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A Comparative Histopathological Study of Heparin Coated and Uncoated Polytetrafluoroethylene Shunts in Children With Congenital Heart Defect

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Abstract

Objective: Recently, heparin coated polytetrafluoroethylene (PTFE) shunts are available and are believed to improve inherent shunt problems such as thrombosis and excessive and incomplete neointima formation or occlusion. We aimed at comparing the potential histopathological differences in the neointima (in) between uncoated (UCS) PTFE shunts and heparin coated (HCS) PTFE shunts. **Materials and Methods:** Thirteen shunts (six UCS and seven HCS) were analyzed. The specimens were fixed in formalin, embedded in paraffin or in methylmethacrylate, and characterized by standard and immunohistochemical staining. The thickness of pseudointima proliferation was graded as follows: 0 = no cell layers, 1 = few layers <100 μm, 2 = partial layers >100 μm, 3 = complete layers <300 μm, 4 = complete layers >300 μm, and 5 = occlusion. **Results:** Mean shunt size was 3.4 ± 0.2 mm in UCS and 3.1 ± 0.2 mm in HCS ($P = .053$). Mean time of implantation was 163 ± 75 days in UCS and 97 ± 52 days in HCS ($P = .091$). There were no significant differences in the proportion of patients with functionally single ventricle, body surface area, age at implantation, or implantation type, between both groups. Shunt occlusion did not occur. Unplanned shunt explantation due to cyanosis was performed in one patient in each group. Partial thrombus formation was observed in one UCS ($P = .462$). There was complete endothelialization in 50% of UCS and 86% of HCS ($P = .266$). The grade of pseudointima proliferation was 1.8 ± 0.4 in UCS and 1.7 ± 0.5 in HCS ($P = .646$). **Conclusions:** The histopathological workup of PTFE shunts revealed equally partial endothelialization and discrete pseudointima proliferation in both the groups. The process of endothelialization may be faster in HCS.

Keywords

congenital heart disease, univentricular heart, congenital heart surgery, shunts, systemic to pulmonary artery, endothelium

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Introduction

Surgical implantation of a systemic to pulmonary artery shunt is a standard procedure to control the pulmonary blood supply in children presenting with congenital heart defects. This palliative procedure is used prior to corrective surgery by means of separating the systemic and pulmonary circulation or prior to definitive palliation by means of a Fontan circulation. Early mortality of patients following a modified Blalock-Taussig shunt operation[1] for any indication declined during the last 60 years from 16% in the first half of this time period to 9% in the second half.[2] This significant early mortality rate is accompanied by a high interstage mortality, which ranges between 14% and 26%.[3-5] Shunt thrombosis is the main cause of death in the interstage period.[6]

Recently, heparin coated shunts are available and are believed to reduce interstage morbidity and mortality due to thrombosis, excessive neointima formation, and occlusion.

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We aimed at comparing the potential histopathological differences in the neointima in uncoated cardiopulmonary bypass system (UCS) and heparin coated cardiopulmonary bypass system (HCS) polytetrafluoroethylene (PTFE) grafts.

Methods

Shunt Implantation and Explantation

All shunts were implanted via a midline sternotomy. For placement of a modified Blalock-Taussig shunt, the brachiocephalic trunk was mobilized. A vascular clamp was placed on the brachiocephalic trunk proximal to the carotid artery to exclude the vessel. A longitudinal incision was made in the vessel. The anastomosis with the polytetrafluorenthylen tube was performed using a nonresorbable monofilament suture 7/0. The vascular clamp was removed and the shunt temporarily occluded. The distal anastomosis was performed with continuous 7/0 nonresorbable monofilament suture. The vascular clamp was then removed. The technique for placement of a central aortopulmonary shunt was identical compared to the technique for placement of a modified Blalock-Taussig shunt except for the fact that the proximal anastomosis was accomplished by partial clamping of the ascending aorta. Cardiopulmonary bypass was used for placement of all shunts.

All children were treated with heparin at a starting dose of 5,000 IU/m^2 of body surface area and per day from six to eight hours postoperatively with heparin dose adjustment keeping the partial thromboplastin time two times higher of normal range until the central venous line was removed followed by acetylsalicylic acid at a dose of 3 to 5 mg/kg of body weight until the shunt was removed.

