Influence of Different Antithrombotic Regimens on Platelet-Mediated Thrombin Generation in Patients with Left Ventricular Assist Devices

Filippo Consolo,*† Loris Pozzi,‡ Marina Pieri,§ Patrizia Della Valle,‡ Alberto Redaelli,¶ Armando D'Angelo,‡ and Federico Pappalardo*†

We characterized the biologic background of prothrombotic platelet function in the setting of durable left ventricular assist devices (LVADs) evaluating the role of different antithrombotic regimens. Platelet-mediated thrombin generation was quantified using the Platelet Activity State (PAS) Assay and the Thrombin Generation Test (TGT) in 78 patients implanted with the HeartMate II (n = 10, 13%), the HeartMate 3 (HM3) (n = 30, 38%), or the HVAD (n = 38, 49%) and managed with oral anticoagulation plus aspirin (n = 46, 59%) or anticoagulation alone (n = 32, 41%). Coagulation parameters (platelet count, International Normalized Ratio (INR), activated Partial Thromboplastin Time, Fibrinogen and D-Dimer levels) and hemolysis (lactate dehydrogenase levels [LDH]) were also recorded to comprehensively characterize the hemostatic profile in the two groups. In patients without aspirin, the PAS assay revealed low-intensity increase in platelet prothrombinase activity (1.11-fold, $p = 0.03$). Similarly the TGT revealed moderate higher platelet reactivity when compared with patients receiving aspirin, consistent with reduction in lag time (0.87-fold, $p < 0.001$), increase in peak of thrombin generation (1.5-fold, $p = 0.002$) and thrombin generation rate (2-fold, $p = 0.02$), but comparable endogenous thrombin potential ($p = 0.50$). Coagulation parameters and LDH were comparable in the two groups ($p > 0.05$). Moreover, no differences were noted in platelet prothrombinase activity of patients implanted with the HM3 or HVAD. Our results suggest that, in the setting of durable LVADs, aspirin minimally modulates the biochemical pathway of platelet-mediated thrombin generation. Accordingly, re-evaluation of current antithrombotic management criteria in patients stratified according to bleeding/thromboembolic risk might be safe and beneficial to prevent adverse events. *ASAIO Journal* 2020; 66:415–422.

Key Words: mechanical circulatory support, left ventricular assist device, platelet, thrombin generation, antiplatelet therapy, aspirin

Mechanical Circulatory Support (MCS) with continuous-flow left ventricular assist devices (LVADs) has emerged as a viable therapeutic option for patients affected by end-stage heart failure (HF) refractory to medical management.[1] To date, over 25,000 devices have been implanted in the United States[2], and over 2,500 in the EU[3].

Worldwide volume of durable LVAD implants for definitive destination therapy increased enormously in the last few years[4] and is expected to further increase, consistent with the reported actual and projected annual incidence of HF[5].

LVADs provide optimal hemodynamic recovery and improve end-organ perfusion, functional status, and quality of life.[1] Survival at 2 years is competitive with heart transplantation and, noteworthy, significantly higher with respect to medical therapy.[6–9]

Nevertheless, despite clinical efficacy of these devices, LVAD therapy remains limited by postimplant morbidity, primarily device thrombosis, thromboembolic events (stroke and neurologic complications), and bleeding episodes, which severely affect long-term survival.[4]

Thrombotic and thromboembolic events generally occur due to overactivation of the hemostatic system. The trigger of these complications is multifactorial and involves intra- and extra-device issues that severely impair platelet function and physiologic hemostasis.[10–12] In particular, shear-mediated platelet activation has been addressed as a major contributing element to LVAD thrombosis. Indeed, different sites that impair platelet function and induce a prothrombotic state due to abnormal shear stress have been identified in the LVAD system.[13]

To prevent thrombosis, current antithrombotic strategies routinely include oral anticoagulants (vitamin-K antagonist), typically warfarin targeted to an INR of 2–3, and antiplatelet agents, usually aspirin, with the aspirin dose varying with the specific implanted LVAD.[14,15] On the other hand, previous studies have suggested that bleeding events are triggered by acquired von Willebrand factor (vWf) deficiency, as a result of the shear flow-related degradation of vWf high-molecular-weight multimers.[16]

On top of that, anticoagulation and antiplatelet therapy *per se* also bears a risk for bleeding.[14,17,18] Reduced antithrombotic strategy, specifically aspirin discontinuation, has been explored to mitigate bleeding complications in this clinical

From the *Università Vita Salute San Raffaele; †Advanced Heart Failure and Mechanical Circulatory Support Program, San Raffaele Scientific Institute; ‡Coagulation Service and Thrombosis Research Unit, San Raffaele Scientific Institute; §Anesthesia and Cardiothoracic Intensive Care, San Raffaele Scientific Institute; and ¶Department of Electronics, Information and Bioengineering, Politecnico di Milano, Milano, Italy.

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Correspondence: Filippo Consolo, Università Vita Salute San Raffaele, Via Olgettina 58, 20132, Milano (MI), Italy. Email: consolo.filippo@unisr.it.

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scenario and showed to be beneficial to reduce bleeding diathesis.[19–23] These studies suggest that aspirin might exacerbate pathophysiology of bleeding disorders in LVAD patients.

On clinical grounds, according to the international MCS guidelines,[24] the effect and efficacy of anticoagulants is monitored by INR measurement. Conversely, to date, platelet function in LVAD patients remains poorly characterized. In particular, no data on prothrombotic platelet function in patients managed with different antithrombotic protocols are available that might support clinical decision on how to optimize LVAD antithrombotic regimen to prevent bleeding events or to treat bleeding episodes.

Aim of this study was to characterize the biologic background of platelet-mediated thrombin generation in LVAD patients and to evaluate the influence of different antithrombotic regimens. In detail, we sought to quantify and compare the platelet thrombin generation profile in patients managed with or without aspirin.

