Continuous-Flow LVAD Support Causes a Distinct Form of Intestinal Angiodysplasia

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Rationale: The objective of this autopsy study was to determine whether gastrointestinal angiodysplasia develops during continuous-flow left ventricular assist device (LVAD) support.

Objective: LVAD support causes pathologic degredation of von Willebrand factor (vWF) and bleeding from gastrointestinal angiodysplasia at an alarming rate. It has been speculated that LVAD support itself may cause angiodysplasia. The relationship to abnormal vWF metabolism is unknown. We tested the hypothesis that abnormal gastrointestinal vascularity develops during continuous-flow LVAD support.

Methods and Results: Small bowel was obtained from deceased humans, cows, and sheep supported with a continuous-flow LVAD (n=9 LVAD, n=11 control). Transmural sections of jejunum were stained with fluorescein isothiocyanate–conjugated isolectin-B4 for endothelium to demarcate vascular structures and quantify intestinal vascularity. Paired plasma samples were obtained from humans before LVAD implantation and during LVAD support (n=41). vWF multimers and degradation fragments were quantified with agarose and polyacrylamide gel electrophoresis and immunoblotting. Abnormal vascular architecture was observed in the submucosa of the jejunum of human patients, cows, and sheep supported with a continuous-flow LVAD. Intestinal vascularity was significantly higher after LVAD support versus controls (5.2±1.0% versus 2.1±0.4%, P=0.004). LVAD support caused significant degradation of high–molecular-weight vWF multimers (–9±1%, P<0.0001) and accumulation of low–molecular-weight vWF multimers (+40±5%, P<0.0001) and vWF degradation fragments (+53±6%, P<0.0001).

Conclusions: Abnormal intestinal vascular architecture and LVAD-associated vWF degradation were consistent findings in multiple species supported with a continuous-flow LVAD. These are the first direct evidence that LVAD support causes gastrointestinal angiodysplasia. Pathologic vWF metabolism may be a mechanistic link between LVAD support, abnormal angiogenesis, gastrointestinal angiodysplasia, and bleeding. (Circ Res. 2017;121:963-969. DOI: 10.1161/CIRCRESAHA.117.310848.)

Key Words: angiodysplasia ▪ bleeding ▪ left ventricular assist device ▪ von Willebrand factor

Gastrointestinal bleeding is the number one cause of hospital readmission in outpatients undergoing left ventricular assist device (LVAD) support.1 Approximately 20% to 40% of patients with a continuous-flow LVAD bleed from the gastrointestinal tract.2–5 The most common source of bleeding is angiodysplasia.3–6

For more than a decade, it has been speculated that LVAD support itself may cause gastrointestinal angiodysplasia.7 Emerging evidence has demonstrated that continuous-flow LVADs cause abnormal angiogenesis8 and mucosal vascularization.9 These studies suggested that LVAD support itself alters systemic angiogenic signals, which may contribute to the development of angiodysplasia and bleeding.

In parallel, there is an emerging body of evidence that von Willebrand factor (vWF) regulates multiple vascular pathways,10,11 including angiogenesis.12 It is clear that LVAD support causes pathologic degradation of vWF and significantly alters systemic vWF metabolism. Therefore, it is possible that abnormal vWF metabolism may play a major role in altering angiogenic signals and promoting angiodysplasia.

In an autopsy study, we tested the hypothesis that abnormal vascular architecture develops during continuous-flow LVAD support. We examined intestine from humans, cows, and sheep supported with a continuous-flow LVAD. In parallel, plasma samples from 41 continuous-flow LVAD patients were evaluated for pathologic vWF degradation.

Methods

Human blood samples were obtained from LVAD patients with approval from the Hospital of the University of Pennsylvania Institutional Review Board (No. 818944, Novel Model of LVAD-Like von Willebrand Factor Degradation for the Investigation of Bleeding in

Clinical Track
Novelty and Significance

What Is Known?
- Patients undergoing artificial circulation with an implantable left ventricular assist device (LVAD) develop intestinal bleeding from angiodysplasia at an alarming rate.
- LVADs cause abnormal degradation of von Willebrand factor (vWF), a clotting protein, which circulates in the blood.
- It is unknown whether LVAD support itself causes angiodysplasia or whether there is a relationship between angiodysplasia and vWF degradation.

