo replacement of a stenotic mitral valve.45 The findings of this study confirm that the mean vWF:Ag level in a group of patients with mitral stenosis is significantly elevated compared with that in a group of patients with aortic stenosis or other cardiac disorders. A direct, linear correlation between the vWF:Ag concentration and PVR existed among all 43 patients in this study, with or without valvular disease. The elevations of both mean vWF:Ag level and PVR in the cohort of 17 patients with mitral stenosis were greater than those in the 26 catheterized patients without mitral stenosis.

Therefore, elevation of the mean vWF:Ag level in patients with mitral stenosis can be related to the hemodynamic changes that may occur in some patients with mitral stenosis15 rather than to the presence of the valvular lesion itself. This conclusion is supported by several findings of this study. That the correlation between elevations in PVR and the vWF:Ag concentration can occur in patients with other cardiopulmonary disorders is exemplified by the fact that the patient with one of the highest PVRs in our study, and the highest measured vWF:Ag level,
suffered from congestive cardiomyopathy without stenotic valvular disease. Furthermore, patients with mitral stenosis but minimal elevations of PVR had only mildly elevated vWF:Ag levels; prominently elevated vWF:Ag levels were identified in patients with large pressure gradients across the mitral valve or small calculated valve areas only if the PVR was also elevated. Thus, severity of the stenosis based on mitral valve area or gradient did not correlate with the vWF:Ag level. This finding does not discount the possibility that elevation of the vWF:Ag level participates in thromboembolism in these patients as there is no simple correlation between the incidence of thromboembolism and the size of the mitral orifice; however, cardiac output is inversely related to both the tendency for embolization and the level of vWF:Ag (present study).

Previous reports have demonstrated that the large multimeric forms of vWF participate in platelet aggregation induced by shear stress in vitro and thus could contribute to the thrombotic tendency in patients with rheological abnormalities, such as those with valvular heart disease. In this study, however, the proportion of large multimers was generally lower in patients with cardiovascular disease than in a healthy control population, and the proportion of large multimers did not correlate with cardiac output, PVR, or the severity of mitral valve stenosis. Such large vWF multimers normally do not circulate, but are released by stimulants such as catecholamines, thrombin, and vasopressin and its derivatives and then are rapidly cleared. The coupling of a high vWF:Ag concentration and a normal or slightly decreased proportion of large multimers is in contrast to the high vWF:Ag concentration and high percentage of large multimers in patients undergoing surgical procedures. It is possible that a greater proportion of these HMW multimers are released locally by the (pulmonary?) endothelium, but their presence cannot be detected in the systemic circulation by current methods.

In resting vascular endothelial cells and platelets, vWF is contained in storage depots (Weibel-Palade bodies and platelet alpha granules, respectively). The cellular source of the circulating vWF:Ag measured in this study is uncertain, although it is estimated that platelet-derived vWF:Ag accounts for only 5% of the circulating total. In this study, the vWF:Ag level did not vary with the patient’s platelet count and there was no significant difference between vWF:Ag levels in patients taking aspirin versus those who had not received aspirin. Plasma levels of the platelet release product β-TG, which is also contained in platelet alpha granules, did not correlate with the vWF:Ag level, and thus a similar origin of these products is not likely. Although no relation was found between PVR and the concentration of circulating tPA, which is chiefly (but not exclusively) derived from endothelial cells, tPA has not been localized to the same storage site as vWF, and thus these proteins might not be released concurrently. The lack of correlation of the plasma tPA antigen concentration with cardiovascular hemodynamic parameters suggests that the unknown stimuli that effect vWF release in patients with altered hemodynamics may be relatively specific for vWF. Alternatively, this discrepancy may be due to differences in plasma clearance and circulating half-times; this possibility is strengthened by the inverse correlation of cardiac output with vWF:Ag concentration, suggesting that the clearance of vWF from the circulation might be decreased.

Thus, it seems most likely that the vWF:Ag measured in these patients is derived from the vascular endothelium. One mechanism to explain the correlations seen in this study is that both vWF release and altered hemodynamics might be affected by a common hormonal stimulus (e.g., elevated concentrations of catecholamines or vasopressin) that accompanies heart failure. An alternate and more interesting possibility is that vWF release, and possibly synthesis, by the vascular endothelium might be caused by the increased vascular resistance; the results of this study suggest that this effect may be continuous from a normal to a markedly elevated PVR, rather than being a threshold effect. Increasing shear stress enhances the synthesis or release of prostacyclin and tPA from cultured endothelial cells, and the pulmonary endothelium from patients with pulmonary hypertension contains increased amounts of intracellular vWF, suggesting that increased synthesis or decreased degradation can be associated with hemodynamic abnormalities. Thus, these observations and the findings of the present study suggest that increased vascular pressures or resistance might enhance endothelial synthesis or release of vWF, which then could contribute to the thrombotic tendency in patients with altered hemodynamics.

Acknowledgments

The authors deeply appreciate the considerable efforts of Dr. Patricia C. Come, Mary Ann Lee, Linda Robertson, and the staff of the Echocardiography Laboratory at Beth Israel Hospital for their assistance in a preliminary study. The authors would like to thank Leslie Chute for technical assistance, the staff and fellows of the Beth Israel Cardiac Catheterization Laboratory for help with sample acquisition, and Mary Hartmann for assistance with preparation of the manuscript.

References


**KEY WORDS** • mitral stenosis • pulmonary vascular resistance
Correlation of circulating von Willebrand factor levels with cardiovascular hemodynamics.
W F Penny, M Weinstein, E W Salzman and J A Ware

_Circulation_. 1991;83:1630-1636
doi: 10.1161/01.CIR.83.5.1630

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1991 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/83/5/1630

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

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

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