For this aim, we utilized two innovative diagnostic tests of platelet function that we have recently introduced: 1) the Platelet Activity State (PAS) assay[25] and 2) a modified experimental protocol of the standard Thrombin Generation Test (TGT).[26] These assays demonstrated optimal clinical competence to evaluate platelet prothrombinase activity in the setting of LVAD support and influence of antithrombotic regimen.[25–28] The PAS assay allows selective measurement of the platelet thrombin generation rate as a surrogate marker of shear-mediated platelet activation and prothrombotic state.[25,27,28] Concerning the TGT, our group has modified the standard experimental protocol of the test[29] to account selectively for the platelet contribution on plasmatic thrombin generation, also excluding any influence of oral anticoagulants (*i.e.*, warfarin) on the test.[27]

Here, we used the PAS assay and the modified-TGT to characterize the pathophysiology of platelet prothrombinase activity in LVAD patients on different antithrombotic regimens.

Materials and Methods

Study Design

Platelet-mediated thrombin generation was measured in 78 patients implanted with the HeartMate II (HMII; Throatec Corp. Pleasanton, CA; n = 10, 13%), HeartMate 3 (HM3, Abbott Laboratories; Chicago, IL, n = 30, 38%) or HeartWare Ventricular Assist Device (HVAD, Medtronic Inc. Framingham, MA; n = 38, 49%) in the setting of end-stage HF between March 2011 and February 2019. The study was conducted at San Raffaele Scientific Institute in Milan, Italy. The study complies with the Declaration of Helsinki. Institutional Review Board approval was obtained before patient enrollment and all patients provided informed consent to participate in the study.

The PAS assay and the TGT were performed in 46 patients (59%) managed with oral anticoagulation (warfarin, INR target 2.0–2.5) and aspirin. The aspirin dose varied according to the pump: specifically, patients implanted with HMII and those with HM3 were prescribed low-dose aspirin (100 mg/day; n = 21, 45%); patients implanted with the HVAD were prescribed high-dose aspirin (300 mg/day aspirin; n = 25, 55%). The tests were also performed in patients managed with oral anticoagulation alone (n = 32, 41%), including 7 patients (22%) discharged without aspirin because of postoperative bleeding (cardiac tamponade, n = 2 HM3 patients) or due to significant risk of hemorrhagic complications consistent with preoperative HAS-BLED[30] score ≥ 4 (n = 5 HM3 patients), and 25 patients (78%) discontinued from aspirin following a later bleeding event (left hemothorax n = 3 HM3 patients, intracranial hemorrhage n = 2 [HM3: n = 1, HVAD: n = 1], gastro-intestinal bleeding n = 8 [HMII: n = 1; HM3: n = 2; HVAD: n = 5], epistaxis n = 9 [HMII: n = 1, HM3: n = 4, HVAD: n = 4]; other minor bleeding events n = 3). Median time to event was 107 (34–657) days.

Study end-points included measurement and comparison of PAS and TGT values in the two groups as well as of coagulation parameters (platelet count, INR, activated Partial Thromboplastin Time, Fibrinogen, and D-Dimer levels) and index of hemolysis (lactate dehydrogenase, LDH). Moreover, we compared platelet-mediated thrombin generation in patients implanted with the HM3 and the HVAD.

To perform the analysis, blood samples were collected at follow-up visits performed at the LVAD outpatient clinic of our Institute, according to the clinical plan of patients' follow-up. For patients discontinued from aspirin following a bleeding event, blood samples were collected following at least 30 days from aspirin discontinuation, to ensure complete platelet turnover and to prevent potential confounding factors associated with the therapeutic medical interventions that followed the event (*e.g.*, blood transfusion).

Data are reported at the longest available follow-up.

Analysis of Platelet Prothrombinase Activity: the Platelet Activity State Assay

The PAS assay was performed according to the procedures previously described.[25,27] To perform the test, 10 ml of whole blood were withdrawn from patients through venipuncture or *via* central venous catheter and collected in anticoagulant citrate dextrose solution (ACD-A, 10:1, v/v). Upon collection, samples were immediately processed for analysis of platelet prothrombinase activity. Whole blood was centrifuged (170*g*, 10 min, room temperature) to separate platelet-rich-plasma (PRP). Purified platelets were obtained through PRP gel-column filtration in Sepharose2B gel (Sigma-Aldrich, St. Louis, MO). Purified platelets were then diluted to a standard concentration of 20,000/μL in a HEPES-modified Ca^{2+}-free Tyrode's platelet buffer containing 0.1% fatty acid-free bovine serum albumin, with 3 mMCa^{2+} added 10 min before experiments. Platelets were incubated with a modified prothrombin precursor reagent (acetylated prothrombin, Ac-FII)[31] for 10 min at 37°C, in the presence of FXa and Ca^{2+} (final concentrations: 5,000 platelets/μL, 200 nM Ac-FII, 100 pM FXa, and 5 mM Ca^{2+}). The platelet thrombin generation rate was quantified through spectrophotometric analysis (Multiskan FC Microplate Photometer, ThermoFisher Scientific Inc., Waltham, MA) using Chromozym-TH (Roche Diagnostics, Rotkreuz, Switzerland) as the thrombin-specific chromogenic peptide substrate. Kinetic absorbance readings were performed at 37°C at 405-nm wavelength for 7 min. The PAS value was calculated as the slope of the linear fitting of the absorbance-time data points, over the 7-min kinetic reading. PAS values were normalized against those obtained by sonicating a platelet sample from the same donor (Misonix Microson microprobe sonicator Qsonica Llc, Newtown, CT; sonication conditions: 10 W, 10 sec). The sonication step yields platelets with maximal prothrombinase activity; thus, normalized PAS values represent

the bulk activity as a fraction of the maximal thrombin-generating potential of sonicated platelets (PAS = 100%). For each PAS measurement, multiple (n = 8) readings were performed to evaluate data reproducibility.