What New Information Does This Article Contribute?
- LVAD support causes a distinct form of angiodysplasia.
- LVAD support causes significant degradation of vWF into small protein fragments that circulate in the blood during LVAD support.
- Abnormal vWF degradation may play an important role in the development of intestinal angiodysplasia and bleeding in LVAD patients.

Mechanical circulatory support with an LVAD is a standard therapy for patients with advanced, life-threatening heart failure. However, outcomes are limited by gastrointestinal bleeding, which occurs in 20% to 40% of patients, most frequently from angiodysplasia. Abnormal degradation of vWF by the LVAD may play an important role. This is the first study to demonstrate that LVAD support caused angiodysplasia. Patients also developed significant degradation of vWF protein into small fragments that circulated in the blood. These findings support the novel hypothesis that vWF fragments are a trigger for intestinal angiodysplasia in patients undergoing LVAD support. If this proves to be the case, vWF degradation may be a clinical target for therapy.

Nonstandard Abbreviations and Acronyms

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Patients With an LVAD. Whole human blood was obtained from patients before and during LVAD support. Human tissues were obtained from deceased human donors with approval from the Hospital of the University of Pennsylvania, Department of Pathology. Families of the deceased provided consent for an autopsy and tissue donation. Large animal LVAD implants were performed, and tissue samples were obtained with Institutional Animal Care and Use Committee approval.

Small Bowel Tissue Collection and Preparation
Continuous-flow LVADs were implanted in humans, calves, and sheep (Table). Circumferential samples of mid-jejunum were obtained from deceased human patients (n=4 LVAD, 247±36 days support, 52±12 years old; n=4 control, 69±10 years old), euthanized cows (n=2 LVAD, 90±0 days support, 4–6 months old; n=4 control, 4–6 months old), and euthanized sheep (n=3 LVAD, 38±2 days support, 18±18 months old; n=3 control, 18±18 months old).

Tissue samples were stored in 10% neutral buffered formalin (Sigma-Aldrich, St. Louis, MO) overnight at room temperature. Samples were cut in cross-section at ≈4 mm thickness and stored in 70% ethanol.

Histopathologic Staining
Fixed cross-sections of jejunum were embedded in paraffin, sectioned at 5 μm, deparaffinized, rehydrated, and stained as previously described.\(^1\) Masson trichrome staining (Sigma-Aldrich, St. Louis, MO) was performed to define general tissue architecture. Fluorescein isothiocyanate–conjugated isoclin-B4 staining for endothelium (Vector Laboratories, Burlingame, CA) was performed to confirm and demarcate vascular structures to quantify vascularity.

Quantification of Vascularity
Histologic stains were viewed with epifluorescence microscopy (Leica DM IL LED; Leica Microsystems Inc, Wetzlar, Germany). Three random fields were photographed and analyzed per tissue section. Values were averaged to obtain a single representative mean value. Intestinal vascularity was determined as the ratio of area occupied by isoclin-B4 stain/area of small intestine sampled. Images were analyzed with ImageJ software (National Institutes of Health, Bethesda, MD).

Blood Collection
Paired samples of whole human blood were obtained before LVAD implantation and during LVAD support (n=41). Blood was anticoagulated in sodium heparin blood collection tubes (BD Biosciences, Franklin Lakes, NJ). Plasma was frozen and stored at −80°C before gel electrophoresis and immunoblotting for vWF was performed.

Agarose Gel Electrophoresis and Immunoblotting for vWF Multimers
High–molecular-weight plasma vWF multimers were resolved by standard electrophoresis and immunoblotting techniques as previously described.\(^1\)\(^4\)\(^–\)\(^9\) Plasma samples were diluted 1:40 in NuPAGE lithium dodecyl sulfate Sample Buffer (Invitrogen). Samples were heated at 70°C for 10 minutes. Samples were loaded into 10% vertical agarose/SDS gels (0.1% SDS, 0.375 mol/L Tris). Electrophoresis was performed at 60 V for 2.5 hours at 4°C in Tris-Acetate SDS running buffer (Invitrogen, Carlsbad, CA) in an XCell SureLock Mini-Cell Electrophoresis System (Invitrogen).

