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Subclinical thrombus formation in bioprosthetic pulmonary valve conduits

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ABSTRACT

Objectives: Bioprosthetic pulmonary valve conduits have been reported with an increased risk of endocarditis. Thrombus formation is considered as source of these serious and life-threatening infections. We reviewed a series of explanted valved pulmonary conduits for histological evidence for thrombus formation.
Materials and methods: Explanted bioprosthetic pulmonary valves were fixed in formalin and embedded in paraffin or in methylmethacrylate. Standard staining as well as immunohistochemical staining techniques were applied. Native pulmonary valves of German domestic pigs served as controls.
Results: 47 valved pulmonary conduits (Hancock n = 23, Homograft n = 7, Contegra n = 7, Melody n = 7, other n = 3) were analyzed histologically. Average time of implantation had been 63 months (6 to 342 months). Indications for explantation included significant obstruction (n = 45), regurgitation (n = 7), and/or endocarditis (n = 6). In 44/47 (93%) specimen, we found accumulation of thrombotic material at the basis of the semilunar valve sinus to a variable degree. 11 patients had been treated with antiplatelet agents, 2 had received anticoagulants at the time of explantation. There was no suspicion of thrombus formation clinically or echocardiographically prior to explantation in any of the patients.
Control porcine pulmonary valves (n = 5) did not show any evidence of accumulation of thrombotic material.
Conclusions: In a large series of explanted valved pulmonary conduits, formation of subclinical, mostly non-infectious thrombotic material was an almost ubiquitous finding. We speculate that high incidence of endocarditis in bioprosthetic valves may in part be explained by thrombus apposition at the basis of conduit valve sinus.

1. Introduction

The right ventricular outflow tract (RVOT) and/or the pulmonary valve (PV) are affected in a significant number of congenital heart defects [1]. Thus, many patients require therapeutic interventions of the RVOT and the PV often resulting in the necessity of pulmonary valve replacement early in their life. Accordingly, pulmonary valved conduit implantation or conduit replacement has become one of the most frequent surgical procedures in adolescents and adults with congenital heart disease [2].

Implanted pulmonary valved conduits are subject to progressive degeneration over time. Furthermore, incidence of endocarditis is significantly higher compared to native heart valves. Thrombus formation with subsequent infiltration by infective agents circulating in the bloodstream is considered as source for development of endocarditis [3]. Reason for high frequency of endocarditis in patients with pulmonary valved conduits, however, is not fully understood yet.

Recently, high incidence of subclinical thrombus formation related to bioprosthetic valves in aortic position has been reported [4,5]. Since thrombus formation seems to play a key role in the development of endocarditis, we aimed to screen explanted pulmonary valved conduits for subclinical thrombus formation as a possible risk factor for endocarditis.

2. Materials and methods

A total of 47 explanted bioprosthetic pulmonary valve conduits sent to our laboratory for histopathologic analysis were processed as follows: conduits were flushed with normal saline after surgical explantation and preserved in formalin (phosphate buffered 4%) to prevent autolysis. For extended histopathological and immunohistological work-up specimen were embedded in a synthetic resin (methylmethacrylate, Technovit 9100, KULZER&Co, Wehrheim, Germany), hardened, and subsequently sectioned in slices of

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Table 1
Clinical data.

Type of conduit		Age at implantation	Implantation time	Indication for explantation			Presence of thrombus material at the basis of valve sinus	Anticoagulation at time of explantation	Thrombus despite anticoagulation	Anti-platelet-therapy at time of explantation	Thrombus despite anti-platelet-therapy
				PS	PR	Endocarditis					
	n	Years Mean (min./max.)	Months Mean (min./max.)	n	n	n	n (%)	n	n	n	n
Hancock	23	1.7 (0/7.1)	51.2 (9.1/189.2)	22	7	1	21 (91.3)	1[a]	1	6	5
Homograft	7	10.4 (2.0/22.9)	91.8 (6.2/182.6)	7	1	/	7 (100)	/	/	2	2
Contegra	7	3.2 (0.5/6.0)	66.9 (14.9/141.8)	6	2	1	7 (100)	/	/	2	2
Melody	7	17.9 (12.4/23.5)	40.2 (22.1/62.9)	6	1	4	7 (100)	1	1	1	1
Other	3	29.7 (3.2/58.6)	40.9 (30.4/51.1)	3	/	/	2 (66.6)	/	/	/	/
All	47	5.6 (0/58.6)	57.3 (6.2/189.2)	44	11	6	44 (93.6)	2	2	11	10

Abbreviations: PS = pulmonary stenosis; PI = pulmonary valve insufficiency.
[a] INR not in therapeutical range at time of explantation.

