Evaluation of Bioprosthetic Valve-Associated Thrombus in Ventricular Assist Device Patients

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Background. Thromboembolic events may be related to thrombotic deposition on prosthetic valves. In a left ventricular assist device (LVAD) that contains two porcine pericardial bioprosthetic valves in addition to significant associated biomaterial placement, this may be particularly true. Thrombotic deposits on valves removed from LVADs at autopsy or heart transplantation were scored to determine (1) the nature and location of valvular deposition, (2) whether deposition was related to thromboembolic events, (3) correlations between deposition and patient hemodynamic and coagulation parameters, and (4) implant time dependency.

Methods and Results. Novacor LVADs were implanted in 23 patients as a bridge to transplantation for 1 to 303 days. Photographs of the concave (downstream) and convex (upstream) side of the inflow and outflow valve were made at explant and later scored for (1) total thrombus area (10=equivalent of cusp area), (2) percent of cusp area occupied by solid thrombus, (3) thrombus color (10=dark red, 0=white), and (4) average percent of valve strut height involved with thrombus (from a side view). The inflow valve was shown to have heavier and redder deposition than the outflow valve. This was also true for the concave versus the convex side. Heaviest deposition was seen on the inflow valve concave side, which rests within the LVAD pumping sac and may be subject to poor convection. Patients with neurological thromboembolic events (8/23) during implantation had heavier deposition on the inflow valve concave side (5.7±2.7 versus 4.6±2.2, P<.05). Pump volumetric output was also found to negatively correlate with thrombus area on this valve and side (r=-.61, P=.002). Platelet release (platelet factor 4) was correlated with thrombus involvement on the upstream (convex) side of the inflow valve (r=.82, P=.002). No significant dependence of deposition on the implant time was found.

Conclusions. Valve thrombus deposition was related to thromboembolic events. Pump volumetric output and platelet release were found to be related to deposition. These results may have implications for the role of hemodynamics and platelet activation in thromboembolism associated with prosthetic valve placement in general. (Circulation. 1993;88[part 1]:2023-2029.)

Key Words • thromboembolism • left ventricular assist device • bioprosthesis • Novacor

The placement of prosthetic or bioprosthetic valves in humans is associated with a finite risk of thromboembolism. In several left ventricular assist device (LVAD) designs, two valves are located between the pumping sac and the inflow and outflow conduits, respectively. Since the biomaterial surfaces of the conduits and pumping sac may impart a thromboembolic risk or platelet activation potential of their own, and since LVAD patients have been shown to have elevated indices of coagulation and platelet activation, it seems reasonable to expect that the valves in these patients would be subject to increased deposition and embolization. Thromboembolism has indeed been a major complication faced over the course of LVAD implantation, and the valves associated with these devices are considered likely sources for the generation of emboli. Reducing the incidence of such thromboembolism in LVAD patients has been the motivation for many alterations in device design and anticoagulation protocols. At the University of Pittsburgh, 23 patients received the Novacor LVAD (Novacor Division, Baxter Healthcare Corp) as a bridge to transplantation between March 1988 and August 1991. The valves used in this LVAD are 21-mm modified Carpentier Edwards bioprosthetic pericardial valves (American Edwards Laboratories, Irvine, Calif). At the time of device explant, the inflow and outflow valves were retrieved and photographed from side, upstream (convex side of valve), and downstream (concave side of valve) perspectives. An analysis of valve morphology was carried out and correlated with clinical, hemodynamic, and coagulation data to address the following questions: (1) What is the nature and location of valvular deposits? (2) Is deposition related to thromboembolic events? (3) Does deposition correlate with hemodynamic and coagulation parameters? (4) Is there a temporal trend in valve thrombus deposition?