Protein was transferred to polyvinylidine difluoride membranes using the iBlot dry transfer device (Invitrogen). Membranes were blocked for 1 hour and incubated with rabbit anti-human vWF primary antibody (1:500; Dako, Glostrup, Denmark) overnight. The following day, membranes were incubated with goat anti-rabbit IgG horseradish peroxidase–conjugated secondary antibody (1:3000; Cell Signaling, Danvers, MA) for 2 hours at room temperature, developed with Luminata Forte Western Blot HRP Substrate (Millipore, Billerica, MA), and imaged with an ImageQuant LAS 4000 (GE Healthcare, Piscataway, NJ). ImageJ (National Institutes of Health) was used to perform densitometric quantification of vWF multimers.

Polyacrylamide Gel Electrophoresis and Immunoblotting for vWF Degradation Fragments
Plasma vWF degradation fragments were resolved by standard electrophoresis and immunoblotting techniques as previously described.\(^1\)\(^4\)–\(^9\) Plasma samples were diluted 1:40 in NuPAGE lithium dodecyl sulfate Sample Buffer (Invitrogen). Samples were heated at 70°C for 10 minutes and loaded into NuPAGE 3% to 8% Tris-Acetate SDS gels. MagicMark XP Western Protein Standard (Invitrogen) and HiMark Pre-Stained Protein Standard Ladder (Invitrogen) were loaded. Electrophoresis was performed at 150 V for 1 hour and 25 minutes in Tris-Acetate SDS running buffer (Invitrogen). Protein was transferred, blocked, probed for vWF, and imaged as described above.

Each membrane was probed for human plasma albumin as a loading control with a goat anti-human albumin horseradish peroxidase–conjugated antibody (1:5000 Abcam, Cambridge, MA). Blots were washed, developed, and imaged as described above. ImageJ (National Institutes of Health) was used to perform densitometric quantification of vWF fragment bands.

vWF Immunoblotting Analysis
Relative amounts of high–molecular-weight vWF multimers, low–molecular-weight vWF multimers, and vWF degradation fragments were
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quantified. ImageJ (National Institutes of Health) was used to measure the chemiluminescent intensity of immunoblots pre-LV AD and during LV AD support. Images, which contained paired pre- and LV AD samples, underwent differential contrast enhancement to highlight biological changes.17

Statistics
GraphPad, version 5.00, (Prism; GraphPad Software, Inc, La Jolla, CA) was used to perform statistical analyses and plot data. The average intestinal vascularity in 9 subjects with an LV AD (4 humans, 2 cows, 3 sheep) was compared with 11 controls (4 humans, 4 cows, 3 sheep) via unpaired Student t test. Paired Student t tests compared high–molecular-weight vWF multimers and vWF degradation fragments between pre-LV AD and LV AD samples. A P<0.05 was considered statistically significant. All data were presented as mean±SE.

Results
Intestinal Vascular Architecture After LVAD Support
Control small intestine exhibited normal vascular architecture and vascularity. Submucosal arteriole/venule pairs appeared normal in size and structure. In comparison, after LVAD support, large, dilated angiodysplastic lesions were noted within the intestinal submucosa. The ectatic, thin-walled vascular structures were not appropriately paired with an arteriole (Figure 1A). The lesions occupied a large total area of the submucosa and did not seem to cross through the intestinal muscularis.

Intestinal vascularity was significantly higher in jejunum from subjects with a continuous-flow LVAD versus control subjects (Figure 1B, all samples 5.2±1.0% versus 2.1±0.4%, P=0.004: human samples, 5.0±1.1% versus 2.3±0.3%; bovine samples 8.9±1.4% versus 2.9±0.5%; ovine samples 3.0±0.5% versus 0.5±0.2%).

Pathologic vWF Metabolism During Continuous-Flow LVAD Support
Continuous-flow LVAD support caused degradation of high–molecular-weight vWF multimers and accumulation of low–molecular-weight multimers and vWF degradation fragments (Figure 2). High–molecular-weight vWF multimers decreased by 9±1% (P<0.0001). Low–molecular-weight vWF fragments increased by 40±5% (P<0.0001). vWF degradation fragments increased by 53±6% (P<0.0001). The profile of degradation was consistent with the profile that we15,17,19 and others20–25 have previously reported.

Discussion
In an autopsy study, we observed abnormal intestinal vascular architecture in humans, cows, and sheep supported with a continuous-flow LVAD. Pathologic vWF degradation was observed in plasma samples. These are the first evidence to demonstrate that angiodysplasia may be a consistent pathologic finding during continuous-flow LVAD support and raise the question of a mechanistic association between pathologic vWF degradation and angiodysplasia.

