European Journal of Cardio-Thoracic Surgery 2022, 62(1), ezab474
https://doi.org/10.1093/ejcts/ezab474 Advance Access publication 31 October 2021

ORIGINAL ARTICLE

Cite this article as: Consolo F, Marasi A, Della Valle P, Bonora M, Pieri M, Scandroglio AM *et al.* Bleeding in patients with continuous-flow left ventricular assist devices: acquired von Willebrand disease or antithrombotics? Eur J Cardiothorac Surg 2022; doi:10.1093/ejcts/ezab474.

Bleeding in patients with continuous-flow left ventricular assist devices: acquired von Willebrand disease or antithrombotics?

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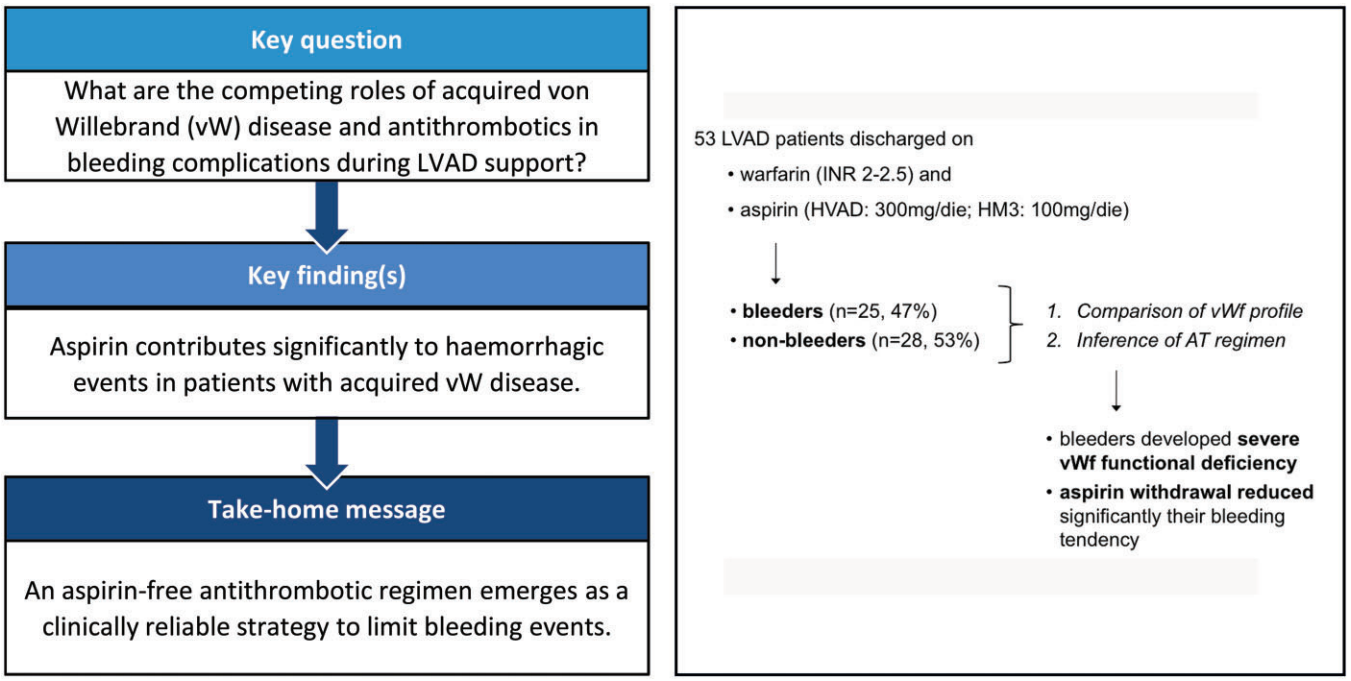
Received 16 June 2021; received in revised form 23 September 2021; accepted 3 October 2021



Abstract

OBJECTIVES: To evaluate the competing pro-haemorrhagic contribution of acquired von Willebrand (vW) disease and antithrombotic therapy in patients implanted with continuous-flow left ventricular assist devices (LVADs).

Presented at the 66th ASAIO Annual Conference, Washington DC, USA, 10–12 June 2021.

METHODS: We compared the extent of vW factor (vWf) degradation [vWf antigen (vWf:Ag)] and a decrease of functional activity of large vWf multimers [vWf collagen binding (vWf:CB)] in LVAD patients who did and did not suffer from bleeding. Data were measured pre-implant, at short-term (t1: <3 months) and long-term (t2: >12 months) follow-up. The occurrence of primary bleeding events, as well as bleeding recurrence, was correlated with patient-specific vWf profile and antithrombotic regimen. Indeed, patients were discharged on warfarin (international normalized ratio: 2–2.5) and aspirin, with the latter withhold after a first bleeding episode.

