Circulating von Willebrand Factor Antigen as a Predictor of Short-term Prognosis in Pulmonary Hypertension*

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Study objective: To determine the potential value of plasma von Willebrand factor antigenic activity (vWF:Ag) and other commonly measured clinical variables for predicting which patients with precapillary pulmonary hypertension would be unlikely to survive 1 year.

Design: Prospective clinical study. The data obtained at the beginning of the study were analyzed at the end of the first year of follow-up.

Patients and methods: Forty patients aged 1.2 to 45 years (median, 24 years) entered the study. Eleven patients had primary pulmonary hypertension, and in the remaining ones, pulmonary vascular disease was associated with antiphospholipid syndrome (n = 1), collagen vascular disease (n = 1), schistosomiasis (n = 3), or congenital heart defects (Eisenmenger’s syndrome) (n = 24). Plasma vWF:Ag was determined by electroimmunodiffusion, and the results were expressed as the percentage of activity.

Results: Seven of 11 patients with primary pulmonary hypertension but only 4 of 29 patients with secondary pulmonary hypertension died during the follow-up period (p < 0.005). Initial vWF:Ag values were significantly higher in the nonsurvivor group in comparison with the survivors (256.6 ± 85.3% and 132.0 ± 59.3% activity, respectively; p < 0.0001). The likelihood of fatal outcome as a function of plasma vWF:Ag levels was estimated for primary and secondary pulmonary hypertensive patients using logistic regression analysis. Decreased life expectancy was significantly correlated to high vWF:Ag levels and an established diagnosis of primary pulmonary hypertension. A plasma vWF:Ag of >240% (p = 0.003) was 54% sensitive and 93% specific for identifying patients who were unlikely to survive 1 year, with an overall predictive value of 75%. No other variables correlated significantly with survival.

Conclusion: Plasma vWF:Ag seems to be a useful biochemical index for predicting short-term prognosis in patients with pulmonary hypertension. In contrast to hemodynamic and histopathological predictors of survival, vWF:Ag does not require invasive techniques to be determined. In light of the possibility of false-negative results, serial measurements should be performed over time in patients with vWF:Ag of <240%. This observation proved helpful in two patients in this study.

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Key words: von Willebrand factor; pulmonary hypertension; endothelial cells

Abbreviations: vWF:Ag = von Willebrand factor antigenic activity

A large multimeric glycoprotein, von Willebrand factor (vWF), synthesized exclusively by endothelial cells and megakaryocytes. It is found in the endothelial basement membrane, where it serves to anchor platelets and endothelial cells to the matrix.

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vWF also is present in plasma, where it acts as a carrier for coagulation factor VIII, and within platelet α-granules as an adhesive protein. Within endothelial cells, vWF has been identified in the cytoplasm, on the plasma membrane, in the endoplasmic reticulum, and, particularly, in cell-specific organelles called Weibel-Palade bodies.1

In physiologic conditions, vWF is released from endothelial cells into the plasma and to the abluminal cell surface, where it binds to the subendothelium. After secretion into the plasma, endothelial cell-derived vWF is converted by cleaving activities present in granulocytes into a series of multimers with molecular masses from 500 kd to as high as
In pathological conditions, endothelial cell stimulation by a number of agents, such as thrombin, fibrin, histamine, epinephrine, vasopressin, endotoxin, cytokines, components of the complement system, and shear stress, is followed by a rapid release of vWF from storage granules into the circulation. For this reason, plasma antigenic activity of vWF (vWF:Ag) has been used widely as a marker of endothelial cell injury. Increased plasma vWF:Ag has been reported in several disorders associated with microvascular damage, including scleroderma, thrombotic thrombocytopenic purpura, nephritis, pregnancy-induced hypertension, myocardial infarction, diabetic angiopathy, and pulmonary hypertension. During the course of bacterial, viral, and parasitic infections, vWF participates as an acute-phase reactant. In these instances, a significant elevation occurs in plasma vWF:Ag in addition to the hepatic components of the acute-phase response. Moreover, plasma vWF:Ag has been used as a predictor of lung involvement in nonpulmonary sepsis syndrome. Elevated plasma vWF:Ag has been shown to be predictive of mortality in this disorder.