At the time of explantation, the shunt was dissected carefully in order to avoid any squeezing of the shunt. After excluding the pulmonary arteries with vascular clamps, the shunt was proximally clipped. In the presence of pulmonary artery stenosis at the distal shunt anastomosis, the distal part of the shunt was completely explanted and the pulmonary artery was enlarged with a patch. In the absence of stenosis, the shunt was clipped distally and the medial part of the shunt was explanted. The mid portion of the shunts were analyzed in all specimens except for specimen 1389 where the distal anastomosis was analyzed.

Tissue Preparation

After surgical removal, the shunt was briefly flushed with saline. Subsequently, macroscopic evaluation and documentation were accomplished and the specimen was placed in formalin for fixation. One part of each specimen was embedded in a synthetic resin (methylmethacrylate, Technovit 9100; Kulzer and Co, Wehrheim, Germany), hardened, and subsequently sectioned in slices of 0.8 mm using a diamond cutter (300 CP; Exakt GmbH, Norderstedt, Germany). These slices were ground down to 10 to 30 μm using a rotational grinder (400 CS; Exakt GmbH). The other parts of the specimen were dehydrated and put in paraffin wax according to routine protocols. Standard staining was performed with Richardson blue for the resin-embedded specimen. Paraffin wax-embedded specimen was stained with hematoxylin and eosin. For immunohistochemical staining, binding of first antibodies was detected using horseradish peroxidase-conjugated secondary antibodies. The sections were counterstained with hemalaun. The medial part of all shunts was analyzed. The thickness of pseudointima proliferation was graded as follows: 0 = no cell layers, 1 = few layers <100 μm, 2 = partial layers >100 μm, 3 = complete layers <300 μm, 4 = complete layers >300 μm, and 5 = occlusion.

Statistical Analysis

Frequencies are given as absolute numbers and percentages. Continuous data are expressed in terms of the mean and standard deviation. Fisher's exact test was performed to detect significant differences between the groups. For comparison of continuous variables between two groups, the *t*-test was used (two-tailed tests were used for all analyses). Analyses were performed with SPSS 20.0 for Windows (SPSS Inc, Chicago, Illinois).

Results

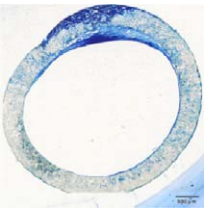
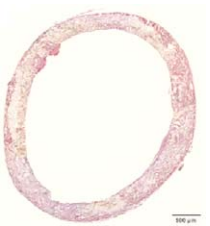
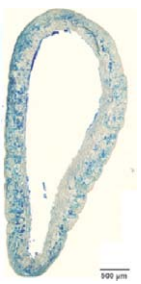
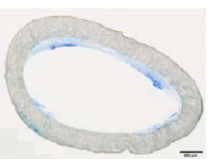
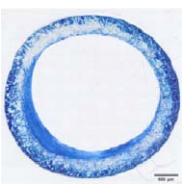
Histopathological workup was performed on a total of six UCS and seven HCS shunts that were explanted between February 2011 and January 2012. Table 1 depicts clinical and histopathological findings of all 13 specimen.

Two UCS and one HCS were excluded from further comparisons because a stent was implanted in the shunt. Stent implantation was performed at days 13 and 66 after UCS implantation and at day 20 after HCS implantation due to stenosis at the distal anastomosis.

Demographic data, diagnoses, and implantation times of the remaining six UCS and seven HCS are listed in Table 2: mean shunt size was 3.4 $\pm$ 0.2 mm in UCS and 3.1 $\pm$ 0.2 mm in HCS (P = .053). Mean time of implantation was 163 $\pm$ 75 days in UCS and 97 $\pm$ 52 days in HCS (P = .091). There were no significant differences in the proportion of patients with functionally single ventricle, body surface area, age at implantation, or implantation type, between both the groups.