Analysis of Platelet Thrombin Generation: the Thrombin Generation Test

Experimental protocol of the modified-TGT was reported in Consolo *et al*.[26] Specifically, the test was performed according to a modification of the procedure described by Hemker.[29] Purified platelets were obtained from LVAD patients according to the protocol described earlier for PAS assay and diluted to a standard concentration of 100,000 pl/μL in platelet buffer. Platelets were then diluted 1:2 (final concentration 50,000 platelets/μL) in a platelet-free plasma pool (PFP) previously obtained from healthy donors. Blood was collected from these donors in 0.1 mol/L sodium citrate with addition of corn trypsin inhibitor (final concentration 18.3 μg/mL, Hematologic Technologies, Essex Junction, VT) to avoid contact phase activation of coagulation factor XII. PFP was obtained through two centrifugation steps, the first one at 2,500 rpm for 10 min and the second one at 12,500 rpm for 5 min. To perform the test, 20 μL of tissue factor (final concentration 0.5 pmol/L, PRP reagent, Stago, Asnières-sur-Seine, France) were added to the reconstituted platelet + PFP sample. Following the addition of a fluorogenic substrate reagent (FluCa-Kit, Stago, Asnières-sur-Seine, France), the reaction was monitored in a Fluoroscan Ascent plate reader (Thermo Labsystem, Helsinki, Finland), using ThrombinoscopeTM software (Synapse BV, Maastricht, the Netherlands); the fluorescent signal was converted to a thrombin concentration by continuous comparison with the signal generated by a thrombin calibrator (Thrombin Calibrator, Stago, Asnières-sur-Seine, France) added to a separate PFP sample.[32] Triplicate measurements were carried out. Kinetic fluorescence readings were performed at 37°C (excitation filter: 390 nm; emission filter: 460 nm) for 60 min. The TGT curves (or thrombograms, TGs)—reporting the thrombin generation rate over the 60-min kinetic reading—were obtained for purified platelets (*i.e.*, basal) and sonicated samples. Sonication conditions were set according to the experimental protocol of PAS assay (10 W, 10 sec). From the TGs (basal and sonicated) the following parameters were extracted: 1) lag time (LT [min]), that is, the time to a thrombin concentration of 10 nmol; 2) peak of thrombin generation (Peak [nmol]); 3) time-to-peak (ttPeak [min]), that is, the time-to-peak of thrombin generation; 4) endogenous thrombin potential (ETP [nmol × min]), that is, the total thrombin generated in the sample (area under the curve); 4) Acceleration (ACC [nmol/min]), that is, the slope of thrombin generation to Peak. For each parameter, the basal-over-sonicated ratio was calculated and used in the analysis, to normalize basal thrombin generation against maximal thrombin generation.

Statistical Analysis

Categorical data are presented as absolute numbers and percentages and were compared by two-tailed χ^2 test or Fisher exact test, when appropriate. Numerical data are presented as medians and interquartile range (25th–75th percentiles). Comparison between groups was performed throughout the parametric T-test of Student or the nonparametric Mann-Whitney *U* test for normally and non-normally distributed data, respectively. A p-value < 0.05 was considered statistically significant. Statistical analyses were performed with the STATA software v.13 (StataCorp LLC, College Station, TX).

Results

Demographic and preimplant patient characteristics are reported in **Table 1**. Overall median duration of LVAD support at the time of the test was 331 (145–673) days.

Table 1. Preoperative Characteristics of Patients Discharged with and without Aspirin

Variable	Overall (n = 78)	Warfarin and Aspirin (n = 46, 59%)	Warfarin Monotherapy (n = 32, 41%)	*p*
Age at implant (years)	66 (62–70)	66 (59–68)	69 (64–72)	0.009
Females	5 (6%)	2 (4%)	3 (9%)	0.39
Ischemic HF etiology	45 (57%)	27 (58%)	18 (56%)	>0.99
INTERMACS profile*				
1	14 (18%)	7 (15%)	7 (22%)	0.13
2	19 (24%)	13 (28%)	6 (19%)	
3	25 (32%)	11 (24%)	14 (44%)	
4	20 (26%)	15 (33%)	5 (15%)	
Intention to treat				
DT	59 (76%)	33 (72%)	26 (81%)	0.42
BTT/BTC	19 (24%)	13 (28%)	6 (19%)	
LVEDD (mm)	71 (64–77)	74 (64–78)	70 (64–74)	0.11
Diabetes	23 (29%)	14 (30%)	9 (28%)	>0.99
BMI (kg/m^2)	23.5 (21.8–27.4)	23.4 (20.9–27.4)	24.1 (22.7–27.8)	0.34
Creatinine (mg/dL)	1.25 (0.98–1.75)	1.22 (0.93–1.69)	1.36 (1.06–1.95)	0.14
Hemoglobin (g/dL)	11.5 (10.4–12.6)	11.7 (10.4–12.9)	11.2 (10.4–12.2)	0.24
Temporary MCS	46 (59%)	18 (39%)	28 (100%)	<0.001
HM2	10 (13%)	8 (18%)	2 (7%)	
HM3	30 (38%)	13 (28%)	17 (53%)	
HVAD	38 (49%)	25 (54%)	13 (40%)	

* INTERMACS with temporary circulatory support-modifier.

BMI, body mass index; BTC, bridge to candidacy; BTT, bridge to transplant; DT, destination therapy; HF, heart failure; HM3, heartmate 3; LVEDD, left ventricular end-diastolic diameter; MCS, mechanical circulatory support (Intra Aortic Balloon Pump, Impella, Extracorporeal Membrane Oxygenation).

Table 2. Comparison of Coagulation Parameters and Hemolysis Index in Patients Managed with and without Aspirin in the Background of Warfarin Administration

	Warfarin and Aspirin (n = 46)	Warfarin Monotherapy (n = 32)	*p*
Platelet count (10^9/L)	199 (155–247)	202 (166–252)	0.80
INR	2.22 (1.9–2.54)	2.04 (1.84–2.33)	0.16
aPTT	1.16 (1.07–1.24)	1.14 (1.08–1.24)	0.89
Fibrinogen (mg/dL)	351 (309–461)	428 (307–500)	0.27
D-Dimer (μg/mL)	2.08 (1.21–3.32)	2.35 (1.32–3.80)	0.26
LDH (unit/L)	288 (244–355)	256 (217–324)	0.13

aPTT, activated partial thromboplastin time; INR, International Normalized Ratio; LDH, lactate dehydrogenase; PT, prothrombin time.