Endothelial cell injury and dysfunction have been well documented in pulmonary hypertension. From the morphologic point of view, structural changes found using scanning and transmission electron microscopy have suggested a phase of enhanced metabolic function of endothelial cells. From the biochemical point of view, evidence for endothelial cell dysfunction in pulmonary hypertension comes from reports showing abnormal circulating levels of endothelin, thrombomodulin, and vWF that are associated with increased immunostaining of the pulmonary vascular endothelium for endothelin and vWF. Reduction of endothelium-derived vasodilators has been reported as well. A possibility exists that the magnitude of endothelial cell dysfunction is correlated with the extent and severity of pulmonary microvascular damage in pulmonary hypertension. If so, the assessment of endothelial cell function using biochemical markers might have prognostic implications. We therefore planned the present study to examine prospectively whether a correlation exists between circulating levels of vWF and short-term prognosis in patients with pulmonary hypertension. Other variables such as patient sex, age, mean pulmonary arterial pressure, and the etiology of pulmonary hypertension also were analyzed to determine their predictive value in identifying which patients would be unlikely to survive over the short term.

Materials and Methods

Patients and Study Protocol

This study involved 40 patients with precapillary pulmonary hypertension who were under medical treatment at the Heart Institute of the School of Medicine of the University of São Paulo, Brazil. Patients entered the study consecutively, and patients with primary and secondary pulmonary hypertension were included. Levels of mean pulmonary arterial pressure higher than 25 mm Hg at rest in the presence of normal pulmonary wedge pressure at routine cardiac catheterization were considered necessary for patient inclusion. The diagnosis of primary pulmonary hypertension was established only in the absence of disorders that are known to be associated with pulmonary vascular disease, such as chronic airway obstruction or fibrotic lung disease, pulmonary thromboembolism, congenital heart disease, chronic liver disease, portal hypertension, collagen vascular disease, schistosomiasis, HIV infection, and antiphospholipid syndrome. After initial biochemical determinations, which were performed no longer than 15 days after hemodynamic measurements, patients were followed for 1 year. At the end of the follow-up period, the results of the initial determinations were compared in nonsurvivors and survivors to identify possible predictors of prognosis. Patients waiting for heart-lung transplantation were included if they were not subjected to any kind of surgical treatment during the period of the study. Patients or their parents were informed about the research purpose of blood sampling and gave their consent. The control group consisted of 20 healthy volunteers with an age range similar to that of the patients. The study protocol was approved by the Scientific Committee of the Heart Institute at the University of São Paulo.

Blood Sampling and Inhibition of Proteolysis In Vitro

Peripheral venous blood was collected in 1:10 volumes of 3.8% sodium citrate, in the presence of the following protease inhibitors: 5 mM edetic acid, 6 mM N-ethylmaleimide, 1 mM phenylmethylsulfonyl fluoride, 0.25 mM of leupeptin, and 20 U/mL of aprotinin. Immediately after, plasma was separated by centrifugation at 3,000g for 20 min and was stored at −70°C until use. Samples were thawed only once for use.

Plasma von Willebrand Factor Antigenic Activity

Plasma vWF:Ag was measured by electroimmuno-diffusion using a kit obtained from Diagnostica Stago (Asnières, France). Samples always were processed in duplicate. In patients with exceedingly high vWF:Ag, plasma was diluted 1:2 or 1:4 and was processed again. Results were compared with a standard curve and were expressed as a percentage of activity.

Plasma von Willebrand Factor Biological Activity

The biological activity of von Willebrand factor was determined by the ristocetin cofactor assay. Washed normal platelets (5 × 10⁷ cells) were subjected to agglutination in the presence of either normal plasma or plasma from pulmonary hypertensive patients (50 µL) and of threshold ristocetin concentrations ranging from 0.35 to 0.5 mg/mL. The agglutination response (ristocetin cofactor activity) was determined by the change in light transmission at 4 min. Results were expressed as a percentage of the response obtained with normal plasma in the same assay.
Analysis of Data

In general, results are expressed as the mean (± SD), although median values and ranges are also provided when appropriate. Comparisons of data obtained at the beginning of the study between patients and control subjects or between patient groups were made using the Student’s t test. Differences between patient subgroups were tested by analysis of variance with appropriate multicomparison tests. To identify possible indicators of poor short-term prognosis, variables were subjected to univariate and multivariate analyses. Possible correlations of sex, age at the beginning of the study (< 14 vs > 14 years), and the etiology of pulmonary hypertension with patient status at 1 year were tested using the χ2 statistics and Fisher’s exact test. Differences between survivors and nonsurvivors regarding age, mean pulmonary arterial pressure, and plasma vWF:Ag were tested using the Student’s t test. Variables that were identified as possible predictors of prognosis by these initial methods then were subjected to logistic regression analysis (SAS/STAT® pack; SAS Institute Inc; Cary, NC). Selection was performed by a stepwise procedure, and estimated parameters were used for curve fitting. A significance level of 0.05 was assumed.