RESULTS: Fifty-three patients were enrolled. The median follow-up was 324 (226–468) days. We recorded 25 primary bleeding events (47% of patients). All primary events occurred in patients on warfarin and aspirin. Both vWf:Ag and vWf:CB decreased significantly post-implant ($P = 0.0003$ and $P < 0.0001$), and patients showing pathological vWf:CB/vWf:Ag ratio (<0.7) increased progressively over the time of support (pre-implant = 26%, t1 = 58%, t2 = 74%; $P < 0.0001$). Of note, activity of large vWf multimers of bleeders was significantly lower at t2 with respect to non-bleeders (vWf:CB: 61 (36–115) vs 100 (68–121), $P = 0.04$; vWf:CB/vWf:Ag ratio: 0.36 (0.26–0.61) vs 0.58 (0.33–0.96), $P = 0.04$). Despite these marked differences in the vWf profile, following aspirin discontinuation only 3 patients had bleeding recurrence.

CONCLUSIONS: Aspirin contributes significantly to haemorrhagic events in the background of acquired vW disease; its discontinuation significantly reduces bleeding recurrence.

Clinical trial registration: https://clinicaltrials.gov/ct2/show/NCT03255928; ClinicalTrials.gov Identifier: NCT03255928.

Keywords: Left ventricular assist device • Bleeding • Von Willebrand factor • Antithrombotic therapy • Aspirin

ABBREVIATIONS

AT	Antithrombotic
LVAD	Left ventricular assist device
HM3	HeatMate 3
HVAD	HeartWare Ventricular Assist Device
INR	International normalized ratio
PAS	Platelet Activity State
PRE	Pre-implant
vW	von Willebrand
vWf	von Willebrand factor
vWf:Ag	von Willebrand factor antigen
vWf:CB	von Willebrand factor collagen binding

INTRODUCTION

The clinical success of left ventricular assist devices (LVADs) has grown enormously. The 2-year survival of patients implanted with modern LVADs is competitive with heart transplant [1, 2].

However, non-surgical bleeding remains a major clinical concern. The ReVOLVE trial reported that bleeding was the most common adverse event in patients implanted with the HeartWare Ventricular Assist Device (HVAD) (Medtronic Inc., USA), occurring in 45% of patients [3]. The final report of the MOMENTUM 3 trial reported that 44% of patients implanted with the HeartMate 3 (HM3; Abbott Laboratories, USA) suffered from bleeding over a 2-year follow-up, and 10% of them required surgery [4].

Accordingly, thorough mechanistic evaluation of the pathophysiology of spontaneous bleeding in this specific setting and identification of reliable strategies to prevent haemorrhagic events are urgently needed.

Patients supported with continuous-flow LVADs develop shear-induced degradation of von Willebrand factor (vWf) high-molecular weight multimers, leading to acquired von Willebrand (vW) disease, impaired haemostatic function, and ultimately, increased risk of spontaneous bleeding [5–7]. Nevertheless, not all patients with abnormal vWf activity experience bleeding, suggesting that other factors likely contribute to the development of haemorrhagic events.

Recent studies questioned whether routine dual antithrombotic (AT) therapy with vitamin K antagonist and aspirin contributes to bleeding complications [8–13]. A multicentre randomized trial is ongoing to evaluate the efficacy and safety of an antiplatelet-free AT regimen to reduce the incidence of bleeding with the HM3 [14].

To date, data providing combined evaluation of vWf profile and AT regimen in LVAD patients and correlation of these 2 elements with bleeding events are missing.

We hypothesized that—in the background of acquired vW disease—aspirin magnifies the bleeding risk. To test our hypothesis, we evaluated the competing role of acquired vW disease and aspirin in the development of bleeding complications.

METHODS

Ethical statement

The study conforms to the ethical guidelines of the Declaration of Helsinki and was approved by local IRB (protocol ID: BIOSUOVAD, approved: February 2015, and PASVAD, approved: June 2017; ClinicalTrials.gov ID: NCT03255928). All patients signed informed consent to participate to the study.

Study design

The study was performed in patients implanted with continuous-flow LVADs from November 2015 to February 2020 at San Raffaele Scientific Institute (Milan, Italy). Data were prospectively collected.

Acquired vW disease following LVAD implantation was evaluated measuring (i) the plasma concentration of vWf antigen (vWf:Ag) and (ii) vWf collagen-binding capacity (vWf:CB), a surrogate marker of the functional haemostatic activity of large (high-molecular weight) vWf multimers. Then, the vWf activity-to-antigen ratio (vWf:CB/vWf:Ag ratio) was calculated, and values <0.7 were considered as indicative of pathological vWf activity (impaired haemostatic function) according to literature [15]. Table 1 further describes the methods we used to evaluate the vWf degradation and activity.

Data were measured at different time points: (i) pre-implant (PRE) and (ii) short-term (t1: <3 months of support) and (iii)

Table 1: Test methods of von Willebrand factor degradation and deficiency

vWf assay	Description
vWf:Ag	• Quantitative assessment of vWf protein level in patient's plasma • Indicative of vWf protein degradation • Performed with an automated assay kit (STA—LIATEST VWF:Ag assay; STAGO, France) using the STA R Max® haemostasis analyzer (STAGO)
vWf:CB	• Quantitative assessment of the functional haemostatic activity of vWf high-molecular weight multimers • Based on the evaluation of the capacity of large vWf multimers to bind collagen • Performed with ASSERACHROM VWF:CB ELISA kit (STAGO)
vWf:CB/vWf:Ag	• Allows normalization of large vWf multimers functional activity over the total vWf protein level • Values <0.7 are indicative of vW disease [15]

vW: von Willebrand; vWf: von Willebrand factor; vWf:Ag: von Willebrand factor antigen; vWf:CB: von Willebrand factor collagen binding.

long-term (t2: >12 months of support) follow-up, and compared in patients who (i) did or (ii) did not suffer from bleeding. This way, we evaluated potential differences in the vWf profile of bleeders and non-bleeders that might explain different susceptibility to bleeding over the course of support.