Coagulation parameters and hemolysis index (LDH) were not different in the two groups (**Table 2**; $p > 0.05$).

Median PAS value measured in patients managed with warfarin and aspirin (median time of support 373 [128–688] days) was equal to 0.45% (0.36%–0.57%), indicating low platelet prothrombinase activity. Median PAS value measured in patients on warfarin monotherapy (median duration of support: 315 [157–665] days; 85 [54–289] days of aspirin-free antithrombotic regimen) was 0.50% (0.46%–0.57%). Comparison between the two groups revealed low-intensity increase of PAS in patients not on aspirin (1.11-fold higher, $p = 0.03$). However, although higher PAS values were measured in patients not on aspirin, these values were significantly lower than PAS cutoff values characteristics of overt thromboembolic events (>3%)[27] or indicative of high risk of thromboembolic complications (>1%),[27] suggesting that the aspirin-free antithrombotic regimen was not associated to an actual—higher—thrombotic risk.

Analysis of the thrombograms of the two groups as measured *via* the modified-TGT revealed comparable kinetics of thrombin generation (**Figure 1**) and moderate increase of platelet reactivity in patients not on aspirin. In detail, LT was 0.87-fold lower in patients managed without aspirin (**Table 3**; $p < 0.001$); in addition, peak of thrombin generation was 1.5-fold higher in these patients (**Table 3**; $p = 0.002$); likewise, patients not on aspirin exhibited a 2-fold higher rate of thrombin generation (ACC; **Table 3**; $p = 0.01$). On the other hand, we recorded comparable ttPeak and ETP in the two groups (**Table 3**; $p > 0.05$).

Detailed analysis of PAS values stratified according to the implanted LVAD system (*i.e.*, the HMII, HM3 and HVAD) revealed comparable platelet prothrombinase activity, in patients managed with or without aspirin (**Table 4**). As far as the analysis of the TGT parameters is concerned, limited differences were recorded in the thrombin generation profile of patients implanted with the HVAD (**Table 4**).

Furthermore, patients implanted with the HM3 or the HVAD were not characterized by a different platelet prothrombinase activity. In detail, comparable PAS values ($p = 0.58$) were recorded in patients managed with aspirin. Concerning the TGT, LT and ttpeak were significantly lower in the HM3 group ($p = 0.04$ and 0.003, respectively). However, Peak and ACC were comparable ($p = 0.25$ and 0.83, respectively). Furthermore, total amount of thrombin generated by platelets was lower in HM3 patients (ETP: $p = 0.002$). No differences were observed in patients managed with anticoagulation alone (PAS: $p = 0.76$; LT: $p = 0.84$; Peak: $p = 0.38$; ttpeak; $p = 0.83$; ETP: $p = 0.87$; ACC: $p = 0.47$).

Consistent with the low measured platelet prothrombinase activity, despite reduced antithrombotic regimen, patients discharged on warfarin monotherapy—that is, who did never receive aspirin—as well as those discontinued from aspirin following a bleeding event did not suffer from thrombotic/thromboembolic complications over a current follow-up of 426 (203–753) days. Furthermore, irrespective of aspirin use, a similar results profile was recorded at the longest available follow-up in patients with aspirin (928 [594–1457] days). Remarkably, aspirin discontinuation avoided bleeding recurrence in 97% of the patients who suffered from previous bleeding episodes. Indeed, following aspirin withdrawal, only one patient implanted with the HMII suffered from intracranial hemorrhage in the background of concomitant systemic infection. Conversely, we recorded seven episodes of major bleeding events (intracranial hemorrhage) in patients managed with warfarin and aspirin (15% of the patients; HMII: n = 2; HM3: n = 1; HVAD: n = 4). All these patients died due to bleeding-related complications.

Discussion

The current study addressed, for the first time, the biologic background of platelet prothrombinase activity in patients implanted with durable LVAD systems on different

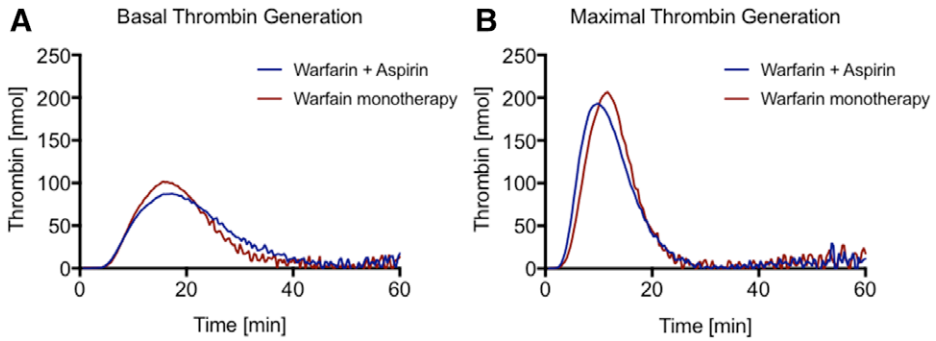

Figure 1. Average thrombograms measured in LVAD patients on warfarin and aspirin ($n = 46$) and warfarin monotherapy ($n = 32$). Basal thrombin generation of isolated platelets (**A**) was normalized against maximal prothrombinase activity of sonicated samples (**B**). Sonication was performed before assay of thrombin generation to yield platelets with maximal prothrombinase activity. LVAD, left ventricular assist device. full color online

Table 3. Comparison of Normalized Values (Basal Over Sonicated) of the Parameters Extracted from the Thrombograms of Patients Managed with and without Aspirin in the Background of Warfarin Administration

