Journal of the American College of Cardiology
© 2009 by the American College of Cardiology Foundation
Published by Elsevier Inc.

Vol. 53, No. 23, 2009
ISSN 0735-1097/09/$36.00
doi:10.1016/j.jacc.2009.02.048

Severely Impaired von Willebrand Factor-Dependent Platelet Aggregation in Patients With a Continuous-Flow Left Ventricular Assist Device (HeartMate II)

Jolanta Klovaite, MD,* Finn Gustafsson, MD, PHD,† Svend A. Mortensen, MD, DMSC,† Kåre Sander, MD, DMSC,‡ Lars B. Nielsen, MD, PHD, DMSC*§

Copenhagen, Denmark

Objectives This study investigated the influence of the mechanical blood pump HeartMate II (HMII) (Thoratec Corporation, Pleasanton, California) on blood coagulation and platelet function.

Background HMII is an implantable left ventricular assist device used for the treatment of heart failure. Patients treated with HMII have increased bleeding tendencies.

Methods We measured agonist-induced platelet aggregation in 16 patients on HMII support.

Results The von Willebrand factor (vWF)-dependent ristocetin-induced platelet aggregation was impaired in 11 of the 16 patients, of which 12 had experienced at least 1 minor or major bleeding episode. The impaired ristocetin-induced platelet aggregation was associated both with decreased specific activity of plasma vWF, presumably due to lack of high molecular weight vWF multimers, as well as with attenuated function of the platelets themselves.

Conclusions The results imply that HMII treatment is associated with impaired platelet aggregation, which may contribute to an increased tendency to bleed. (J Am Coll Cardiol 2009;53:2162–7) © 2009 by the American College of Cardiology Foundation

Left ventricular assist devices (LVADs) are mechanical blood pumps used for the treatment of severe heart failure (e.g., as a bridge to heart transplantation, myocardial recovery, or as long-term destination therapy). HeartMate II (HMII) (Thoratec Corporation, Pleasanton, California) is among the most commonly used LVAD systems (1,2).

Exposure of the blood to foreign surfaces may activate the coagulation system. Hence, systemic anticoagulation with vitamin K and platelet inhibitors are recommended in HMII-treated patients (2). However, an increased tendency to bleed above what is normally observed in patients receiving anticoagulation treatment has been observed at both early and late stages after HMII implantation (3).

The HMII contains an axial-flow pump that draws blood from the left ventricle and continuously pumps it into the ascending aorta, exposing the blood to high shear stress (4). High shear stress increases the susceptibility to proteolysis of the largest von Willebrand factor (vWF) multimers (5,6), decreasing the ability of vWF to induce platelet aggregation. Hence, exposure of the blood to high shear stress might impose an acquired form of von Willebrand disease with qualitative vWF defects and increased bleeding tendencies. Such a pathogenic mechanism may explain the tendency to bleed of patients with aortic stenosis (7–9) or defects of cardiac septae (10). vWF-dependent platelet aggregation involves the binding of vWF to the GPIb-IX-V glycoprotein receptor complex on platelets. Exposure of the blood to shear stress (e.g., during hemodialysis) also results in defective GPIb-IX-V receptor function (11,12), which increases bleeding tendency. The aim of this study was to investigate the putative impact of HMII treatment on blood coagulation and specifically to test the hypothesis that HMII may impair vWF-dependent platelet aggregation.

Methods

Patients. We studied 12 men and 4 women, 19 to 63 years of age, in whom a HMII had been implanted 17 to 459 days (mean 151 days) previously. The indication for HMII treatment was end-stage dilated (n = 10) or ischemic (n = 6)

From the Departments of *Clinical Biochemistry, †Cardiology, and ‡Thoracic Surgery, Rigshospitalet, and the §Department of Biomedical Sciences, University of Copenhagen, Copenhagen, Denmark.