Data were also compared between patients implanted with the HVAD or the HM3 to characterize the impact of the 2 pumps.

Preoperative patient characteristics were also compared in the 2 groups.

The AT regimen was recorded for each patient and correlated with the incidence of (i) primary bleeding events and (ii) bleeding recurrence. Indeed, all patients were initially managed with warfarin targeted to international normalized ratio (INR) of 2.0–2.5 and aspirin (HVAD = 300 mg/die; HM3 = 100 mg/die), but aspirin was discontinued following a bleeding event. Patients discontinued from aspirin were later managed with warfarin monotherapy (INR target = 2.0–2.5).

We also evaluated the pro-thrombotic risk of patients discontinued from aspirin comparing (i) coagulation parameters and haemolysis indexes (haemoglobin, platelet count, INR, activated partial thromboplastin time ratio, lactate dehydrogenase levels, D-dimer and fibrinogen levels) and (ii) pro-thrombotic platelet activity measured via the Platelet Activity State (PAS) assay in the 2 groups. The PAS assay provides a quantitative measure of actual platelet pro-thrombotic profile and associated risk of thrombotic complications [16–18]. The rate of thrombotic/thromboembolic complications was also recorded and compared according to the AT regimen.

All data were retrieved at the longest available follow-up.

INTERMACS definitions for major bleeding and thrombotic/thromboembolic events were applied (version 3.0, www.intermacs.org); bleeding complications not meeting INTERMACS criteria were defined as minor events. Events occurred during patients postoperative in-hospital stay were excluded.

Experimental protocols of vWf:Ag, vWf:CB and PAS assays are reported as Supplementary Material.

Statistical analysis

Categorical data are presented as absolute numbers and percentages and were compared by two-tailed χ^2 test or Fisher's exact test. In detail, the χ^2 test (with Yates' continuity correction) was applied for contingency tables with >2 rows or columns when no cells had an expected frequency count of <1, and no more than 20% of the cells <5, according to [19]. Numerical data are presented as medians and interquartile range (25th–75th percentiles). Comparison of longitudinal data (PRE versus t1 versus t2) was performed with the analysis of variance (ANOVA) test for correlated samples using the linear mixed-effects model (restricted maximum likelihood estimation method): the model accounts for both fixed effects (different time points) and random effects (different observations within a patient); the Geisser–Greenhouse correction was applied; the Tukey post hoc test was applied to evaluate differences between groups (PRE versus t1 and t1 versus t2). Comparison between groups (bleeders versus non-bleeders and HVAD versus HM3) was performed throughout the parametric *T*-test of Student or the non-parametric Mann–Whitney *U*-test for normally and non-normally distributed data, respectively. A *P*-value of <0.05 was considered statistically significant. Statistical analyses were performed with GraphPad PRISM v.8.2.0 (GraphPad Software, San Diego, CA, USA).

RESULTS

Patient characteristics and incidence of bleeding events

We analysed 53 patients. The median duration of LVAD support was 324 (226–468) days. We recorded 25 primary non-surgical bleeding events (47% of the patients). Of those 20% were gastrointestinal; minor bleeding events included 11 epistaxis and 2 other mucocutaneous (52%). The median duration of support at the time of a primary bleeding event was 155 (61–388) days. Supplementary Material, Table S1 further describes primary bleeding events, including indication of time of occurrence and the implanted device. All events were associated with warfarin and aspirin AT regimen and triggered aspirin discontinuation.

PRE patient characteristics are reported in Table 2, showing no significant differences between bleeders and non-bleeders, except for age, with bleeders being older.

Analysis of the coagulation profile at the time of a bleeding event showed that patients were in target INR (2.51 [1.98–2.97]) and activated partial thromboplastin time range (ratio 1.15 [1.10–1.30]).

Analysis of vWf profile and at regimen and correlation with bleeding events

Post-implant, both vWf:Ag and vWf:CB decreased significantly (Fig. 1; vWf:Ag: PRE: 248 [203–292]%, t1: 182 [138–229]%; t2: 170 [125–211]%; $P < 0.0001$; vWf:CB: PRE: 167 [131–226]%; t1: 107 [88–152]; t2: 82 [55–117]; $P < 0.0001$).

Significant reduction in vWf:Ag levels was already evident at t1 and did not further change at t2 (Fig. 1A; PRE versus t1: $P = 0.003$; PRE versus t2: $P = 0.0004$; t1 versus t2: $P = 0.91$).

