Thrombogenicity of Mechanical Aortic Valves in an Animal Model: Site Specific Testing Is Crucial

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We evaluated a new trileaflet prosthesis and a control bileaflet prosthesis in the mitral and aortic positions in 27 calves. The prototype trileaflet valve (TV1) functioned satisfactorily in the mitral position ($TV1_m$, n = 7) but later yielded thrombogenic complications in the aortic position ($TV1_a$, n = 4). The valve was redesigned (TV2) and retested in the mitral ($TV2_m$, n = 4) and aortic ($TV2_a$, n = 5) positions, along with control valves (C_m, n = 4; C_a, n = 3). At necropsy, the valves were graded on a scale of 0 (no visible thrombi) to 4 (thrombi greater than 5 mm and/or obstructed leaflets). The $TV1_m$, $TV2_m$, and C_m animals, respectively, had implant durations of 215 ± 112, 140 ± 63, and 159 ± 89 days and thrombus grades of 0.71 ± 0.76, 0.33 ± 0.58, and 1.50 ± 0.58. The $TV1_a$, $TV2_a$, and C_a animals had implant durations of 18 ± 12, 159 ± 61, and 108 ± 62 days and thrombus grades of 2.75 ± 1.00, 0.50 ± 0.58, and 0.67 ± 0.58 ($p < .005$; $TV2_a$ *vs.* $TV1_a$). Three $TV1_a$ calves died early of valve related complications. A design irregularity, undetected in the mitral position but revealed in the aortic position, caused a high early mortality in the $TV1_a$ animals. Redesigning the prosthesis eliminated valve related mortality and significantly reduced the thrombus grade. Because satisfactory performance in the mitral position does not guarantee safety or efficacy in the aortic position, site specific preclinical testing is crucial for mechanical heart valves. *ASAIO Journal* 2004; 50:376–380.

Although modern mechanical heart valves (MHVs) are highly durable, they entail a lack of central flow across the valve annulus, resulting in heightened resistance to blood flow. This may cause high shear stress, turbulence, and local stagnation,[1] leading to activation of the coagulation or complement systems. Therefore, MHVs present a risk of thrombosis and require lifelong anticoagulation therapy.

One of our research goals has been to produce a prosthetic valve that combines the central flow characteristics and low thrombogenicity of a bioprosthesis with the durability of an MHV. *In vitro* computational fluid dynamics testing of a newly designed MHV with three leaflets (Trileaflet Valve, Model TV1; Triflo Medical, Inc., Costa Mesa, CA) showed that the valve has minimal regurgitant flow, a minimal pressure decrease, and a natural flow passage without stagnation or separation. This valve has a large effective orifice area and favorable central flow characteristics. These features may allow the trileaflet valve to closely emulate the natural heart valve with regard to favorable hemodynamics and reduced risk of thrombogenesis.

New standards for preclinical testing of MHVs suggest that site specific evaluation is needed to assess performance characteristics such as thrombogenicity, wear, and hemodynamics in all clinically relevant anatomic valve positions.[2–4] For this reason, we tested the trileaflet valve in both the mitral and the aortic position in growing calves. We chose the bovine model because calves have been used in our research laboratory to assess mechanical circulatory support devices preclinically for more than 20 years and to evaluate and test MHVs for more than 10 years.[2,5–7]

Materials and Methods

The Valve

The trileaflet MHV has pyrolytic carbon leaflets that are aerodynamically shaped to minimize flow separation on their surfaces and to enhance early closure during the deceleration phase. The valve's design is expected to reduce impact loads, cavitation potential, blood trauma, and noise production at closure. A report concerning the preclinical evolution of the MHV is being published separately.

