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Higher soluble thrombomodulin and angiogenic markers in continuous flow left ventricular assist device–supported patients associated with arteriovenous malformation and nonsurgical bleeding

Kavitha Muthiah, MBChB, PhD, FRACP, FCSANZ,[a,b,*] Louise L. Dunn, BSc(Hons), PhD,[b] Hunter Eckford, BMedSci,[a] David Connor, BMedSci(Hons), PhD,[c,d] Desiree Robson, RN,[a] Peter S. Macdonald, MBBS, FRACP, MD, PhD,[a,b] and Christopher S. Hayward, MD, FRACP[a,b]

[a]*Heart Failure and Transplant Unit, St. Vincent's Hospital, Sydney, Australia*
[b]*Victor Chang Cardiac Research Institute, Sydney, Australia*
[c]*St. Vincent's Centre For Applied Medical Research, Sydney, Australia*
[d]*Department of Hematology, St. Vincent's Hospital, Sydney, Sydney, Australia*

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Bleeding complications are a bane of continuous flow left ventricular assist devices (cfLVAD); gastrointestinal bleeding (GIB) from arteriovenous malformation (AVM) predominating. We hypothesized that shear stress disrupts vascular endothelium altering angiogenesis and contributing to bleeding. We profiled markers of endothelial dysfunction (soluble thrombomodulin [sTM]) and angiogenesis (angiopoietin-1 [Ang-1], angiopoietin-2 [Ang-2]) in 21 patients implanted with a centrifugal cfLVAD. Bleeding episodes were documented in 11 patients, 8 had GIB, 4 of whom had AVMs. We observed a dynamic change in sTM and Ang-2/Ang-1 ratio following cfLVAD support ($p = 0.030$ and $p = 0.025$, respectively). Bleeding patients had higher sTM and Ang-2/Ang-1 ratios than patients with no bleeding ($p = 0.04$ and $p = 0.06$, respectively). At D180, patients with AVMs had significantly higher Ang-2/Ang-1 ratios vs patients without proven AVMs ($p = 0.006$). We conclude that bleeding in cfLVAD-supported patients is associated with alteration in endothelial/vascular homeostasis, possibly contributing to AVM formation.

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Background

Continuous flow left ventricular assist devices (cfLVAD) constitute the majority of mechanical circulatory support in patients with end-stage heart failure as a bridge to cardiac

*Corresponding author: Kavitha Muthiah, MBChB, PhD, FRACP, FCSANZ, Heart and Lung Transplant Unit, St Vincent's Hospital, 390 Victoria Street, Sydney, NSW 2010, Australia.
E-mail address: kavitha.muthiah@svha.org.au.

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transplant and destination therapy.[1] However, centrifugal cfLVAD support is associated with complications resulting from altered physiology.[2] With increasing demand for LVADs, it is imperative we understand the effects of hemodynamic changes on end-organ function to improve outcomes.

Nonsurgical bleeding (NSB) is a well-established complication of long-term mechanical circulatory support.[2,3] In cfLVAD support, NSB is thought to be a byproduct of pathological shear stress as the change in flow waveform from pulsatile to continuous flow damages the microvasculature.[4] Consequently, cell-signaling pathways in the vascular endothelium are dysregulated influencing smooth muscle cell proliferation and altering protein expression by endothelial cells.[5] One consequence is an increased angiogenic potential evidenced by Tabit et al,[6] in which the endothelial cells of cfLVAD-implanted patients exhibited heightened angiopoietin-2 (Ang-2) expression outweighing the protective effects of angiopoietin-1 (Ang-1). Subsequently, the investigators found that patients with Ang-2 levels above the mean had greater NSB events than patients with levels below the mean. We and others have found excess bleeding angiodysplasia in the gastrointestinal tract of cfLVAD patients manifesting as immature, ectatic, and structurally compromised blood vessels which we theorize to be a result of shear-induced endothelial dysfunction.[7,8] Further, we posit that soluble thrombomodulin (sTM), a marker of endothelial damage by hemodynamic turbulence, may exacerbate angiodysplasia-driven gastrointestinal bleeding (GIB) in cfLVAD support by impairing platelet aggregation.[9] Our aim was to link these markers to NSB and arteriovenous malformations (AVM) in cfLVAD support to inform therapy.