0.8 mm using a diamond cutter (300CP, Exakt GmbH, Norderstedt, Germany). These slices were ground down to 10–30 μm using a rotational grinder (400CS, Exakt GmbH, Norderstedt). Standard staining was performed with Richardson blue. For immunohistochemical staining, deplastification of the ground sections was performed as previously described by our group [6]. Binding of primary antibodies was detected using horseradish peroxidase conjugated secondary antibodies. Sections were counterstained with hemalaun.

Images were documented with a microscope camera and a corresponding software system (CCD-color camera Color View II and software Analysis 3.2, Olympus Europa Holding GmbH, Hamburg, Germany). In addition, slides were scanned with an automatic virtual microscope scanning system for data storage and for enabling of virtual microscopy (Olympus AS110 Fluorescence Virtual Slide Microscope Scanner, Olympus Europa Holding GmbH, Hamburg, Germany).

In order to compare findings with native valves, five porcine pulmonary valves of healthy and untreated German domestic pigs served as controls. All native porcine valves were fixed in formalin and prepared for histologic evaluation with paraffin embedding and haematoxylin/eosin staining.

Clinical data of the patients were obtained from medical records. Informed consent was obtained from every patient and data were processed and documented according to the Declaration of Helsinki.

3. Results

3.1. Patients

The study included 47 bioprosthetic pulmonary valve conduits from 46 patients sent from three German and one Dutch center (Göttingen, München, Stuttgart, and Leiden). All conduits had been implanted into the right ventricular outflow tract (RVOT) in patients with congenital

Fig. 1. Gross pathology of pulmonary conduits. Panels a–d displaying conduits with histologic evidence of thrombus material within the sinus of the valve cusps but without a history of endocarditis showing no macroscopic evidence of fibrin condensations, thin valve cusps, and only few pseudointima formation lining the conduit wall (a and c: Melody valves; b and d: Hancock conduits); panels e and f displaying conduits from patients with endocarditis showing partial thickening of valve cusps but no apparent vegetations at the valve edges or macroscopic thrombus formations (e and f: Melody valves); panels g and h displaying conduits without histologic or macroscopic evidence of subclinical thrombus formation (g: Biocor conduit; h: Contegra conduit).

heart disease and concomitant stenosis and/or insufficiency of the pulmonary valve.

We analyzed 23 Hancock conduits (Medtronic Inc., Minneapolis, USA), 7 homografts, 7 Contegra conduits (Medtronic Inc., Minneapolis, USA), 7 Melody transcatheter valves (Medtronic Inc., Minneapolis, USA), two Freestyle bioprostheses (Medtronic Inc., Minneapolis, USA), and one Biocor Stented Heart Valve System (St. Jude Medical Inc., St. Paul, MN, USA), respectively. For our study, two conduits had been obtained from one patient. This patient had initially received a Hancock conduit (12 mm) for repair of truncus arteriosus. At age 5.5 years he had exchange to a 20 mm Hancock conduit which needed to be replaced by a homograft 6 months later due to endocarditis and progressive stenosis unresponsive to antibiotic treatment. Clinical data were taken from medical records. Information on time between implantation and explantation ("implantation time") as well as indications for explantation are summarized in Table 1. Graft stenosis was the leading cause for conduit explantation in our series (44/47). Almost a quarter of all patients (11/47) displayed conduit valve insufficiency in addition. Six of the patients had suffered from endocarditis at the time of explantation of the valved conduit. Detailed echocardiographic evaluation prior to explantation had not shown any signs of thrombus apposition related to the valved conduit in any of the patients.

3.2. Macroscopic evaluation

Macroscopically, all valve cusps from patients without a history of endocarditis (n = 41) appeared thin and intact. There was no evidence of thrombus apposition at the outer surface or at the edges of the semilunar valves (Fig. 1). In contrast, all six specimen from patients with endocarditis showed partial valve thickening and typical macroscopic pattern of endocarditic lesions.

3.3. Histology: thrombus apposition

In 44 of 47 specimen there was evidence of superficial apposition of thrombotic material at the base of the otherwise histologically regular semilunar valve sinus (Fig. 2a–f). This material showed the typical thrombus pattern of a fibrin network, partially with enclosed blood cells. Presence of fibrin was confirmed by immunohistochemistry (Fig. 3a–d). No further thrombus apposition was found at the remaining conduit walls or valve cusps in specimen *without* endocarditis.