	Warfarin and Aspirin (n = 46)	Warfarin Monotherapy (n = 32)	*p*
LT (min)	1.51 (1.35–1.61)	1.32 (1.18–1.45)	<0.001
Peak (nmol)	0.38 (0.27–0.47)	0.56 (0.33–0.65)	0.06
ttPeak (min)	1.89 (1.42–2.12)	1.38 (1.26–1.76)	0.002
ETP (nmol × min)	0.84 (0.66–0.89)	0.86 (0.67–0.91)	0.50
ACC (nmol/min)	0.16 (0.13–0.24)	0.34 (0.15–0.49)	0.02

ACC, acceleration; ETP, Endogenous Thrombin Potential; LT, lag time; ttPeak, time-to-peak.

antithrombotic regimens. According to recent clinical[19–23] and mechanistic[33,34] studies questioning effectiveness of current antiplatelet strategies in the prevention of LVAD thrombosis, and suggesting a contributory role of intensive (*i.e.*, dual) antithrombotic regimen in enhancing bleeding diathesis, we focused our analysis on: 1) evaluating the effect of different aspirin regimens on platelet thrombin generation and associated prothrombotic state and 2) identifying a possible correlation between different aspirin therapeutic strategies and the occurrence of bleeding and thrombotic complications. We have also previously questioned the role of aspirin in continuous-flow rotary LVADs, as we showed that aspirin did not prevent the development of thrombotic complications in patients characterized by a specific platelet-related prothrombotic background.[27]

According to our results, the PAS assay and the TGT revealed low-intensity increase in platelet reactivity in patients not on aspirin, suggesting no differences in the thrombotic risk of these patients with respect to those managed with warfarin and aspirin. The analysis was conducted in patients implanted with different LVAD technologies, including the HMII axial pump, the centrifugal fully magnetically levitated HM3, and the hydrodynamically levitated HVAD centrifugal pump. Consistent with experimental data, clinical observations confirmed previous findings that in patients with no significant differences in coagulation parameters (**Table 2**), aspirin-free antithrombotic management might be beneficial to: 1) limit incidence of bleeding events over the course of support, 2) avoid hemorrhage recurrence in patients who suffered from previous bleeding episodes, and, interestingly, 3) might not portend to an increased thrombotic risk.[19–23]

In detail, our results support previous findings showing: 1) the beneficial effect of aspirin discontinuation following a bleeding episode in patients implanted with the HMII, further expanding the 2-year endpoint of the EU-TRACE study,[21] 2) the safety of warfarin monotherapy in patients implanted with the HM3[22], and 3) reduced bleeding tendency in high-risk HM3 patients managed with primary warfarin monotherapy.[23]

These results provide a contributory explanation for the poor background of aspirin therapy in LVAD patients.

The results in the HM3 group are further supported by the studies by Netuka *et al.*[35] and Bansal *et al.*,[36] which reported greater preservation of vWf in HM3 patients compared with patients implanted with the HMII but comparable rate of bleeding complications with the two devices, suggesting that other factors, including antithrombotic management, may be predominant than acquired vWf deficiency in determining bleeding while on the HM3. In addition, Colombo *et al.* recently showed higher risk of hemorrhagic stroke in HM3 patients managed with aspirin *versus* patients on oral anticoagulation (86% *vs.* 57%, respectively) and comparable risk of thromboembolic events, namely ischemic stroke in patients managed with or without aspirin.[37] Taken together, these results indicate that, on the one hand, aspirin might exacerbate pathophysiology of bleeding disorders in HM3 patients and that, on the other hand, anticoagulation alone might be effective and safe to limit thromboembolic complications. Bleeding complications while on the HM3 are a major issue and a real clinical need to improve hemocompatibility with this device exists. The Multicenter Study of MagLev Technology in Patients Undergoing Mechanical Circulatory Support Therapy with HeartMate 3 (MOMENTUM 3) trial revealed an incidence of bleeding complications of 43% in the HM3 population (bleeding requiring surgery: 12%; gastro-intestinal bleeding: 27%).[9] This was further confirmed by the 2-year results of the HM3 CE mark study, which reported an incidence of 16% of bleeding events requiring surgery.[38] Evaluation of low-intensity anticoagulation (INR 1.5–1.9 plus aspirin) has been also exploited to reduce bleeding events in HM3 patients (the MAGENTUM 1 study[39]). However, first results demonstrated that this strategy did not eliminate bleeding recurrence. Moreover, data reported by Colombo *et al.*[37] indicate that reduced anticoagulation might portend to a higher risk of ischemic stroke. Here, we suggest that

Table 4. Prothrombotic Platelet Function and Platelet Thrombin Generation Profile in Patients Managed with Aspirin and Without Aspirin in the Background of Warfarin Administration Stratified According to the Specific Implanted LVAD System

	HMII (*n* = 10)			HM3 (*n* = 30)			HVAD (*n* = 38)		
	Warfarin and aspirin (*n* = 8)	Warfarin monotherapy (*n* = 2)	*p*	Warfarin and aspirin (*n* = 13)	Warfarin monotherapy (*n* = 17)	*p*	Warfarin and aspirin (*n* = 25)	Warfarin monotherapy (*n* = 13)	*p*
PAS (%)	0.40 (0.36–0.45)	0.42 (0.28–0.57)	0.67	0.46 (0.39–0.62)	0.51 (0.46–0.65)	0.26	0.45 (0.37–0.59)	0.49 (0.45–0.59)	0.26
LT (min)	1.53 (1.51–1.63)	1.41 (1.21–1.62)	0.19	1.41 (1.29–1.51)	1.33 (1.14–1.45)	0.21	1.52 (1.32–1.66)	1.32 (1.2–1.45)	0.01
Peak (nmol)	0.37 (0.27–0.43)	0.25 (0.15–0.35)	0.14	0.33 (0.26–0.49)	0.54 (0.32–0.65)	0.14	0.38 (0.32–0.59)	0.65 (0.36–0.68)	0.11
ttPeak (min)	2.13 (1.42–2.25)	1.74 (1.16–2.32)	0.55	1.48 (1.34–1.81)	1.40 (1.24–1.78)	0.65	2.02 (1.68–2.15)	1.37 (1.30–1.66)	0.004
ETP (nmol × min)	0.85 (0.70–0.88)	0.55 (0.25–0.86)	0.18	0.69 (0.53–0.84)	0.86 (0.67–0.90)	0.07	0.88 (0.72–0.94)	0.89 (0.74–0.94)	0.98
ACC (nmol/min)	0.16 (0.13–0.20)	0.13 (0.12–0.14)	0.29	0.18 (0.14–0.30)	0.32 (0.16–0.49)	0.32	0.16 (0.13–0.36)	0.46 (0.16–0.50)	0.04