Manuscript received November 11, 2008; revised manuscript received February 6, 2009, accepted February 10, 2009.

cardiomyopathy. After HMII implantation, low molecular weight heparin and warfarin were initiated when postoperative bleeding was acceptable, typically on day 2 after surgery. In patients with underlying ischemic heart disease, aspirin was added, if tolerated. In case of manifest thromboembolic events, further platelet inhibition with clopidogrel was initiated. The international normalized ratio (INR) was monitored closely, and if it decreased below 1.8, low molecular weight heparin was initiated until the INR was >2. At the time of the present study, all patients received warfarin, 5 received aspirin, and 1 of these patients also received clopidogrel. Five patients underwent heart transplantation (HTx) after 166 to 392 days (mean 295 days) of treatment with HMII.

We also investigated hospitalized patients with congestive heart failure (CHF) (ejection fraction ≤20%; New York Heart Association functional class III to IV) due to dilated (n = 4) or ischemic (n = 5) cardiomyopathy and 6 outpatients who were receiving warfarin therapy because of previous venous thrombosis (INR 2.4 to 4.1). Seven of the patients with CHF received aspirin, with 1 of these patients also receiving clopidogrel and 1 warfarin. Clinical data were obtained from patient medical records. Control samples were obtained from healthy staff members at the Department of Clinical Biochemistry, Rigshospitalet, Copenhagen.

Biochemical analyses. Blood was anticoagulated with 3.8% sodium citrate (Vacuette, Greiner Bio-One GmbH, Kremsmünster, Austria). Platelet-rich plasma (PRP) was prepared by centrifugation at 200 *g* for 10 min at 21 to 23°C. The remaining blood was recentrifuged at 2,400 *g* for 15 min to obtain platelet-poor plasma (PPP). Plasma for biochemical analysis was analyzed immediately, or stored at −80°C within 2 h after blood collection.

Laboratory analyses were conducted at the Department of Clinical Biochemistry, Rigshospitalet. Plasma von Willebrand factor antigen (vWF:Ag) was measured with sandwich enzyme-linked immunosorbent assay by the use of antibodies from DAKO-Cytomation, SA (Trappes, France). The von Willebrand factor ristocetin-cofactor activity (vWF:RCo) was measured with reagents and the BCT instrument from Dade Behring (Marburg, Germany). Multimeric patterns of vWF were determined by sodium dodecyl sulfate-agarose gel electrophoresis and western blotting at the Department of Clinical Biochemistry, Malmö University Hospital, Malmö, Sweden. Scanned images were analyzed with ImageJ (National Institutes of Health, Bethesda, Maryland). We measured a disintegrin and metalloproteinase with a thrombospondin type 1 motif, 13 (ADAMTS-13) activity with reagents from Pepta Nova (Sandhausen, Germany). Glycocalicin was measured with an enzyme-linked immunosorbent assay (Glycocalicin EIA kit, Takara Biomedical, Ohtsu, Japan).

Platelet aggregation. PRP was mixed with autologous PPP to obtain a final platelet count of ~250 × 10^9/l. Platelet aggregation after addition of agonists was measured at 37°C in a Whole Blood Lumi-Aggregometer 560 CA (Chrono-log Corporation, Havertown, Maryland) after the addition of adenosine diphosphate (ADP) (5 μmol/l), collagen (2 μg/ml), arachidonic acid (1 mmol/l), or ristocetin (1.25 mg/ml) (Triolab, Copenhagen, Denmark). We measured the release of adenosine triphosphate with CHRONO-LUME reagent (Triolab). Aggregation was expressed as percent of the maximal aggregation obtained 8 min after the addition of agonists. Patient samples were always analyzed in parallel with platelets from a healthy control subject. When ristocetin-induced platelet aggregation was impaired, patient PRP was mixed with normal PPP (1 + 3), and normal PRP was mixed with patient PPP (1 + 3) before the addition of ristocetin (1.25 mg/ml). Final platelet counts in mixtures were 109 ± 31 × 10^9/l and 122 ± 22 × 10^9/l when patient platelets and normal platelets were used, respectively.

