

Mechanical Valve Thrombosis in a Chronic Animal Model: Differences between Monoleaflet and Bileaflet Valves

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Background and aim of the study: The occurrence of mechanical valve thrombosis was studied in a chronic sheep model of valve implantation in the pulmonary position.

Methods: Six monoleaflet and six bileaflet mechanical valves were implanted in young sheep. Anticoagulation was stopped at one week postoperatively, after which weekly fluoroscopic evaluation was performed. Animals were sacrificed at the moment of any abnormality on the fluoroscopic image or, if no abnormality occurred, at fixed time intervals of two and three months. After explantation, valves were examined macroscopically, by stereomicroscopy, histology and scanning electron microscopy. Lung biopsies were taken.

Results: All monoleaflet valves remained functionally intact for up to three months without anticoagulation, but explantation after two or three months revealed thrombi attached to struts, cuspal surface

and valve ring. All bileaflet valves, except one, showed obvious dysfunction on fluoroscopy (one or both cusps fixed) within a time frame of three to eight weeks after implantation. Histology and scanning electron microscopy showed primary thrombotic material in one or both hinges, obstructing further cusp movement. Lung embolism was detected in only one animal among biopsy specimens.

Conclusion: The low-pressure environment of the pulmonary position caused primary thrombotic changes in all implanted mechanical valves. The thrombosis caused severe mechanical dysfunction only in the bileaflet valves, probably due to their delicate hinge mechanism. These findings may be used in the further development of an animal model of mechanical valve thrombosis.

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Improvements in both materials and design have led to better hemodynamic performance and mechanical durability of mechanical heart valves. Nonetheless, all mechanical valves still share a common disadvantage, namely the risk of valve thrombosis and associated thromboembolic complications. In the case of biological valves, new valve designs and materials are tested in a variety of animal models, ranging from subcutaneous rat models to valve implantations in larger animals. Some of these models have become widely accepted as a standard for preclinical valve testing. In mechanical valves however - where one of the main issues is thrombogenicity - the reliability of valve test-

ing towards valve thrombosis remains questionable (1).

Efforts have been made to study the occurrence and characteristics of mechanical valve thrombosis in different large animal models, but the development of a reliable and reproducible model of valve thrombosis is difficult. Short-term implantations in the mitral or aortic positions in sheep or pig models did not result in systematic valve thrombosis, even when all anticoagulation treatment was stopped (2-5). Long-term implants (>3 months) in these models revealed sporadic valvular dysfunction due to fibrous tissue overgrowth with secondary thrombotic changes, but no systematic primary valve thrombosis occurred (6-8). When anticoagulation was included in the protocols, fatal hemorrhagic complications complicated the study course (8).

From the clinical standpoint, it is known that right-sided mechanical valves carry a higher risk of thrombotic complications, due to the low-pressure environment (9). This has been suggested for the tri-

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cuspid position, and limited experience with mechanical valves in the pulmonary position seems to show an even higher thrombosis ratio (10).

The study aim was to investigate the possibility of designing a large animal model that produces primary mechanical valve thrombosis, within a relative short time. Such a model would enable the evaluation of different valve types (or new valve designs) with regard to their thrombogenicity. Thus, the behavior of clinically used mechanical valves (both monoleaflet and bileaflet) was studied in the pulmonary position in sheep. To the best of the present authors' knowledge, this is the first report of an experimental implantation of mechanical valves in the pulmonary position in sheep.

Materials and methods

Animals

Young adult sheep of bodyweight range 45-55 kg were selected. All animals were housed for a minimum of one week before the planned valve implantation. Preoperative assessment included a thorough physical examination, laboratory sampling (blood count, acid-base status, ionogram, coagulation profile), vaccinations and medication to establish eligibility for the study.

All sheep (n = 12) were cared for by a veterinarian in accordance with the *Guide for the Care and Use of Laboratory Animals* as published by the National Institute of Health. The study was approved by the Ethics Committee of the Katholieke Universiteit Leuven.

Mechanical valves

The mechanical valves used included six monoleaflet (Björk-Shiley tilting disc, size 19 mm (n = 4) and 21 mm (n = 2)), and six bileaflet (CarboMedics, size 19 mm (n = 4) and St. Jude Medical, size 21 mm (n = 2)).