Methods

Clinical

A schematic demonstrating the flow geometry of the Novacor LVAD as well as the labeling conventions used for the valves in this study is seen in Fig 1. The concave
side of both valves is downstream, but for the inflow valve, this concave side rests within the pumping sac. The conduits leading to and from the pumping sac are made of Dacron fabric, which is preclotted with the patient's blood, cryoprecipitate, and thrombin immediately before implantation. The pumping sac is composed of Biomer (Ethicon Corp, Somerville, NJ). More extensive information regarding the design, placement, and postoperative management of the Novacor LVAD has previously been published.9-11

Valves evaluated for this study were obtained from 23 patients implanted with Novacor LVADs over a 4-year period commencing in March 1988. Of these 23 patients, 18 had successful transplants and were discharged. In 2 patients, one or both valves were changed over the course of implantation in an attempt to prevent further thromboembolic events. This occurred in the patient implanted for the longest period (303 days total implant; both valves changed at postoperative day 245) and a patient implanted for 72 days (inflow valve changed at 69 days). The median valve implant duration was 33 days (range, 1 to 245 days; inter-50% range, 7 to 86 days).

The anticoagulation routine followed in these patients varied. The percentages of patients receiving antithrombotic drugs alone or in combination were aspirin 39%; persantine 78.3%; coumarin 60.9%; and heparin 73.9%. Current protocol calls for postsurgical heparinization to a level capable of maintaining an activated partial thromboplastin time >48 seconds (1.5×upper normal). When oral drug administration is possible, coumarin, aspirin (80 mg/d), and persantine (225 mg/d) are initiated. Coumarin dosages are adjusted to yield a prothrombin time >19 seconds (1.5×upper normal). Once the prothrombin time reaches therapeutic levels, heparin is discontinued.

Eight patients suffered neurological events that were considered to be thromboembolic in nature while on the device. The majority of these events were transient, and 6 of these 8 patients received transplants and were discharged. An experimental protocol to examine plasma parameters for coagulation (prothrombin fragment F1.2, fibrinopeptide A, and thrombin-antithrombin complex) and platelet secretion (β-thromboglobulin and platelet factor 4) was initiated in time for the 10 last patients. Hemodynamic data, however, were collected for all but 1 patient and included arterial pressure, heart rate, pump rate, pump fill volume, pump residual volume, pump volumetric output, and the cardiac index.

Valve Retrieval and Scoring

At the time of heart transplantation or autopsy, the LVAD was explanted in an assembled state from the body. Saline was gently passed through the device to remove excess blood, and the conduits were unscrewed from the pumping sac to allow valve retrieval. The conduits and pumping sac were generally found to be free of deposition. The inflow and outflow valves were photographed individually against a uniform cloth background for convex (upstream) and concave (downstream) views. A side view, used for strut thrombotic involvement evaluation, was photographed while the valve was held by the support ring.

Slides made from the photographs were viewed and scored independently by four individuals who were blinded to the patient of origin but were made aware of whether the valve was from the inflow or outflow site. Each valve side was scored in three categories: thrombus area, percent of valve cusp area involved with thrombus, and red cell involvement or “redness.” Parameter scores from the four individuals were averaged.

As indicated in Fig 2, the thrombus area measurement was in arbitrary units, with a value of 10 units given to the area within the support ring for reference. It would thus be theoretically possible to have values >10 if the valve were completely occluded. Thrombus was also covered some of the support ring area. The measurement of percent cusp area involvement applied only to the deposition on the three porcine valve leaflets. This measurement was performed to separate the cusp deposition from support ring deposition, since different mechanisms might be at work on these surfaces. Red cell involvement was assessed on a scale from 0, indicating entirely white deposition, to 10, indicating uniformly dark red deposition. Darker red deposition may be indicative of lower shear deposition. Scorers were shown slides with examples of color reflecting 10, 5, and 0 on the arbitrary scale before scoring and could access these slides during the scoring procedure. A fourth measurement, the average percent of valve strut length involved with thrombus, was made from the side view of each valve. In these determinations, for each

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**Fig 1.** Schematic of Novacor LVAD. Valve side designations are shown in the context of blood flow through the device.

**Examples:**

<table>
<thead>
<tr>
<th>Thrombus Area:</th>
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<tbody>
<tr>
<td>Entire valve area (not ring) has a value of 10 units.</td>
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<tr>
<td>0</td>
</tr>
<tr>
<td>3</td>
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<tr>
<td>7</td>
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**Fig 2.** Diagram of thrombus area measurement technique. A value of 10 units was assigned to an area of deposition equivalent to that occupied by the three cusps from an overhead perspective. Scorers were asked to visually transform the area seen into a solid area in the cusp region and assign an area value.
valve the percent of the strut length containing deposition for each of the three struts was averaged.