RESULTS

Patient age ranged from 1.2 to 45 (median 24) years, and the mean pulmonary arterial pressure was 27 to 112 (median 65) mm Hg. All but one patients had New York Heart Association class III or IV symptoms. According to the above criteria, the diagnosis of primary pulmonary hypertension was established in 11 patients. In the remaining patients, pulmonary vascular disease was associated with antiphospholipid syndrome (n = 1), collagen vascular disease (n = 1), schistosomiasis (n = 3), or congenital heart defects (Eisenmenger’s syndrome) (n = 24). During the period of the study, all patients received antiplatelet drugs, and most patients were under anticoagulant treatment with warfarin. Patients with Eisenmenger’s syndrome were subjected to hemodilution procedures to maintain an hematocrit level below 56%. Medical therapy was not significantly changed during the follow-up period. Moreover, there were no significant differences in therapeutic measures between survivors and nonsurvivors, except that drugs for cardiovascular support were administered to patients of the nonsurvivor group at the very end of their illness.

Laboratory procedures that were performed at the beginning of the study showed that vWF:Ag was significantly increased in patients with pulmonary hypertension compared with control subjects, with respective activity values of 166.3 ± 87.0 and 87.3 ± 22.7% (p < 0.001). Despite the high circulating levels, the biological activity of vWF was decreased. In patients, the ristocetin cofactor activity, expressed as a percentage of the control value obtained in the same assay, was 73.9 ± 36.6% (95% CI, 57.8% to 90.0%). Patients with primary pulmonary hypertension had higher vWF:Ag levels than patients with secondary pulmonary hypertension (231.1 ± 89.1 vs 141.7 ± 73.7% activity, respectively; p = 0.003), although pulmonary artery pressure did not differ significantly between the groups.

Eleven patients died during the first year of follow-up. Seven patients had primary pulmonary hypertension, and the remaining patients had either congenital heart disease (n = 3) or collagen vascular disease (n = 1). Survival was not influenced by patient age, sex, or mean pulmonary arterial pressure (Tables 1 and 2). However, the proportion of patients with primary pulmonary hypertension who died in the interval was significantly higher than that for patients with secondary pulmonary hypertension (p < 0.005, Table 1). In addition, the nonsurvivors had higher vWF:Ag values at the beginning of the follow-up period than the survivors, and this was highly significant (p < 0.0001, Table 2). In the subgroup of patients with secondary pulmonary hypertension, vWF:Ag was significantly higher in the nonsurvivors than in the survivors (247.5 ± 78.7% and 124.8 ± 58.3% activity, respectively; p < 0.05). In patients with primary pulmonary hypertension, a clear trend toward higher vWF:Ag levels for the nonsurvivors also was observed (261.8 ± 94.6% activity for nonsurvivors vs 177.2 ± 49.7% activity for survivors). Thus, vWF:Ag values were similar for the nonsurviving patients from both subgroups (p = NS). For the whole group of 40 patients, a vWF:Ag of > 240% (p = 0.003) was 54% sensitive for and 93% specific for identifying patients who were unlikely to survive 1 year, with an overall predictive value of 75%. The influence of plasma vWF:Ag levels on the likelihood of fatal outcome during the first year of follow-up in patients with

Table 1—Correlation of Sex, Age, and Etiology of Pulmonary Hypertension With 1-Year Survival

<table>
<thead>
<tr>
<th>Variable</th>
<th>Category</th>
<th>Deceased</th>
<th>Alive</th>
<th>p, χ²</th>
<th>p, Fisher</th>
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<td>7</td>
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<td>0.455</td>
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<tr>
<td></td>
<td>(%)</td>
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<tr>
<td></td>
<td>Female</td>
<td>7</td>
<td>22</td>
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<td>Age (yr)</td>
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<td>3</td>
<td>8</td>
<td>0.984</td>
<td>1.000</td>
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<tr>
<td>(%)</td>
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<td>(27.27)</td>
<td>(72.73)</td>
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<tr>
<td>≥14</td>
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<td>8</td>
<td>21</td>
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<tr>
<td>(%)</td>
<td></td>
<td>(27.59)</td>
<td>(72.41)</td>
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<td>Etiology of pulmonary hypertension</td>
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<tr>
<td>PPH*</td>
<td></td>
<td>7</td>
<td>4</td>
<td>0.002</td>
<td>0.004</td>
</tr>
<tr>
<td>(%)</td>
<td></td>
<td>(63.64)</td>
<td>(36.36)</td>
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<tr>
<td>SPH</td>
<td></td>
<td>4</td>
<td>25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(%)</td>
<td></td>
<td>(13.79)</td>
<td>(86.21)</td>
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</tr>
</tbody>
</table>