Implantation

Before surgery, each animal received an intramuscular injection of antibiotics (ampicillin 800 mg; Albipen LA, Mycofarm, Belgium). Anesthesia was induced with ketamine 10-20 mg/kg intramuscular (Imalgene) and halothane. After endotracheal intubation and ventilatory support, general anesthesia was maintained with a combination of halothane (1.5-2%) and nitrous oxide, with further bolus doses of fentanyl as necessary. Both a peripheral venous line and a central venous catheter (jugular vein) were placed. A gastric tube was inserted for decompression, and electrocardiography leads were placed in position.

A left thoracotomy was performed, entering the left

pleural cavity through an incision at the second intercostal space. The pericardium was opened with a T-shaped incision and cradled with sutures. The phrenic nerve was always clearly seen and spared. The main pulmonary artery was dissected free from the underlying ascending aorta, in such a way that complete mobilization of the pulmonary was reached over at least 7-8 cm. The animal was systemically heparinized (heparin sodium, 250 IU/kg bodyweight; Rhone-Poulenc Rorer, Brussels, Belgium). An activated clotting time of more than 999 s was reached and maintained, in order to ensure adequate anticoagulation. An arterial cannula (22 Fr) was placed into the main pulmonary artery, immediately proximal to the pulmonary bifurcation, at a previously placed purse-string suture. A venous cannula was placed into the right atrium via the purse-string suture at the right atrial appendage. Both cannulae were connected to a pneumatic right ventricular assist device (Medos-HIA VAD, Medos, Aachen, Germany) with a 54-ml ventricle, primed with physiological saline. This circuit functioned only as a right-heart-bypass system: there was no need to arrest the heart, there was no cooling, and ventilation was continued in the normal manner. There was no requirement of blood transfusion during either the priming or the procedure.

When the assist device reached an adequate flow to bypass the right ventricle, the pulmonary artery was clamped proximally (immediately above the level of the native valve) and distally (immediately proximal to the cannula). The main pulmonary artery was opened using a longitudinal incision, after which a mechanical valve was implanted using continuous 4-0 Prolene sutures.

After removal of the clamps, the native pulmonary valve was destroyed by tearing two cusps with a clamp introduced through a purse-string suture placed at the level of the sinuses. This maneuver allowed the implanted valve to bear the entire load in the pulmonary position. Next, the Medos assist device was stopped and all cannulae were removed. Good valvular function was obtained in every implantation, as assessed by palpation and clearly audible valve clicking.

The chest was closed in layers, and a chest drain placed in the left pleural space. Immediately before extubation, the first X-radiographic assessment was performed and recorded, to ensure optimal valvular function (complete opening and closing of the valve) at the end of the procedure. On recovery from the anesthesia, the animal was extubated and transferred to the recovery room.

Postoperative care

The chest drain was removed at approximately 1-2 h

postoperatively. Feeding was allowed immediately. The animals received analgesics (piritramide, 10-20 mg i.m.; Dipidolor®, Janssen Pharmaceuticals, Beerse, Belgium), diuretics (furosemide, 20 mg i.m.; Lasix®, Hoechst Marion Roussel) and antibiotics (ampicillin, 300 mg/day i.m.) during the first two days after surgery. During the first postoperative week, low molecular-weight heparin (enoxaparin, 20 mg twice daily; Clexane®, Rhone-Poulenc Rorer) was administered by

subcutaneous injection. At the end of the first week, all medication and anticoagulation was stopped and the second X-radiographic assessment was performed. Afterwards, the animals were returned to the controlled animal facility, where their general health status was checked on a daily basis.

