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Alterations of von Willebrand Factor and Ristocetin Cofactor Activity During Atrial Fibrillation

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The aim of this study was to assess the plasma levels of von Willebrand factor antigen and ristocetin cofactor activity, which are well-known markers of endothelial function, in atrial fibrillation (AF) with or without mitral stenosis (MS). Forty-two patients (16 patients with MS and AF [MS(+)AF(+)], 13 patients with nonvalvular AF [MS(–)AF(+)], and 13 patients with MS and sinus rhythm [MS(+)AF(–)]) were included. Von Willebrand factor antigen levels and ristocetin cofactor activities in all participants were assessed. Overall, von Willebrand factor antigen levels and ristocetin cofactor activities in the AF(+) patients were higher than in the AF(–) patients ($P = .003$ and $P = .002$, respectively). Von Willebrand factor antigen levels and ristocetin cofactor activities in the 3 groups were found to be different ($P = .012$ and $P = .01$, respectively). Von Willebrand factor antigen levels were similar between the MS(+)AF(+) and MS(–)AF(+) groups and were higher than that of the MS(+)AF(–) group. Ristocetin cofactor activity in the MS(–)AF(+) group was significantly higher than in the other 2 groups. The ristocetin cofactor activity and von Willebrand factor antigen levels were significantly higher in diabetic or hypertensive patients than in nondiabetic or normotensive patients. According to the results of this study, circulating von Willebrand factor antigen levels and plasma ristocetin cofactor activities are affected by the presence of AF, MS, and associated comorbidities including type 2 diabetes mellitus and systemic hypertension. Further studies are needed to assess the role of von Willebrand factor antigen and ristocetin cofactor activity in predicting vascular thrombotic events in AF, MS, systemic hypertension, and diabetes mellitus.

Keywords: von Willebrand factor; ristocetin cofactor activity; atrial fibrillation; mitral stenosis

Von Willebrand factor (vWF) is a multimeric glycoprotein synthesized by endothelial cells and megakaryocytes. Von Willebrand factor carries Factor VIII in the circulation and is required for its stability. It also mediates platelet adhesion to the vessel wall by formation of a link between specific platelet membrane receptors (glycoprotein Ib–IX complex) and subendothelium.[1] Circulating vWF is used as a marker of endothelial injury or dysfunction.[2-7] Plasma vWF levels are elevated in patients with nonvalvular atrial fibrillation (AF) associated with endothelial dysfunction.[8-10] Ristocetin cofactor (RiCOF) activity assay is used as a reliable surrogate test for the adhesive function of vWF and allows identification of the qualitative abnormalities of the molecule.[11]

The mechanism of increased thrombogenesis in AF remains poorly understood. Although abnormality in blood flow within the atria is a major determinant of thrombosis in AF, the increased risk of thromboembolism associated with the presence of complex aortic plaque remote from the left atrial appendix (LAA)[12] and diabetes[13] supports the possibility of additional mechanisms such as a widespread abnormal endothelial dysfunction and subsequent thrombotic

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Table 1. Essential Characteristics of the Studied Patients[a]

Patient Groups	MS(+)AF(+)	MS(−)AF(+)	MS(+)AF(−)	*P*
Age (years)	57.5 (29-80)	65 (55-75)	39 (32-51)	<.001[b]
Number of patients	16	13	13	
Female/male	13/3	10/3	13/0	.197[c]
Left atrial dimension (cm)	5.2 (4.2-7.8)	4.3 (3.2-5.4)	4.4 (3.3-5.8)	.001[b]
End-diastolic dimension (cm)	4.9 (4-5.9)	5 (4.1-6.2)	4.8 (4-5.2)	>.05[b]
Ejection fraction (%)	64.5 (56-71)	65 (35-75)	68 (63-73)	>.05[b]
Mitral valve area (cm^2)	1.55 (0,9-2,5)	NA	1.7 (1.2-2.5)	<.001[d]
Mitral mean gradient (mmHg)	6.5 (3-10)	NA	5 (0-9)	<.001[d]
Pulmonary artery pressure (mmHg)	45 (20-70)	30 (20-70)	25 (20-50)	.052