Conversely, activity of large vWf multimers showed a progressive trend of significant decline over time (Fig. 1B; PRE versus t1:

Table 2: Demographics and preoperative patient characteristics

	Overall (*n* = 53)	Bleeders (*n* = 25, 47%)	Non-bleeders (*n* = 28, 53%)	*P*-value
Age at implant (years), median (IQR)	66 (64–72)	71 (66–73)	65 (60–69)	*0.001*
Female sex, *n* (%)	4 (8)	2 (8)	2 (7)	>0.99
Ischaemic HF aetiology, *n* (%)	26 (49)	11 (44)	15 (53)	0.59
INTERMACS class[a], *n* (%)				0.26
1–2	17 (32)	6 (8)	11 (25)	
3–4	36 (68)	19 (16)	17(14)	
Intention to treat, *n* (%)				
DT	44 (83)	22 (88)	22 (79)	
BTT/BTC	9 (17)	3 (12)	6 (21)	0.47
Creatinine (mg/dl)	1.40 (1.12–1.89)	1.36 (1.05–1.76)	1.53 (1.14–2.09)	0.22
Haemoglobin (g/dl)	11.4 (10.5–12.3)	11.3 (10.3–12.0)	11.4 (10.8–12.5)	0.70
Platelet count ($\times 10^9$/l)	164 (125–200)	164 (126–204)	161 (122–209)	0.65
temporary MCS, *n* (%)	47 (89)	21 (84)	26 (89)	0.40
HVAD, *n* (%)	28 (53)	14 (56)	14 (50)	0.78
HM3, *n* (%)	25 (47)	11 (44)	14 (50)	

BTC: bridge to candidacy; BTT: bridge to transplant; DT: destination therapy; HF: heart failure; IQR: interquartile range; MCS: mechanical circulatory support.
[a]INTERMACS with TCS modifier.
Italics emphasis indicates statistical significance ($p < 0.05$).

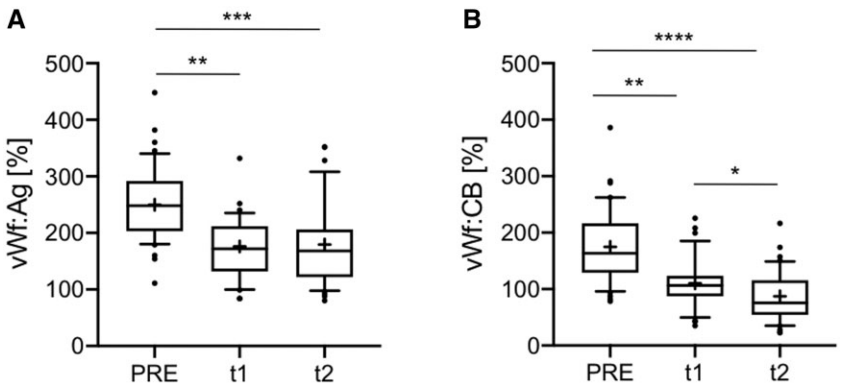


Figure 1: (**A**) plasma concentration of von Willebrand factor antigen (vWf:Ag) and (**B**) activity of large von Willebrand factor multimers (von Willebrand factor collagen binding, vWf:CB) over the time of left ventricular assist device support in the overall population. PRE: pre-implant; t1: short-term follow-up (<3 months of support); t2: long-term follow-up (>12 months of support). Bars indicate 25th, 50th and 75th percentiles; whiskers indicate 10th and 90th percentiles; + indicates mean values; • indicates outliers. **** indicates $P < 0.0001$; *** indicates $P < 0.001$; ** indicates $P < 0.01$; * indicates $P < 0.05$.

$P = 0.005$; PRE versus t2: $P < 0.0001$; t1 versus t2: $P = 0.03$), and the number of patients with pathological vWf activity (vWf:CB/vWf:AG ratio <0.7) increased progressively with increasing time of support (PRE: 26%, t1: 58%, t2: 74%; $P < 0.0001$).

Interestingly, degradation of vWf was comparable in the 2 groups (vWf:Ag; Fig. 2 and Table 3).

On the other hand, PRE vWf functional activity was lower in bleeders (vWf:CB: $P = 0.004$; vWf:CB/vWf:Ag ratio: $P = 0.002$; Fig. 2 and Table 3) and marked differences were also evident at t2 (vWf:CB: $P = 0.04$; vWf:CB/vWf:Ag ratio: $P = 0.04$; Fig. 2 and Table 3).

Our data did not show any statistically significant differences in the vWf profile of patients implanted with the HVAD or the HM3 (Fig. 3), and a similar rate of bleeding events was observed with the 2 pumps (HVAD: $n = 14$, 50%; HM3: $n = 11$, 44%; $P = 0.43$).

Following aspirin discontinuation only 3 patients (12%) had bleeding recurrence over a median follow-up of 328 (121–488) days (2 intracranial, 1 epistaxis), indicating that aspirin withdrawal was largely effective to prevent further bleeding events.

Analysis of AT regimen and pro-thrombotic profile

Coagulation parameters and haemolysis indexes in patients on standard AT regimen and in those discontinued from aspirin are reported in Table 4 (median follow-up: patients without aspirin = 328 [121–488] days; patients with aspirin = 237 [79–392] days).