X-radiographic assessment

All implanted valves had radio-opaque leaflets,

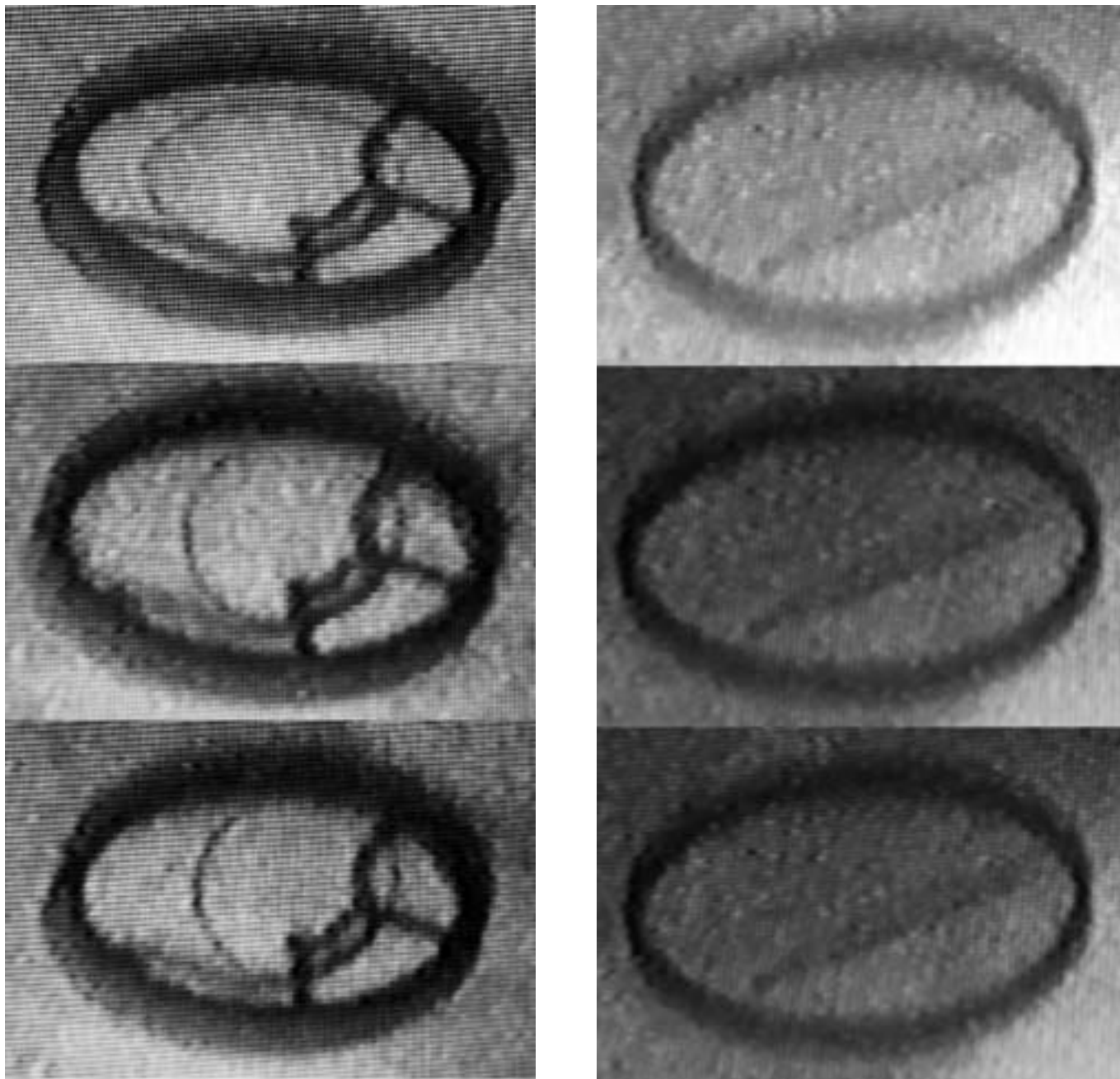


Figure 1: Representative images from the fluoroscopic valve evaluation. Still images were taken from the recorded X-radiograph video. The images show three consecutive photographs within a time frame of 1 s. A) Three consecutive images of a normal functioning monoleaflet valve. B) Three consecutive images of a bileaflet valve with obstruction of one cusp; the blocked cusp is always in the same position.