*PPH = primary pulmonary hypertension; SPH = secondary pulmonary hypertension.
primary and secondary pulmonary hypertension is shown in Figure 1. Multivariate analysis showed that the etiology of pulmonary hypertension (i.e., primary vs secondary form) and plasma vWF:Ag levels influenced patient outcomes independently. The equation that represents the probability of death as a function of initial vWF:Ag values was:

$$\log\left(\frac{\text{probability of death}}{\text{probability of survival}}\right) = -3.95 + 0.02$$

$$\text{vWF:Ag} - 1.74(P \text{ or } S)$$

where $P$ and $S$ are 0 and 1, respectively, for primary and secondary pulmonary hypertension.

In three of our patients, the results of two determinations of plasma vWF:Ag were available. In two patients (a 37-year-old woman with primary pulmonary hypertension and a 40-year-old woman with a persistent ductus arteriosus), heightened vWF:Ag in the second sample relative to the first one (80 and 49% increases, respectively) was followed by a fatal outcome a few months later. The third patient (an 8-year-old boy) was the only case of regression of primary pulmonary hypertension that we have ever seen among our pediatric patients. At the beginning of the study, this patient had a mean pulmonary arterial pressure at rest of 45 mm Hg. Plasma vWF:Ag was 226%. One year later, the mean pulmonary arterial pressure was 20 mm Hg and vWF:Ag was 130%.

**DISCUSSION**

This study involved pediatric and adult patients with precapillary pulmonary hypertension. Most of the patients had New York Heart Association class III or IV symptoms that were compatible with moderate to severe pulmonary vascular disease. Despite the fact that the patient population was mixed in terms of the etiology of pulmonary hypertension, the overall mortality ratio of 27% at 1 year was within the range (23 to 32%) reported for primary pulmonary hypertension.24 It has been suggested that children with primary pulmonary hypertension have...
a higher mortality within 1 year of follow-up than do patients older than 14 years.25 In this study, short-term prognosis was not significantly influenced by patient age. In particular, young patients did not have a higher mortality ratio, probably because 7 of 11 patients younger than 14 years of age had secondary pulmonary hypertension, which was associated with a better prognosis. Pulmonary artery pressure has been demonstrated to impact on survival in patients with primary pulmonary hypertension.26 Such a correlation was not observed in our patients. A likely explanation for this apparent discrepancy is that the impact of hemodynamic parameters on survival, which has been reported for patients with primary pulmonary hypertension, may not be as evident in mixed patient populations that include patients with congenital heart defects as was the case in the present study.

Essentially, our study demonstrates that plasma vWF:Ag, a biochemical marker of endothelial cell dysfunction, may be a useful index for identifying patients with pulmonary hypertension who are unlikely to survive 1 year, suggesting a pathophysiological relationship between endothelial cell dysfunction and the severity of pulmonary vascular disease. In addition, exceedingly high vWF plasma levels in patients with primary pulmonary hypertension seemed to be particularly worrisome. The value of elevated vWF:Ag in identifying patients who are unlikely to survive also has been demonstrated in acute disorders such as sepsis syndrome.18 An important limitation of this study is that in many instances parameters previously identified as predictors of survival in pulmonary hypertension were not available for analysis. Consequently, we were unable to establish how much survival was influenced by variables such as cardiac index, right atrial pressure, and histopathological features of pulmonary arteries. Our results should, therefore, be interpreted cautiously in light of the number of variables other than endothelium dysfunction that might have influenced patient outcome in such a complex disorder.

Another point of caution is the small number of patients with pulmonary hypertension secondary to disorders other than congenital heart disease. Although in a preliminary analysis, these patients seemed to behave exactly as did patients with congenital cardiac defects, in terms of outcome, further studies obviously are necessary for a better understanding of vWF changes and their prognostic implications in different forms of precapillary pulmonary hypertension.

