Activation of Endothelial and Coagulation Systems in Left Ventricular Assist Device Recipients

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**Background.** The paucity of organ donors has necessitated redirecting research toward finding alternative means to a heart transplant, such as left ventricular assist devices (LVADs) that will serve not merely as bridge-to-transplant but also as destination therapy. To better understand hemorrhagic and thromboembolic complications that currently limit the use of such devices, we studied the endothelial and coagulation system changes in LVAD recipients with time.

**Methods.** We studied these markers of endothelial dysfunction: circulating endothelial cells and expression of E-selectin, vascular cell adhesion molecule, intercellular adhesion molecule, and tissue factor on circulating endothelial cells, thrombin generation (prothrombin fragments 1,2 and thrombin/antithrombin), and fibrinolysis (D-dimer). Our study group consisted of 21 LVAD recipients (on day 0 and on postoperative days 1, 7, 30, 90, and 180) and 7 control patients undergoing non-LVAD cardiac surgery.

**Results.** Baseline values of intercellular adhesion molecule, E-selectin, tissue factor, thrombin/antithrombin, and D-dimer were significantly higher in LVAD recipients than the normal range. Markers of thrombin generation (thrombin/antithrombin and prothrombin fragments 1,2) and fibrinolysis (D-dimer) peaked postoperatively and declined to baseline levels or below by 3 months. But the expression of inducible endothelial markers (intercellular adhesion molecule, E-selectin, tissue factor) on circulating endothelial cells increased postoperatively, then decreased but remained elevated above preoperative levels for up to 6 months. In our control patients, baseline levels of intercellular adhesion molecule, E-selectin, tissue factor, D-dimer, and thrombin/antithrombin were lower and decreased significantly by day 7, as compared with LVAD recipients ($p < 0.05$).

**Conclusions.** Left ventricular assist device recipients experienced significant baseline activation of endothelial and coagulation systems, further accentuated in the early postoperative period. Left ventricular assist device recipients also had prolonged activation of the endothelial and coagulation systems, suggesting activation of the extrinsic (tissue factor) pathway of thrombosis mediated by sustained endothelial dysfunction in these patients. Further studies are needed to determine the clinical influence of such changes in LVAD recipients.
repeated exposure to cardiovascular risk factors or the presence of cardiovascular disease can ultimately exhaust the protective effect of endogenous antiinflammatory systems within endothelial cells. As a result, not only does the endothelium become dysfunctional but also endothelial cells can lose integrity and detach into the circulation. Circulating markers of such endothelial cell damage include endothelial microparticles (derived from activated or apoptotic cells) and whole endothelial cells. A broader appreciation of the numerous functions of the endothelium can be obtained by studying the levels of molecules of endothelial origin in circulating blood [13].

As one of the fundamental homeostatic mechanisms of mammalian biology, the blood coagulation system establishes a delicate balance between the procoagulant and anticoagulant functions of blood and of the vessel wall, thereby guarding against excesses in either direction and, normally, preventing unwanted hemorrhage or thrombosis. The vascular endothelium and its various components, such as tissue factor (TF), play an integral role in this homeostasis. We herein report our study of the alterations in the endothelial and coagulation systems in LVAD recipients. The objectives of our study were to (1) identify changes in the endothelial system in LVAD recipients, (2) identify changes in their coagulation system, (3) determine trends in such changes during the course of LVAD support, and (4) compare such changes with those occurring in control patients who underwent non-LVAD cardiac surgery.

Material and Methods

Patients

We performed a prospective study of 21 unselected recipients undergoing LVAD placement at the University of Minnesota Medical Center, from July 1, 2006, through December 30, 2007. The study was approved by the University of Minnesota Institutional Review Board for Research on Human Subjects and conducted according to the Declaration of Helsinki. All patients provided written informed consent. Our exclusion criteria for the LVAD study recipients were a history of renal failure requiring dialysis, a requirement for ventilatory support, hepatic dysfunction resulting in coagulopathy, and biventricular failure requiring biventricular assist device support. We also studied a control group of 7 patients undergoing non-LVAD cardiac surgery.