3.4. Histology: endocarditis and inflammatory reactions

Despite the above mentioned thrombus material at the cusps basis, only specimen from patients *with* endocarditis also presented with additional thrombus material in terms of vegetations at the conduit wall and/or the remaining parts of the valve cusps. No gross endocarditic adhesions at the valve edges or tearing of valve cusps were found. Local distribution of endocarditic lesions was varying among affected specimen. Only endocarditic specimen showed typical histological signs of acute inflammation with granulocyte infiltration of thrombotic material, valve tissue, and conduit wall. No further cellular inflammatory reactions were noted throughout our series apart from sporadic lymphocytic infiltrations.

3.5. Histology: neo-endothelialization

Endothelialization of conduit walls as well as valve cusps in all specimen was demonstrated by immunohistochemistry (IHC) with binding of antibodies against CD-31 (Fig. 3e–h).

3.6. Histology of control native porcine valves

Careful histological examination of five native porcine pulmonary valves showed no evidence for any thrombotic deposits. Complete endothelialization as well as absence of any inflammatory reaction was documented.

3.7. Anticoagulation and antiplatelet therapy

At the time of conduit explantation, 11 patients had received antiplatelet therapy and another two individuals had had anticoagulation with vitamin K antagonists (VKA). In 10 of the 11 patients with antiplatelet therapy, fibrin-platelet fibrin condensations were found as well as in both patients on VKA. One of the patients receiving VKA (Hancock conduit) had an INR in a non-therapeutic range (INR < 2) at

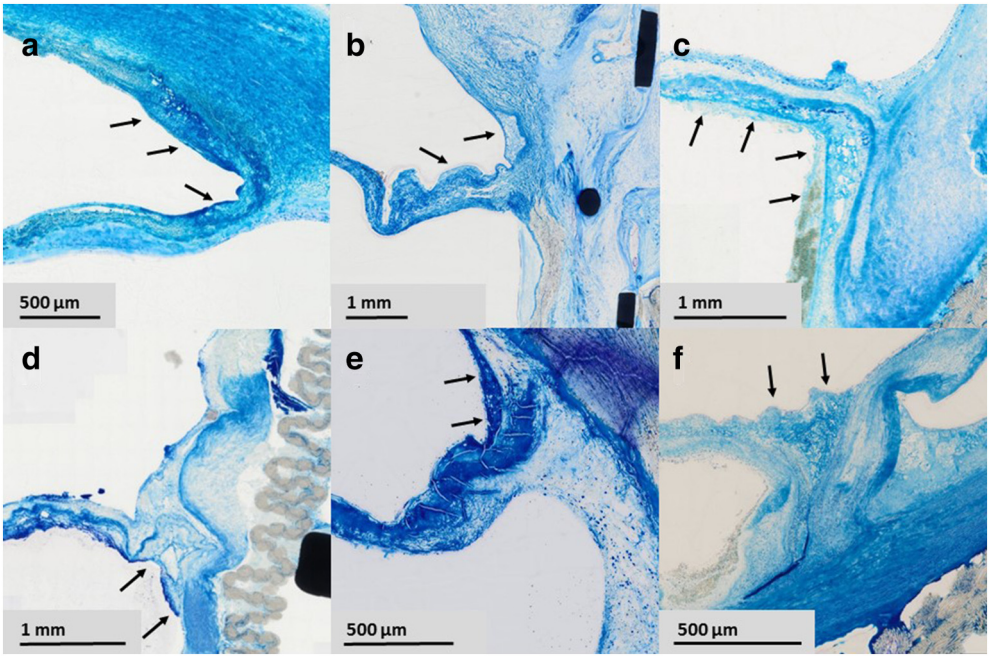

Fig. 2. Histology – thrombus formation. Subclinical thrombus formation (fibrin condensations indicated by arrows) at the base of the valvular sinus (Richardson staining; black parts: stent struts). Valve types and time intervals between implantation and explantation: a Contegra/65 months; b Melody/63 months; c Hancock/13 months; d Hancock/18 months; e Hancock/38 months; f Hancock/14 months.