Preoperative Care

All of the calves received humane care in compliance with the Principles of Laboratory Animal Care, formulated by the National Society for Medical Research and the Guide for the Care and Use of Laboratory Animals (National Institutes of Health Publication No. 85–23, revised 1996). The calves were assigned to either the mitral or the aortic valve group. Preoperatively, they were given routine care and feeding until 24 hours before surgery, when food was withdrawn, and hair was removed from the surgical sites with a depilatory. On the morning of the operation, the animals were premedicated with glycopyrrolate (0.02 mg/kg subcutaneously) and xylazine (0.10 mg/kg intramuscularly). Approximately 30 minutes later, intravenous access was obtained via a triple lumen catheter in the right jugular vein. Ketamine (10 mg/kg intravenously [IV]) was administered to induce anesthesia. The calves were then intubated under direct vision with a cuffed endotracheal tube, and an orogastric tube was inserted for abdominal decompression. When surgical preparation was complete, the animals were transported to the operating room.

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Operative Procedure

Mitral Valve Implantation. General anesthesia was maintained with vaporized 0.5–3.0% isoflurane. Ventilatory support was continued with a volume cycled ventilator. Arterial blood gas analysis was performed periodically, and ventilatory adjustments were made to maintain blood gases within normal limits. For broad spectrum antibiotic coverage, cefonicid sodium (2 g IV) was given immediately before surgery. Pancuronium bromide (10 mg/kg IV) was given for muscle relaxation.

The calf was placed in the right lateral recumbent position. The left carotid artery and jugular vein were isolated and prepared for cardiopulmonary bypass cannulation. An incision was made over the left fifth rib, the muscles and pleura were incised, and the fifth and sixth ribs were removed. The internal thoracic artery was isolated and a pressure line inserted. Heparin (3 mg/kg) was administered, and an arterial cannula was inserted in the left carotid artery, followed by a venous cannula in the left internal jugular vein. Another venous cannula was inserted in the right atrial appendage and advanced into the inferior vena cava. The coronary sinus was cannulated for administration of retrograde cardioplegia and blood. Cardiopulmonary bypass was initiated, and the animal's body temperature was lowered to 32°C. Once the aorta was crossclamped, a Bangash needle was inserted into the aortic root. Antegrade cardioplegic solution was administered until the heart was completely arrested; then retrograde cardioplegia was begun. A left atriotomy was performed, and the atrial walls were retracted for exposure. The mitral apparatus was removed, and a 29 mm Triflo trileaflet valve or a 29 mm St. Jude Medical valve (St. Jude Medical, Inc., St. Paul, MN) was sewn into place with interrupted, pledgeted sutures.

Upon completion of the mitral valve replacement, the calf's body temperature was raised to 37.5°C. The atriotomy was then closed, the aortic crossclamp was removed, and the heart was de-aired. The heart was defibrillated at 50–100 J and, if necessary, was paced at 100 bpm. After hemodynamic stabilization occurred, the Bangash needle was removed, and the animal was weaned from cardiopulmonary bypass. The bypass cannulas were then removed, protamine was administered to reverse the effects of heparin, and the internal thoracic arterial line was transferred to the carotid artery. Epicardial echocardiography was conducted to verify proper valve function, a satisfactory valve gradient, and the absence of perivalvular leakage. A 40 Fr chest tube was inserted into the chest cavity for drainage. The ribs were approximated with steel wire, and the muscle and skin were closed in layers in the standard fashion. The neck incision was also closed in layers. Butorphanol (0.01–0.10 mg/kg intramuscularly) was given for initial postoperative analgesia. The animal was moved to the lift table and then to a stanchion for transfer to the intensive care unit.

Aortic Valve Implantation. Our technique of aortic valve implantation has been described elsewhere.[2] In this study, the procedures for general anesthesia, arterial blood gas analysis, and ventilatory support were the same as for mitral valve implantation. For broad-spectrum antibiotic coverage, cefazolin (2.0 g IV) was given immediately before surgery. Pancuronium bromide (9.5 mg IV) was given for muscle relaxation. The calf was placed in the right lateral recumbent position, and the previously described cardiopulmonary bypass procedure was followed. In this case, however, only the fifth rib was removed. After the aortic and venous cannulas were inserted in standard fashion, two concentric 2–0 polyester nonabsorbable pursestring sutures were placed in the descending aorta, and an additional aortic cannula was inserted into the descending aorta. Once cardiopulmonary bypass was initiated, the brachiocephalic artery was clamped, and the descending aorta was occluded above the cannula. Antegrade cardioplegia was then given to arrest the heart. An aortotomy was performed, and the aortic walls were retracted for exposure. A 21 mm Triflo trileaflet or St. Jude Medical valve (St. Jude Medical, Inc., St. Paul, MN) was sewn into place with pledgeted, interrupted 2–0 polyester nonabsorbable sutures or running 2–0 polypropylene sutures. The aortotomy was then closed, the aortic crossclamp was removed, and the heart was de-aired. Echocardiography was performed, and the rest of the procedure was the same as for mitral valve implantation.