Statistical analysis. The results are mean ± SD unless otherwise indicated. Statistical analyses were conducted with Prism software (version 2.0, Graph Pad, San Diego, California). Two-group comparisons were made with the Student *t* test.

Abbreviations and Acronyms

ADAMTS-13 = a disintegrin and metalloproteinase with a thrombospondin type 1 motif, 13
ADP = adenosine diphosphate
CHF = congestive heart failure
HMII = HeartMate II
HTx = heart transplantation
INR = international normalized ratio
LVAD = left ventricular assist device
PPP = platelet-poor plasma
PRP = platelet-rich plasma
vWF = von Willebrand factor
vWF:Ag = von Willebrand factor antigen
vWF:RCo = von Willebrand factor ristocetin-cofactor activity

Results

Of 16 HMII-treated patients, 12 had major or minor bleeding (Table 1). None had any history of increased bleeding tendency before HMII implantation. Two patients had suspected thromboembolic events (Table 1). Two other patients exhibited temporary bilateral loss of vision and lateralized motor deficits in the post-operative phase, respectively. In both cases, cerebral computed scans were normal and did not reveal cerebral infarction (or bleeding) (Table 1).

Changes in blood coagulation parameters could not explain the increased bleeding tendency (Table 2). However, ristocetin-induced platelet aggregation was severely impaired in patients with a bleeding history (Table 1, Fig. 1A). We found that ADP-induced platelet aggregation was moderately reduced in 7 patients, of whom 2 received aspirin (Fig. 1A). Collagen- and arachidonic acid-induced platelet aggregation (and release of adenosine triphosphate) was only abnormal in 1 patient who received both aspirin and clopidogrel (Fig. 1A).

JACC Vol. 53, No. 23, 2009
June 9, 2009:2162–7

Table 1 Laboratory Values, Anticoagulation, Bleeding, and Thrombotic/Neurological History in HMII-Treated Patients Before and After HTx

ID #	Ristocetin-Induced Platelet Aggregation (%)	ADP-Induced Platelet Aggregation (%)	Specific Activity of vWF*	Lack of High Molecular Weight vWF Multimers†	Anticoagulation	Bleeding History	Neurological and Thrombotic History
Before HTx							
1	3	98	0.5	Yes	W	Minor	Watershed infarct
2	3	46	0.4	Yes	W	Minor	TIA
3	4	22	0.4	Yes	W, A	Major‡	None
4	4	ND	0.5	Yes	W, A	Minor	None
5	5	ND	0.3	Yes	W, A	Major‡	None
6	5	46	0.5	Yes	W	Major§	None
7	5	48	0.8	Yes	W	None	None
8	8	40	0.4	Yes	W	Major§	None
9	17	ND	0.6	Yes	W, A	Major‡	None
10	19	23	0.5	Yes	W, A, C	Minor	None
11	44	59	0.8	Yes	W	None	None
12	64	42	0.8	Yes	W	None	Retinal artery embolus
13	72	76	0.8	Yes	W	None	TIA
14	73	74	1.0	Yes	W	Minor	None
15	75	90	0.6	Yes	W	Minor	None
16	79	64	0.6	Yes	W	Minor	None
After HTx							
3	62	50	1.0	No	No	None	None
4	72	77	1.0	No	No	None	None
5	87	71	1.0	No	A, D	None	None
7	91	77	0.9	No	No	None	None
9	72	59	0.9	No	No	None	None

*von Willebrand factor ristocetin-cofactor activity (vWF:RCo) divided by von Willebrand factor antigen (vWF:Ag). †Assessed by the use of sodium dodecyl sulfate-agarose gel electrophoresis. ‡Major bleeding occurred in relationship with operative procedures. Patient 3 received 11 portions, patient 5 received 17 portions, and patient 9 received 15 portions of packed red blood cells within 24 h after heart transplantation (HTx). Patient 5 also had major bleeding after implantable cardioverter-defibrillator insertion. §These individuals had both major and minor bleeding episodes during HeartMate II (HMII) treatment. Patient 6 received blood transfusions after epistaxis; patient 8 received blood transfusion after gastrointestinal bleeding.