Pro-thrombotic platelet activity as measured via the PAS assay was significantly higher in patients discontinued from aspirin (0.45 [0.33–0.59]% vs 0.51 [0.46–0.59]%; $P = 0.015$; Fig. 4).

No differences in the rate of thrombotic/thromboembolic complications were recorded in patients treated with or without aspirin (with aspirin: 1 event of pump thrombosis; without aspirin: 2 events: 1 ischaemic stroke; 1 pump thrombosis; $P = 0.60$).

DISCUSSION

This study investigated the competing pro-haemorrhagic contribution of acquired vW disease and AT therapy in the setting of

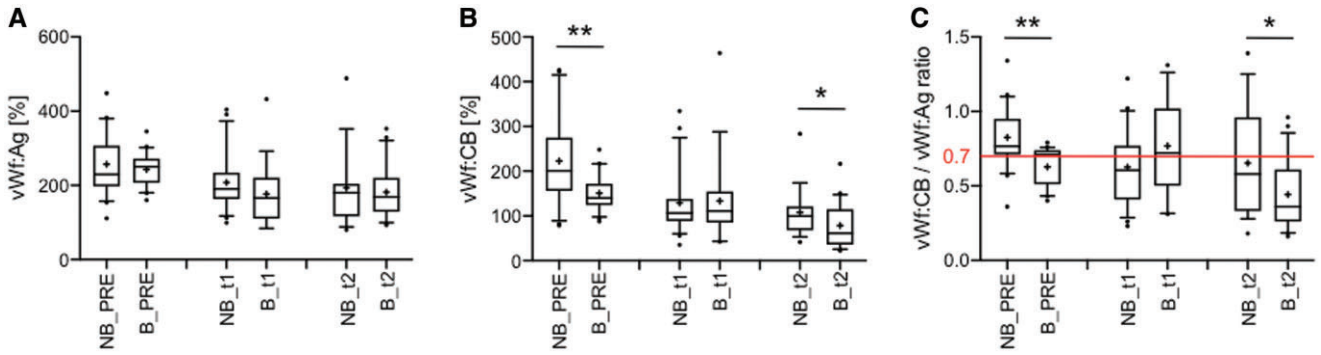


Figure 2: Comparison of von Willebrand factor profile at short-term (t1: <3 months of support) and long-term (t2: >12 months of support) follow-up in patients who did versus did not suffer from bleeding. (**A**) Plasma concentration of von Willebrand factor antigen (vWf:Ag); (**B**) functional activity of von Willebrand factor high-molecular weight multimers (von Willebrand factor collagen binding, vWf:CB); and (**C**) von Willebrand factor activity to antigen ratio (vWf:CB/vWf:Ag). B: bleeders; NB: non-bleeders. Bars indicate 25th, 50th and 75th percentiles; whiskers indicate 10th and 90th percentiles; + indicates mean values; • indicates outliers. ** indicates $P < 0.01$; * indicates $P < 0.05$.

Table 3: Comparison of von Willebrand factor degradation (von Willebrand factor antigen, vWf:Ag) and deficiency of large von Willebrand factor multimers [von Willebrand factor collagen binding (vWf:CB) and von Willebrand factor collagen binding/von Willebrand factor antigen (vWf:CB:vWf:Ag)] in bleeders vs non-bleeders at different time points over the time of left ventricular assist device support

	Bleeders (n = 25, 47%)	Non-bleeders (n = 28, 53%)	P-value
vWf:Ag, median (IQR)			
PRE	250 (206–272)	229.5 (197–307)	0.50
t1	166 (110–221)	190 (163–234)	0.21
t2	168 (128–220)	180 (116–204)	0.84
vWf:CB, median (IQR)			
PRE	140 (124–172)	200 (156–275)	*0.004*
t1	110 (85–155)	106 (88–138)	0.82
t2	61 (36–115)	100 (68–121)	*0.04*
vWf:CB/vWf:Ag, median (IQR)			
PRE	0.71 (0.51–0.74)	0.76 (0.71–0.95)	*0.002*
t1	0.72 (0.50–1.02)	0.60 (0.41–0.77)	0.13
t2	0.36 (0.26–0.61)	0.58 (0.33–0.96)	*0.04*

IQR: interquartile range; PRE: pre-implant; t1: <3 months follow-up; t2: >12 months follow-up; vWf: von Willebrand factor; vWf:Ag: von Willebrand factor antigen; vWf:CB: von Willebrand factor collagen binding.

