

# Increased von Willebrand Factor in the Endocardium as a Local Predisposing Factor for Thrombogenesis in Overloaded Human Atrial Appendage

Mitsumasa Fukuchi, MD,\* Jun Watanabe, MD,\* Koji Kumagai, MD,\* Yukio Katori, MD,†  
Shigeo Baba, MD,\* Koji Fukuda, MD,\* Takuya Yagi, MD,\* Atsushi Iguchi, MD,‡  
Hitoshi Yokoyama, MD,‡ Masahito Miura, MD,\* Yutaka Kagaya, MD,\* Shigekazu Sato, MD,§  
Koichi Tabayashi, MD,‡ Kunio Shirato, MD\*

Sendai, Japan

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<b>OBJECTIVES</b>	We investigated immunoreactive von Willebrand factor (vWF), a platelet adhesion molecule, in the endocardial endothelium and its relationship to thrombogenesis in the human atrial appendage.
<b>BACKGROUND</b>	Intra-atrial thrombogenesis is generally thought to be induced by blood stasis in the atrial appendage involved with atrial fibrillation (AF). Little attention has been paid to alterations of the endocardial endothelium on which the thrombus develops.
<b>METHODS</b>	Atrial appendage tissue was obtained at heart surgery or at autopsy from AF and non-AF cardiac patients and from noncardiac patients. Immunohistochemistry for endothelial cell markers including vWF, CD31, CD34 and endothelial nitric oxide synthase (eNOS) and platelet glycoprotein Ib/IX or IIb/IIIa was performed and semiquantitatively graded.
<b>RESULTS</b>	In contrast to the apparent immunostaining for CD31, CD34 and eNOS, only focal or little immunoreactive vWF was seen in the endocardium of noncardiac patients. Immunoreactive vWF in the endocardial endothelium was increased in most cardiac patients, particularly in the left, but not in the right, atrial appendage of patients with mitral valvular disease, irrespective of whether AF was present. Platelet adhesion/thrombus formation in the endocardium was found in limited sites in which the overlying endothelium was deficient in eNOS and CD34. When warfarin-treated cases were excluded, there was a significant correlation between the immunohistochemical grade for vWF and the degree of platelet adhesion/thrombus formation in the endocardium.
<b>CONCLUSIONS</b>	Immunoreactive vWF in the endocardial endothelium was increased in overloaded human atrial appendage, which may be a local predisposing factor for intraatrial thrombogenesis. (J Am Coll Cardiol 2001;37:1436–42) © 2001 by the American College of Cardiology

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Intra-atrial thrombus formation in patients with atrial fibrillation (AF) is known to be a potent risk factor for thromboembolic events such as stroke (1). Thrombus formation is preferentially found in the atrial appendage and has been thought to be induced by blood stasis associated with AF (2,3). However, the incident rates of cardioembolic complications have been reported to vary among AF patients depending on the presence of coexistent heart diseases and clinical risk factors for thromboembolism, including age, hypertension, diabetes mellitus and previous ischemic attack (4,5). In addition, in patients with AF alone who were <65 years old, the incident rate of stroke was similar to that in people without AF (<1%/year for both) (4–6).

Little attention has been paid to alterations of the

endocardial surface on which intra-atrial thrombus develops (2,3). Platelet adhesion to the deendothelialized vessel wall is an initiating event of thrombus formation (7) and is mediated through the platelet adhesion molecule von Willebrand factor (vWF) present in the plasma and/or subendothelium (8–10). von Willebrand factor is synthesized and stored in endothelial cells and platelets and can be secreted locally at the site of injured vessels (11). Recently, elevated plasma or endothelial levels of vWF have been shown in pulmonary hypertension (12), congestive heart failure (13) and chronic AF (14), implying the possible role of vWF in the thrombotic predilection of these hemodynamic abnormalities. Therefore, we have speculated that altered expression of vWF in the endocardial endothelium may affect local thrombogenicity in the human atrial appendage.

We examined immunoreactive vWF in the endocardial endothelium of atrial appendages of AF and non-AF patients with a variety of underlying cardiac pathogeneses and compared the data with those of noncardiac patients without any clinical predictors for thromboembolic risk. In

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From the \*Department of Cardiovascular Medicine, †Otolaryngology and ‡Thoracic and Cardiovascular Surgery, Tohoku University, Graduate School of Medicine, Sendai, Japan; and the §Department of Cardiovascular Surgery of Tohoku Kosai Hospital, Sendai, Japan.