A = aspirin; ADP = adenosine-induced platelet aggregation; C = clopidogrel; D = dipyridamole; ND = not determined; TIA = transient ischemic attack; vWF = von Willebrand factor; W = warfarin.

To differentiate between plasma and platelet defects in patients with impaired ristocetin-induced platelet aggregation, we mixed platelets from the patients treated with HMII with normal plasma (or vice versa) before studying platelet aggregation. Seven of the 11 patients displayed signs of impaired ability of both plasma and platelet factors to mediate ristocetin-induced platelet aggregation (Fig. 1B). Two patients displayed normal ristocetin-induced platelet aggregation when their platelets were mixed with normal plasma, and 2 patients had normal platelet aggregation when their plasma was mixed with normal platelets (Fig. 1B).

Lack of high-molecular weight vWF multimers in HMII-treated patients. The average plasma concentrations of vWF protein (vWF:Ag) and activity (vWF:RCo) were 1.6 ± 0.7 kIU/l and 1.0 ± 0.6 kIU/l, respectively. Hence, although vWF:Ag was elevated, as previously observed in patients with CHF (13), 11 of 16 patients treated with HMII had decreased specific activity of plasma vWF (vWF:RCo/vWF:Ag) (Fig. 2A), and vWF specific-activity was lowest in patients with a history of severe bleeding (Fig. 2A). The patients treated with HMII had fewer large vWF multimers than healthy control patients (Figs. 2B and 2C). This finding was not due to increased plasma ADAMTS-13 levels, which were normal

Table 2 Basic Laboratory Values of HMII-Treated Patients Before and After HTx and Patients With CHF

Laboratory Values	Patients With CHF (n = 9)	Patients With HMII (n = 16)	HMII Patients After HTx (n = 5)	Reference Interval
Hemoglobin (mmol/l)	8.3 ± 1.3	7.3 ± 1.4	7.6 ± 0.6	7.0–10.0
Platelet count ($\times 10^9$/l)	230 ± 93.4	264 ± 83.7	299 ± 69.5	150–400
INR	1.2 ± 0.3	2.3 ± 0.5	1.1 ± 0.1*	<1.3
APTT (s)	29 ± 3	38 ± 7	25 ± 3	23–35
Fibrinogen (μmol/l)	13.4 ± 4.7	15.2 ± 5	12 ± 1.4	5.3–10.3
D-dimer (mg/l)	0.8 ± 0.6	2.2 ± 2.4	0.4 ± 0.4	<0.5
C-reactive protein (mg/l)	15 ± 15	35 ± 48	15 ± 18	<10
Factor VIII (kIU/l)	1.82 ± 0.8	1.53 ± 0.6	2.15 ± 0.7	0.5–1.49

Values are mean ± SD. *Warfarin was stopped after HTx.

APTT = activated partial thromboplastin time; CHF = congestive heart failure; INR = international normalized ratio; other abbreviations as in Table 1.