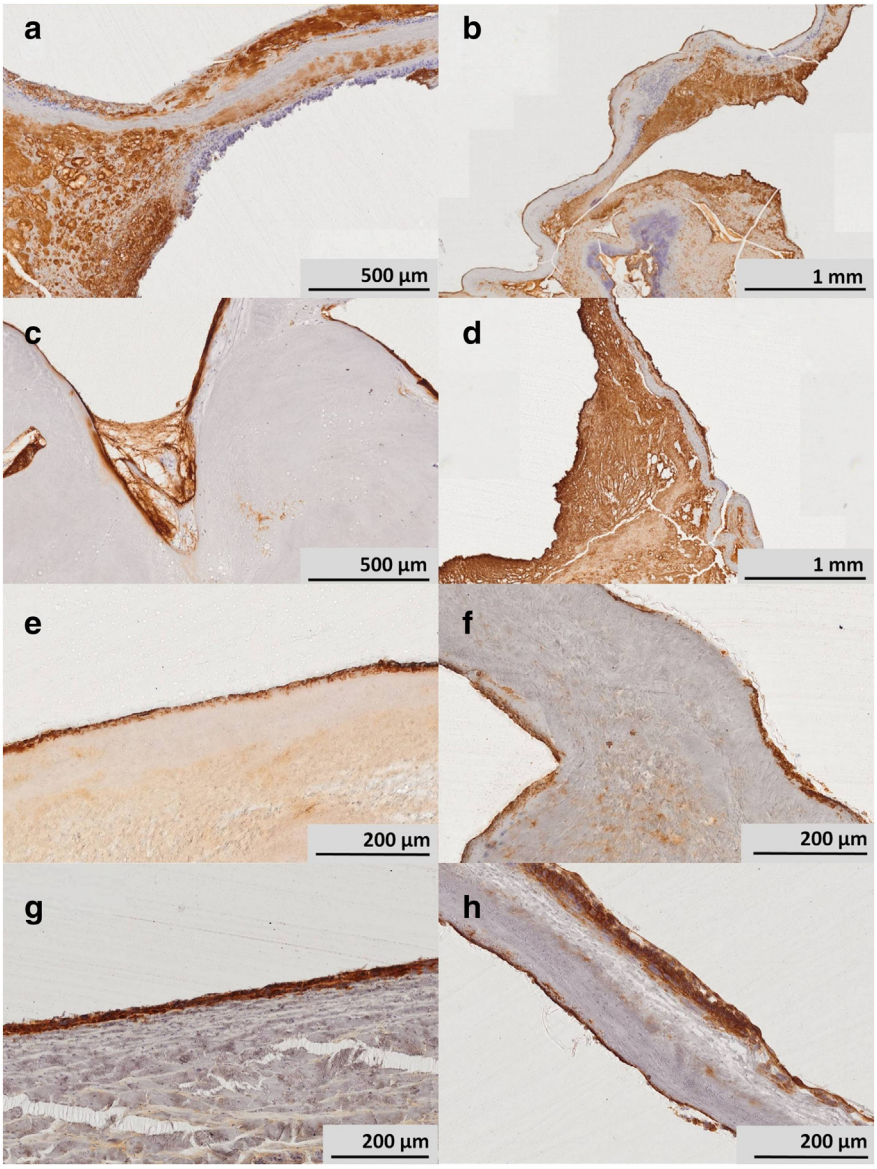

Fig. 3. Immunohistochemistry – fibrin deposits and endothelium. Immunohistochemical staining with antibodies against fibrin indicating thrombus formation (Fig. 2a–d; positive staining indicated by brown color). Immunohistochemical staining with antibodies against CD-31 indicating presence of endothelium (Fig. 2e–h; positive staining indicated by brown color). Valve types and time intervals between implantation and explantation: a Hancock/12 months; b Homograft/6 months; c Melody/49 months; d Melody/63 months; e Freestyle/41 months; f Melody/22 months; g Contegra/15 months; h Hancock /15 months.

hospital admission prior to conduit explantation. The other patient (Melody valve) receiving VKA had no medical record of subtherapeutic INR prior to explantation of the pulmonary graft.

4. Discussion

4.1. High prevalence of thrombus formation

Valve thrombosis and thromboembolic events in patients with a bioprosthetic pulmonary valve conduits have been thought to be very rare and usually only to be found postoperatively until graft tissue surfaces are entirely overgrown by autologous endothelial cells [7–9]. In our series of 47 unselected pulmonary valve conduits we were able to demonstrate a surprisingly high prevalence of subclinical thrombus formation at the sinus of the valves.

We identified 4 studies describing histopathological findings of explanted bioprosthetic valved conduits in pulmonary position [10–13]. In his review on pathology of prosthetic heart valves, Schoen [13] reported on “large thrombotic deposits usually present on the outflow aspects of the cusps”. It remains unclear if the base of the valve sinus had also been involved. Images were not provided. Thrombus formation within the valve sinus was not addressed or shown in the remaining studies.