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**Abbreviations and Acronyms**

AF	= atrial fibrillation
eNOS	= endothelial nitric oxide synthase
GP Ib/IX	= glycoprotein Ib/IX
GP IIb/IIIa	= glycoprotein IIb/IIIa
IgG	= immunoglobulin G
vWF	= von Willebrand factor

In addition, to explore the possible role of vWF in thrombogenesis, we investigated the relationship between immunoreactive vWF in the endocardium and platelet adhesion/thrombus formation detected at the tissue level.

**METHODS**

**Patients.** This study was performed with the permission of the Ethics Committee for clinical and experimental investigations at Tohoku University. Atrial appendages were obtained at heart surgery from patients with mitral (n = 15) or aortic valvular disease (n = 11), coronary artery disease (n = 6) and atrial septal defect (n = 7), and at autopsy (within 3 h after death) from patients with end-stage heart failure (n = 4) and noncardiac patients (seven men/three women, average age 59 ± 5.1 years) with no clinical high-risk predictors for thromboembolism (4,5). The clinical data of cardiac patients are shown in Table 1. Although most patients with valvular heart disease had one or more episodes of congestive heart failure, heart surgery was performed in a well-controlled condition showing nearly normal ranges for the cardiac index and left ventricular ejection fraction. Atrial fibrillation accompanied 12 of 15 patients with mitral valvular disease, but only four of 28 other cardiac patients. Two patients with valvular heart disease had a history of cerebral infarction, which was confirmed by a computed tomographic scan of the head.

Full-dose warfarin and/or low-dose aspirin were administered in 10 of 15 patients with mitral valvular disease and in nine of 16 patients with AF. These antithrombotic drugs were usually discontinued three days before heart surgery. Both right and left atrial appendages were obtained from some operative cases that received Maze operation and from all autopsy cases. Only the right atrial appendage was available in the other cases.

**Immunohistochemistry.** Isolated atrial tissue was immediately fixed in 4% paraformaldehyde for 6 h at room temperature. The tissue was sectioned every 3 mm to survey the immunoreactivity for vWF and platelet adhesion/thrombus formation in the endocardium. Immunohistochemistry was performed in cryostat sections (4 μm thick) by the avidin-biotin-immunoperoxidase method as previously described (15). Briefly, sections were incubated serially with the following solutions: 1) 2% hydrogen peroxide for 30 min to block endogenous peroxidase activity; 2) 0.3% Triton-X 100 for 15 min to permeabilize the membrane; 3) 10% normal goat serum for 60 min to reduce nonspecific binding of the antiserum; 4) primary antisera for 16 h at 4°C; 5) biotinylated goat antimouse or goat antirabbit immunoglobulin G (IgG) at a dilution of 1:200 for 45 min; and 6) avidin-biotinylated horseradish peroxidase complex (Vectastain, Vector Laboratories, Burlingame, California) at a dilution of 1:100 for 45 min. Immunoreactive sites were visualized by incubation with 0.025% 3,3-diaminobenzidine and 0.01% hydrogen peroxide for 3 min. Phosphate-buffered saline (pH 7.4) was used to dilute each solution and to wash the sections three times between each step. Finally, tissue sections were counterstained with hematoxylin.

As primary antisera, polyclonal and monoclonal anti-vWF antibodies (both Dako, Glostrup, Denmark) at a dilution of 1:2,000 and 1:300, respectively, were used to evaluate vWF in the endocardial endothelium. To confirm

**Table 1.** Biographical and Hemodynamic Data of Cardiac Patients

Diagnosis	Mitral VD	Aortic VD	IHD	ASD	Heart Failure
Men/women	7/8	7/4	4/2	5/2	2/2
Age (yrs)	58 ± 3.2	53 ± 5	62 ± 3.8	36 ± 10.3	68 ± 6.7
CI (l/min)	2.5 ± 0.18	3.5 ± 0.17	3.7 ± 0.40	NA	NA
LVEF (%)	63 ± 3.3	68 ± 4.3	57 ± 8.9	67 ± 1.6	39 ± 6.2
LVEDD (mm)	55 ± 2.8	59 ± 3.4	54 ± 2.3	38 ± 0.8	62 ± 4.8
AF (+/-)	12/3	1/10	0/6	1/6	2/2
Hypertension (%)	13	27	17	14	0
Diabetes (%)	13	9	17	0	25
Medications					
Warfarin (%)	53	9	0	0	25
Aspirin (%)	40	27	50	0	25
Digoxin (%)	87	0	17	29	50
ACE inhibitor (%)	20	18	50	0	50
Ca antagonist (%)	13	36	50	14	25
Nitrates (%)	13	9	83	0	0
Diuretics (%)	53	9	0	0	100