Postoperative Management

Once the calf was in the intensive care unit, the chest drainage catheter was attached to an underwater suction container set at 20 cm of water vacuum. The calf was weaned from mechanical ventilation in accordance with its alertness and ability to breathe spontaneously. When swallowing and chewing were observed and arterial blood gases were within normal limits, the calf was extubated (mean extubation time, 1.5 hours postoperatively). It was then encouraged to stand as soon as possible. Vital signs, urine output, and chest tube drainage were monitored and recorded hourly. Intravenous fluids, blood products, and pharmacologic support were administered according to the animal's needs. Food and water were given freely. Anticoagulation treatment consisted of a continuous heparin infusion (50 mg/d) for the first week of the study.

Necropsy and Pathology

At the end of the study period, the surviving calves were euthanized, and a complete necropsy was performed. A midline incision was made from the mandible to the pubic symphysis. The heart was removed *en bloc* and was transected perpendicular to the long axis in 1 cm circumferential rings from the apex to the base.

The explanted valves were graded according to a semiquantitative scale ranging from 0–4: grade 0, no visible thrombi; grade 1, thrombi less than 5 mm; grade 2, thrombi greater than 5 mm and/or one leaflet obstructed; grade 3, thrombi greater than 5 mm and/or 2 leaflets obstructed; and grade 4, thrombi greater than 5 mm and/or 3 leaflets obstructed.

The original version of the trileaflet valve (TV1) (**Figure 1**, top) was initially placed in the mitral position and then in the aortic position. The redesigned version (TV2) **(Figure 1**, bottom) was retested in both the mitral and the aortic position, along with bileaflet control valves.

Statistical Analysis

The study's end-points were the duration of the implant in days and the thrombus grade. The results were expressed as the mean ± 1 standard deviation. Student's t-test was used to compare the variables, and $p < 0.05$ was considered significant.

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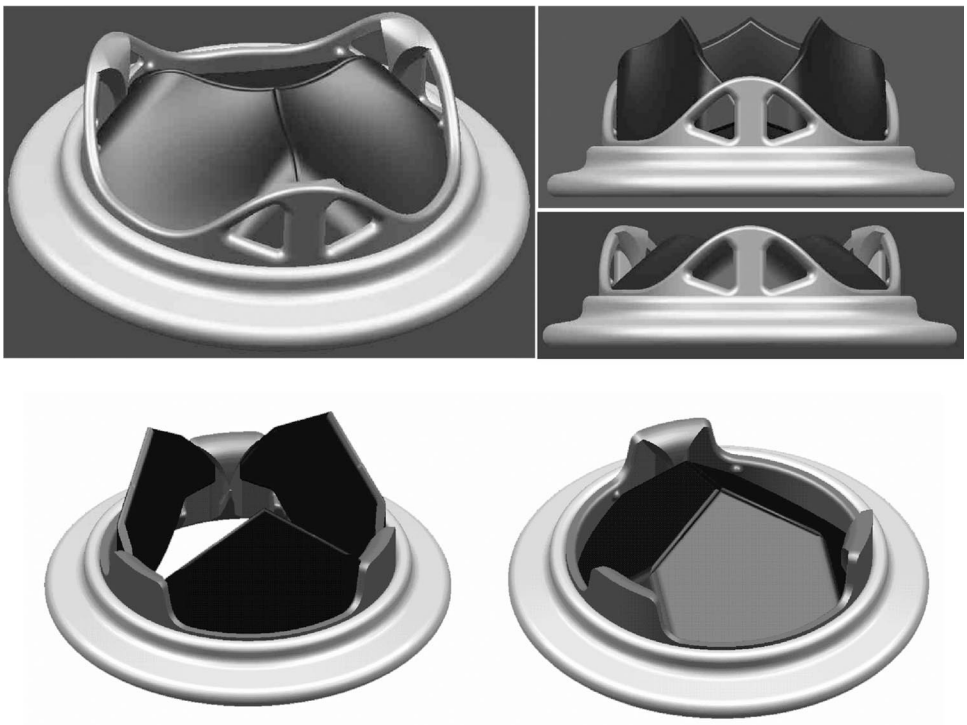


Figure 1. Top, the original valve (TV1). Bottom, the redesigned valve (TV2).