Methods

Study population and sample collection

Patients were consecutively enrolled upon implantation with a centrifugal cfLVAD. Informed consent was sought from eligible patients. Patients were on anticoagulant therapy with warfarin (international normalised ratio 2-3) and antiplatelet therapy. Blood sampling was performed before centrifugal cfLVAD implantation (D0) and at D7, 30, 90, and D180 or until cardiac transplant or death.

Antigen immunoassays

Plasma was collected in citrate tubes and centrifuged at 2°C to 8°C for 15 minutes at 1000 × *g* within 30 minutes of collection. Ang-1, Ang-2, and sTM were measured. For Ang-1, the samples were centrifuged again for 10 minutes to create platelet-poor plasma. Cell lysis, preparation of reagents and working standards, dilutions, and loading of sample wells were conducted for antigens per their Quantikine immunoassay manuals (RnD Systems, Inc.). Standards and plasma samples were run as single assays. Upon completion, sample wells were read on a microplate reader at 450 nm with background subtraction at 540 nm. Standard curves were sTM (0, 62.5, 500, 4,000 pg/ml), Ang-1 (0, 62.5, 500, 4,000 pg/ml), and Ang-2 (0, 46.9, 375, 3,000 pg/ml).

Nonsurgical bleeding events

Episodes of bleeding were documented. Days from device implantation to GIB events were calculated and then segregated by closest time point of blood collection, that is, day 0, 7, 30, 90, and 180. Potential GIB episodes were assessed with gastroscopy, colonoscopy, and/or mesenteric angiography to locate and record the origin of bleeding. The presence of AVM was documented. Other adverse events were documented and defined per the 2021 Interagency Registry for Mechanically Assisted Circulatory Support (INTERMACS). Bleeding events were characterized as overt bleeding unrelated to the ventricular assist device implant operation, resulting in hospitalization, blood cell transfusion, reoperation, or death. Thromboembolic events comprised intracorporeal device thrombosis, venous thromboembolism, and arterial thromboembolism indicated by non-CNS arterial perfusion deficit. Routine gastroscopy and colonoscopy were not performed before left ventricular assist device implantation.

Statistical analysis

Analyses were exploratory in nature. A comparison of antigen presence/concentration over time was performed with analysis of variance. Multivariable analysis and Mauchly's Test of Sphericity were used to adjust the association of sTM and Ang-2/Ang-1 with bleeding and AVM formation. Continuous data were summarized as mean (standard deviation) and categorical data as absolute numbers.

Results

Patient population

Twenty-one patients implanted with a cfLVAD (HeartWare Continuous Flow Ventricular Assist Device) over a 24-month period were included. Two patients (9.5%) had an additional centrifugal cfLVAD implanted in the right ventricle for biventricular support. Thirty-three percent of patients were in INTERMACS 1, 62% in INTERMACS 2%, and 5% in INTERMACS 3 at the time of implant. Baseline demographic and clinical characteristics after stratifying patients into bleeding and AVM categories are presented in Table 1. Of the 21 patients, 18 were males and 3 females. Dilated cardiomyopathy was the most prevalent etiology followed by ischemic and hypertrophic cardiomyopathies (n = 10, 8, and 3, respectively). Over the study period, 8 patients were transplanted and 8 died. Sixteen patients were on angiotensin-converting enzyme inhibitors, 6 on angiotensin receptor blockers, 19 on beta-blockers, and 18 on