ACC, acceleration; ETP, endogenous thrombin potential; HMII, heartmate II; HM3, heartmate 3; HVAD, heartware ventricular assist device; LT, lag time; LVAD, left ventricular assist device; PAS, platelet activity state; ttPeak, time-to-peak.

discontinuation of antiplatelet therapy might be more effective in this scenario. Moreover, according to previous reports,[23,36] we highlight the need for a randomized study addressing the issue of antiplatelet agents with the HM3.

This study is also the first clinical report that comprehensively analyzed and linked platelet prothrombinase activity and clinical outcomes in patients implanted with the HVAD. Antithrombotic recommendations with the use of aspirin on top of oral anticoagulants were reinforced in the case of HVAD implantation, as clinical evidences showed that high aspirin dose (325 mg/day) in HVAD patients was associated with a decrease in device thrombosis and neurologic events.[40] However, the optimal dose of aspirin in HVAD patients remains controversial[41]: as a matter of fact, many centers adopt lower aspirin dose (81 mg/day) to prevent bleeding complications. Nevertheless, there is no consensus as to whether discontinuation of aspirin might be safely pursued to treat HVAD bleeding events. In this study, despite differences in the platelet thrombin generation profile were observed in HVAD patients not on aspirin, which were not noted in patients implanted with the HMII and the HM3 (**Table 4**), our data indicate low-intensity prothrombinase platelet function with this device. Accordingly, aspirin-free antithrombotic regimen might have potential benefit in selected HVAD patients to limit/prevent bleeding complications, with no significant implications as far as the thromboembolic risk is concerned.

Interestingly, in this study, we did not record major differences in platelet prothrombinase activity in patients implanted with the HM3 or the HVAD. Potential differences in the thrombogenic potential of the two devices can not be ruled out; however, our findings suggest that a patient-specific predisposition to develop thromboembolic and/or bleeding complications may play a relevant a role and may not be suppressed (thromboelic events) or even may be amplified (bleeding events) by the therapeutic protocol.

Our results shed light on the need to re-evaluate current antithrombotic management criteria in selected LVAD patients prospectively stratified according to significant bleeding/thromboembolic risk, consistent with clinical evidences that the biochemical pathway of LVAD-related platelet thrombin generation is of low intensity and it is minimally modulated by aspirin. Indeed, the definition of patient-tailored antithrombotic regimens might contribute to prevent HRAEs and to improve LVAD therapeutic outcomes. In this regard, we underline the importance of a strict and systematic monitoring of prothrombotic platelet function in patients not on aspirin, in order to timely identify potential major alterations in platelet reactivity requiring prompt modification of the antithrombotic regimen to prevent thrombosis. For this aim, analysis of platelet function *via* the diagnostic assays we utilized here might have enhanced clinical value in the setting of MCS, as they were designed to selectively account for shear-mediated platelet injury and prothrombotic activity. In particular, the PAS assay was extensively validated in previous preclinical studies that analyzed prothrombotic platelet response to mechanical stimulation (shear-forces).[42–47] Conversely, other platelet function tests might have limited consistency in this specific clinical setting. For example, Light Transmission Aggregometry and the Multiple Electrode Analyzer use chemical agonists to evaluate platelet aggregation and thromboelastogram (TEG) and rotational thromboelastometry use an excessively high amount of tissue factor or of contact pathway activators to evaluate the platelet contribution to maximum clot firmness. As such, they might not be related to the pathophysiology of MCS-thrombosis. Indeed, the literature describes extensively that the specific stimulating environment (chemical *versus* mechanical) induces a different platelet response.[33,34] Furthermore, a recent study showed that analysis of prothrombotic platelet function *via* TEG measurements do not correlate with LVAD thrombosis.[48] We suggest that those tests should be at least recalibrated to define normal *versus* abnormal cutoff values that effectively account for shear-mediated prothrombotic platelet function in MCS patients.

Importantly, we underscore that the results of our study only apply to patients on MCS. Indeed, the biologic response of platelets sustaining repeated exposure to LVAD shear stresses is likely to be different from that of patients with cardiovascular disease (*e.g.*, coronary artery disease or acute myocardial infarction). As such, the findings of this study might not be generalized to different clinical settings where the cardioprotective benefit of aspirin is well established.

Study Limitations

This is a single-center study limited by its relatively small number of patients. Further multicentric investigations on a larger patient cohort are warranted to validate our results, that is, to exclude that increasing the sample size might reveal higher differences in the prothrombotic profile of the two groups that remained unnoticed in our cohort. In particular, data on HVAD patients not on aspirin should be treated with caution, as, to the best of our knowledge, no studies with larger cohort of patients exist that support our findings. Moreover, patient-specific analysis of prothrombotic platelet function before and after aspirin discontinuation was not performed: a systematic longitudinal characterization of these phenomena (*i.e.*, pre- *versus* post aspirin withdrawal) is warranted to confirm the results presented here. Accordingly, findings from this small, non-randomized retrospective study should be interpreted as "hypothesis generating" and not to change clinical practice until they will be validated in larger—randomized—observational studies.

Analysis of the impact of preoperative temporary circulatory support (TCS) on bleeding complications was not performed. However, median time of bleeding occurrence was 107 (34–657) days postimplant, suggesting no correlation between bleeding events and preoperative TCS. On the other hand, TCS included different (and sometimes concomitant) devices (IABP, Impella, ECMO) with potential different "hemorrhagic impact".