Results

This study used 27 Corriente crossbred calves that had a mean weight of 96 ± 4 kg and a mean age of 100 ± 25 days. The prototype valve (TV1) was initially tested in the mitral position ($TV1_m$) in 7 calves and functioned satisfactorily. Subsequent placement in the aortic position ($TV1_a$) in four calves yielded thrombogenic complications. The redesigned valve (TV2) was tested in the mitral position ($TV2_m$) in four calves and the aortic position ($TV2_a$) in five calves. Bileaflet control valves were tested in the mitral position (C_m) in four animals and the aortic position (C_a) in three animals.

Table 1 shows the implant durations and thrombus grades. The $TV1_m$, $TV2_m$, and C_m animals, respectively, had implant durations of 215 ± 112, 140 ± 63, and 159 ± 89 days and valve thrombus grades of 0.71 ± 0.76, 0.33 ± 0.58, and 1.50 ± 0.58. The $TV1_a$, $TV2_a$, and C_a animals, respectively, had implant durations of 18 ± 12, 159 ± 61, and 108 ± 62 days and valve thrombus grades of 2.75 ± 1.00 **(Figure 2)**, 0.50 ± 0.58 **(Figure 4)**, and 0.67 ± 0.58 **(Figure 3)**.

Before the end of the study, two of the three C_a calves were killed because they had shortness of breath and pulmonary edema, suggesting that the valve's effective orifice area was too small for the animal's size. At necropsy, these calves exhibited concentric left ventricular hypertrophy and patchy areas of transmural necrosis in the anterior septum. A report concerning these hemodynamic problems is being published separately. Three of the four $TV1_a$ animals died on postoperative days 11, 13, and 15, respectively, because of complications related to partial or full thrombosis of the prosthetic valve **(Figure 2)**. In the fourth calf, the valve was rotated by 60° at implantation. That animal survived for 33 days, and at necropsy, grade 2 thrombus was present, but the valve leaflets were fully moveable.

Because of high mortality in the first four aortic implants of the TV1, the valve was redesigned. The hinges of the new TV2 valve were left solid, without perforations (windows). This correction significantly reduced the thrombus grade in the $TV2_a$ *versus* the $TV1_a$ group ($p < 0.005$) and significantly

Table 1. Implant Durations and Thrombus Grades

	Mitral Position			Aortic Position		
Variable	$TV1_m$	$TV2_m$	C_m	$TV1_a$	$TV2_a$	C_a
Implant duration (d)	215 ± 112	140 ± 63	159 ± 89	18 ± 12	159 ± 61	108 ± 62
Valve thrombus grade*	0.71 ± 0.76	0.33 ± 0.58	1.50 ± 0.58	2.75 ± 1.00	0.50 ± 0.58	0.67 ± 0.58
n =	7	4	4	4	5	3

$TV1_a$, prototype valve, aortic; $TV2_a$, redesigned valve, aortic; C_a, control valve, aortic; $TV1_m$, prototype valve, mitral; $TV2_m$, redesigned valve, mitral; C_m, control valve, mitral.

* Grade 0, no visible thrombi; grade 1, thrombi <5 mm; grade 2, thrombi >5 mm and/or 1 leaflet obstructed; grade 3, thrombi >5 mm and/or 2 leaflets obstructed; grade 4, thrombi >5 mm and/or 3 leaflets obstructed.