Abbreviations: NA, not assessed; MS, mitral stenosis; AF, atrial fibrillation; MS(+)AF(+), MS and AF; MS(−)AF(+), nonvalvular AF; MS(+)AF(−), MS and sinus rhythm.
a. Data show median with minimum and maximum values.
b. Kruskal-Wallis test.
c. Chi-square test.
d. Mann-Whitney *U* test.

process. Accordingly, many studies focused on various endothelial markers, prothrombotic factors, and indicators of platelet activation in patients with AF.[2,6,7,14-18]

Atrial fibrillation and mitral stenosis (MS) coexist in a substantial number of patients. Plasma vWF levels and vWF expression in atrial endocardium were increased in MS associated with pulmonary hypertension[19] and left atrial enlargement,[18] respectively. Plasma vWF levels were correlated with ultrastructural damage to the surface of the LAA endocardium in patients with MS (many of whom had AF). These data support that endothelial damage or dysfunction (or vWF itself) may serve as an indicator of the increased thrombotic risk in patients with MS.[7]

The aim of this study was to assess the effects of AF and MS, independently or in combination, on the plasma levels of vWF antigen and RiCOF concurrently. These markers are widely available and easy to assess, and relations between these markers and AF and/or MS might result in use of vWF as a predictor of risk of thrombosis in these patient groups. We intended to test the hypothesis that endothelial dysfunction due to AF and MS, alone or in combination, could affect vWF and RiCOF.

Patients and Methods

Forty-two patients (36 women and 6 men), admitted to the Department of Cardiology at the Hacettepe University School of Medicine, were enrolled in the study. The study group included 16 patients with MS and AF (MS(+)AF(+)), 13 patients with nonvalvular AF (MS(−)AF(+)), and 13 patients with MS and sinus rhythm (MS(+)AF(−)). Von Willebrand factor antigen levels and RiCOF activity in all participants were assessed along with blood counts. Von Willebrand factor and RiCOF levels were measured by using commercially available hemostasis assays developed by Diagnostica Stago (Taverny, France). All patients with AF and/or MS underwent conventional 2-dimensional transthoracic echocardiography and Doppler examination.

Statistical analyses were performed using the SPSS statistical software package (SPSS Software; SPSS Inc, Chicago, Illinois). Patient characteristics were compared between groups, with chi-square test for categorical variables and Mann-Whitney *U* test or Kruskal-Wallis test for numeric variables. Results were expressed as median values with minimum and maximum values. A *P* value of less than .05 was considered statistically significant.

Results

Patient characteristics are summarized in Table 1. The age of the patients in the MS(+)AF(−) group was significantly lower than in the other 2 groups ($P = .001$). Von Willebrand factor levels and RiCOF activities in the 3 groups were found to be different (Kruskal-Wallis test; $P = .012$ and $P = .01$, respectively) (Table 2). Von Willebrand factor levels were similar between the MS(+)AF(+) and MS(−)AF(+) groups and were higher than that of the MS(+)AF(−) group (Figure 1). RiCOF activity in the MS(−)AF(+) group was significantly higher than in the other 2 groups (Figure 2). Although RiCOF activity in the

Table 2. vWF Levels and RiCOF of the Studied Patient Groups[a]

	MS(+)AF(+)	MS(–)AF(+)	MS(+)AF(–)	*P*[b]
RiCoF (%)	98.2 (62-265)	171 (78-380)	87 (46-102)	.001
vWF (IU/dL)	179 (52-384)	137 (102-390)	105 (50-123)	.012

Abbreviations: MS, mitral stenosis; AF, atrial fibrillation; RiCOF, ristocetin cofactor activities; vWF, von Willebrand antigen; MS(+)AF(+), MS and AF; MS(–)AF(+), nonvalvular AF; MS(+)AF(–), MS and sinus rhythm.
a. Data show median with minimum and maximum values.
b. Kruskal-Wallis test.

Table 3. Characteristics of the Studied Patients and vWF and RiCOF Values[a]

Patient Groups	DM(+)	DM(–)	HT(+)	HT(–)
Number of patients	9	33	16	26
Female/male	9/0	27/6	14/2	22/4
RiCOF (%)	188 (80-380)	91.5 (46-296)	131 (78-380)	90 (46-265)
vWF (IU/dL)	192 (67-390)	110 (50-394)	169 (67-390)	110 (50-394)

Abbreviations: DM, diabetes mellitus; HT, hypertension; RiCOF, ristocetin cofactor activities; vWF, von Willebrand antigen.
a. Data are presented as median with minimum and maximum values.