Makkar et al. performed cardiac CT scans in patients after implantation of bioprosthetic aortic valves and reported reduced motion of the valve leaflets. Subclinical thrombus formation related to the valves was supposed to account for these findings since leaflet motion returned to normal after initiation of anticoagulation therapy [4]. A comparable case series published recently covering a significant number of patients supported these findings [5]. In accordance with the findings in our series patients had no clinical or echocardiographic evidence for thrombus formation.

Reason for the high prevalence of fibrin condensations found at bioprosthetic valves remains elusive. According to Virchow's triad, thrombus formation can be caused by changes in (a) blood composition, (b) endothelial alterations, or (c) stasis.

Hypercoagulation or abnormalities of blood counts had not been studied in detail in our series of patients. Ubiquitous finding of thrombus formation, however, make clotting abnormalities as the underlying disorder very unlikely. As endothelial surface was intact on standard staining with positive immunohistochemical staining, endothelial damage can be ruled out. It is, however, an important finding that 4D-MRI-studies on flow patterns demonstrated blood stasis within the sinus of semilunar bioprosthetic valves when compared to native valves [14]. Therefore, we speculate that stasis was the main reason for thrombus formation as observed in our study.

4.2. Implication of thrombus formation on pathogenesis of endocarditis

Up to now, endothelial damage followed by thrombus formation and subsequent settling of infective agents was considered responsible for development of endocarditis [3]. This theory was deducted from animal experiments demonstrating formation of fibrin condensations and subsequent infiltration with infective agents after artificial endothelial laceration and incubation with bacteria [15–20].

To the best of our knowledge, it has never considered before whether thrombus formation may occur even in the absence of endothelial damage. Our findings of almost ubiquitous non-bacterial thrombus formation within the sinus of bioprosthetic valves give rise to the assumption that fibrin condensation due to stasis may be the primary nidus for endocarditis. To the best of our knowledge, thrombus formation has not been described so far in any native pulmonary valve of individuals without previous surgery or intervention.

4.3. Therapeutic modulation of thrombus formation

Given the high incidence of thrombus formation and its potential impact on endocarditis, it seems reasonable to advocate the use of preventive measures such as anti-platelet therapy or even anticoagulation for patients with bioprosthetic valves.

Eleven of our 46 patients had received anti-platelet therapy with acetylsalicylic acid (ASA) until explantation of their valved pulmonary conduit. Only one of these 11 patients had valve sinus free from thrombotic material. Accordingly, it seems unlikely that ASA alone may serve as effective prevention against thrombus formation as reported by Chakravarty et al. [5] using even dual antiplatelet therapy. Another study from Egbe et al. [21] again did not report any reduction of thrombus formation at bioprosthetic valves by antiplatelet treatment early after surgical implantation as assessed by echocardiography.

Vitamin K antagonists (VKA) have been shown to be highly effective in the prevention of thrombus formation. Only two of the patients had been on VKA at the time of conduit explantation. Both had thrombus formation.

5. Conclusions

We found almost ubiquitous subclinical thrombus formation at the base of valve cusps in bioprosthetic pulmonary conduits. Findings were substantiated by means of histology and immunohistochemistry. Damage of the endothelial surface was not evident in any of the conduits studied.

Thus, we assume that stasis with subclinical thrombus formation alone – without endothelial damage - and subsequent infiltration with infective agents is the most probable cause for the high incidence of endocarditis in patients with bioprosthetic pulmonary valve conduits.

It is of note that antiplatelet therapy with ASS in almost a quarter of our patients did not prevent formation of fibrin condensations.

In order to confirm our findings, further studies including histological analysis of explanted bioprosthetic pulmonary conduits have to be conducted. Finally, prospective studies on the effect of anticoagulation therapy in order to prevent thrombus formation are needed.

6. Limitations

We report on an unselected patient group with a limited number of patients. When compared to other histologic studies on bioprosthetic pulmonary valves, however, number of conduits available for analysis in our study is significant. A total of 6 different types of pulmonary conduits were studied. Therefore, numbers are too small to allow comparative analysis of the different conduit types.

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Conflict of interest

H. S., S. S., A. E. and T. P. have received travel grants from Medtronic Inc., A. E. has served as a proctor for Medtronic Inc. in regard to the Melody valve. R. B., J. H., J. C., R. F. A. P. H., P.J. and M. S. declare to have no conflicts of interest.

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