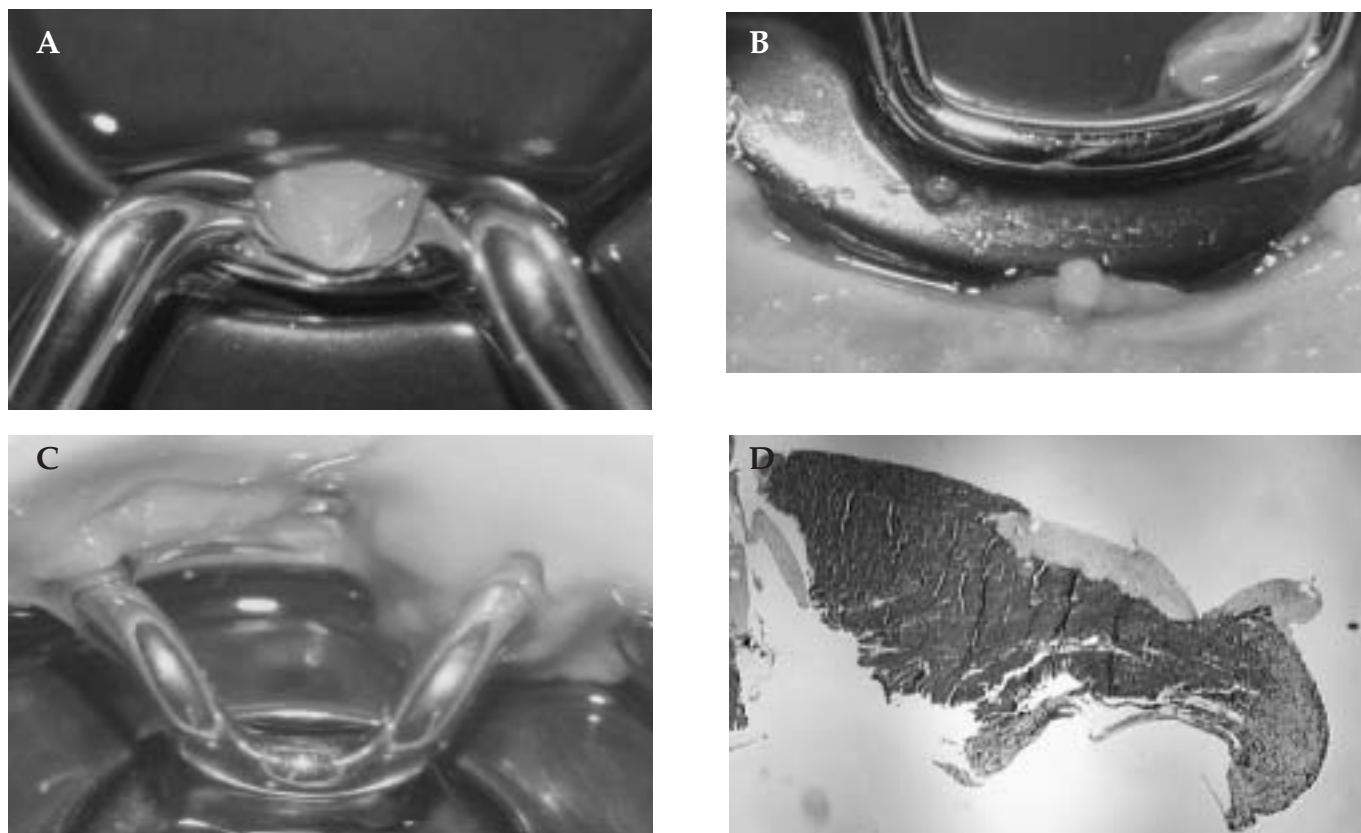


Figure 2: Representative stereomicroscopical images of three different explanted monoleaflet valves (Björk-Shiley). A) Small thrombus located at the outflow strut. B) Thrombus underneath the inflow strut. C) Thrombus, overgrown by fibrous tissue around the outflow strut. Macroscopically, the valve seems to be affected by fibrous tissue overgrowth, but the corresponding histology (D) revealed a central core of pure thrombus, with cellular ingrowth and thrombus organization.

which made it possible to evaluate valvular function by fluoroscopic X-radiographic assessment. Functional evaluation of mechanical valves using fluoroscopy has been described, both in experimental and clinical settings (11-13). Fluoroscopy was performed at the end of the operative procedure and at one week postoperatively, immediately before the animal was returned to the controlled animal facility. Anticoagulation was stopped after the first week. Subsequently, the animals underwent weekly fluoroscopic X-radiographic control to evaluate valvular function.

Plan of sacrifice

When the weekly fluoroscopic X-radiographic evaluation revealed an abnormality in valve function (absence of a normal opening and closing movement), the animal was sacrificed immediately. If no such abnormality was identified, it was planned to sacrifice the animals at two fixed time intervals, namely eight or 12 weeks after implantation.