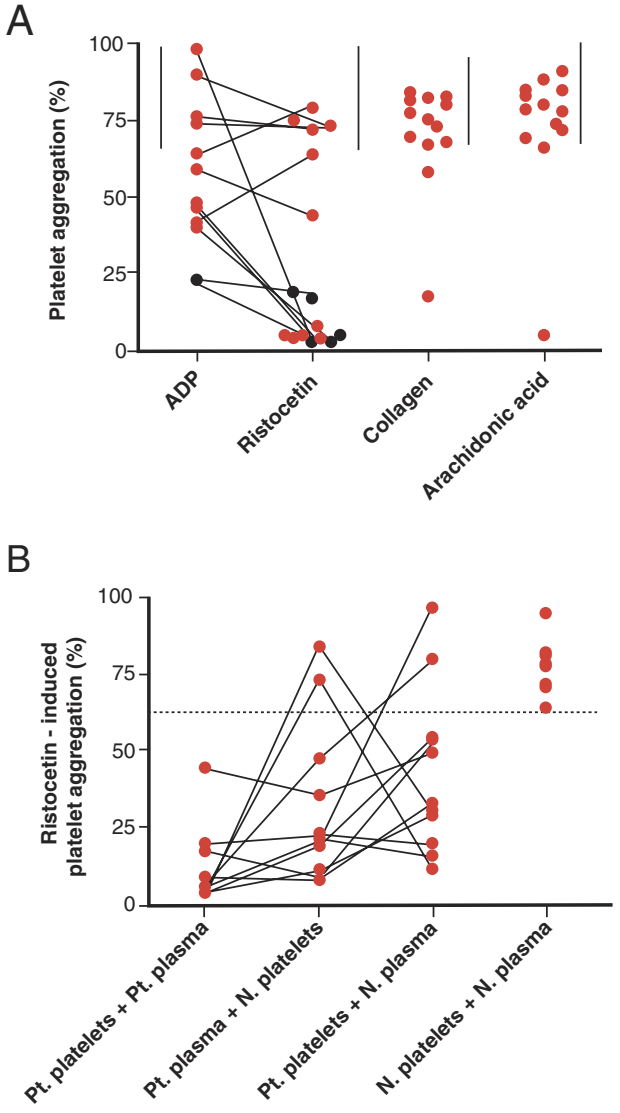

Figure 1 **Agonist-Induced Platelet Aggregation in HMII-Treated Patients**

(A) Platelet aggregation after stimulation with adenosine diphosphate (ADP), collagen, arachidonic acid, or ristocetin. Platelet-rich plasma (PRP) was incubated for 8 min at 37°C with the indicated agonists. Results are expressed as percentages of the maximal aggregation. Reference intervals are shown as **vertical bars** (64% to 98%, 72% to 94%, 66% to 100%, and 64% to 100% for ADP-, collagen-, arachidonic acid-, and ristocetin-induced platelet aggregation, respectively). A sample from a healthy individual (laboratory staff) was always analyzed in parallel with patient samples. **Lines** connect data from the same patient. **Black circles** indicate patients receiving aspirin. **(B)** Ristocetin-induced platelet aggregation in mixtures of plasma and platelets from patients (Pt.) and healthy control patients (N). To discriminate between the effect of plasma and platelet factors in patients with impaired ristocetin-induced platelet aggregation, patient platelets were mixed with plasma from a healthy person, or patient plasma was mixed with platelets from a healthy person, before the measurement of ristocetin-induced platelet aggregation (see the Methods section for details). **Lines** connect data from the same patient. The **dotted horizontal line** indicates the lower boundary of the reference interval in healthy individuals. HMII = HeartMate II.