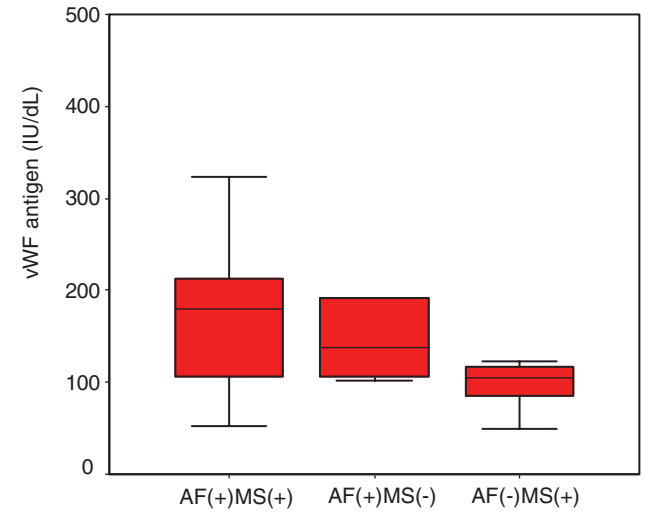

Figure 1. The vWF levels of the patient groups. MS, mitral stenosis; AF, atrial fibrillation; vWF, von Willebrand factor.

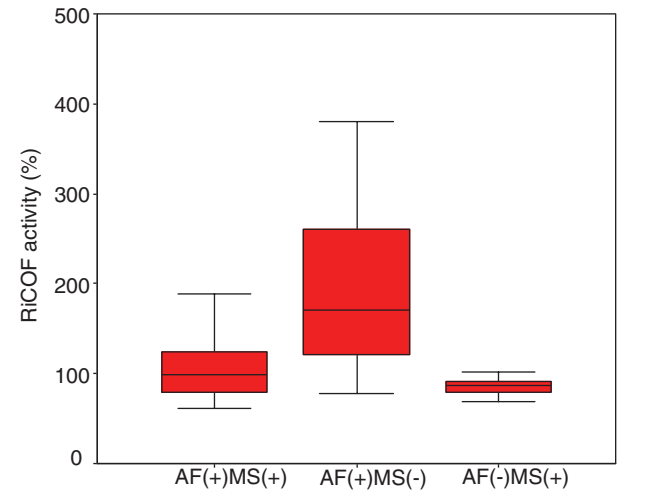

Figure 2. The RiCOF activities in the patient groups. MS, mitral stenosis; AF; atrial fibrillation; RiCOF, ristocetin cofactor.

MS(+)AF(+) group was not significantly higher than in the MS(+)AF(–) group, *P* value approached significance (*P* = .052). Von Willebrand factor levels and RiCOF activities in the AF(+) patients were higher than in the AF(–) patients (*P* = .003 and *P* = .002, respectively) (Table 3).

Among all the studied patients, vWF levels and RiCOF activities were inversely correlated with ejection fraction (vWF antigen: $r = -0.458$, $P = .003$; RiCOF: $r = -0.361$, $P = .022$). No significant correlation was detected between vWF and RiCOF and mitral valve area, mitral mean gradient, pulmonary arterial pressure, hemoglobin concentration, and platelet count.

Among the 42 patients, 9 were diabetic and 16 were hypertensive. Median RiCOF activity was 188% (range: 80%-380%) and vWF antigen level was 192 IU/dL (range: 67-390 IU/dL) in diabetic patients. The RiCOF activity and vWF antigen levels in diabetic patients were significantly higher than in nondiabetic