LVAD-Associated Gastrointestinal Bleeding Is a Major Problem
Bleeding is the number one cause of hospital readmission and a major obstacle to improving outcomes, reducing costs, and expanding the public health impact of mechanical circulatory support.1,26 Thirty percent to 75% of patients experience nonsurgical bleeding.20–25 Approximately 18% to 40% of patients develop gastrointestinal bleeding.2–4 In greater than 60% of patients with gastrointestinal bleeding, the source is angiodysplasia.5,6

Importantly, abnormal vWF metabolism contributes to LVAD-associated bleeding.20–25 Supraphysiologic shear stress from continuous-flow LVADs accelerates vWF degradation and causes a vWF deficiency characterized by loss of high–molecular-weight vWF multimers and accumulation of low–molecular-weight vWF multimers and vWF degradation fragments.15,20–25 As a result, patients acquire a bleeding diathesis.

LVAD-Associated Angiodysplasia May Be a Distinct Pathology
There is indirect evidence that LVAD-associated gastrointestinal bleeding is a distinct pathophysiology unrelated to preexisting gastrointestinal lesions, antiplatelet therapy, or

| Table. Characteristics of Human Patients, Cows, and Sheep Supported With a Continuous-Flow LVAD |
|-----------------|------------------|---------------------|------------------|
| Control         | LVAD             |
| Age             | Cause of Death   | Age                | LVAD Duration of Support | Cause of Death |
| Human           |                  | Human              | HeartMate II       | 319 d           | Intracranial hemorrhage |
| 66 y            | Multigorgan failure | 55 y               | HeartMate II       | 148 d           | Intracranial hemorrhage |
| 73 y            | Pulmonary embolism | 27 y               | HeartMate II       | 260 d           | Intracranial hemorrhage |
| 93 y            | Ruptured aortic aneurysm | 74 y               | HVAD               | 260 d           | Intracranial hemorrhage |
| 45 y            | Sepsis           | 50 y               | HeartMate II       | 260 d           | Intracranial hemorrhage |
| Bovine          |                  | Bovine             | MVAD              | 90 d            | Elective euthanasia     |
| 4–6 mo          | Elective euthanasia | 4–6 mo             | MVAD              | 90 d            | Elective euthanasia     |
| Ovine           |                  | Ovine              | HeartMate II       | 42 d            | Elective euthanasia     |
| 18 mo           | Elective euthanasia | 18 mo              | HeartMate II       | 37 d            | Elective euthanasia     |
| 18 mo           | Elective euthanasia | 18 mo              | HeartMate II       | 36 d            | Elective euthanasia     |
| LVAD indicates left ventricular assist device. |
systemic anticoagulation. Indeed, the majority of patients who develop bleeding do not have a previous history of gastrointestinal bleeding.\(^27,28\) And a previous history of bleeding is not a risk factor for bleeding during LV AD support.\(^3\) Furthermore, antiplatelet therapy is not a risk factor for LV AD-associated bleeding,\(^28\) and most patients who bleed present with a therapeutic or subtherapeutic INR.\(^3\) In fact, gastrointestinal bleeding occurs far more frequently in LV AD patients than expected from anticoagulation alone (anticoagulated LV AD patients, 63 events per 100 patient-years versus anticoagulated mechanical valve patients, 4.6 events per 100 patient-years).\(^29\) Together, these clinical observations suggest that LV AD-associated gastrointestinal bleeding is not related to preexisting lesions or antithrombotic therapy but that continuous-flow LV AD support may independently contribute to the development of gastrointestinal angiodysplasia.

The currently accepted pathophysiologic mechanism for age-related angiodysplasia is incongruent with the clinical presentation of LV AD patients and inconsistent with our findings. In humans, age is a major risk factor for gastrointestinal angiodysplasia.\(^30,31\) However, we observed abnormal intestinal vascularity in relatively young subjects. It is unusual that abnormal vascularization was noted in a 27-year-old patient and in healthy 8-month-old calves implanted with an LVAD (Table).

Mechanistically, age-related angiodysplasia develops from transient increases in bowel lumen pressure and wall tension that cause episodic venous outflow obstruction and dilation of venules where they penetrate the muscularis.\(^32,33\) Over years, precapillary sphincters dilate, lose competency, and produce arteriovenous communications. This mechanism accounts for the highest incidence of age-related angiodysplasia in the right colon, which has the greatest luminal diameter and wall tension.