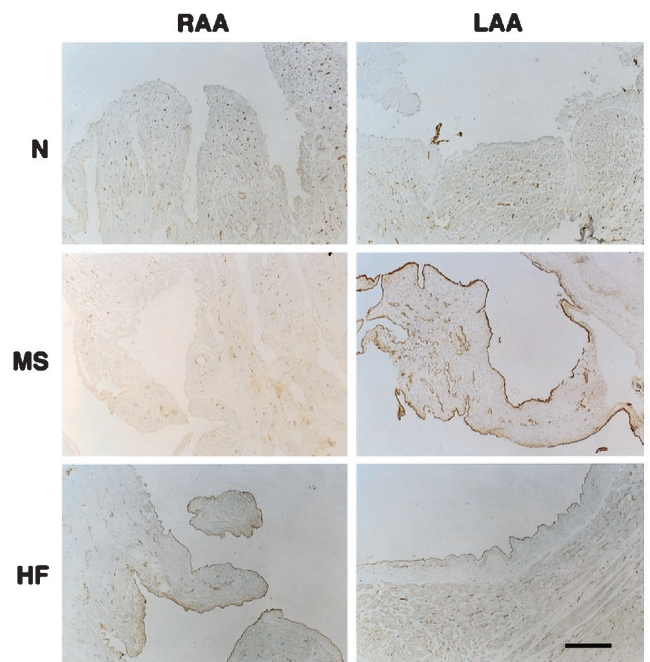
Age and hemodynamic data are expressed as means ± SEM.

ACE = angiotensin-converting enzyme; AF = atrial fibrillation; ASD = atrial septal defect; Ca = calcium; CI = cardiac index; IHD = ischemic heart disease; LVEDD = left ventricular end-diastolic dimension; LVEF = left ventricular ejection fraction; NA = not available; VD = valvular disease.

the integrity of the endothelium, serial sections were also immunostained for other endothelial cell markers using monoclonal antibodies against CD31 (Dako), CD34 (Nichirei, Tokyo, Japan) and eNOS (Transduction Laboratories, Lexington, Kentucky). Platelets on the endocardium were detected by immunostaining with monoclonal antibodies against the membrane receptors for vWF; glycoprotein (GP) Ib/IX (PharMingen, San Diego, California) and GP IIb/IIIa (Chemicon International Inc., Temecula, California). The concentrations of these primary antisera were determined by using isolated normal human platelets and human coronary arteries isolated at autopsy. The specificity of immunostaining was assessed by incubation with nonimmunized rabbit or mouse serum instead of the primary antiserum.

**Immunoelectronmicroscopy.** Atrial tissue was fixed in a mixture of 4% paraformaldehyde and 0.2% glutaraldehyde for 4 h at room temperature. After the fixation, the tissue was dehydrated with two washes (60 min each) in 70% ethanol and was placed into LR White resin (Agar Scientific Ltd., Essex, U.K.). After 1 h the LR White resin was changed and the tissue left in it was placed on a rotary mixer overnight. Following an additional change of resin for 1 h, the tissue was embedded in a small gelatin capsule to avoid oxygen and polymerized at 50°C for 24 h. Ultrathin resin sections (110 nm) were cut on a Leica Ultracut R Microtome and collected on 200-mesh nickel grids. The immunogold labeling method was employed as previously described (16). Briefly, grids were incubated serially on droplets of the following solutions: 1) 5% normal goat serum in phosphate-buffered saline containing 1% bovine serum albumin (1% BSA/PBS, pH 8.2) for 10 min; 2) polyclonal anti-vWF antibody (Dako) diluted at 1:500 in 1% BSA/PBS at 4°C overnight; 3) a series of three fresh droplets of 1% BSA/PBS for 5 min each; 4) goat antirabbit IgG, conjugated to 10-nm gold particles, diluted at 1:100 in 1% BSA/PBS for 1 h (British BioCell International, Cardiff, United Kingdom); 5) a series of three fresh droplets of 1% BSA/PBS for 5 min each followed by washing in distilled water; and 6) 2% aqueous uranyl acetate for 20 min followed by washing with distilled water and air drying. The sections were examined using a JEM-1010 electron microscope (JEOL Ltd., Tokyo, Japan) operated at an accelerating voltage of 80 kV.