Explantation

Sheep were premedicated and anesthetized as described above. The left thoracotomy was reopened

and, after heparinization, the valve was excised. The lungs were also excised, examined and sliced to check for major damage due to lung embolism. A total of 18 random lung biopsies was taken from each animal for further histological analysis.

Valve analysis

After explantation, valves were examined macroscopically using stereomicroscopy, with the appearances being recorded photographically. All attached material was carefully dissected from the valvular surface and subsequently fixed in formaldehyde for histological analysis. After embedding in paraffin, 4 μ m-thick sections were stained with hematoxylin and eosin. After removal of the most prominent thrombotic material, the cusps were prepared for scanning electron microscopy of the valve surface.

Statistical analysis

The proportion of dysfunctional valves in both groups was compared using chi-square testing.

Results

All 12 sheep survived the operative procedure with-

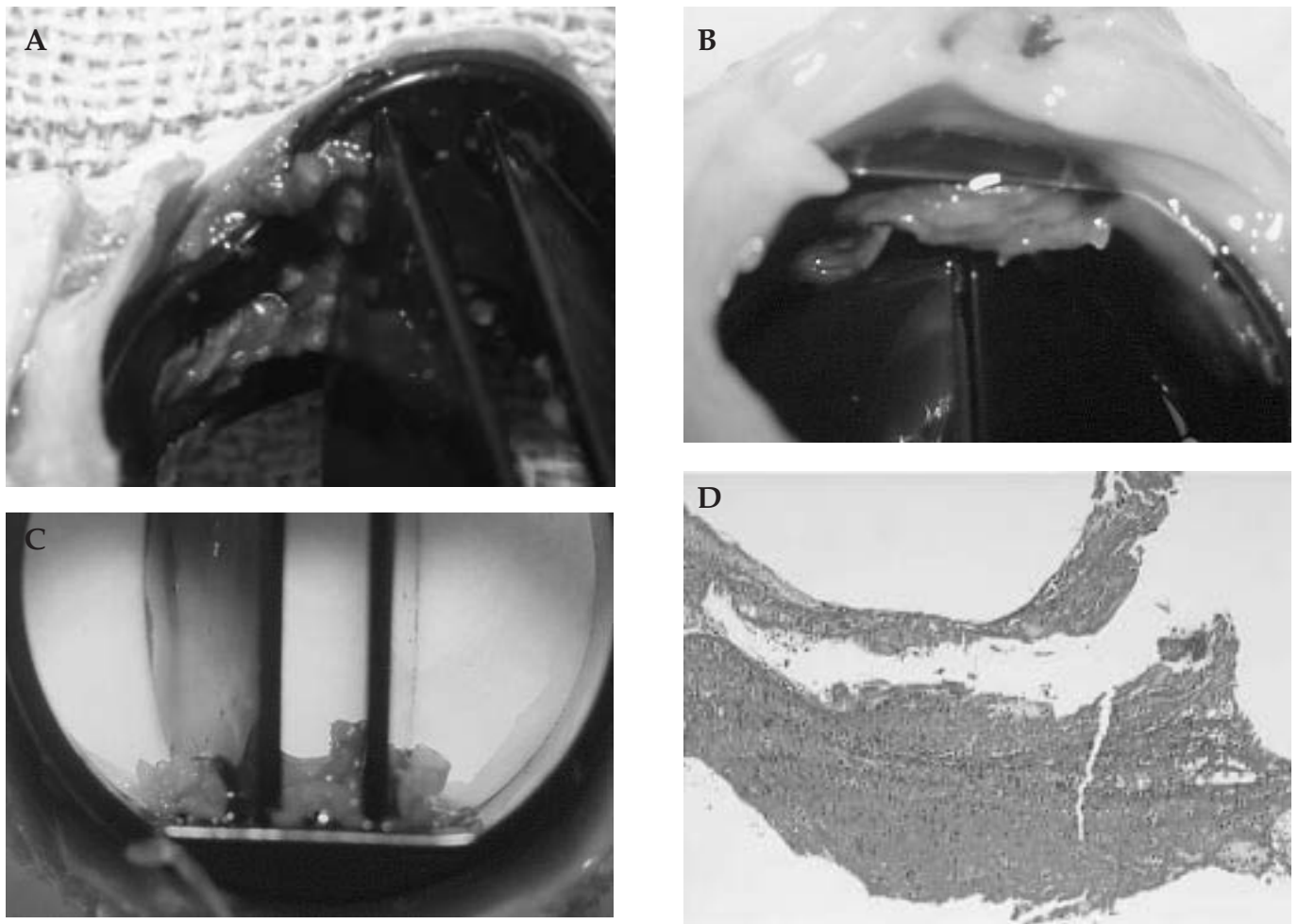


Figure 3: Representative stereomicroscopical images of three different explanted bileaflet valves. A) Thrombus located precisely at the hinge region of the valve, causing immediate obstruction of normal cusp movement. B) Thrombus covering the inflow side of the hinge. C) Larger thrombus at the hinge region. The corresponding histology revealed pure thrombus (D).

out any problems. The animals remained in good physical condition, until the day of sacrifice, and there were no problems with infection. The results for each of the explanted valves from the monoleaflet and bileaflet groups are listed in Tables I and II, respectively. Representative images from the fluoroscopic evaluation are shown in Figure 1.

Monoleaflet valves

No X-radiographic abnormalities were observed in the monoleaflet group. All implanted monoleaflet valves retained a good valve function for up to eight (n = 3) or 12 weeks (n = 3) after implantation. However, at the time of explantation, all of the valves revealed small thrombi attached to the valvular surface (Fig. 2). The eight-week explants all showed thrombi mainly located at the outflow strut, and sometimes extending towards the valve ring. When tested manually after implantation, the disc in all of these valves still had normal mobility, there being no obstruction of disc

movement by the accumulated thrombi. The 12-week explants were more severely affected, and revealed thrombi attached to both outflow and inflow struts, frequently extending towards the ring. In two of these valves, complete disc closure was impossible due to interposing thrombus. As the defect in closure was only a few degrees, the X-radiographic analysis was clearly not sufficiently sensitive to detect the incomplete valve closure.