In the LVAD recipients, we measured all markers as follows: on day 0 (preoperative or baseline levels) and on postoperative days 1, 7, 30, 90, and 180; in the control patients, on day 0, and on postoperative days 1, 7, and 30.

Treatment

Anesthetic and surgical care was given per our institutional protocols. Monitoring included standard modalities (electrocardiogram, temperature, invasive blood pressure, pulse oximetry, and gas monitoring) plus central venous pressure or pulmonary artery catheter monitoring and transesophageal echocardiography. Apoptinin was used for repeat sternotomy procedures; ε-aminocaproic acid, for first-time sternotomy procedures. Anticoagulation for non-LVAD cardiac surgery and LVAD placement consisted of 400 U/kg unfractionated porcine heparin. Standard techniques were used both for LVAD placement and for non-LVAD cardiac surgery. At the conclusion of cardiopulmonary bypass, anticoagulation was reversed with protamine. All 28 study patients were monitored with continuous telemetry until their discharge from the hospital.
Left Ventricular Assist Devices

HEARTMATE II LEFT VENTRICULAR ASSIST DEVICE. The HeartMate II (Thoratec Corp, Pleasanton, CA) consists of an internal blood pump with a percutaneous lead that connects the pump to an external system driver and power source. The pump has an implant volume of 63 mL and generates up to 10 L/min of flow at a mean pressure of 100 mm Hg. The inflow cannula is connected to the left ventricular apex; the outflow graft is connected to the ascending aorta. The pump has a rotor that is mobilized by an electromotive force generated by the motor. Pump output depends on the speed of the rotor and on the difference in pressures between the inflow and outflow cannulas.

VENTRASSIST LEFT VENTRICULAR ASSIST DEVICE. The VentrAssist (Ventracor, Sydney, Australia) is a third-generation centrifugal pump with hydrodynamic bearings and an electromagnetically driven impeller. The pump is treated with a diamond-like carbon coating on blood-contacting surfaces to enhance neointimalization. The pump is small and measures 67 mm in diameter and 298 grams; it can provide flows from 2 to 10 L/min with average pressure from 50 to 160 mm Hg. Similar to the HeartMate II, the inflow cannula is connected to the left ventricular apex; the outflow graft is connected to the ascending aorta.

Device Management

Per our local practice at the University of Minnesota, we usually adjust the fixed-rate speed of the continuous-flow LVADs (both HeartMate II and VentrAssist) to maximize left ventricular decompression and to improve cardiac output, simultaneously allowing for at least a 1:3 ratio of

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**Table 1. Patient Demographics**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total</th>
<th>HM II</th>
<th>VentrAssist</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>21</td>
<td>11</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Age (y)</td>
<td>55.9 ± 13.5</td>
<td>56.2 ± 14.9</td>
<td>55.6 ± 12.7</td>
<td>0.92</td>
</tr>
<tr>
<td>Sex</td>
<td>Males: 81% (17/21)</td>
<td>Males: 82% (9/11)</td>
<td>Males: 80% (8/10)</td>
<td>0.31</td>
</tr>
<tr>
<td>Diagnosis</td>
<td>Ischemic cardiomyopathy (86%)</td>
<td>100%</td>
<td>70%</td>
<td>0.05</td>
</tr>
<tr>
<td>Baseline EF</td>
<td>0.206 ± 0.0883</td>
<td>0.227 ± 0.109</td>
<td>0.186 ± 0.060</td>
<td>0.34</td>
</tr>
<tr>
<td>Baseline creatinine (mg/dL)</td>
<td>1.46 ± 0.57</td>
<td>1.5 ± 0.6</td>
<td>1.4 ± 0.6</td>
<td>0.62</td>
</tr>
</tbody>
</table>

EF = ejection fraction; HM = HeartMate.
aortic valve opening. We optimize the revolutions per minute speed, both hemodynamically and echocardiographically, at the time of LVAD placement, before the patient is discharged from the hospital (ie, after admission for LVAD placement), and if clinical events (eg, new symptoms or suction events) warrant further adjustment.