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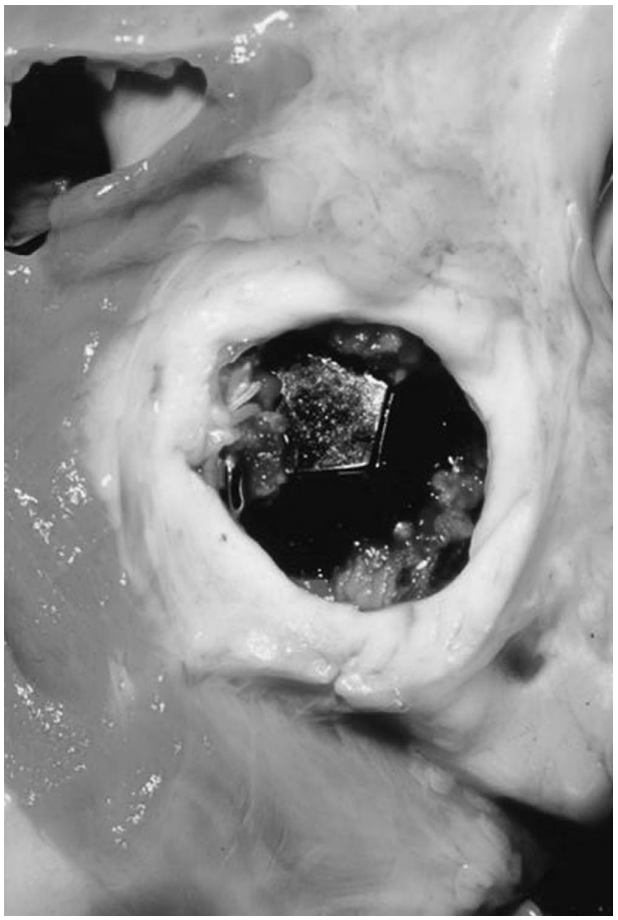


Figure 2. Severely thrombosed (grade 4) original valve (TV1) in the aortic position, as seen from the outflow side.



Figure 3. Minimally thrombosed (grade 1) control valve (St. Jude Medical) in the aortic position, as seen from the outflow side.

increased the survival time in the $TV2_a$ group. All of the animals that received the redesigned valve survived for the full term of the study.

Discussion

Our study showed that it is crucial to examine and validate new valve models in well designed *in vivo* animal studies even if *in vitro* methods such as computational fluid dynamics and digital particle image velocimetry show satisfactory valve function. Computerized valve studies[8–11] do not consider blood viscosity, coronary flow, blood coagulability, thrombogenicity, valve performance during arrhythmias, large hemodynamic changes, or turbulence behind various valve structures. All of these factors may affect valve performance.[11] Previous computational fluid dynamics testing had suggested that the design of our first (TV1) trileaflet valve might reduce thrombogenicity related to the presence of "windows" (openings) in the hinge areas; these windows were supposed to increase wash out over a critical surface and, hence, to reduce stagnation induced thrombosis. In the mitral position, this theory was persuasive owing to the presence of a larger orifice area, lower filling pressures, and lower blood flow velocities through the valve and the hinge windows. Indeed, our prototype trileaflet valve was quite successful in the mitral position *in vivo*, and it had a lower thrombus grade than the control valve (**Table 1**). Similarly, using the same trileaflet prototype in the calf mitral position, Sato *et al.*[12] reported excellent blood compatibility with the exception of one explanted trileaflet valve showing fibrinous tissue ingrowth in the valve "window." In the setting of aortic valve replacement, however, the hemodynamic conditions are quite different;[13–14] the aortic valve orifice area is smaller than that of the mitral valve, so higher velocity is produced with the same cardiac output through the valve prosthesis. This high velocity creates severe turbulence and flow separation behind small openings, such as the hinge windows in our prototype (TV1) prosthesis. In addition, there are more areas of stasis or counter flow behind the hinges of the aortic valve prosthesis that may compound the adverse flow conditions in the TV1 prototype valve. The hinge can directly affect valve durability and functionality.[15–18] Because of increased mortality after the first four aortic implants of this valve and because all of the thrombus seen in our cases originated behind the windows in the hinges, we concluded that the windows created these adverse hemodynamic effects. We retested the TV1 valve with more detailed computational fluid dynamics studies and, indeed, found flow separation and turbulence behind the hinge windows. We reevaluated the valve's design and modified it by leaving the hinges unperforated. Subsequently, when tested in both the mitral and aortic positions, using the St. Jude Medical valve as a control device **(Figure 3**), the redesigned valve (TV2) **(Figure 4**) performed satisfactorily, and all of its recipients survived to the end of the study.