Despite the limited number of variables that we analyzed, the highly significant correlation observed between elevated vWF:Ag levels and death during the first year of follow-up suggests that the altered metabolic function of endothelial cells is an important determinant of patient outcome in precapillary pulmonary hypertension. The etiology of the disease also was shown to influence survival, ie, patients with primary pulmonary hypertension had a higher mortality rate in interval. Although vWF:Ag and the etiology of pulmonary hypertension were shown to be statistically independent variables, from a biological point of view they seemed to be somewhat related to each other. Indeed, patients with primary pulmonary hypertension had relatively higher vWF:Ag levels. We therefore speculate that these patients had a poorer short-term prognosis in part because they had more advanced endothelial cell dysfunction than patients with secondary pulmonary hypertension with similar levels of pulmonary artery pressure. This is not to say that a high vWF:Ag level is characteristic of primary pulmonary hypertension, because vWF:Ag levels in nonsurvivors with the secondary form were similarly high. Elevated vWF plasma levels seem to be a characteristic of patients with advanced disease. The possibility is raised that some patients with primary pulmonary hypertension who had relatively lower vWF:Ag levels have a more insidious disease and a better survival rate.

In our opinion, the rationale for measuring plasma vWF:Ag in patients with pulmonary hypertension seems to be the possibility of identifying patients with vWF:Ag levels of >240%, because these levels clearly were associated with decreased life expectancy in our patients. Despite the excellent specificity of 93%, however, a plasma vWF:Ag level of >240% was only 54% sensitive in identifying patients who are unlikely to survive 1 year. To deal with this problem, it seems reasonable to perform serial measurements of vWF:Ag in patients with moderate to severe pulmonary hypertension and a plasma vWF: Ag level of <240%. Plasma levels of vWF do seem to increase over time as the disease worsens. In three of our patients, changes in vWF:Ag levels correlated better with patient outcome than did initial vWF:Ag levels. A recommendation for serial measurements may not be warranted at this time because our data are limited, but this remains a possibility for future testing.

The exact pathophysiological linkage between increased vWF plasma levels and decreased short-term survival in patients with pulmonary hypertension remains to be determined. One possibility is a direct involvement of vWF in the disease process. High circulating concentrations of vWF in these patients could be associated with an increased risk of thrombotic events. As an adhesive protein, vWF is adsorbed rapidly on the surface of activated platelets and participates in platelet sequestration.28 This
abnormal “clearance” of active species of vWF may explain partly the decreased biological activity of plasma vWF that is observed in patients with pulmonary hypertension. Consistent with this hypothesis is the observation that the larger multimers of vWF are lacking when patient plasma is analyzed in agarose gels.\textsuperscript{16,20} Preliminary data obtained from a smaller group of patients in our laboratory suggest that structural abnormalities of vWF also are associated with a worrisome prognosis in patients with pulmonary hypertension.\textsuperscript{30} The second possibility is that vWF does not participate directly in the progression of the disease. In this case, vWF would be coexpressed with another molecule that is relevant from the pathophysiological point of view. Endothelin-1 is a likely candidate, because it acts as a dual-function mediator promoting vasoconstriction and smooth muscle cell growth and is found in large amounts in arteries in patients with pulmonary hypertension.\textsuperscript{22} The third possibility is that a worsening of the disease process is determined by a complex and unpredictable association of biological events. In this case, the increased release of vWF from Weibel-Palade bodies could be a result of the nonspecific elevation of cytosolic free calcium within dysfunctional endothelial cells.

In conclusion, a group of patients with precapillary pulmonary hypertension with significantly decreased short-term life expectancy exists, to whom specific attention should be paid. These patients would be the logical candidates for heart-lung transplantation. Several predictors of survival have been proposed for identifying such patients, including clinical, hemodynamic, and histopathological parameters. In our opinion, as many variables as possible should be taken into consideration for estimating the probability of survival in such a complex clinical situation. An advantage of including biochemical parameters in the determination of prognosis for these patients seems to be the possibility of repeated measurements by noninvasive procedures during the course of the disease. Based on our findings, we would like to suggest that biochemical indicators of endothelial dysfunction may be used as prognostic indicators in patients with pulmonary hypertension. In particular, a plasma vWF:Ag level of > 240% seems to be very specific in the identification of patients who are unlikely to survive 1 year. In addition, serial measurements of vWF:Ag levels over time may increase the likelihood of detecting such high levels. Finally, we speculate that biochemical markers might be useful for monitoring endothelial cell function during pharmacological interventions such as intravenous prostacyclin administration.

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