Table 1 Patient Characteristics Compared to Prevalence of Nonsurgical Bleeding

Demographics	All patients; $n = 21$	Patients with bleeding; $n = 11$	Patients with no bleeding; $n = 10$	p-value
Age in years (median)	47	47	47.5	0.748
Sex (male), *n* (%)	18 (85.7)	10 (47.6)	8 (38.1)	0.476
Race/ethnicity (%)				
Caucasian	17 (81)	9 (42.9)	8 (38.1)	0.916
Middle eastern	1 (4.8)	0	1 (4.8)	0.283
Pacific Islander	2 (9.5)	1 (4.8)	1 (4.8)	0.943
Asian	1 (4.8)	1 (4.8)	0	0.329
Etiology of heart failure, *n* (%)				
Ischemic cardiomyopathy	8 (38.1)	4 (19)	4 (19)	0.864
Hypertrophic cardiomyopathy	3 (14.3)	1 (4.8)	2 (9.5)	0.476
Dilated cardiomyopathy	10 (47.6)	6 (28.6)	4 (19)	0.505
Outcomes (%)				
HTX during study period	8 (38.1)	2 (9.5)	6 (28.6)	0.049
Died during study period	8 (38.1)	5 (23.8)	3 (14.3)	0.072
INTERMACS[1]	7 (33.3%)	5 (23.8)	2 (9.5)	0.216
Duration of cfLVAD support, days (median, [IQR])	273 [280]	273 [189]	296.5 [678]	0.368
Prior MCS support	14 (66.6)	9 (42.9)	5 (23.8)	0.122
Dialysis during study	6 (28.6)	5 (23.8)	1 (4.8)	0.072
BiVAD	2 (9.5)	2 (9.5)	0	0.156
Previous GIB	1 (4.8)	1 (4.8)	0	0.329
Aortic valve function during study period (%)				
Closed throughout	14 (66.7)	8 (38.1)	6 (28.6)	0.537
Open/intermittently open	4 (19)	2 (9.5)	2 (9.5)	0.916
Closed progressing to open/intermittently open	2 (9.5)	0	2 (9.5)	0.119
RV status pre-cfLVAD insertion (%)				
Normal to mild RV dysfunction	2 (9.5)	1 (4.8)	1 (4.8)	0.811[a]
Moderate to severe RV dysfunction	17 (81)	10 (47.6)	7 (33.3)	
Normal to mild tricuspid regurgitation	7 (33.3)	3 (14.3)	4 (19)	0.135[a]
Moderate to severe tricuspid regurgitation	6 (28.6)	5 (23.8)	1 (4.8)	
Average RV function post-cfLVAD insertion (%)				
Normal to mild RV dysfunction	2 (9.5)	2 (9.5)	0	0.156[a]
Moderate to severe RV dysfunction	19 (90.5)	9 (42.9)	10 (47.6)	

Abbreviations: BiVAD, biventricular assist device; cfLVAD, continuous flow left ventricular assist device; GIB, gastrointestinal bleeding; HTX, heart transplant; INTERMACS, Interagency Registry for Mechanically Assisted Circulatory Support; IQR, interquartile range; MCS, mechanical circulatory support; RV, right ventricle.

All other *p-values were obtained using a paired t*-test.

[a]*p*-values performed using the chi-square test.

mineralocorticoid receptor antagonists. One patient had an endoscopy performed before cfLVAD implantation to investigate for bleeding and was found to have areas suggestive of angiodysplasia. This patient also experienced further GIB in the study.

Nonsurgical bleeding and AVM formation

Eleven patients had 1 or more episodes of bleeding (median 29 days from device implantation to first bleeding episode); 8 with GIB. Four patients had AVM formation. AVMs are manifested in both the stomach and bowel.

Of the 8 subjects that experienced GIB, 1 presented with bleeding on day 7 after cfLVAD implantation, 5 patients on day 30, and 3 patients on day 90. Of these patients, 1 patient experienced 2 bleeding events at approximately day 90, while another experienced 3 events; 2 events on day 30 and 1 event on day 90.