**Histological analysis.** Immunoreactivity for vWF and platelet adhesion/thrombus formation in the endocardium was semiquantitatively assessed by light microscopic observation. Immunostaining intensity for vWF in the endocardium was graded in comparison with that in the endothelium of intramyocardial vessels of the same sections (grade 1, only focal or little staining; grade 2, diffuse, weaker staining; grade 3, similar staining and grade 4, stronger staining). The grading was performed independently by two observers without prior knowledge of the case histories. In cases in which there was a discrepancy between the two observers, the final score was determined by joint observation. Grades for different sections from the same atrial appendage were averaged to give a single value. The degree



**Figure 1.** Typical examples of immunohistochemistry for von Willebrand factor in right and left atrial appendages from patients with noncardiac disease (N; 63 years old, male), mitral valvular stenosis (MS; 53 years old, female), and end-stage heart failure (HF; 80 years old, male), respectively. The latter two cases were accompanied by atrial fibrillation. RAA and LAA indicate right and left atrial appendages, respectively. Bar = 200  $\mu$ m.

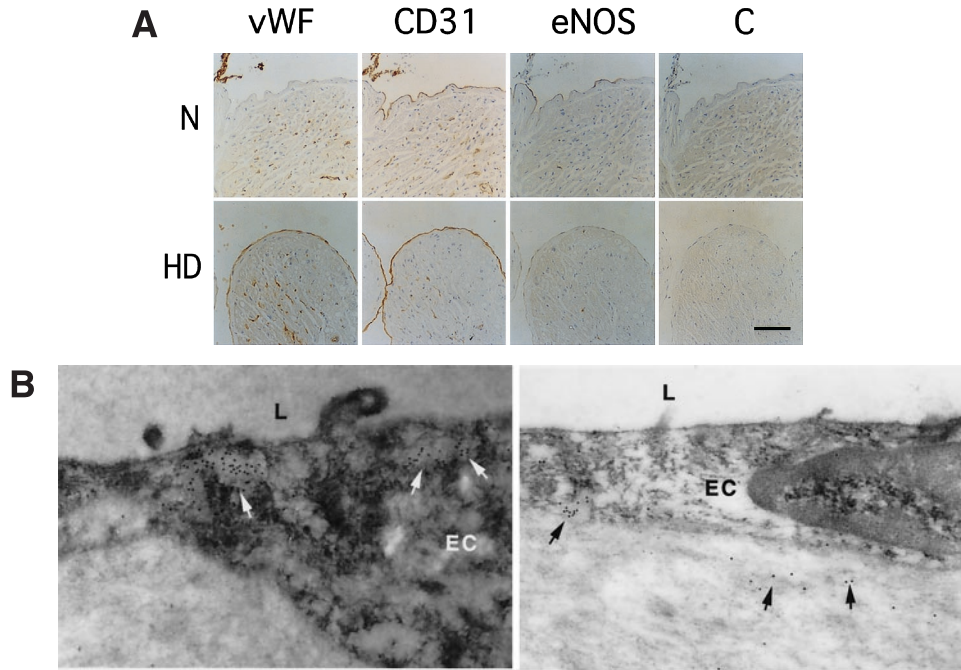
of platelet adhesion/thrombus formation in the endocardium was graded according to the distribution and nature of adhered platelets positive to GP Ib/IX and GP IIb/IIIa in each atrial appendage (grade 1, no adhesion; grade 2, a few patchy adhesions; grade 3, multiple and/or widespread adhesions and grade 4, thrombi containing other blood cell types). Clotted blood with no attachment to the endocardium was sometimes found in autopsy cases and was excluded as a postmortem artifact.