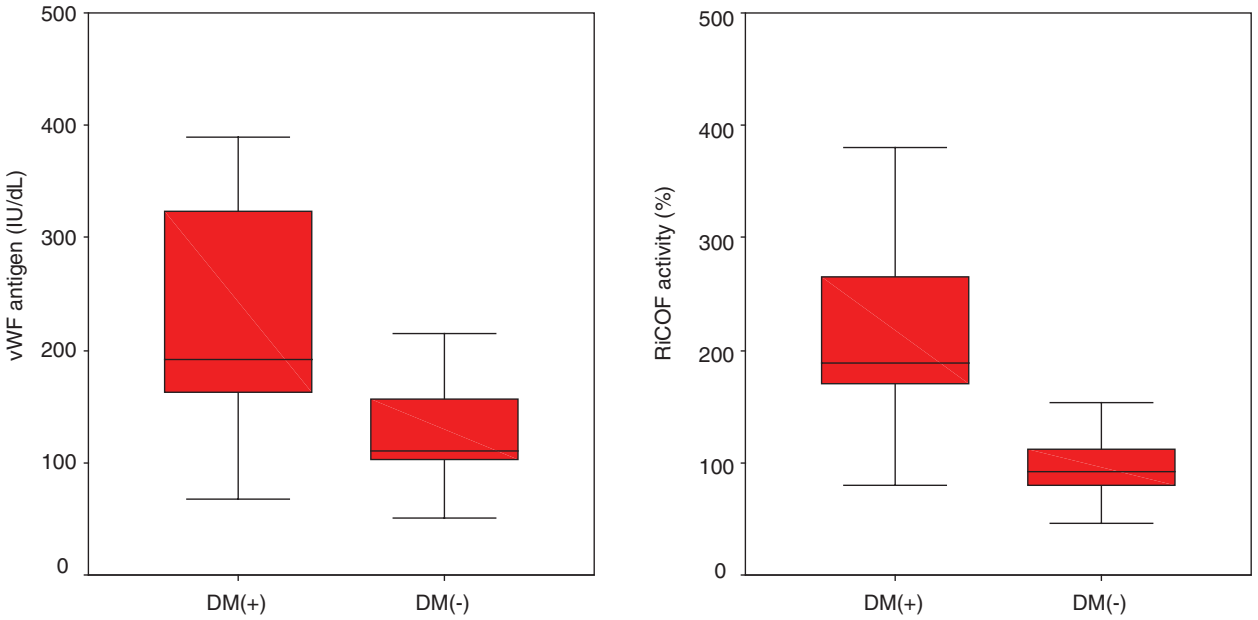

Figure 3. The von Willebrand factor (vWF) levels (A) and ristocetin cofactor (RiCOF) activities (B) of diabetic and nondiabetic patients. DM, diabetes mellitus.

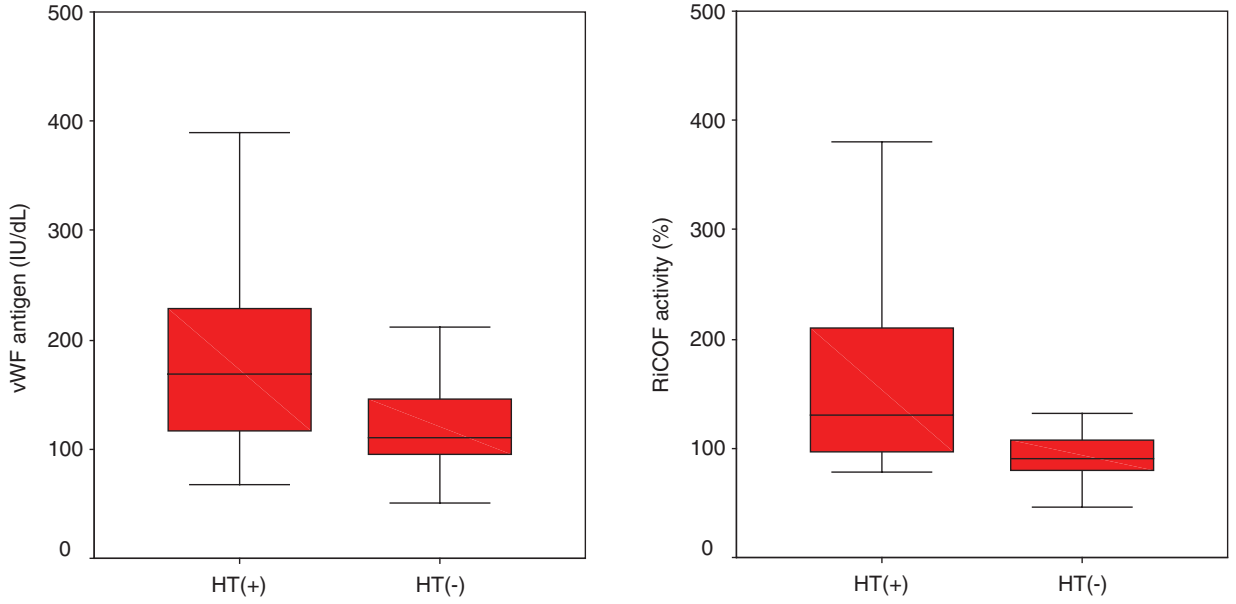

Figure 4. The von Willebrand factor (vWF) levels (A) and ristocetin cofactor (RiCOF) activities (B) of hypertensive and nonhypertensive patients. HT, hypertension.