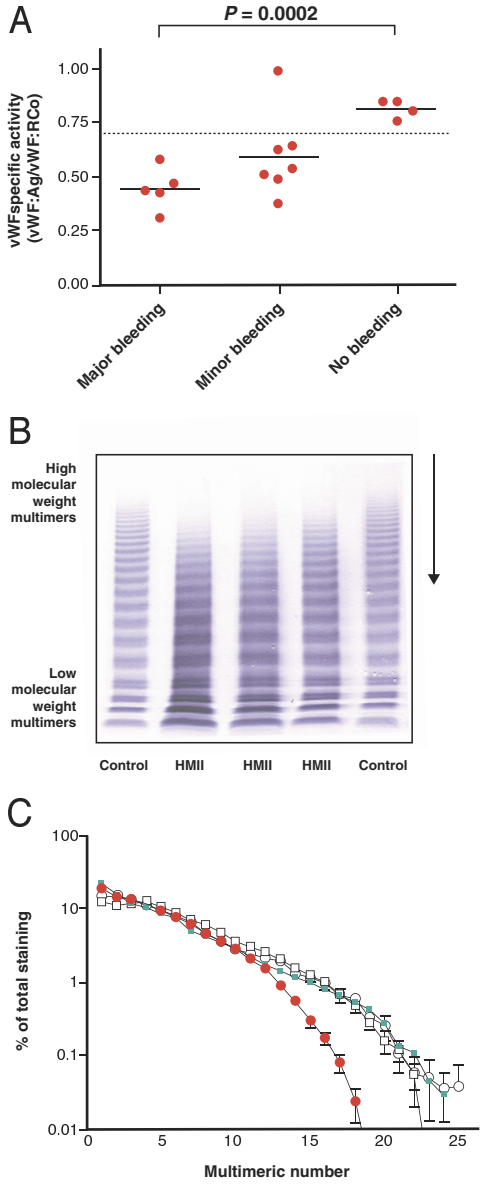

Figure 2 **Specific Activities and Multimeric Patterns of Plasma vWF in HMII-Treated Patients**

(A) Specific activities of plasma vWF in HMII-treated patients. Patients were divided into those with a history of major, minor, or no bleeding. The **dotted horizontal line** indicates the lower boundary of the reference interval of vWF:RCo/vWF:Ag in healthy individuals. The specific activity of plasma vWF was calculated as vWF:RCo divided by vWF:Ag. The p value is derived from a Student *t* test comparing patients with major bleeding with patients with no bleeding. **(B)** Western blot of plasma vWF multimers in 3 representative HMII-treated patients before HTx (2 healthy controls subjects with normal vWF multimeric pattern are shown for comparison). The **arrow** indicates the direction of protein migration during gel electrophoresis. **(C)** Distribution of vWF staining between high and low molecular weight multimers (assessed by densitometry of vWF bands) in HMII-treated patients (**solid circles**, n = 16), HMII-treated patients after HTx (**open circles**, n = 5) and patients with CHF (**solid squares**, n = 9), and healthy control patients (**open squares**, n = 8). Values are expressed as a percentage of total staining and shown as mean ± SEM. CHF = congestive heart failure; HTx = heart transplantation; vWF = von Willebrand factor; vWF:Ag = von Willebrand factor antigen; vWF:RCo = von Willebrand factor ristocetin-cofactor activity; other abbreviations as in Figure 1.

JACC Vol. 53, No. 23, 2009
June 9, 2009:2162–7

(1.2 ± 0.4 AU/l; reference interval: 0.75 to 1.40 AU/l) in the HMII-treated patients.

Normalized vWF-dependent platelet aggregation after HTx. Ristocetin-induced platelet aggregation (Fig. 3A), plasma vWF-specific activity (Fig. 3B), and vWF multimeric distribution (Figs. 2C and 3C) were all normalized after HTx. Also, patients with CHF and patients receiving warfarin because of a previous venous thrombosis had normal ristocetin-induced platelet aggregation (Fig. 3A) and plasma vWF-specific activity (Fig. 3B). Patients with CHF also had normal vWF multimeric distribution (Fig. 2C).

Discussion

The present results imply that HMII treatment is associated with impaired platelet aggregation, which may contribute to an increased tendency of bleeding in patients. The most striking finding was that 11 of 16 HMII-treated patients had impaired ristocetin-induced platelet aggregation and that 9 of the 10 patients with severely impaired ristocetin-induced platelet aggregation had a history of minor or major bleedings. The impaired platelet aggregation was most likely due to the HMII treatment because each of the 5 patients examined had normalized platelet aggregation after HTx. We examined whether the defect ristocetin-induced platelet aggregation was due to plasma and/or platelet factors. The results suggest that both platelet and plasma factors and often a combination of the 2 contributed toward the observed abnormality.

The specific activity of vWF was decreased in HMII-treated patients. This finding was probably at least in part due to the lack of HMW vWF multimers. As such, HMII appears to induce a secondary VWD type 2 similar to what has previously been observed in patients with aortic stenosis (7–9) and other cardiac defects (10). This finding is in agreement with those recently reported by Geisen et al. (14). Although the specific activity of plasma vWF was decreased in HMII-treated patients, the vWF activity was still within the normal range. Therefore, the lack of HMW multimers may not fully account for the plasma factor leading to impaired ristocetin-induced platelet aggregation. Hence, it is conceivable that the plasma from HMII-treated patients might contain 1 or more factors that actually inhibit the ristocetin-induced platelet aggregation. One such putative factor could be fragments of vWF generated during the loss of HMW multimers (15,16).