**Statistical analysis.** Immunohistochemical grades for vWF in the endocardium were categorized into four ranks (1,  $\leq 2$ ,  $\leq 3$ , and  $\leq 4$ ) in each atrial appendage and were compared among different patient groups by the Kruskal-Wallis rank test. After global testing, a multiple-comparison procedure with Student-Newman-Keuls test was performed in each pairwise comparison. The relationship between the immunohistochemical grade for vWF and the degree of platelet adhesion/thrombus formation in the endocardium was examined by Spearman rank correlation. The incidence was compared among different groups by chi-square analysis. Values of  $p < 0.05$  were considered significant.

## RESULTS

Immunoreactivity for vWF in the endocardium varied widely among individual atrial appendages, whereas apparent immunoreactivity for vWF was consistently seen in the endothelium of the intramyocardial vessels of the same atrial tissues as well as in isolated platelets (Fig. 1). In noncardiac



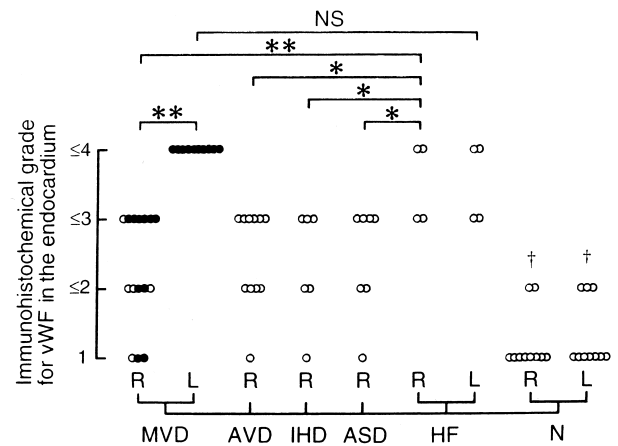


**Figure 2.** (A) Typical examples of immunohistochemistry for endothelial cell markers including von Willebrand factor, CD31 and eNOS in atrial appendages from noncardiac (N) and cardiac patients (HD), between which the immunoreactivity for vWF in the endocardium differed greatly. No specific staining was seen in control sections incubated with nonimmunized immunoglobulin (C). Bar = 100  $\mu$ m. (B) Electron micrographs of left atrial appendage of a cardiac patient, which had been stained with the immunogold method using polyclonal anti-vWF antibody. Gold particles (**arrows**) are present in vesicles within the endothelial endothelium and in the subendothelium. EC = endothelial cell; L = lumen. Original magnification  $\times 12,000$ .

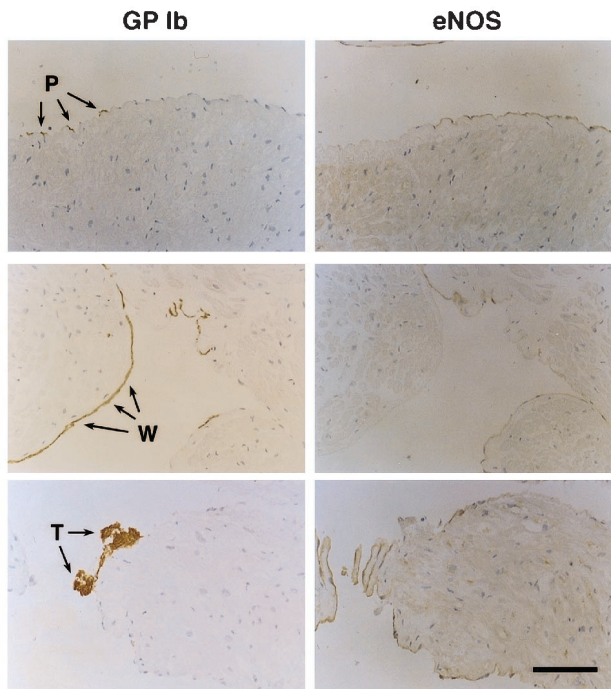
patients with none of the high-risk predictors for thromboembolism, only focal or little immunoreactivity for vWF was seen in the endocardium of both right and left atrial appendages (Fig. 1, N). On the other hand, apparent immunoreactivity for vWF was extensively seen in the endocardium of atrial appendages of most cardiac patients examined. Particularly, stronger immunoreactivity for vWF in the endocardium compared with that in the endothelium of intramyocardial vessels was seen in the left, but not in the right, atrial appendages of patients with mitral valvular disease (Fig. 1, MS), and in both right and left atrial appendages of patients with end-stage heart failure (Fig. 1, HF). In contrast to the varying immunoreactivity for vWF in the endocardium, an apparent immunoreactivity for CD31 and CD34 in the endocardium was preserved in most cases of both cardiac and noncardiac patients (Fig. 2A; vWF vs. CD31). Immunoreactivity for endothelial nitric oxide synthase (eNOS) in the endocardium was apparently seen in most atrial appendages of noncardiac patients, but was inconsistent in atrial tissues of cardiac patients (Fig. 2A; eNOS). Immunoelectron microscopy showed that positive gold labeling for vWF antigen was observed mainly in vesicles within the endothelial endothelium and, to a lesser extent, in the subendothelium (Fig. 2B). Few gold particles were observed alongside the luminal surface of the plasma membrane of the endothelium. No specific staining was seen in the negative control sections (data not shown).