Bileaflet valves

The bileaflet valves showed a completely different result, with five of the six valves having to be explanted early - that is, before the planned explantation date - due to clear valve dysfunction on fluoroscopy. Valve dysfunction was seen as a complete or incomplete block in the movement of one or both cusps of the valve. Explantation of these valves showed the presence of thrombotic material in and around the hinge regions of the valves (Fig. 3). In some valves, the

thrombi were very small, but their strategic location near or inside the hinge, caused valve dysfunction. All thrombosed cusps were blocked either in an open or a half-open position, which explains why none of the animals died from the valvular thrombosis that occurred. One bileaflet valve, which was planned to be explanted at eight weeks, was still functioning. This valve showed small thrombi near the hinge region, but these were not large enough to obstruct cusp movement.

Histology

All macroscopically viewed material attached to the monoleaflet and bileaflet valves resembled thrombus, and histological analysis confirmed this in all removed fragments (Figs. 2 and 3). Histology showed the presence of primary thrombotic tissue, with the typical image of a fibrin clot containing captured platelets and other blood elements. Some thrombi showed signs of early clot organization, and some showed positive iron staining in macrophages. Thrombi retrieved from the valve ring showed signs of fibrous tissue overgrowth extending from the pulmonary artery wall, though the

core of organizing clot was still recognizable.

Among the lung biopsies, only one showed a clear image of ischemic damage due to a small embolism. This occurred in an animal with a monoleaflet valve, explanted at 12 weeks. All other biopsies (18 in each animal) showed normal and healthy lung tissue.

Scanning electron microscopy of the discs and cusps, after removal of the most prominent thrombotic material, revealed the presence of very small fibrin aggregates and clots, with scattered red blood cells and platelets.

Proportion of dysfunctional valves in each group.

Bileaflet valves became dysfunctional after respectively three (n = 1), four (n = 1), five (n = 2) and six (n = 1) weeks, with one or both cusps blocked (Fig. 4). All monoleaflet valves retained their global valve function until the planned explantation date. Thus, five of six bileaflet valves became dysfunctional during the implantation period, compared to none of the monoleaflet valves (p = 0.034).