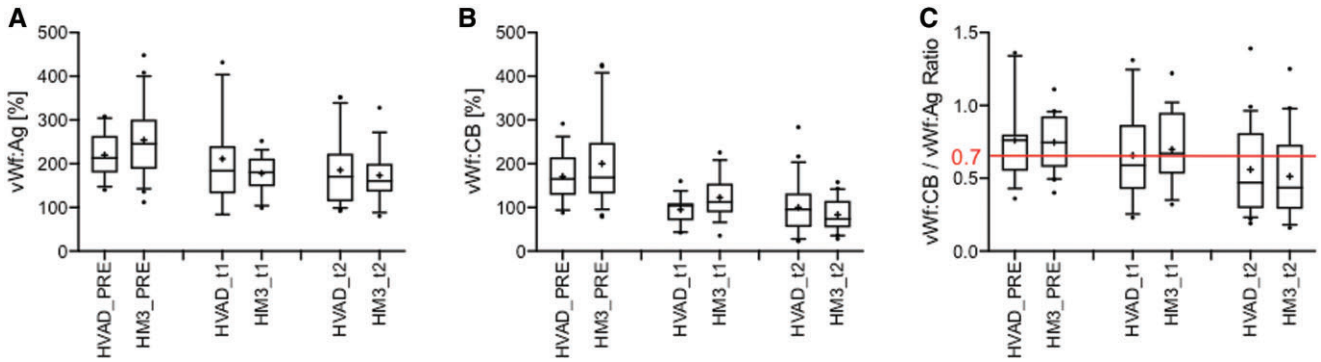


Figure 3: Comparison of von Willebrand factor profile in patients implanted with the HeartWare Ventricular Assist Device or the HeatMate 3. (**A**) plasma concentration of von Willebrand factor antigen (vWf:Ag); (**B**) plasma von Willebrand factor activity (vWf:CB); and (**C**) von Willebrand factor activity to antigen ratio (vWf:CB/vWf:Ag). PRE: pre-implant; t1: short-term follow-up (<3 months of support); t2: long-term follow-up (>12 months of support). Bars indicate 25th, 50th and 75th percentiles; whiskers indicate 10th and 90th percentiles; + indicates mean values; • indicates outliers.

LVAD support and provided further mechanistic insights into the pathophysiology of bleeding in LVAD patients, which correlate with observed clinically outcomes.

Our data suggest that acquired vW disease is not pivotal to the development of bleeding complications while on LVAD and that aspirin has an important pro-haemorrhagic contribution, which

Table 4: Comparison of coagulation parameters and haemolysis index in patients managed with and without aspirin in the background of warfarin administration

	Warfarin and aspirin ($n = 28$)	Warfarin monotherapy ($n = 25$)
Haemoglobin (g/dl), median (IQR)	13.1 (11.3–14.0)	10.5 (11.3–13.2)
Platelet count (10^9/l), median (IQR)	199 (173–219)	203 (167–280)
LDH (U/l), median (IQR)	242 (231–266)	337 (306–461)
INR, median (IQR)	2.20 (1.89–2.38)	2.51 (1.98–2.97)
aPTT ratio, median (IQR)	1.16 (1.10–1.24)	1.15 (1.10–1.30)
Fibrinogen (mg/dl), median (IQR)	389 (309–462)	428 (307–500)
D-dimer (μg/ml), median (IQR)	1.44 (1.25–1.74)	2.21 (1.15–3.03)

aPTT: activated partial thromboplastin time; INR: international normalized ratio; IQR: interquartile range; LDH: lactate dehydrogenase.

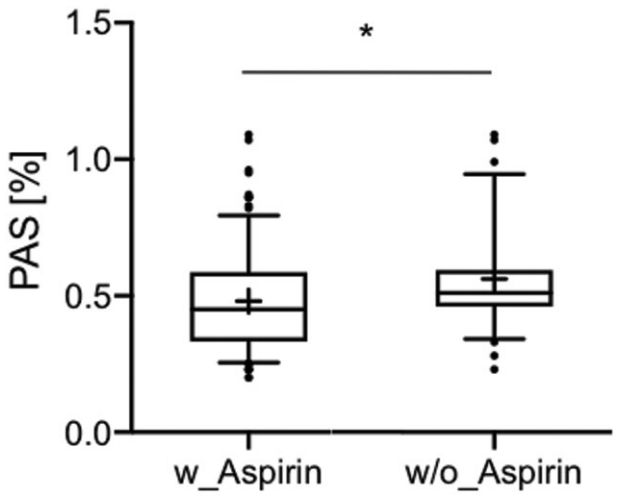


Figure 4: Comparison of platelet activation measured via the Platelet Activity State assay in patients treated with ($n = 27$) or without aspirin ($n = 25$). Bars indicate 25th, 50th and 75th percentiles; whiskers indicate 10th and 90th percentiles; + indicates mean values; • indicates outliers; * indicates $P = 0.015$.

adds up to vWf deficiency and eventually magnifies the bleeding risk.

This is consistent with the observation that (i) despite marked differences in the vWf profile of bleeders, pathological vWf activity was observed in bleeders and non-bleeders, particularly at t2 (vWf:Ag/vWf:CB < 0.7; Fig. 2 and Table 3), i.e. when the majority of bleeding events (68%) occurred; and (ii) aspirin-free AT regimen allowed to significantly reduce the incidence of bleeding.

Our results are in agreement with previous studies that showed that acquired vW disease following LVAD implantation does not univocally lead to bleeding episodes, thus supporting our hypothesis that other factors likely contribute to enhanced bleeding tendency in some patients.