Anticoagulation involved a combination of warfarin and aspirin for the continuous-flow groups. After LVAD placement, we did not change defibrillator and biventricular pacing settings. All patients underwent a standard postoperative rehabilitation program.

**Blood Sampling and Biochemical Assays**

Blood samples were drawn from patients on day 0 (baseline or preoperative levels) and on postoperative days 1, 7, 30, 90, and 180. After discarding the initial blood obtained from venipuncture, venous blood was collected in Vacutainer tubes (BD Vacutainer Systems, Franklin Lakes, NJ) containing EDTA (for endothelial cell analysis) and sodium citrate (for serum markers). We processed the blood samples immediately for study.

For endothelial studies, we used immunohistochemical examination of buffy-coat smears to enumerate circulating endothelial cells, and evaluated the surface phenotype by applying immunofluorescence microscopy to preparations of circulating endothelial cells. The panel of antibodies used included a specific anti–endothelial cell antibody (detecting CD-148), P1H12, polyclonal blocking antibody to TF (Fig 1; provided by Dr. Ron Bach, Veterans Affairs Administration Medical Center, Minneapolis, MN), fluorochrome-labeled murine monoclonal antibodies against intercellular adhesion molecule 1 (ICAM-1) or vascular-cell adhesion molecule 1 (VCAM-1; South Biotechnology, Birmingham, AL); and murine monoclonal antibodies against E-selectin (Genzyme, Cambridge, MA) We used secondary antibodies as required: goat antimouse immunoglobulin conjugated to Lissamine rhodamine (Jackson IRL, Westgrove, PA) or fluorescein isothiocyanate (Sigma Chemical Co, St. Louis,
rhodamine-conjugated goat anti-rabbit immunoglobulin (Jackson IRL); and alkaline phosphatase–conjugated antimouse immunoglobulin (Sigma or Chemicon International, Temecula, CA).

The serum markers measured included prothrombin fragment 1.2 monoclonal antibody (Behring kit); von Willebrand factor (BCS VWA LIA ASSAY; using STAGO KIT:LIATEST); D-dimers, (Diagnostica Stago STA-R); and thrombin/antithrombin III complexes (enzyme-linked immunosorbent assay test). These four tests were performed at the clinical laboratory of the University of Minnesota Medical Center, Fairview, MN, according to standard methods.

The measured study markers in relation to the coagulation cascade have been depicted in Figure 2.

### Statistical Analysis

The changes in the biomarker levels with time were studied by using a linear mixed model with random
effects. The advantage of the mixed model over standard analysis of variance is to take into account the correlations between repeated measurements within the same subject and estimate the effects of covariates (cases versus controls, device) using all available data. Linear mixed model analysis was carried out by using PROC MIXED procedures of SAS software V8.0 (SAS Institute, Inc, Cary, NC). Linear, quadratic, and cubic curves were fitted to the biomarker levels to model the changes with time. F tests were used to determine the specific parametric forms (horizontal line indicating constant with time, linear line with slope not equal to zero, and quadratic or cubic curves) as well as the significance of covariate effects (cases versus control and device difference among cases). Statistical tests with probability values less than 0.05 were considered significant.

Paired Student’s t test was used to compare postoperative levels in cases with their baseline (preoperative) levels. Because there were five postoperative measurements (days 1, 7, 30, 90, 180), five paired Student’s t tests were conducted for each marker. Bonferroni procedure was used to adjust for multiple hypothesis testing. Specifically, a paired Student’s t test was considered significant only if its probability value was less than 0.05/5, or 0.01.