There was no shunt occlusion (Table 3). All shunts were explanted electively except for one shunt in both groups: specimen 1315 was explanted 78 days after implantation due to a stenosis at the distal anastomosis. Specimen 1307 was explanted nine days after implantation due to a kinking at the proximal end of the shunt. Histologically, there were no thrombi in the examined shunts except for one wall adherent thrombus in a UCS (Figure 1A, specimen 1360; P = .462). Evaluation of inflammatory reactions revealed a uniform pattern in both groups with only few foreign body giant cells locally related to the PTFE–tissue interface (Figure 1B). No lymphocytic or granulocytic infiltrations were seen in any of the specimen. Endothelialization was examined by means of immunohistochemical staining with antibodies against endothelium-specific antigens (von Willebrand factor and CD-31). Complete endothelialization was demonstrated in all

Table 1. Grading of Pseudointima.[a]

Nonheparin Coated							
Specimen #	1217	1224	1282	1315	1357	1360	
Days implanted	141	200	166	78	287	104	
Endothelialization	Partial	Complete	Partial	Partial	Complete	Complete	
Thrombus formation	No	No	No	No	No	Yes	
Grading pseudointima	2	2	2	1	2	2	
Heparin Coated							
Specimen #	1281	1307	1358	1379	1389	1391	1416
Days implanted	92	11	122	112	182	85	78
Endothelialization	Complete	Partial	Complete	Complete	Complete	Complete	Complete
Thrombus formation	No	No	No	No	No	No	No
Grading pseudointima	2	1	2	2	2	1	2

[a] 0 = no cell layers, 1 = few layers <100 μm, 2 = partial layers >100 μm, 3 = complete layers <300 μm, 4 = complete layers >300 μm, and 5 = occlusion.

Table 2. Patients' Characteristics by Type of Shunt.

Parameter	Noncoated Shunt, n = 6	Coated Shunt, n = 7	*P* Value
Gender male	6	3	.070
BSA at implantation	0.20 ± 0.02	0.20 ± 0.02	.946
SV	4	3	1.000
PA VSD	0	3	
PA IVS	2	1	
TA	0	2	
DORV TOF	1	0	
DORV TGA PS	0	1	
DORV SV	1	0	
HLHS	1	0	
ccTGA VSD PS	1	0	
Modified Blalock-Taussig vs central shunt	5/1	2/5	.103
Shunt 3 vs 3.5 mm	1/5	5/2	.103
Shunt size, mm	3.4 ± 0.2	3.1 ± 0.2	.053
Time implanted, days	163 ± 75	97 ± 52	.091

Abbreviations: BSA, body surface area; ccTGA, congenitally corrected transposition of the great arteries; DORV, double outlet right ventricle; HLHS, hypoplastic left heart syndrome; IVS, intact ventricular septum; PA, pulmonary atresia with ventricular septal defect ; PS, pulmonary stenosis; TA, tricuspid atresia; SV, functionally single ventricle; TGA, transposition of the great arteries; TOF, tetralogy of Fallot.

Table 3. Clinical and Histopathological Findings by Type of Shunt.

Parameter	Noncoated Shunt, n = 6	Coated Shunt, n = 7	*P* Value
Not elective explantation	1	1	1.000
Occlusion	0	0	
Partial thrombus formation	1	0	.462
Total vs partial endothelialization	3/3	6/1	.266
Inflammatory response	6	7	
Neointima proliferation grade	1.8 ± 0.4	1.7 ± 0.5	.646

specimen except for two NCS (specimen 1217 and 1315) and one HCS (specimen 1307; Figure 1C; $P = .559$ for comparison of UCS vs HCS). All shunts (both UCS and HCS) showed some degree of tissue proliferation at the inner (lumen sided) surface of the PTFE material. These proliferations consisted of myofibroblasts surrounded by extracellular matrix (Figure 1D). There was no significant difference in the grade of pseudointima formation between UCS and HCS (Table 3; 1.8 ± 0.4 in UCS vs 1.7 ± 0.5 in HCS, $P = .646$).

Discussion

A variety of complex congenital cardiac malformations in newborns require the placement of a systemic to pulmonary artery shunt to control the pulmonary blood flow. However, this palliative procedure is associated with a significant mortality prior to definitive correction or palliation.[2-5] Shunt occlusion due to thrombosis is the main cause of death in the interstage period.[6] The present histopathological workup of PTFE shunts revealed equally partial endothelialization and discrete pseudointima proliferation in both the groups. We observed only one thrombus in an UCS.

There are many factors involved in shunt thrombosis, which may be assigned to Virchow's triad: first, stasis of blood flow may occur due to low cardiac output or due to technical difficulties during implantation. Second, hypercoagulability may be present after an excess of procoagulatoric agents administered to control bleeding after surgery or in case of pathological high hematocrit after diuretic therapy. Third, endothelial injury is present at the anastomosis. In addition, the blood is exposed to foreign material at the inner surface of the shunt.