In contrast, the majority of LVAD-associated angiodysplasia (and bleeding) occurs in the small bowel and upper gastrointestinal tract,\(^3,5,34,35\) not the colon. In the small bowel and upper gastrointestinal tract, luminal diameter and wall tension are low, and so venous obstruction is not responsible for angiodysplasia. To this point, we observed angiodysplasia in the small bowel. Lesions did not seem to cross through the intestinal muscularis (Figure 1A). As such, it is unlikely that venous outflow obstruction caused the angiodysplasia that we observed. These findings suggest that LVAD-associated angiodysplasia may be its own distinct pathology with different pathophysiologic triggers than age-related angiodysplasia.

### Abnormal vWF Metabolism During LVAD Support May Alter Angiogenesis and Contribute to Angiodysplasia

Recent studies have suggested that LVAD support alters vascular proliferation and stability. In vitro experiments have demonstrated abnormal levels of angiogenic peptides\(^8,36\) and angiogenesis\(^4\) with plasma from LV AD patients. In vivo,
patients develop nasal mucosal hypervascularity and, as we have shown, intestinal angiodysplasia. The upstream trigger is unknown but may be related to abnormal vWF metabolism.

Indeed, there is an emerging body of evidence that vWF plays critical regulatory roles in multiple vascular pathways that include regulation of angiogenesis. As previously noted, supraphysiologic shear stress from continuous-flow LVADs accelerates vWF metabolism. As a result, vWF degradation fragments accumulate in LVAD patients. Systemic consequences of elevated vWF fragments circulating in the blood are unknown. However, Heyde syndrome, the constellation of aortic stenosis, vWF degradation, and bleeding from gastrointestinal angiodysplasia highlights the important biological relationship between sheer stress, pathologic vWF metabolism, and gastrointestinal angiodysplasia.

Similarly, a variety of patients with an abnormal vWF profile develop gastrointestinal angiodysplasia. Approximately 37% of patients with congenital type IIA von Willebrand disease present with gastrointestinal bleeding, frequently from angiodyplasia. The prevalence of angiodyplasia in acquired type IIA von Willebrand disease is an alarming 12%. These clinical observations again suggest a causal relationship between an abnormal vWF and angiodyplasia.

Recently, we observed that vWF degradation fragments (both from LVAD patients and generated in an in vitro LVAD model) altered angiogenesis (data not shown, currently under peer-review for publication). Endothelial cells cultured with vWF fragments exhibited altered endothelial cell migration, tubule formation, proliferation, and apoptosis. These mechanistic findings suggested that vWF degradation may be an important trigger of abnormal angiogenesis and thereby of angiodysplasia. Interestingly, cGMP and angiopoietin-2, which are downstream effectors of vWF, are elevated in LVAD patients. In fact, vWF exerts its regulatory role over angiogenesis through cGMP and angiopoietin-2. Thus, these findings support the hypothesis that abnormal vWF metabolism contributes to the development of angiodyplasia by altering systemic angiogenic signaling through cGMP and angiopoietin-2. Although this hypothesis was not specifically tested, it is conceptually appealing and supported by multiple recent reports from our group and from others.

Limitations
This study was performed with small sample sizes from multiple species. The analysis compared the average intestinal vascularity in 9 subjects with an LVAD (4 humans, 2 cows, 3...
sheep) to 11 controls (4 humans, 4 cows, 3 sheep). We combined samples from multiple species because it is difficult to obtain fresh tissues from human LVAD patients and chronic large animal LVAD studies are expensive. Abnormal vascularization was observed in each species group, which suggested that the biological response was strong and conserved across species. It is also reassuring that biological changes were consistent and without great variability within the individual species groups. Importantly, the statistical significance across control versus LVAD intestinal vascularity was strong ($P=0.004$).

**Conclusions**

LVAD-associated VWF degradation and intestinal angiodysplasia were consistent findings in multiple species supported with a continuous-flow LVAD. LVAD-associated gastrointestinal angiodysplasia may be a distinct pathology. Mechanistic relationships between LVAD-associated VWF degradation, abnormal angiogenesis, and angiodysplasia remain to be further defined.

**Disclosures**

None.

**References**


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