patients ($P = .002$ and $P = .019$, respectively) (Figure 3). Similarly, the RiCOF activity and vWF levels in 16 hypertensive patients were significantly higher than in nonhypertensive patients ($P = .019$ and $P = .049$, respectively) (Figure 4).

Discussion

According to the results of this study, circulating vWF levels and plasma RiCOF activities are affected by the presence of AF, MS, and associated comorbidities

including type 2 diabetes mellitus (DM) and systemic hypertension (HT). Those observations are in accordance with previous investigations,[2,5-7,9,18,20-22] indicating that endothelial damage or dysfunction (or vWF itself) may play an important role in the pathobiology of vascular outcome in AF, MS, DM, and HT. Furthermore, vWF might represent an adjunctive aid to the clinical risk stratification in the patients suffering from those disorders.

Disease presentations with respect to the prothrombotic state are heterogeneous in AF, MS, DM, and HT.[2,5,7,18,22-27] Shear stresses at the vessel wall affect endothelial cell integrity and function, leading to the secretion of vWF in these disorders. Platelets are concentrated at the vessel wall, where they can be activated by high shear stresses by interacting with vWF. Areas of recirculating blood flow under distinct shear stresses predispose to intracardiac thromboembolism as observed in AF.[28] Altered vWF and RiCOF activities in our patient groups in this study seem to be in relation to those pathogenetic mechanisms.

In our study, diabetes (Figure 3) and hypertension (Figure 4) represented significant contributing and precipitating factors to both vWF and RiCOF activities in the patient groups. Previous studies also revealed that vWF may serve as a molecular link between endothelial dysfunction and vascular events of DM.[26,27,29-31] A unique prothrombotic state associated with insulin resistance and increased vWF release, which could lead to a higher risk for the development of thrombotic events in hypertensive diabetic patients, has been described.[26] Our results confirmed that DM and HT contribute to the pathological hemostasis of both AF and MS by further increasing the vWF in the studied patient groups. Congestive heart failure may also contribute to hypercoagulability and thrombotic risk in AF through increased endothelial damage and dysfunction.[23] Accordingly, in our study, vWF levels and RiCOF activities were inversely correlated with ejection fraction in all the studied patients.

In the presence of activated platelets and the complement of vWF antigen, the antibiotic ristocetin causes glycoprotein Ib/vWF-dependent platelet agglutination in vitro. Because vWF levels reflect the ongoing endothelial dysfunction, RiCOF as a surrogate test for the adhesive function of vWF could also be used for the identification of the endothelial injury or dysfunction in a wide variety of disorders including AF, MS, DM, and HT. In our present study, endothelial dysfunction due to AF and MS, alone or in combination, affected vWF and RiCOF mainly inharmoniously. Von Willebrand factor levels were similar between the MS(+)AF(+) and MS(−)AF(+) groups and were higher than that in the MS(+)AF(−) group (Figure 1). However, RiCOF activity in the MS(−)AF(+) group was significantly higher than in the other 2 groups (Figure 2). Therefore, although AF is a major pathological event affecting both vWF and RiCOF, sometimes the presence of other contributing factors such as MS may change their levels inharmoniously. Hence, vWF and RiCOF should simultaneously be searched to seek out ongoing endothelial dysfunction in a given clinical disorder.

The search of endothelial dysfunction in relation to the vWF kinetics is not just academic, because the subject is directly related to the pathogenesis and management of patients with AF, MS, DM, and HT.[2-4,14-16,22] The antithrombotic strategy may rely on the severity of ongoing vascular endothelial function in those diseases. Therefore, further prospective studies are needed to assess the prognostic role of vWF and RiCOF in AF, MS, HT, and DM in relation to vascular thrombotic events to predict cardiovascular mortality as well as their role in complementing clinical risk stratification schemas.

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