There are important technical considerations to avoid stasis: the choice between a modified Blalock-Taussig shunt[1] and a central aortopulmonary shunt[7]; the selection of the appropriate size and length; and the possibility to allow for additional pulmonary flow via the native pulmonary artery or via the collateral arteries. Hypercoagulability can be prevented by medical treatment using anticoagulation with heparin, acetylsalicylic acid,[5] or coumadin.[8] The problem of lack of intact endothelium within the lumen of the shunt immediate after implantation cannot be solved in neonates who need an urgent shunt operation. However, recently, HCSs are available and are believed to reduce shunt occlusion due to thrombosis.

The potential influence of shunt type, size, and length of shunt thrombosis cannot be elucidated in the present series due to the limited statistical power. We observed only one thrombus. The potential influence of medical treatment cannot be assessed in the present series due to the methodology of the study. The anticoagulation regime was similar in all patients. However, to the best of our knowledge, the present study is the first attempt to investigate the potential histopathological differences in the neointima in UCS and HCS in children.

Every synthetic material, which is implanted into the body, leads to reactions in the surrounding tissue. Four reactions can be observed when prosthetic material is placed into the blood

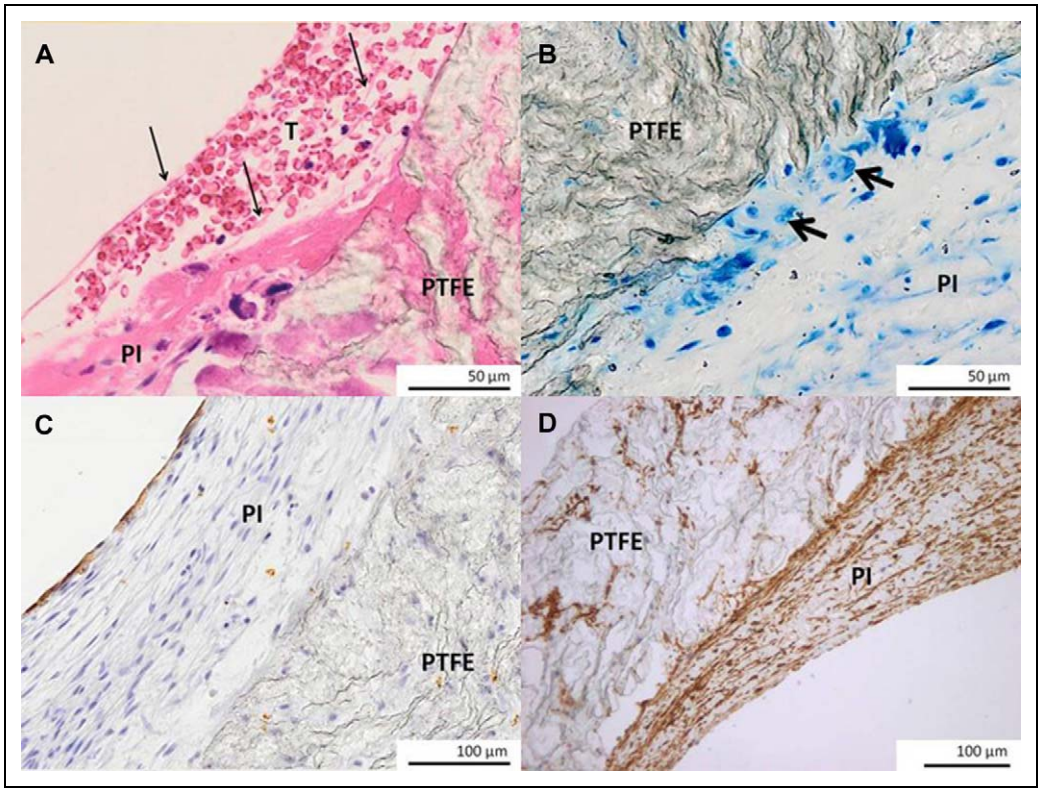


Figure 1. A, Superficial adherence of thrombotic material (T) to the polytetrafluoroethylene (PTFE) membrane (PTFE) with typical fibrin fibers (slim arrows) with included blood cells next to pseudointima (PI)—patient #1360; time implanted 104 days; hematoxylin–eosin stain. B, Few histiocytes (macrophages) forming foreign body giant cells (bold arrows) adjacent to the PTFE material—patient #1379; time implanted 112 days; Richardsons' stain. C, Immunohistochemical staining with antibodies against CD-31 (endothelium—brown color)—patient #1315; time implanted 78 days. D, Positive staining (brown color) of cells with antibodies against smooth muscle actin demonstrating typical myofibroblasts forming the pseudointima—patient #1224, time implanted 200 days.