Baghai *et al.* [20] reported that 85% out of 39 patients implanted with the HeartMate II (Thoratec Corp., Pleasanton, CA, USA) developed acquired vW disease after 2 weeks of LVAD support, but 'only' 43% of them suffered from bleeding in the short postoperative period, with this percentage increasing to 75% in the late period [20]. The same group later observed on a larger cohort of patients ($n = 198$) a 60% overall rate of bleeding events (HeartMate II: 41%, HM3: 19%) with all patients suffering from post-implant degradation of vWf [21].

Bansal *et al.* [22] reported that the HM3 pump provides greater preservation of the vWf multimeric structure with respect to axial HeartMate II pump following 3 months of support; however, incidence of bleeding was comparable with the 2 pumps, further supporting our hypothesis of other contributing factors—addictive to vWf degradation—in determining bleeding complications.

The same study showed significant differences in the vWf profile in HM3 patients who experienced bleeding events [22]: these data corroborate our observation of post-implant differences in vWf functional activity of bleeders (Table 3).

On the other hand, none of these studies analysed the association between vWf profile, changes in AT regimen and clinical outcomes.

Moreover, our study further expands data from [22], extending the analysis of vWf activity over long-term support: in this regard, we show further deterioration of haemostatic activity of large vWf multimers over prolonged time of LVAD support (>12 months).

We also observed baseline altered vWf profile in patients who suffered from bleeding events (Table 3), which is in agreement with previous studies [20–22]. Bansal *et al.* [22] suggested that the heart failure severity might establish the conditions for baseline vWf alterations, as they found PRE significant differences in the vWf profile in patients on INTERMACS 1–2, regardless the presence of temporary mechanical circulatory support with intra-aortic balloon pump. However, we believe that further studies selectively focusing on the actual contribution of different or concomitant temporary mechanical circulatory support devices as well as on potential inferences of PRE anticoagulation strategies on vWf activity are needed to properly interpret baseline vWf activity. As a matter of fact, predisposing factors and/or the underlying biological mechanisms that lead some patients to experience more severe impairment of vWf activity prior to or during LVAD support are not fully clear and warrant further investigation.

Our study also provides direct comparison of the vWf profile in patients implanted with the HVAD or the HM3. We show that both pumps elicit degradation of vWf and impact vWf activity, but no differences were noted between the 2 devices (Fig. 3). Furthermore, the rate of bleeding was similar with both pumps (Table 1).

Klaeske *et al.* [23] reported improved preservation of the vWf multimeric structure (high- versus intermediate- versus low-molecular weight multimers) in HM3 patients with respect to the

HVAD. However, those differences did not correlate with a reduced rate of bleeding complications with the HM3 [23]; accordingly, in our opinion, the translational impact and clinical significance of these findings are limited, and evaluation of large vWf multimers activity seems sufficient to 'clinically' evaluate bleeding risk in the LVAD population.

Our analysis on the correlation between change in AT regimen and bleeding recurrence corroborates previous studies showing beneficial effect of aspirin discontinuation to prevent bleeding recurrence in patients with continuous-flow pumps [8–13].

Despite we acknowledge that different types of bleeding (major versus minor or mucosal versus non-mucosal) might deserve different treatments, our results further support the current evidences, which suggest efficacy of aspirin discontinuation irrespective of the bleeding type. We indeed speculate that minor events might be a signal of impeding major events whose clinical manifestation might be prevented by prompt changes in the AT therapeutic protocol (i.e. aspirin withdrawal). Furthermore, a switch to aspirin-free AT regimen seems to be consistent to prevent bleeding with both the HM3 and HVAD.

The concept of reduced AT strategy to prevent bleeding has also been approached in terms of low-intensity anticoagulation (INR target: 1.5–1.9) in patients with the HM3) [24]. On the other hand, this approach was suggested for only few selected patients rather than for the general LVAD population [24].

Our hypothesis of pro-haemorrhagic effect of aspirin is in accordance with reported platelet dysfunction and impaired aggregation capacity of heart failure patients secondary to prolonged intake of drugs to treat heart failure (diuretics, anticoagulants/antiplatelets, angiotensin-converting enzyme (ACE) inhibitors, β-blockers, calcium channel blockers, etc.), kidney and liver dysfunction, haemodynamic decompensation, and platelet α- and δ-granule secretion defects following LVAD implant [21].

As such, it is reasonable to assume that antiplatelets might further contribute to haemostatic imbalance and higher susceptibility to spontaneous bleeding. It is worth noting that only aspirin was discontinued following a bleeding event. Warfarin was not stopped nor the INR changed. This allows us to isolate the effect of aspirin on bleeding events.

Our results also confirm previous evidence of poor efficacy of aspirin to mediate pro-thrombotic platelet activity driven by continuous-flow LVADs. Indeed, the median absolute increase in PAS values of patients discontinued from aspirin was extremely low (1.13-fold; Fig. 4), and PAS values remained two-fold lower than cut-off values indicative of high risk of thromboembolic complications (>1% [17]). Consistent with PAS analysis, similar rate of thrombotic events was recorded in patients treated with or without aspirin ($P = 0.60$). Previous studies showing no inferences of antiplatelet drugs in the rate of ischaemic stroke support our findings [4, 25, 26]. Moreover, our data indicate that the coagulation profile is not influenced by aspirin (Table 4), which is also consistent with the comparable pro-thrombotic risk we observed in patients with or without aspirin.