The immunohistochemical grade for vWF in the endocardium was summarized according to the underlying heart

disease (Fig. 3). There was a significantly higher immunohistochemical grade for vWF in the endocardium of both right and left atrial appendages of all cardiac patient groups



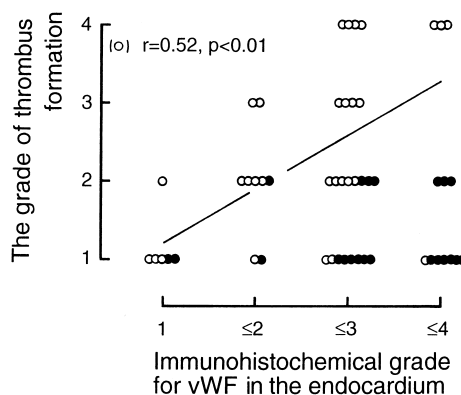
**Figure 3.** Distribution of immunohistochemical grades for von Willebrand factor in the endocardium of atrial appendages according to the underlying cardiac pathogenesis. Immunohistochemical grades for von Willebrand factor were averaged to give a single value to each atrial appendage and were categorized into four ranks (1,  $\leq 2$ ,  $\leq 3$  and  $\leq 4$ ). **Closed symbols** indicate cases of mitral valvular disease in which right and left atrial appendages were paired. There was a significant difference among different groups by Kruskal-Wallis rank test ( $p < 0.01$ ). Each pairwise comparison was performed by Student-Newman-Keuls test for multiple comparisons. † $p < 0.01$  vs. all groups with underlying cardiac pathogenesis other than IHD ( $p < 0.05$ ). \* $p < 0.05$ ; \*\* $p < 0.01$ . ASD = atrial septal defect; AVD = aortic valvular disease; HF = end-stage heart failure; IHD = ischemic heart disease; MVD = mitral valvular disease; N = nondiseased heart; NS = not significant.



**Figure 4.** Immunohistochemical localization and patterns of platelet adhesion/thrombus formation in atrial appendages of cardiac patients. Immunohistochemistry for glycoprotein Ib/IX and endothelial nitric oxide synthase in serial sections is shown in three typical cases. P = patchy platelet adhesion; T = thrombus; W = widespread platelet adhesion. Bar = 100  $\mu$ m.

than in those of noncardiac patients. Furthermore, among the cardiac patient groups, the left, but not the right, atrial appendages of patients with mitral valvular disease and both right and left atrial appendages of patients with end-stage heart failure showed a significantly higher immunohistochemical grade for vWF in the endocardium compared with that of the right appendages of any other patient groups. However, a similar immunohistochemical grade for vWF in the endocardium was seen in the atrial appendages of cardiac patients with and without concomitant AF (mean values; 2.33 [n = 16] vs. 2.44 [n = 25] for right atria, 3.63 [n = 12] vs. 3.25 [n = 4] for left atria). In addition, this vWF grade was not correlated with any clinical and hemodynamic variables including age, hypertension, diabetes, cardiac index, left ventricular ejection fraction, and left ventricular end-diastolic dimension or with any medications before heart surgery.

No macroscopic thrombus formation was observed in any atrial appendages examined. Immunohistochemistry for platelet GP Ib/IX or GP IIb/IIIa showed various degrees of platelet adhesion/thrombus formation in more than half the atrial appendages of cardiac patients with and without AF (Fig. 4, shown for GP Ib/IX). Adhered platelets in the endocardium also had apparent immunoreactivity for vWF and CD31, but were found only in limited sites with the endocardial endothelium deficient in immunoreactive eNOS and CD34 (Fig. 4, shown for eNOS). Only patchy or



**Figure 5.** Relation between the immunohistochemical grade for von Willebrand factor (vWF) and the degree of platelet adhesion/thrombus formation in the endocardium. Each symbol indicates a right or left atrial appendage obtained from a cardiac patient. There was a significant correlation between the two variables ( $r = 0.52$ ,  $p < 0.01$  by Spearman rank correlation) only when the cases who had received warfarin-treatment (closed symbols) were excluded.

no platelet adhesions were found in the endocardium of atrial appendages from noncardiac patients.