Conclusions

We characterized the biologic background of platelet prothrombinase activity in LVAD patients managed with different antithrombotic regimens and provide mechanistic insights into actual platelet-related prothrombotic risk in patients managed with/without aspirin. Our results suggest that aspirin minimally modulates the biochemical pathway of platelet thrombin generation in the setting of durable MCS. This study also suggests patient-specific tendency to hemostatic disorders. As such, the definition of patient-tailored pharmacological strategies supported by systematic analysis of platelet function might

prospectively contribute to improve LVAD hemocompatibility and long-term outcomes. Indeed, the possibility to identify *a priori* those patients characterized by a specific activated prothrombotic profile and to prospectively stratify those who might/might not benefit from an antiplatelet might significantly innovate current criteria for postoperative pharmacological management to reduce HRAEs.

References

1. Pinney SP, Anyanwu AC, Lala A, Teuteberg JJ, Uriel N, Mehra MR: Left Ventricular assist devices for lifelong support. *J Am Coll Cardiol* 69: 2845–2861, 2017.
2. Kormos RL, Cowger J, Pagani FD, et al: The society of thoracic surgeons intermacs database annual report: Evolving indications, outcomes, and scientific partnerships. *J Heart Lung Transplant* 38: 114–126, 2019.
3. de By TMMH, Mohacsi P, Gahl B, *et al*; EUROMACS members: The European Registry for Patients with Mechanical Circulatory Support (EUROMACS) of the European Association for Cardio-Thoracic Surgery (EACTS): second report. *Eur J Cardiothorac Surg* 53: 309–316, .2018. doi: 10.1093/ejcts/ezx320.
4. Mancini D, Colombo PC: Left ventricular assist devices: A rapidly evolving alternative to transplant. *J Am Coll Cardiol* 65: 2542–2555, 2015.
5. Lam CS, Donal E, Kraigher-Krainer E, Vasan RS: Epidemiology and clinical course of heart failure with preserved ejection fraction. *Eur J Heart Fail* 13: 18–28, 2011.
6. Slaughter MS, Rogers JG, Milano CA, et al; HeartMate II Investigators: Advanced heart failure treated with continuous-flow left ventricular assist device. *N Engl J Med* 361: 2241–2251, 2009.
7. Estep JD, Starling RC, Horstmanshof DA, et al; ROADMAP Study Investigators: Risk assessment and comparative effectiveness of left ventricular assist device and medical management in ambulatory heart failure patients: Results from the ROADMAP Study. *J Am Coll Cardiol* 66: 1747–1761, 2015.
8. Rogers JG, Pagani FD, Tatooles AJ, et al: Intrapericardial left ventricular assist device for advanced heart failure. *N Engl J Med* 376: 451–460, 2017.
9. Mehra MR, Goldstein DJ, Uriel N, et al; MOMENTUM 3 Investigators: Two-year outcomes with a magnetically levitated cardiac pump in heart failure. *N Engl J Med* 378: 1386–1395, 2018.
10. Tarzia V, Buratto E, Bortolussi G, et al: Hemorrhage and thrombosis with different LVAD technologies: a matter of flow? *Ann Cardiothorac Surg* 3: 582–584, 2014.
11. Mehra MR, Stewart GC, Uber PA: The vexing problem of thrombosis in long-term mechanical circulatory support. *J Heart Lung Transplant* 33: 1–11, 2014.
12. de Biasi AR, Manning KB, Salemi A: Science for surgeons: understanding pump thrombogenesis in continuous-flow left ventricular assist devices. *J Thorac Cardiovasc Surg* 149: 667–673, 2015.
13. Selmi M, Chiu WC, Chivukula VK, *et al*: Blood damage in Left Ventricular Assist Devices: Pump thrombosis or system thrombosis? *Int J Artif Organs* 42: 113–124, 2019.
14. Teuteberg JJ, Slaughter MS, Rogers JG, et al; ADVANCE Trial Investigators: The HVAD left ventricular assist device: Risk factors for neurological events and risk mitigation strategies. *JACC Heart Fail* 3: 818–828, 2015.
15. Baumann Kreuziger LM, Kim B, Wieselthaler GM: Antithrombotic therapy for left ventricular assist devices in adults: a systematic review. *J Thromb Haemost* 13: 946–955, 2015.
16. Nascimbene A, Neelamegham S, Frazier OH, Moake JL, Dong JF: Acquired von Willebrand syndrome associated with left ventricular assist device. *Blood* 127: 3133–3141, 2016.
17. Nassif ME, LaRue SJ, Raymer DS, Novak E, Vader JM, Ewald GA, Gage BF: Relationship between anticoagulation intensity and thrombotic or bleeding outcomes among outpatients with continuous-flow left ventricular assist devices. *Circ Heart Fail* 9: e002680, 2016.
18. Baghai M, Heilmann C, Beyersdorf F, et al: Platelet dysfunction and acquired von Willebrand syndrome in patients with left ventricular assist devices. *Eur J Cardiothorac Surg* 48: 421–427, 2015.
19. Saeed O, Shah A, Kargoli F, et al: Antiplatelet therapy and adverse hematologic events during heart mate II support. *Circ Heart Fail* 9: e002296, 2016.
20. Litzler PY, Smail H, Barbay V, et al: Is anti-platelet therapy needed in continuous flow left ventricular assist device patients? A single-centre experience. *Eur J Cardiothorac Surg* 45: 55–59; discussion 59, 2014.
21. Netuka I, Litzler PY, Berchtold-Herz M, et al; EU TRACE Investigators: Outcomes in heartmate II patients with no antiplatelet therapy: 2-year results from the European TRACE Study. *Ann Thorac Surg* 103: 1262–1268, 2017.
22. Lim HS, Ranasinghe A, Mascaro J, Howell N: Discontinuation of aspirin in heartmate 3 left ventricular assist device. *ASAIO J* 65: 631–633, 2019.
23. Consolo F, Raimondi Lucchetti M, Tramontin C, Lapenna E, Papplardo F. Do we need aspirin in HeartMate3 patients? *Eur J Heart Fail* 21: 815–817, 2019.
24. Feldman D, Pamboukian SV, Teuteberg JJ, et al; International Society for Heart and Lung Transplantation: The 2013 international society for heart and lung transplantation guidelines for mechanical circulatory support: Executive summary. *J Heart Lung Transplant* 32: 157–187, 2013.
25. Valerio L, Consolo F, Bluestein D, et al: Shear-mediated platelet activation in patients implanted with continuous flow LVADs: A preliminary study utilizing the platelet activity state (PAS) assay. *Conf Proc IEEE Eng Med Biol Soc* 2015: 1255–1258, 2015.
26. Consolo F, Pozzi L, Sferrazza G, et al: Which antiplatelet therapy in patients with left ventricular assist device and aspirin allergy? *Ann Thorac Surg* 105: e47–e49, 2018.
27. Consolo F, Sferrazza G, Motolone G, et al: Platelet activation is a preoperative risk factor for the development of thromboembolic complications in patients with continuous-flow left ventricular assist device. *Eur J Heart Fail* 20: 792–800, 2018.
28. Consolo F, Sferrazza G, Motolone G, *et al*: Shear-mediated platelet activation enhances thrombotic complications in LVAD patients and is reversed after heart transplantation. *ASAIO J* 65: e33–e35, 2019.
29. Hemker HC: The application of thrombin generation in real life clinical situations. *Thromb Res* 136: 3–4, 2015.
30. Kemal HS, Ertugay S, Nalbantgil S, et al: Utility of CHA2DS2-VASc and HAS-BLED scores as predictor of thromboembolism and bleeding after left ventricular assist device implantation. *ASAIO J* 63: 720–724, 2017.
31. Jesty J, Bluestein D: Acetylated prothrombin as a substrate in the measurement of the procoagulant activity of platelets: elimination of the feedback activation of platelets by thrombin. *Anal Biochem* 272: 64–70, 1999.
32. Luddington R, Baglin T: Clinical measurement of thrombin generation by calibrated automated thrombography requires contact factor inhibition. *J Thromb Haemost* 2: 1954–1959, 2004.
33. Valerio L, Tran PL, Sheriff J, et al: Aspirin has limited ability to modulate shear-mediated platelet activation associated with elevated shear stress of ventricular assist devices. *Thromb Res* 140: 110–117, 2016.
34. Valerio L, Sheriff J, Tran PL, et al: Routine clinical anti-platelet agents have limited efficacy in modulating hypershear-mediated platelet activation associated with mechanical circulatory support. *Thromb Res* 163: 162–171, 2018.
35. Netuka I, Kvasnička T, Kvasnička J, et al: Evaluation of von Willebrand factor with a fully magnetically levitated centrifugal continuous-flow left ventricular assist device in advanced heart failure. *J Heart Lung Transplant* 35: 860–867, 2016.
36. Bansal A, Uriel N, Colombo PC, *et al*: Effects of a fully magnetically levitated centrifugal-flow or axial-flow left ventricular assist device on von Willebrand factor: A prospective multicenter clinical trial. *J Heart Lung Transplant* 38: 806–816, 2019.
37. Colombo PC, Mehra MR, Goldstein DJ, et al: Comprehensive analysis of stroke in the long-term cohort of the MOMENTUM 3 Study. *Circulation* 139: 155–168, 2019.