Table I: Valve pathology: Monoleaflet valves.

| Time of sacrifice (weeks) | X-radiograph result | Inflow aspect | Outflow aspect |
|---------------------------|---------------------|---|--|
| 8 | Normal | No adherent material present No disc motion restriction No fibrous tissue overgrowth | Thrombus attached centrally to outlet strut No fibrous tissue overgrowth |
| 8 | Normal | No adherent material present No disc motion restriction No fibrous tissue overgrowth | Thrombus attached centrally to outlet strut, extending to one side (towards valve ring) No fibrous tissue overgrowth |
| 8 | Normal | No adherent material present No disc motion restriction No fibrous tissue overgrowth | Thrombus at both sides of outlet strut No fibrous tissue overgrowth |
| 12 | Normal | Thrombus at one side of inlet strut Complete disc closure impossible No fibrous tissue overgrowth | Large thrombus covering disc surface Thrombus at outlet strut (centrally) Slight fibrous tissue overgrowth at both sides of outlet strut |
| 12 | Normal | Thrombus underneath inlet strut Complete disc closure impossible No fibrous tissue overgrowth | Two small thrombi on disc surface Thrombus at both sides of outlet strut No fibrous tissue overgrowth |
| 12 | Normal | Thrombus underneath inlet strut No disc motion restriction Slight fibrous tissue overgrowth present at valve ring | Thrombus at both sides of outlet strut No fibrous tissue overgrowth |

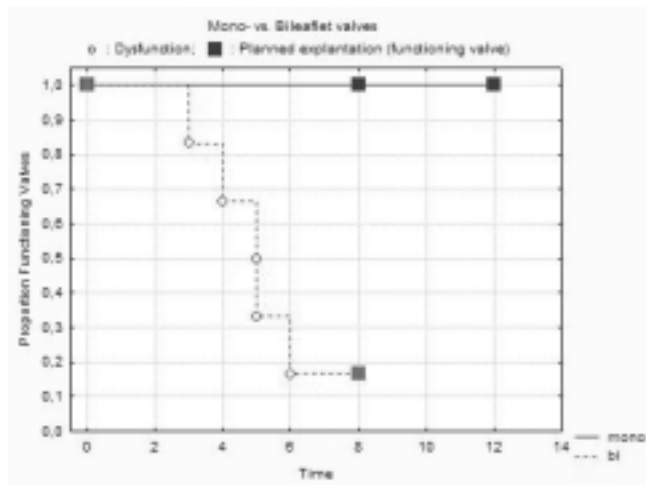


Figure 4: Evolution in time of functioning valves in the mono- and bileaflet groups. All monoleaflet valves retained their valve function until the planned explantation date. Bileaflet valves became dysfunctional (and consequently were explanted) after respectively three ($n = 1$), four ($n = 1$), five ($n = 2$), and six ($n = 1$) weeks, with one or both cusps blocked.

Discussion

Valve implantations in chronic, large animal models have become one of the most important elements in the total preclinical evaluation of new heart valve prostheses. For biological heart valves, tissue calcification and degeneration have been studied in several chronic animal models, with reliable and reproducible results. For mechanical valves, hemodynamic performance and durability have been studied extensively in vitro, combined with valve implantations in animals. Unfortunately, valve thrombogenicity - which is one of the main problematic issues of mechanical valves - remains difficult to investigate in vivo (1).

Many investigators have implanted different mechanical valve types into a variety of animal models, with the main emphasis on mitral and aortic implants in calves, pigs and sheep. Animal left-sided valve implantations are useful for the study of flow characteristics and pressure gradients, but these models do not produce systematic events of valve thrombosis. Given the higher incidence of mechanical valve thrombosis in right-sided clinical implantations, in the present study the performance of two types of mechanical valve was studied in the pulmonary position.