For the two patients implanted with secondary valves, the scores for the secondary valves were used for the characterization of the location and nature of valve deposition as well as how deposition varied as a function of time implanted. The secondary valve scores were not used for the other correlations.

Statistical Methods

An intraclass correlation coefficient for a fixed set of raters was calculated to assess rater reliability for the parameters scored on each valve side, as was the $P$ value indicating whether the correlation coefficient was significantly different from zero. A two-factor repeated-measures ANOVA was performed to test mean value differences in scored parameters from one valve side to another (concave versus convex) and from one valve to another (inflow versus outflow). Interactions between factors were also investigated. Correlations between valve scores and patient hemodynamic or coagulation parameters averaged over the course of implantation were found with CSS:Statistica software (StatSoft, Tulsa, OK).

Results

Scores by Valve and Valve Side

The data are presented to compare the four valve sides of the LVAD in Fig 3. The results given in this figure demonstrate that the concave side of the inflow valve experiences the heaviest thrombus deposition in terms of thrombus area as well as deposition that is the darkest red. The convex side of the inflow valve has the highest percentage of cusp area involved with thrombus. The average percent of the inflow valve strut length involved with thrombus (37±36%) was not significantly different from the outflow valve (46±28%). Examples of light and heavy deposition in terms of thrombus area are seen in Fig 4 for each valve side.

Comparisons by valve or valve side were performed for the four variables scored. (No statistical interactions were found between the scored parameters.) Deposition in terms of thrombus area was heavier on the concave (downstream) side of the valves and preferentially on the inflow valve as opposed to the outflow ($P<.01$). The red cell involvement also followed this trend, with the concave valve side and inflow valve being a darker red ($P<.01$). The percent of cusp area thrombus involvement was higher on the inflow versus the
outflow valve and lower on the concave valve side \((P<.01)\). The outflow valve had a greater average percent strut length involved with thrombus than the inflow valve, but this was not found to be significant \((P>0.05)\).

As a test of the scoring system, the thrombus area on the concave side of the inflow valve was plotted against the average percent of the inflow strut length involved with thrombus. One would anticipate that these variables might be related: an increased involvement on the strut would be seen from the direct photograph of the concave side of the valve and enter into the thrombus area determination, along with any thrombus an involved strut might be connected to on the valve support ring. Fig 5 shows that such a relation does exist between these variables and serves as a qualitative check of the scoring system.

The intraclass correlation for the parameters scored was found to be highly significant in all cases \((P<0.001)\), indicating good agreement between individual scorers. Correlation estimates were >0.8 for the majority of parameters and valve sides scored. A notable exception occurred with scores for the outflow valve convex side, which generally had lower deposition and smaller correlation estimates (range, 0.3 to 0.6).

To look for temporal trends in valve deposition, the dependence of the thrombus area score on the duration of device implant is seen in Fig 6 for the concave side of the inflow valve. This valve and valve side were chosen because thrombotic deposition is heaviest here. The highest deposition levels tended to be seen on valves removed around 50 days after surgery. Coincidentally, among those patients experiencing thromboembolic events, the average time after surgery until the first event was 38±37 days. Further, although no simple mathematical relations could be demonstrated for the entire data set, if one excludes those valves removed before approximately 30 days, thrombus area seems to be decreasing with increasing length of implantation. These data, however, may be biased by the early explant of valves or devices in patients suffering thromboembolic events. The inflow valves removed under such conditions were after implantation periods of 44, 58, 69, and 245 days.

**Grouping by Thromboembolic Event**

Dividing the patient valve score database into thromboembolic event and nonevent groups and comparing scores between these groups showed two interesting differences to be significant. First, patients experiencing thromboembolic episodes had significantly larger areas of thrombus on the concave side of their inflow valve: 5.7±2.7 for event patients and 4.6±2.2 for nonevent patients \((P<0.05\), two-sided Student's \(t\) test). Recall that this valve and valve side had the heaviest deposition in all patients, as was seen in Fig 3A. Second, the average percent of strut length involved with thrombus on the outflow valve was higher for event than nonevent patients: 59±27% versus 27±25% \((P<0.01)\). The two patients with thromboembolic events who had their valves changed had inflow concave thrombus deposition areas of 4.6 and 3.7, both below the average for event patients.