38. Schmitto JD, Pya Y, Zimpfer D, *et al*: Long-term evaluation of a fully magnetically levitated circulatory support device for advanced heart failure-two-year results from the HeartMate3 CE Mark Study. *Eur J Heart Fail* 21: 90–97, 2019.
39. Netuka I, Ivák P, Tučanová Z, et al: Evaluation of low-intensity anti-coagulation with a fully magnetically levitated centrifugal-flow circulatory pump-the MAGENTUM 1 study. *J Heart Lung Transplant* 37: 579–586, 2018.
40. Slaughter MS, Pagani FD, McGee EC, et al; HeartWare Bridge to Transplant ADVANCE Trial Investigators: HeartWare ventricular assist system for bridge to transplant: combined results of the bridge to transplant and continued access protocol trial. *J Heart Lung Transplant* 32: 675–683, 2013.
41. Najjar SS, Slaughter MS, Pagani FD, et al; HVAD Bridge to Transplant ADVANCE Trial Investigators: An analysis of pump thrombus events in patients in the HeartWare ADVANCE bridge to transplant and continued access protocol trial. *J Heart Lung Transplant* 33: 23–34, 2014.
42. Apostoli A, Bianchi V, Bono N, et al: Prothrombotic activity of cytokine-activated endothelial cells and shear-activated platelets in the setting of ventricular assist device support. *J Heart Lung Transplant* 38: 658–667, 2019.
43. Dimasi A, Roka-Moiia Y, Consolo F, et al: Microfluidic flow-based platforms for induction and analysis of dynamic shear-mediated platelet activation-Initial validation versus the standardized hemodynamic shearing device. *Biomicrofluidics* 12: 042208, 2018.
44. Dimasi A, Rasponi M, Consolo F, et al: Microfludic platforms for the evaluation of anti-platelet agent efficacy under hyper-shear conditions associated with ventricular assist devices. *Med Eng Phys* 48: 31–38, 2017.
45. Consolo F, Sheriff J, Gorla S, et al: High frequency components of hemodynamic shear stress profiles are a major determinant of shear-mediated platelet activation in therapeutic blood recirculating devices. *Sci Rep* 7: 4994, 2017.
46. Consolo F, Dimasi A, Rasponi M, et al: Microfluidic approaches for the assessment of blood cell trauma: a focus on thrombotic risk in mechanical circulatory support devices. *Int J Artif Organs* 39: 184–193, 2016.
47. Consolo F, Valerio L, Brizzola S, et al: On the use of the platelet activity state assay for the *In Vitro* quantification of platelet activation in blood recirculating devices for extracorporeal circulation. *Artif Organs* 40: 971–980, 2016.
48. Piche SL, Nei SD, Frazee E, *et al*: Baseline thromboelastogram as a predictor of left ventricular assist device thrombosis. *ASAIO J* 65: 443–448, 2018.