Protein expression

We observed a dynamic increase in sTM and Ang-2/Ang-1 following centrifugal cfLVAD support ($p = 0.030$ and $p = 0.025$, respectively). Patients with bleeding had significantly higher sTM levels compared to patients with no bleeds ($p = 0.04$) (Figure 1). Similarly, there was a trend of higher Ang-2/Ang-1 in patients with bleeding vs no bleeding ($p = 0.06$) (Figure 1). At day 180, patients with documented AVMs had significantly higher Ang-2/Ang-1 ratios compared to patients with no proven AVMs (27.1 ± 20.1 vs 6.4 ± 7.5 ng/ml, $p = 0.006$). This difference was not significant before left ventricular assist device implantation ($p = 0.55$).

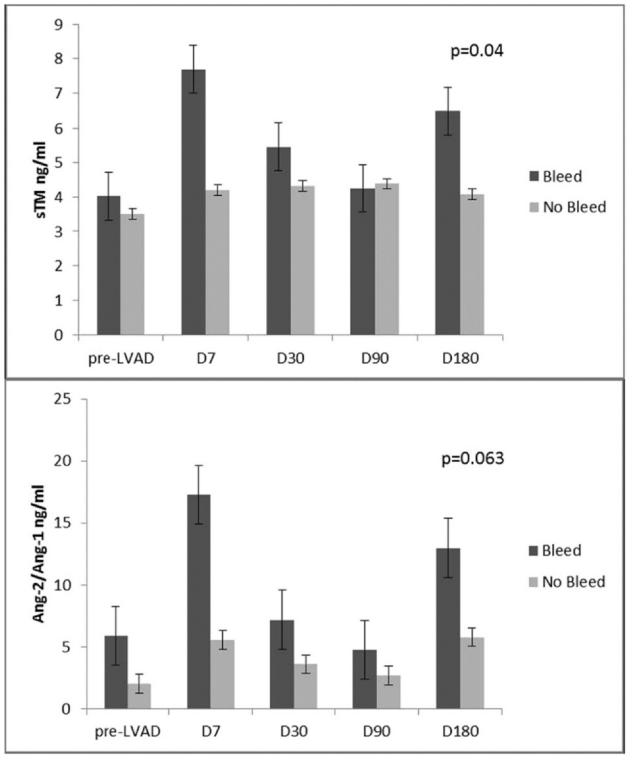

Figure 1 Top: blood concentrations of soluble thrombomodulin (sTM) among "bleed" and "no bleed" patient populations over 180 days (N = 11 and 10, respectively). Bottom: angiopoietin-2/angiopoietin-1 ratio (Ang-2/Ang-1) in "bleed" and "no bleed" groups over 180 days with bleed patients having a higher blood concentration of Ang-2.

Discussion

NSB occurs in 30% to 40% of cfLVAD patients, most often manifesting as GIB, and is a primary cause for readmission.[2,3] Following the model set by Tabit et al, we performed ELISAs (enzyme linked immunosorbent assays) of patient sera and similarly found that endothelial expression of Ang-2 was upregulated in patients implanted with a cfLVAD corresponding with more frequent bleeding episodes.[6] While Tabit et al demonstrated enhanced angiogenesis in vitro by exposing umbilical vein cells to Ang-2-rich serum, our study presents a correlation between Ang-2 expression and AVM formation in patients thereby linking bleeding episodes with device-induced angiodysplasia.[6] Unlike Ang-1 which is synthesized by perivascular cells, Ang-2 is expressed exclusively by endothelial cells whereupon it competitively inhibits Ang-1.[6] Thus, the increases in Ang-2 we observed are likely to have prevented Ang-1 from enabling stable vessel maturation while promoting fragile vascular architecture reminiscent of AVMs described in the intestinal mucosa of cfLVAD recipients.[6,8] Though there is little dispute that Ang-2 gives rise to NSB, the extent to which this altered gene expression is dictated by continuous flow, specifically requires further validation.