These findings suggest that minor design irregularities may be tolerated in the mitral position but not in the aortic one. Therefore, our decision to test the valve in the orthotopic position before considering it for clinical use was crucial and definitely warranted. Without *in situ* orthotopic testing of MHVs, an inap-

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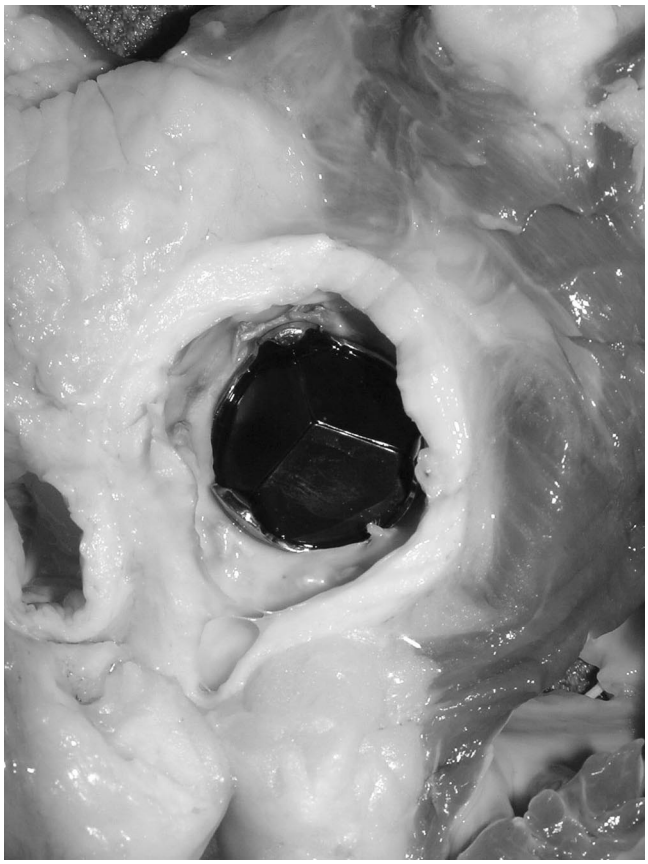


Figure 4. Thrombus free (grade 0) redesigned valve (TV2) in the aortic position, as seen from the outflow side.

propriate design could reach the clinical trial stage. However, it is equally important to use an animal model that presents a "worst case" scenario. The physiologic extremes encountered in the bovine model are ideal for preclinical valve testing, especially of mechanical aortic prostheses, under exaggerated hemodynamic conditions. Moreover, a large increase in body weight, combined with a high cardiac output and stroke volume, generates extreme transvalvular flow and pressure conditions that are ideal for testing MHVs. We prefer the bovine model because we also believe that it has the following advantages over the ovine model:[2,17] rapid growth, strong myocardial contractility, a high cardiac output, and a more extreme hemodynamic state.

Clotting abnormalities in sheep are a source of concern. Compared with human platelet functions, ovine ones are considerably attenuated in their response to biomaterials.[19] The coagulation system of sheep is less active than that of humans,[20] and the factor VIII level is significantly lower in sheep plasma than in human plasma.[21] In addition, during long-term mechanical support, sheep do not grow enough to increase their cardiac output and dP/dt, so device related hemodynamic changes are hard to assess.

Conclusion

Our study proves that a design problem can be detected during evaluation of an MHV in the aortic position after all *in vitro* and animal studies of the same valve in the mitral position had shown satisfactory performance. Strict *in situ* orthotopic valve testing mimics the clinical setting and allows potentially catastrophic complications to be corrected before the valve enters clinical trials. The growing calf, in our opinion, is a superior animal model that allows such testing to be performed under exaggerated physiologic conditions.

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