In order to explore the possible role of increased endocardial vWF in thrombogenicity in atrial appendages of cardiac patients, the degree of platelet adhesion/thrombus formation was plotted as a function of the immunohistochemical grade for endocardial vWF in each atrial appendage (Fig. 5). A significant correlation between both variables was seen only when cases that had been treated with warfarin, alone or in combination with aspirin, (closed symbols in Fig. 5) were excluded ( $r = 0.52$ ,  $p < 0.01$  [n = 30] by Spearman rank correlation). No platelet adhesion/thrombus formation graded at  $3 \leq$  was seen in warfarin-treated cases (n = 22) irrespective of the immunohistochemical grade for vWF. These cases consisted mainly of atrial appendages from mitral valvular disease patients with concomitant AF. On the other hand, the degree of platelet adhesion/thrombus formation was similar in aspirin-treated, but not warfarin-untreated cases nor aspirin or warfarin-treated cases (grade  $\geq 3$ ; 3/10 vs. 10/20,  $p = \text{NS}$ ).

## DISCUSSION

It has been well established that vWF in the subendothelium or plasma is required for platelet adherence to deendothelialized vessel walls (8-10), which is mediated by the interaction with two platelet surface receptors, GP Ib/IX and GP IIb/IIIa (17,18). The present study demonstrated increased immunoreactive vWF in the endocardium of atrial appendages from both AF and non-AF patients with a variety of underlying heart diseases. This increased immunoreactive vWF in the endocardium was significantly correlated with the degree of platelet adhesion/thrombus formation detected microscopically as positive to GP Ib/IX or GP IIb/IIIa. These results suggest a possible role of increased endocardial platelet adhesion molecule vWF in thrombogenesis in the human atrial appendage.

### Immunoreactive vWF in the endocardial endothelium.

In the present study, it is noteworthy that only focal or little immunoreactive vWF was found in the endocardium of noncardiac patients with none of the clinical high-risk predictors for thromboembolism, whereas vWF was apparent in the endothelium of intramyocardial vessels of the same cases. We confirmed that immunoreactivity for other endothelial cell markers, CD31, CD34 and eNOS, was maintained in the endocardial endothelium as well as in the vessel endothelium. Our observations suggest that in noncardiac patients, the endocardial endothelium of the atrial appendage is selectively deficient in vWF, which has been used as an endothelial cell marker in human vessels (19,20). However, this is not surprising because Yamamoto et al. (21) have recently reported that vWF is differentially expressed in endothelial cells among different tissues or organs in mice.

On the other hand, increased immunoreactivity for vWF in the endocardium was found particularly in the left, compared with the right, atrial appendage of patients with mitral valvular disease and in both right and left atrial appendages of patients with end-stage heart failure (Fig. 3), but irrespective of the presence of AF. Previously, Rabinovitch et al. (12) had also shown increased immunostaining for vWF in the pulmonary vessel endothelium of patients with pulmonary hypertension. These immunohistochemical data suggest that increased vWF antigen in the vessel endothelium and atrial endocardium is associated with abnormal hemodynamic conditions. In the present study, the integrity of the overlying endothelium was confirmed in most areas with abundant vWF antigen by immunostaining for several endothelial cell markers (Fig. 2). In addition, an electron microscopic observation showed that vWF antigen was located in vesicles within the endocardial endothelium and subendothelium. This is consistent with evidence that vWF is synthesized in endothelial cells and constitutively secreted into both luminal and abluminal sides of those cells (19,22). Because it has been shown in an *in vitro* study that endothelial cells do not bind or uptake exogenous vWF (23), abundant vWF antigen in the endocardium of overloaded atrial appendage most likely reflects its increased synthesis in the overlying endothelium. Nevertheless, the present study did not determine the exact origin of the vWF deposited in the subendothelium. We cannot exclude the possibility that mechanical overload in the atrial walls may damage the superficial endothelial cell layers, allowing deposition of plasma-derived vWF into the subendothelium.