Another molecule made by endothelial cells is thrombomodulin, synthesized as a response to cell damage particularly in regions of hemodynamic turbulence such as the bifurcation of arteries.[9] Thrombomodulin plays an important role in fibrinogen binding necessary for clotting; however, it can be cleaved by inflammatory molecules into a soluble, less-effective form. Hence, the liberation of thrombomodulin by altered flow coupled with inflammation, such as that induced by the pump material, might lead to increased levels of sTM and ultimately impair clotting in cfLVAD-implanted patients.[4] Even so, some researchers report activation of the coagulant system in cfLVAD support.

As such, the increased Ang-2/Ang-1 ratio and sTM we report following cfLVAD implantation denote an adaptive endothelial phenotype in response to altered hemodynamics. The upregulation of these molecules may reflect similar acute increases in proangiogenic DNA-binding inhibitors observed in this population.[5]

The higher mortality in this study of up to 38% may be explained by the large proportion of patients in INTERMACS categories 1 and 2 (95%) at implant, with 67% of patients bridged from temporary MCS support. In addition, there was no protocol for blood pressure management during the study period which may have impeded optimum treatment. This study is limited by a small sample size at a single center and, therefore, may not be representative of other sites. Further, longer-term follow-up was not reported. It must also be acknowledged that all patients in our cohort were implanted with HeartWare ventricular assist devices, a pump that is no longer widely in use due to recall; however, current-generation devices are also similar to centrifugal continuous flow design.

Currently, our findings strongly indicate that bleeding in cfLVAD patients is associated with alteration in endothelial and vascular homeostasis driven by altered gene expression, possibly contributing to AVM formation. Future research should follow larger sample sizes of cfLVAD patients over several years at multiple sites to validate the current findings and reveal if the observed trends are maintained with an emphasis on endothelial cell adaptation.

Disclosure statement

Kavitha Muthiah reports financial support was provided by the National Heart Foundation of Australia. The other authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Author contributions

K.M., C.H., P.M., and D.R. conceptualized and designed the study. Laboratory tests and ELISAs were performed by L.D., K.M., and D.C. Manuscript was drafted by K.M. and H.E. All coauthors edited and approved the final manuscript.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhlto.2024.100133.

References

1. Cheng A, Williamitis CA, Slaughter MS. Comparison of continuous-flow and pulsatile-flow left ventricular assist devices: is there an advantage to pulsatility? Ann Cardiothorac Surg 2014;3:573-581.
2. Hasin T, Marmor Y, Kremers W, et al. Readmissions after implantation of axial flow left ventricular assist device. J Am Coll Cardiol 2013;61:153-63.
3. Stulak JM, Lee D, Haft JW, et al. Gastrointestinal bleeding and subsequent risk of thromboembolic events during support with a left ventricular assist device. J Heart Lung Transplant 2014;33:60-4.
4. Poredos P, Jezovnik MK, Radovancevic R, Gregoric ID. Endothelial function in patients with continuous-flow left ventricular assist devices. Angiology 2021;72:9-15.
5. Zhang J, Friedman MH. Adaptive response of vascular endothelial cells to an acute increase in shear stress magnitude. Am J Physiol Heart Circ Physiol 2012;302:H983-91.
6. Tabit CE, Chen P, Kim GH, et al. Elevated angiopoietin-2 level in patients with continuous-flow left ventricular assist devices leads to altered angiogenesis and is associated with higher nonsurgical bleeding. Circulation 2016;134:141-52.
7. Muthiah K, Robson D, Macdonald PS, et al. Increased incidence of angiodysplasia of the gastrointestinal tract and bleeding in patients with continuous flow left ventricular assist devices (LVADs). Int J Artif Organs 2013;36:449-54.
8. Kang J, Hennessy-Strahs S, Kwiatkowski P, et al. Continuous-flow LVAD support causes a distinct form of intestinal angiodysplasia. Circ Res 2017;121:963-9.
9. Califano F, Giovanniello T, Pantone P, et al. Clinical importance of thrombomodulin serum levels. Eur Rev Med Pharmacol Sci 2000;4:59-66.