The present findings emphasize a novel aspect of HMII treatment. Thus, it seems important to uncover how impaired platelet function affects the risk of bleeding in HMII-treated patients. The results, albeit based on a rather small number of patients, suggest that the risk of bleeding in HMII-treated patients due to impaired platelet aggregation may be significant, and the authors of future studies should also address whether patients on LVADs with abnormal platelet aggregation would be better off without routine

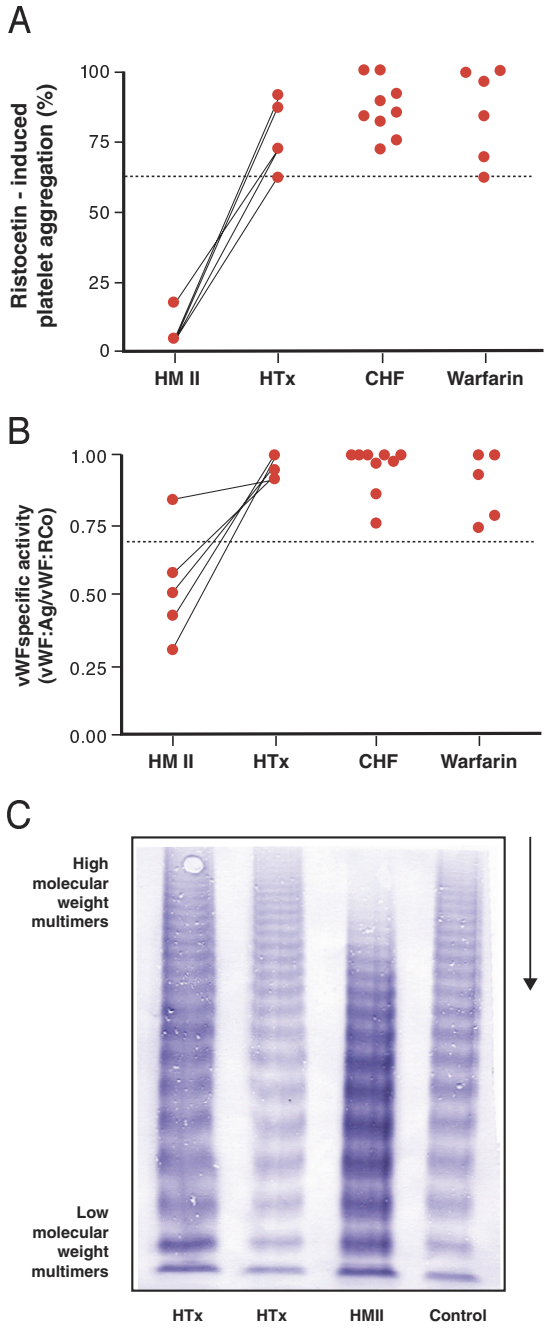

Figure 3 Normalization of the Ristocetin-Induced Platelet Aggregation and Plasma vWF-Specific Activities and Multimeric Patterns After HTx

(A) Ristocetin-induced platelet aggregation and **(B)** specific activity of plasma vWF (calculated as vWF:RCo divided by vWF:Ag) in 5 HMII-treated patients before and after HTx. Nine patients with CHF and 6 outpatients in anticoagulant therapy with warfarin (international normalized ratio 2.4 to 4.1) are shown for comparison. The **dotted horizontal lines** indicate the lower boundaries of the reference intervals in healthy individuals. **Lines** connect data from the same patient. **(C)** Western blot of plasma vWF multimers after HTx in 2 representative HMII-treated patients; a HMII-treated patient lacking high molecular weight multimers and a healthy control patient with vWF normal multimeric pattern are shown for comparison. The **arrow** indicates the direction of protein migration. Abbreviations as in Figure 2.

antiplatelet therapy, especially those in whom ristocetin- and/or ADP-induced platelet aggregation is impaired.