These data confirm the poor evidence for aspirin in LVAD patients and challenge the rationale for its routine use. As a result, aspirin-free AT strategy appears a clinically reliable approach to prevent/limit bleeding while on LVAD. In particular, we speculate that a primary aspirin-free AT strategy might significantly improve outcomes of patients stratified as having a higher bleeding risk. This is consistent with preliminary studies from our group that revealed excellent outcomes in selected HM3 patients with a relevant pro-haemorrhagic profile [13]. In this study age at implant emerged as an important risk factor for bleeding (Table 2), and might therefore be considered as a valid criterion to select patients who should not be treated with aspirin. Nevertheless, standardized criteria allowing patients stratification according to preoperative elevated bleeding risk are missing and urgently needed in the setting of LVAD therapy. Patient-specific genetic predisposition to more severe vWf degradation and/or platelet inhibition in response to antiplatelet therapy may also be evaluated to identify clinically relevant features.

Limitations

This is a single-centre study performed on a relatively low number of patients; yet, the rate of adverse events is comparable with larger multicentre studies. Our data are hypothesis generating and should not drive changes in standard AT protocols of LVAD patients until validated. Further prospective analyses on a larger cohort of patients from different centres are warranted to increase the power of the study.

We did not perform gel electrophoresis analysis of vWf multimers; however, the comparison of our results with respect to available literature [22, 23], leads us to speculate that collagen-binding test (vWf:CB) has higher sensitivity than gel electrophoresis and/or analysis of vWf multimeric structure to characterize impaired vWf haemostatic function that correlates with clinical outcomes.

Furthermore, we did not perform the analysis of vWf ristocetin co-factor activity (vWF:RCo), which is the assay traditionally used to evaluate the binding capacity of vWf with GpB platelet receptors. This decision was driven by evidence reported in the literature showing low specificity and sensitivity of vWf:RCo assay against acquired vW disease, which ultimately translate into significant diagnostic error rate [27]. Finally, while the PAS assay showed lower platelet activation in patients on aspirin versus those discontinued from aspirin (Fig. 4)—suggesting that patients were actually responding to aspirin—evaluation of patient-specific response to aspirin was not performed; in this regard, the analysis of aspirin resistance might contribute to further corroborate the significance of our results. At present, however, there is no a standard monitoring strategy consistently employed among LVAD centres to assess aspirin resistance. We believe that the VerifyNow Rapid Platelet Function Test (Accumetrics, Accriva Diagnostics, USA) might have limited consistency to evaluate pathophysiology of LVAD-related platelet function, as it measures platelet aggregation to fibrinogen-coated beads in response to a chemical agonist (arachidonic acid), rather than to the LVAD-specific stimulating environment (supraphysiological shear stress). Previous studies described that the pathway of platelet activation/aggregation is different when platelets are exposed to chemical versus mechanical (shear stress) agonists [12]. We would, therefore, ultimately challenge the concept of aspirin resistance [28], especially in LVADs, which has been disputed by many groups.

CONCLUSIONS

This study suggests that degradation of vWf does not fully explain spontaneous bleeding in the setting of LVAD support with continuous-flow pumps and supports the hypothesis that pathophysiology of bleeding in this clinical setting is multifactorial. In this regard, we highlight important inferences of aspirin in the development of bleeding events, consistent with further

impairment of platelet haemostatic function produced by aspirin, which adds up to acquired vWf deficiency.

In addition, our study corroborates previous evidence that discontinuation of aspirin is a clinically reliable strategy to prevent the recurrence of bleeding, irrespective of the type and/or severity of bleeding as well as the specific pump implanted. The safety of an aspirin-free AT regimen is consistent with low thrombogenicity of modern LVADs.

Prospectively, the identification of a reliable biological marker able to stratify patients at high bleeding risk and guide-tailored AT regimen is highly desirable.

SUPPLEMENTARY MATERIAL

Supplementary material is available at *EUJCTS* online.

Conflict of interest: none declared.

Data Availability Statement

The data underlying this article cannot be shared publicly for the privacy of individuals that participated in the study. The data will be shared on reasonable request to the corresponding author.

Author contributions

Filippo Consolo: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Validation; Writing—original draft; Writing—review & editing. **Alessandra Marasi:** Data curation; Formal analysis. **Patrizia Della Valle:** Data curation; Formal analysis; Methodology. **Marta Bonora:** Data curation; Formal analysis. **Marina Pieri:** Data curation; Investigation; Writing—review & editing. **Anna Mara Scandroglio:** Investigation; Methodology; Supervision; Validation; Writing—review & editing. **Alberto Redaelli:** Validation; Writing—review & editing. **Alberto Zangrillo:** Investigation; Supervision; Validation. **Armando D'Angelo:** Investigation; Methodology; Supervision; Validation; Writing—review & editing. **Federico Pappalardo:** Conceptualization; Methodology; Supervision; Writing—review & editing.

Reviewer information

European Journal of Cardio-Thoracic Surgery thanks Antonio Miceli and the other, anonymous reviewer(s) for their contribution to the peer review process of this article.

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