Table II: Valve pathology: Bileaflet valves.

| Time of sacrifice (weeks) | X-radiograph result | Inflow aspect | Outflow aspect |
|---------------------------|---------------------|--|---|
| 3* | One cusp blocked | Thrombus in one hinge One cusp restricted from closing No fibrous tissue overgrowth | Thrombus associated with one hinge No fibrous tissue overgrowth |
| 4* | Both cusps blocked | Both hinge regions thrombosed Both cusps restricted from closing No fibrous tissue overgrowth | Both hinge regions thrombosed No fibrous tissue overgrowth |
| 5 | One cusp blocked | One hinge region thrombosed One cusp restricted from closing No fibrous tissue overgrowth | Thrombus at one hinge region, extending to valve ring No fibrous tissue overgrowth |
| 5 | One cusp blocked | One hinge region thrombosed One cusp restricted from closing No fibrous tissue overgrowth | Thrombus present at one hinge, together with smaller thrombi on the leaflet surface No fibrous tissue overgrowth |
| 6 | Both cusps blocked | Both hinge regions thrombosed Both cusps restricted from closing Slight fibrous tissue overgrowth at valve ring | Thrombi at both hinges Slight fibrous tissue overgrowth |
| 8 | Normal | Small thrombus at one hinge No leaflet motion restriction Slight fibrous tissue overgrowth present at valve ring | Several small thrombi on the leaflet surface No fibrous tissue overgrowth |

*St. Jude Medical valve implanted.

Each mechanical valve implanted in this model revealed primary thrombotic changes within a relatively short time frame. The thrombotic events occurred before overgrowing fibrous tissue could obstruct valvular function, suggesting that the events observed could be diagnosed as 'primary valve thrombosis'.

All of the monoleaflet valves accumulated a significant amount of thrombotic material, notably after 12 weeks. The fact that their global valve function remained intact throughout the study period, was most likely due to the robustness of the tilting disc mechanism, which is not easily obstructed by small thrombotic aggregates. However, in two-thirds of explants from the 12-week group, a slight mechanical dysfunction of the valve was already apparent, and a longer implantation time would most likely have resulted in more severe valve dysfunction.

Bileaflet valves failed rapidly in this animal model, probably due to the delicacy of their hinge mechanism. The hinge of several bileaflet valve types can be blocked by even a small thrombus. The rapid failure of this valve model - which is currently seen as the 'gold standard' - implies that it could be interesting to make certain assessments of the thrombogenicity of new mechanical valve designs. A comparison of results obtained for a new experimental valve (new materials and/or new design) with those for bileaflet valves might identify possible improvements in mechanical valve thrombogenicity.

Fluoroscopy failed to detect the presence of thrombotic material in all of the monoleaflet valves. In a clinical setting, echocardiography has been used to detect abnormal valve function and even thrombi or pannus formation on mechanical valves (14,15). In experimental models, transthoracic echocardiography with visualization of implanted valves in the pulmonary position is possible in sheep (16), though most reports relate to biological valves and homografts rather than mechanical valves. Further studies are necessary to evaluate the possibility of using echocardiography in this animal model of valve thrombosis. Clearly, the model would benefit from a more sensitive method for the detection of valve thrombosis.

New diagnostic (acoustic analysis) or therapeutic (thrombolysis) tools could be evaluated in this model. The animals did not die due to valve thrombosis, nor did they suffer from any significant symptoms, most likely due to the open or half-open position in which the valves thrombosed, together with the fact that the resulting valvular regurgitation occurred in the pulmonary position. The low hemodynamic stress in this position could also explain the limited embolic damage to the lungs. These factors are in favor of the model, because it allows the valve to be explanted at

certain planned time intervals, under full heparinization, thereby eliminating the disturbing formation of post-mortem thrombi.

The difference in delay to valve dysfunction between the two valve types in this model was striking and suggests that, in a low-pressure environment, monoleaflet valves are less sensitive to small thrombotic accumulations. Clinical experience with mechanical valves in the pulmonary position is limited, though a report of about 120 clinical implants has suggested similar findings (10). These authors reported a relatively high ratio of mechanical valve failure due to valve thrombosis in bileaflet valves implanted in the pulmonary position (in a well-controlled and well-anticoagulated population), whilst the results with monoleaflet valves were clearly better.

In conclusion, the low-pressure environment of the pulmonary position caused primary thrombotic changes in all implanted mechanical valves. The thrombosis caused severe mechanical dysfunction only in the bileaflet valves, most likely due to their delicate hinge mechanism. The difference in performance between monoleaflet and bileaflet valves was striking, but appeared to have some clinical correlate. It was felt that these experimental results could be used for the further development of an animal model of valve thrombosis. Such a model could be used in the pre-clinical evaluation of new mechanical valve designs and to evaluate new diagnostic or therapeutic tools for mechanical valve thrombosis.

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