Results

Patient Characteristics

Of our 21 LVAD recipients, 11 received the HeartMate II, and 10 received the VentrAssist. Of our 7 control patients, 5 underwent coronary artery bypass grafting, and 2, valve replacement. The mean age of the LVAD recipients was 57 ± 3 years; control patients, 68 ± 2 years (p = 0.01). The percentage of males among the LVAD group was 82% (18 of 22); control group, 85.8% (6 of 7; not significant). Baseline demographic data as well as hematologic data for LVAD recipients for each of the two device types are presented in Table 1 and Figure 3.

Circulating Endothelial Cells and Endothelial Activation

We saw no significant differences in the number of circulating endothelial cells in either our LVAD recipients or our control patients, as compared with the normal range (Fig 4; Tables 2 and 3). However, markers of endothelial activation (including VCAM-1, ICAM, E-selectin, and TF) were all significantly higher at baseline in our LVAD recipients, as compared with our normal range and in our control patients. All these markers further peaked on postoperative day 7 and remained significantly elevated until postoperative day 180. Fur-
there, there appeared to be a secondary rise in E-selectin and TF in LVAD recipients ($p < 0.05$), suggesting a biphasic response (Fig 5; Tables 2 and 3). All endothelial dysfunction markers were significantly higher in LVAD recipients than in control patients at each time point ($p < 0.05$). In our control patients, all markers except E-selectin returned to the normal range by postoperative day 30 (Fig 5).

**Serum Markers**

Serum markers (including von Willebrand antigen, prothrombin fragment 1.2, D-dimers, and thrombin/antithrombin III) were all significantly higher at baseline in our LVAD recipients, as compared with the normal range and our control patients ($p < 0.05$). All these markers peaked on postoperative day 7 (except thrombin/antithrombin III, which peaked on postoperative day 1) and remained significantly elevated until postoperative day 90 (Fig 6; Tables 2 and 3). Similarly, in our control patients, all these markers peaked between postoperative days 1 and 7; however, unlike in our LVAD recipients, all these markers returned to the normal range by postoperative day 30 (Fig 6).

**Comment**

The discrepancy between the limited availability of donor organs and the ever-increasing number of patients with heart failure has led to the increasing use of LVADs [1]. The excellent medium-term results with LVADs have led to the use of permanent LVAD placement for patients with end-stage heart failure [2]. Nevertheless, the clinical success of LVADs has been accompanied by significant complications, including thromboembolic and hemorrhagic events in as many as 30% of recipients.

The development of novel materials used for implant surgery and the increasing use of implanted devices has made it evident that no material is biologically inert. Commonly used biomaterials, so-called inert compounds such as titanium, polytetrafluoroethylene, and acrylics, may trigger an array of iatrogenic effects, including inflammation, fibrosis, coagulation, and infection.

In the case of LVADs, in which the biomaterial is in direct contact with the blood circulation, significant changes in systemic immunologic and thrombotic functions have been well documented. Like most other implanted devices, LVADs activate the coagulation system, resulting in device-related thrombus [7, 14]. The major reasons for this are (1) the contact between blood components and the foreign surfaces of the LVADs and (2) the altered rheologic conditions with different velocities of blood flow and blood stasis in the LVAD recipient heart. Spanier and associates [15] described a phenomenon of a “compensated coagulopathy” underlying the apparent autoanticoagulation in textured-surface HeartMate XVE recipients, attributing this finding to procoagulant stimuli elicited from the LVAD cell-surface environment. Some investigators suggested that such activation of anticoagulation was attributable largely to the continuous contact of blood with the foreign LVAD surface; however, others showed that specific cells that progressively adhere to the textured LVAD surface and become activated may also contribute to the coagulopathy [16, 17].

Our study demonstrated significant activation of both the endothelial and the coagulation systems in patients with end-stage heart failure requiring LVAD placement. In addition, the findings from our current study extend others’ previous observations of significant activation of the procoagulant as well as fibrinolytic pathways in LVAD recipients. We clearly demonstrated that these serum procoagulant and fibrinolytic markers returned to baseline levels by postoperative days 30 to 90. In contrast to the coagulation system, we found persistent activation of the endothelial system up to postoperative day 180.