flow: inflammation, neointima proliferation, endothelialization, and thrombus formation.[9]

The inflammatory response is mediated by the formation of foreign body giant cells at the surface of the synthetic material.[10] The consequence can be a massive inflammatory reaction, leading to early graft failure as it was shown for porcine valves.[11,12] In the present series, the evaluation of inflammatory reactions revealed a uniform pattern in both groups with only few foreign body giant cells locally related to the PTFE.

The inflammatory reaction is accompanied by fibrotic scaring consisting of myofibroblasts surrounded by extracellular matrix, which may be referred to as neointima. The development of this neointima was seen to proceed in a material-dependent pattern.[9] In the present study, we observed little intimal proliferation. The thickness of complete layers was <100 µm and we observed only partial layers with a thickness >100 µm. This translates into a reduction in the cross-sectional areas of 13% for a 3-mm shunt and of 10% for a 3.5-mm shunt. In the present series, there was no significant difference in the grade of pseudointima formation between HCS and UCS. However, there is significant evidence for the beneficial effect of heparin coating of PTFE common carotid artery bypass grafts from a sheep model. Pedersen and colleagues compared coated grafts on one side to noncoated grafts on the other side. They observed a significantly higher patency rate of coated grafts. Moreover, there was significantly less pseudointima formation within the lumen of the coated shunts.[13] These findings were already observed by Lin and colleagues in a baboon model.[14] The authors found that heparin coated PTFE grafts resulted in less intimal hyperplasia, compared with noncoated control PTFE grafts. The difference in the significance between the results observed in the sheep model compared to the present study may be attributed to the longer implantation time in the animals. In addition, comparing shunts in one animal allows for controlling potential influencing factors, which cannot be eliminated in our study. However, it is of note that we observed total endothelialization in six of seven HCS and in only three of six UCS.

There is no endothelium within the lumen of the shunt immediately after implantation. In addition, there is endothelial damage at the position where the arteries were incised for accomplishing the shunt anastomosis. Hence, thrombus formation may occur not only due to a foreign body surface directly exposed to the blood stream but also due to endothelial damage.[15,16] There is a significant early mortality after placement of a systemic to pulmonary artery shunt, especially within the first 30 days, which may be attributed to early thrombotic shunt occlusion.[5] Therefore, early reendothelialization is crucial

because of the antithrombotic nature of endothelial cells.[17] In the present series, we observed endothelial cells as early as 11 days after implantation and a confluent monolayer of endothelial cells three months after implantation. This is in line with scanning electron microscopic findings of a stainless steel coil ten days after interventional closure of a patent ductus arteriosus in a lamb model.[9] This rapid endothelialization may more likely be derived from hematopoetic stem cells than from ingrowth from the vessels proximally and distally to the shunt.[18] It is of note that in the present series only one thrombus occurred in a UCS. However, due to the low number of patients and events, there was no significant reduction in thrombogenicity of UCS compared to HCS.

In conclusion, the present comparison of UCS to NCS in neonates following palliation with a systemic to pulmonary artery shunt reveals equally good clinical outcome of patients with both types of shunts. The histopathological workup of the shunts revealed partial endothelialization and discrete pseudointima proliferation in both the groups. The process of endothelialization may be faster in HCS. The data suggest an early and a late vulnerable period for shunt occlusion. The early period ends with complete endothelialization. This may be as early as one month after implantation. An appropriate shunt flow and treatment with acetlysalicylic acid is crucial in the early period. The late period begins with significant lumen reduction due to intimal proliferation. This is more relevant in very small shunts. Hence, the implantation of 3.0 mm shunts should be avoided.

Limitations

There are multiple potential influencing factors for the performance of shunts, which cannot be considered by means of multivariate testing in a small case series. The patient inclusion was not prospective and randomized. The patients who were treated with a UCS were operated between August 2010 and April 2011. The patients who were treated with a HCS were operated between January 2011 and November 2011. The study does not include all shunts that were implanted between August 2010 and November 2011. Three shunts with stents in the distal anastomosis (two UCS and one HCS) were excluded from further histopathological comparison.

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Declaration of Conflicting Interests

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