**Thrombosis and Hemodynamic Parameters**

Patient valve scores were next correlated with the average implantation values for platelet release and coagulation parameters. Fig 7 shows the relationship found to exist between platelet factor 4 (PF4) and the cusp area thrombus involvement on the convex side of
the inflow valve. This valve side is the first valvular surface seen by platelets as they enter the LVAD pumping sac and is immediately downstream from the Dacron inflow graft, which has been shown at explant to have significant surface-associated thrombin activity. Platelet secretion was not found to be significantly related to the heavy thrombus deposition on the concave side of the inflow valve. Thrombin generation measured in terms of the F1.2 prothrombin fragment was correlated in a manner similar to PF4, with the involvement on the convex side of the inflow valve (r=0.90, P=.04), but only five LVAD patients had F1.2 levels measured. Generally, it was not possible to demonstrate a significant relation between thrombin generation and valve thrombus deposition levels.

Average patient hemodynamic parameters over the course of LVAD implantation were also correlated with inflow valve deposition, since fluid dynamics is known to be a factor in thrombogenesis. A relation was found between pump volumetric output and thrombus area on the concave side of the inflow valve, seen in Fig 8. Lower average volumetric flow rates corresponded to heavier deposition on the concave (pumping sac) side of the inflow valve. Interestingly, in comparing hemodynamic parameters for thromboembolic event and nonevent patient groups, the volumetric pump rate was significantly lower in event than nonevent patients (99±16 versus 105±11, P<.01). Other hemodynamic parameters were not found to be correlated with deposition on the inflow valve.

**Discussion**

Thromboembolism, manifested as transient ischemic attacks and cerebrovascular accidents, is associated with LVAD implantation. With the present increase in the waiting period for heart transplantation and plans for chronic support of transplant-ineligible populations, the need to control LVAD-associated thrombogenesis in devices experiencing this problem will become increasingly critical. Determining the mechanisms of thromboembolism in LVAD recipients will provide a foundation for attempts at controlling this process.

It was observed at device explantation that the bioprosthetic valves of the Novacor LVAD have thrombotic deposition to various degrees but generally in excess of that associated with valve placement alone in the cardiac position. (Implantation periods for valves in the cardiac position, however, are on average much longer than for the LVAD.) The general notion has been that heavy valvular deposition would be associated with an increased risk for thromboembolism in that

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**Fig 5.** Graph shows that the thrombus area on the inflow valve concave side is related to the average percent involvement of the inflow struts with thrombus. This correlation was performed as a qualitative check of the scoring system.

**Fig 6.** Graph showing thrombus area on the concave side of the inflow valve plotted against the number of days after implantation for that valve. Two of the 23 patients had their inflow valves replaced. Both valves for these patients were included in this plot.

**Fig 7.** Graph shows that platelet release as determined by patient platelet factor 4 (PF4) plasma concentrations averaged over the course of implantation is related to the percent of the inflow valve cusp area covered with thrombus on the upstream (convex) side. This is the first valve surface seen by the platelets on entry into the LVAD.
Fig 8. Graph shows that the average volumetric output of the LVAD during implantation is related to the area of thrombotic deposition on the concave side of the inflow valve (the site of heaviest deposition).

et al.6 noted an increase in thrombolytic parameters in the course of total artificial heart implantation. The possibility of a fluctuating deposition dependent on the thrombotic/thrombolytic balance was considered. In this scenario, a peak in deposition would be expected at some point during the implantation followed by thrombolytic degradation to a lower, more stable level. The data in Fig 6 may support this model. A peak does appear to occur, but the data are insufficient to warrant any firm conclusions. It was also noted that the average time until a patient’s first thromboembolic episode was $38 \pm 37$ days, which is approximately the time at which a peak occurs in the data. Here again, a relation is entirely speculative because of the scatter in the data.