Hebbel and colleagues [18] found activation of the vascular endothelium in patients with sickle cell disease. They also showed that an increased number of circulating endothelial cells expressed TF in sickle cell patients; this expression was further increased during vasoocclusive episodes. The TF expressed on the antigen-positive circulating endothelial cell is functional, as demonstrated by a binding assay for factor VIIa and a chromogenic assay sensitive to generation of factor Xa [19, 20]. Before this latter study, the role of the vascular endothelium in activating the coagulation system was uncertain because there is little evidence indicating that endothelial cells in vivo express TF, the system’s triggering mechanism. By establishing that endothelial cells in vivo can express TF, the study demonstrated that the vast endothelial surface can provide an important pathophysiologic trigger for coagulation activation. Until now, studies of the hemostatic alterations associated with LVADs showed elevated thrombin generation in the early postoperative period, with increased thrombin activity and fibrinolysis throughout the course of LVAD support. Those abnormalities were attributed to plasma protein adsorption and intrinsic pathway activation. Our current study showed a late secondary rise in TF, demonstrating for the first time that activation of the extrinsic (TF) pathway of thrombosis mediated by sustained endothelial dysfunction in LVAD recipients may be equally responsible for the coagulation abnormalities. Wilhelm and coworkers [21] previously suggested a role for complement in mediating formation of leukocyte-platelet aggregates, thereby indirectly contributing to thrombin generation through monocyte TF expression.

Endothelial cell activation leads to increased expression of inflammatory cytokines and adhesion molecules that trigger leukocyte homing, adhesion, and migration into the subendothelial space, processes fundamental to cardiovascular disease in general. Well-characterized molecules that can be measured in the circulation with commercial microarrays include E-selectin, VCAM, ICAM, and P-selectin. Similarly, the procoagulant consequences of endothelial activation can be measured and include tissue plasminogen activator and von Willebrand factor. Circulating endothelial cells that detach in the context of endothelial activation and loss of integrity can be measured in the circulation by flow cytometry. Cur-
rent evidence suggests that endothelial function is an integrative marker of the net effects of damage on the cardiovascular system. Importantly, strategies to reverse endothelial function have now been examined in a wide range of patients with cardiovascular disease. Benefit has been shown with a number of pharmacologic interventions, which include drugs that lower lipids and blood pressure, as well as with novel therapies based on new understanding of endothelial biology. These have mostly shown that recovery of endothelial function occurs in response to strategies known to reduce cardiovascular events [22–26]. More recently, it has been shown that clopidogrel may improve endothelial dysfunction in patients with coronary artery disease independent of adverse events after LVAD placement and thereby improve long-term outcomes. An improved understanding of endothelial biology. These have mostly shown that recovery of endothelial function occurs in response to strategies known to reduce cardiovascular events [22–26]. More recently, it has been shown that clopidogrel may improve endothelial dysfunction in patients with coronary artery disease independent of adverse events after LVAD placement and thereby improve long-term outcomes. An improved understanding may even allow potential therapeutic interventions in a timely fashion so as to decrease the incidence of bleeding and thromboembolic complications in high-risk patients.

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References


Notice From the American Board of Thoracic Surgery

The 2009 Part I (written) examination will be held on Monday, November 30, 2009. It is planned that the examination will be given at multiple sites throughout the United States using an electronic format. The closing date for registration was August 1, 2009. Those wishing to be considered for examination must apply online at www.abts.org.

To be admissible to the Part II (oral) examination, a candidate must have successfully completed the Part I (written) examination.

A candidate applying for admission to the certifying examination must fulfill all the requirements of the Board in force at the time the application is received.

Please address all communications to the American Board of Thoracic Surgery, 6333 N St. Clair St, Suite 2320, Chicago, IL 60611; telephone: (312) 202-5900; fax: (312) 202-5960; e-mail: info@abts.org.