**vWF and thrombogenesis in the endocardium.** An intimate relationship between increased immunoreactive vWF in the endocardium and platelet adhesion/thrombus formation (Fig. 5) supports the possible role of the endocardial vWF in increased thrombogenicity in atrial appendages of patients with a variety of underlying cardiac pathogenesises. However, platelet adhesion in the endocardium was found only in limited sites in which the overlying endothelium was

deficient in immunoreactive eNOS and CD34 (Fig. 4). This is consistent with the concept that platelet adhesion to the vessel wall requires endothelial injury, which would induce the secretion of vWF into the lumen and subendothelium and subsequently expose abundant vWF to circulating platelets (8-11,22). Alternatively, in most areas with apparent eNOS staining in the overlying endothelium, the absence of platelet adhesion may be in part explained by the release of endothelium-derived factors, including nitric oxide and prostacyclin, that have been shown to inhibit platelet adhesion/aggregation (24).

Previous studies using transesophageal echocardiography have shown that a rheologic factor, blood stasis, is important in thrombogenesis in the atrial appendages of both AF and non-AF patients with underlying cardiac pathogenesis (2,3). Fatkin et al. (25) have estimated the shear rate of blood at the orifice of the left atrial appendage to be 50 to 150  $s^{-1}$  in patients with AF. Although it has been experimentally demonstrated that vWF plays a major role in platelet adhesion at a high shear rate (8-10), there is evidence suggesting that vWF can mediate platelet adhesion to the subendothelium or vWF-coated coverslips at shear rates as low as 50 to 650  $s^{-1}$  (8,10,18,26,27). In addition, Badimon et al. (26) have observed that platelet adhesion to subendothelium at longer exposure times (20 to 30 min) reaches similar plateau levels at shear rates ranging from 212 to 3,380  $s^{-1}$ . Thus, it is possible that under *in vivo* conditions, chronic exposure of subendothelial vWF to circulating platelets would induce stable platelet adhesion even at low shear rates.

**Effects of antithrombotic therapy.** In the present study, we observed platelet adhesion/thrombus formation at the tissue level in more than half the atrial appendages of the cardiac patients examined. Although the study population was too small to compare the incidence of thrombus formation among different patient groups, it was noted that in cases with warfarin treatment, but not in cases with aspirin treatment alone, neither multiple nor widespread platelet adhesion/thrombus formation (graded at  $\leq 3$ ) was found in any atrial appendages examined (Fig. 5). This observation is consistent with the results of clinical trials in which warfarin was more effective than aspirin in preventing cardioembolic events in patients with nonrheumatic AF (1,5,28). The antithrombotic effect of full-dose warfarin, but not of aspirin, in patients with AF has also been confirmed by the reduced plasma levels of fibrin D-dimer and beta-thromboglobulin (29). Aspirin inhibits platelet aggregation but not direct attachment to the subendothelium, which occurs mostly via fibrinogen (8,30). However, the inhibitory effects of aspirin on platelet aggregation in response to agonists are limited (31), and shear-induced platelet aggregation mediated by vWF is resistant to aspirin (32). Therefore, it is possible that, in aspirin-treated patients, increased vWF in the endocardium may act as an alternative mechanism for aspirin-sensitive, fibrinogen-dependent platelet aggregation.



**Conclusions.** The present study demonstrates, for the first time, increased immunoreactive vWF in the endocardial endothelium and its intimate relationship to thrombogenesis detected microscopically in atrial appendages of patients with underlying cardiac pathogenesis, irrespective of the presence of concomitant AF. The increased vWF in association with the disruption of the endocardial endothelium would contribute to the initiation of thrombogenesis by promoting platelet adhesion/aggregation on the endocardial surface, whereas a rheologic factor is thought to be required for further growth of the thrombus into the atrial lumen. Our results suggest that, independent of the presence of AF, vWF levels in the endocardial endothelium may modulate local thrombogenicity in the human atrial appendage.

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**Reprint requests and correspondence:** Dr. Kunio Shirato, Professor and Chairman, Department of Cardiovascular Medicine, Tohoku University, Graduate School of Medicine, 1-1 Seiryomachi, Aoba-ku, Sendai 980-8574, Japan. E-mail: fukuchi@int1.med.tohoku.ac.jp.

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