Acknowledgment

The authors thank Marianne Johnson for expert technical assistance.

Reprint requests and correspondence: Dr. Lars B. Nielsen, Department of Clinical Biochemistry, KB3011, Rigshospitalet, University of Copenhagen, Blegdamsvej 9, DK-2100 Copenhagen, Denmark. E-mail: larsbo@rh.dk.

REFERENCES

1. Miller LW, Pagani FD, Russell SD, et al. Use of a continuous-flow device in patients awaiting heart transplantation. N Engl J Med 2007;357:885–96.
2. Frazier OH, Gemmato C, Myers TJ, et al. Initial clinical experience with the HeartMate II axial-flow left ventricular assist device. Tex Heart Inst J 2007;34:275–81.
3. Goldstein DJ, Beauford RB. Left ventricular assist devices and bleeding: adding insult to injury. Ann Thorac Surg 2003;75:S42–7.
4. Snyder TA. Preclinical biocompatibility assessment of cardiovascular devices. PhD thesis. University of Pittsburgh, 2006. Available at: http://etd.library.pitt.edu/ETD/available/etd-04062006-102142/unrestricted/snyderta_etd2006_v2.pdf. Accessed April 3, 2009.
5. Tsai HM, Sussman II, Nagel RL. Shear stress enhances the proteolysis of von Willebrand factor in normal plasma. Blood 1994;83:2171–9.
6. Dong JF. Cleavage of ultra-large von Willebrand factor by ADAMTS-13 under flow conditions. J Thromb Haemost 2005;3:1710–6.
7. Pareti FI, Lattuada A, Bressi C, et al. Proteolysis of von Willebrand factor and shear stress-induced platelet aggregation in patients with aortic valve stenosis. Circulation 2000;102:1290–5.
8. Vincentelli A, Susen S, Le TT, et al. Acquired von Willebrand syndrome in aortic stenosis. N Engl J Med 2003;349:343–9.
9. Yoshida K, Tobe S, Kawata M, Yamaguchi M. Acquired and reversible von Willebrand disease with high shear stress aortic valve stenosis. Ann Thorac Surg 2006;81:490–4.
10. Gill JC, Wilson AD, Endres-Brooks J, Montgomery RR. Loss of the largest von Willebrand factor multimers from the plasma of patients with congenital cardiac defects. Blood 1986;67:758–61.
11. Himmelfarb J, Nelson S, McMonagle E, et al. Elevated plasma glycocalicin levels and decreased ristocetin-induced platelet agglutination in hemodialysis patients. Am J Kidney Dis 1998;32:132–8.
12. Yoshida E, Fujimura Y, Ikeda Y, et al. Impaired high-shear-stress-induced platelet aggregation in patients with chronic renal failure undergoing haemodialysis. Br J Haematol 1995;89:861–7.
13. Cooke GE, Eaton GM, Whitby G, et al. Plasma atherogenic markers in congestive heart failure and posttransplant (heart) patients. J Am Coll Cardiol 2000;36:509–16.
14. Geisen U, Heilmann C, Beyersdorf F, et al. Non-surgical bleeding in patients with ventricular assist devices could be explained by acquired von Willebrand disease. Eur J Cardiothorac Surg 2008;33:679–84.
15. Alevriadou BR, Moake JL, Turner NA, et al. Real-time analysis of shear-dependent thrombus formation and its blockade by inhibitors of von Willebrand factor binding to platelets. Blood 1993;81:1263–76.
16. Sugimoto M, Ricca G, Hrinda ME, et al. Functional modulation of the isolated glycoprotein Ib binding domain of von Willebrand factor expressed in *Escherichia coli*. Biochemistry 1991;30:5202–9.

Key Words: heart-assist device ■ hemorrhage ■ platelets.