The valves that were removed from two neurological event patients (at postoperative days 69 and 245) had below-average thrombotic deposition on the concave side of the inflow valve. This may have been a result of the extended time these valves were implanted. Given that substantial thrombotic deposition could occur within 2 days of implantation and given the risks associated with valve replacement, this procedure is not considered worthwhile at this time.

Of prime interest was the difference in thrombotic deposition on the concave side of the inflow valve for thromboembolic event and nonevent patient groups. This result seems to indicate that the relatively heavy deposition seen on this valve might either be the source for thromboemboli or serve as an indicator of hyperaggregable platelets that deposit on this surface as well as forming emboli in the fluid phase. Deposition on the struts of the outflow valve was also significantly higher in the thromboembolic event patient group. These struts are the last valve surface seen by the blood before it exits from the device toward the vasculature. Thromboemboli departing from this surface may not be subject to aggregate breakup or readhesion, as those emboli generated farther upstream in the device would be.

The rate of thromboembolic complications seen in this patient group is comparable to that seen for other ventricular assist devices.17-19 Recently, a substantially lower incidence of thromboembolic complications has been reported with one particular device.20 In terms of overall success in bridging patients to heart transplantation, however, the Novacor LVAD bridged 78% of the patients in this study successfully to heart transplantation and hospital discharge.

Correlation of thrombotic deposition for each patient with coagulation and hemodynamic parameters measured and averaged over the course of implantation identified potentially important relations. An earlier study by the authors examined the relation between hematologic parameters and thromboembolic events in this same patient group.6 The platelet secretion product PF4 was found to be significantly higher in thromboembolic event patients compared with nonevent patients. In the present study, PF4 was found to be positively correlated with the percent of cusp thrombotic coverage on the inflow valve. This is the first valvular surface seen by platelets entering the device. Prefracted platelets in the circulation might be more likely to deposit when they impinge on this surface. Activation of these platelets may occur proximally on the Dacron conduit leading to this valve. Dacron graft implantation alone is associated with chronically high levels of PF4 and
\(\beta\)-thromboglobulin. Additionally, the authors have demonstrated a high thrombin activity on this conduit surface at device explant. (Thrombin is a potent activator of platelets.)

Hemodynamics were also implicated in valvular deposition. The volumetric pump output was negatively correlated with thrombogenic deposition on the concave side of the inflow valve (Fig 7). Lower flow through the pump might lead to reduced convection and longer residence times within the pumping sac. Contact time and platelet agonist concentrations would be increased in this case. The correlation found supports this model. Further, data from the patient population demonstrate that pump output in thromboembolic event patients was lower than for their nonevent counterparts. LVAD hemodynamics are thus related to valvular deposition, which is also related to thromboembolic events. This result is encouraging, since hemodynamics may be relatively easy to control as opposed to the biochemical reactions on the biomaterial surfaces. In support of this concept, recent in vitro flow visualization studies in the present Novacor LVAD demonstrated the effect of modifying pumping parameters on fluid velocity fields in the valvular region. Animal experimentation with an earlier version of this device also qualitatively showed the importance of local flow in valve thrombogenic deposition. Increased control and awareness of the role of pumping parameters and device design from a flow profile perspective may lead to reduced thromboembolic risk in LVAD patients.

Summary

It has been shown that use of a retrospective scoring procedure for a number of characteristics of the porcine trileaflet valves associated with the Novacor LVAD can yield meaningful insights into the thrombogenic process associated with LVADs, despite a relatively limited patient population. The location of heaviest thrombogenic deposition was isolated within the device as the concave side of the inflow valve. Scoring results of the deposition color suggest that this deposition may be characteristic of that found in low-shear environments. Patients who experienced thromboembolic events were shown to have larger deposits on this valve side. Platelet activation was also related to deposition seen on a valve surface (inflow, convex side) experiencing high shear. Finally, hemodynamics, or more precisely the LVAD volumetric pump output, was found to be correlated with valvular deposition, confirming the notion that "good washing" within the pumping sac reduces thrombogenic deposition.

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References

Evaluation of bioprosthetic valve-associated thrombus in ventricular assist device patients.
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