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Adverse pulmonary vascular remodeling in the Fontan circulation

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BACKGROUND: The Fontan circulation is a palliation for patients with a functionally univentricular heart. It is characterized by gradual attrition over time. An increase in pulmonary vascular resistance could be a key factor in the long-term failure of the Fontan circulation. In this study we aimed to identify pulmonary vascular remodeling in patients with a Fontan circulation.

METHODS: Pulmonary vascular histomorphometric analysis and immunohistochemistry were performed in lung tissue obtained at autopsy from 12 Fontan patients. These patients had died either perioperatively (Group A: death during or <15 days after Fontan completion; $n = 5$) or in mid to long-term follow-up (Group B: death >5 years after Fontan completion; $n = 7$). Two age-matched control groups ($n = 10$ and $n = 14$, respectively) were included.

RESULTS: Intra-acinar pulmonary vessels in the Fontan Group B patients showed decreased medial thickness ($p = 0.028$) compared with age-matched controls, whereas intimal thickness was increased ($p = 0.002$). Intimal thickness in the Fontan Group B patients correlated with age at death ($r = 0.964$, $p < 0.001$) and with the length of time that the Fontan circulation had been in place ($r = 0.714$, $p = 0.036$). Immunohistochemistry revealed a reduction of vascular smooth muscles cells in the medial layer of the intra-acinar pulmonary vessels. The eccentric intimal thickening was composed of mainly acellular fibrosis with collagen deposition.

CONCLUSIONS: We observed a unique pattern of adverse pulmonary vascular remodeling in patients with a long-standing Fontan circulation who had died during follow-up. This remodeling pattern may play a major role in long-term attrition of the Fontan circulation.

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The Fontan circulation procedure is performed in patients with congenital heart defects for whom a biventricular correction is not possible. The Fontan circulation is characterized by an un-physiologic pre- and after-load of the single ventricle, chronically increased systemic venous pressures and chronic non-pulsatile flow in the pulmonary vascular bed. Since the Fontan circulation was introduced, survival of these patients has improved.[1,2] However, long-term follow-up is characterized by gradual attrition of the Fontan circulation, eventually resulting in the so-called "failing" Fontan circulation. Several factors have been suggested to play a role in Fontan attrition, including:

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chronic venous congestion; chronic abnormal loading conditions of the single ventricle; arrhythmias; and an increase in pulmonary vascular resistance (PVR), of which the latter has received growing attention recently.[3] In the Fontan circulation, PVR is a major determinant of the single ventricle's pre-load and thus of cardiac output.[4,5] Small increases in PVR in the Fontan circulation can reduce cardiac output significantly.[6–8]

Changes in the pulmonary vasculature may play a role in this PVR increase and thus may be a major factor in the failing Fontan circulation. In experimental studies, non-pulsatile pulmonary flow has been shown to induce endothelial dysfunction and increased apoptosis of vascular smooth muscle cells (VSMCs), and these effects can result in impaired pulmonary vasodilation, adverse pulmonary vascular remodeling and elevation of PVR.[9–15] However, little is known of possible long-term changes in the pulmonary vasculature after Fontan completion.[16,17] Previous histomorphometric studies analyzed lung tissue obtained either during the Fontan operation or post-mortem within 30 days after the Fontan operation.[18–20] In this study, we performed post-mortem histomorphometric and immunohistochemical analyses of the pulmonary vasculature of Fontan patients who died either peri-operatively, at completion of the Fontan circulation or at longer term follow-up (>5 years after Fontan completion). Based on the available experimental evidence showing that non-pulsatile blood flow induces pulmonary endothelial dysfunction and vascular cell apoptosis, we hypothesized that chronic non-pulsatile pulmonary blood flow, as present in the Fontan circulation, is associated with adverse pulmonary vascular remodeling.[9–14]

Methods

Study population

Our study included all patients at the University Medical Center Groningen who died after a Fontan completion between 1975 and 2012, had undergone autopsy, and from who suitable lung tissue specimens were available. In addition, from the institutional pathology archives, lung tissue specimens were collected from an age-matched control group with a sample size twice as large as the Fontan group to serve as controls. This control group consisted of patients who died due to various other causes, all considered not to be associated with abnormal pulmonary hemodynamics.

Tissue preparation

All lung specimens were fixed in 10% phosphate-buffered formalin solution and embedded in paraffin. The slides (4 μm thick) were stained separately with Verhoeff-van Gieson stain and Heidenhain Azan trichrome stain using standard staining protocols.

Histomorphometry

Two lung sections of each subject stained with Verhoeff-van Gieson stain were used for histomorphometric analysis according to a protocol previously reported by van Albada et al.[21] Twenty randomly chosen vessels with an external diameter (ED) <100 μm (intra-acinar) and 6 pre-acinar arteries with an ED of 100 to 300 μm were assessed from each lung section using IMAGESCOPE software, version 11.1.2.752 (Aperio Technologies). Vessels were excluded based on the following criteria: ratio of largest/shortest ED >2; incomplete circular shape or collapse of >25% of the vessel wall; and intra-acinar vessels located adjacent to bronchioles. Three different vascular areas were defined: (1) outer vessel area—area within external elastic lamina; (2) inner vessel area—area within internal elastic lamina; and (3) luminal area—area within luminal border. Areas were transformed into diameter (D) using the formula: $D = 2 \times [\sqrt{(\text{area} / \pi)}]$. The proportional total wall thickness as a percentage of the ED to reflect the occlusion of a vessel was calculated using the formula: % total wall thickness = 100 × [(ED – lumen D) / ED]. This value has been used previously, as it nullifies the effect of vasodilation, vasoconstriction and fixation on measurements.[22–25] The proportional medial thickness was calculated as follows: % medial thickness = 100 × [(ED − internal D) / ED]. The proportional intimal thickness was calculated using the formula: % intimal thickness = 100 × [(internal D − lumen D) / ED]. Intra-acinar vessels without a clearly defined internal elastic lamina combined with luminal occlusion were defined as vessels with intimal lesions (% vessels with intima). Muscularization of the intra-acinar vessels was scored as previously described by van Suylen et al, and as used extensively in our laboratory.[21,26–28] Muscularization is presented as the percentage of vessels per category degree of muscularization. Intra-acinar vessels with a double elastic lamina for >50% of its circumference were defined as completely muscular (% muscularized vessels). Intra-acinar vessels with a double elastic lamina for <50% of its circumference were defined as partially muscular (% partially muscularized). Normal, non-muscular, intra-acinar vessels had a single elastic lamina and no luminal occlusion (% non-muscularized).

Immunohistochemistry

Paraffin-embedded lung sections were microwave heated for 20 minutes with Tris-ethylene-diamine tetraacetic acid (EDTA) buffer for antigen retrieval, followed by pre-incubation with 0.3% hydrogen peroxide. For alpha-smooth muscle actin (αSMA), sections were embedded for 1 hour at room temperature with primary antibody (1:50; αSMA, monoclonal mouse, DAKO) followed by secondary antibody for 30 minutes (1:100; rabbit anti-mouse immunoglobulin G coupled with peroxidase, DAKO). For CD31, sections were embedded for 30 minutes at room temperature with primary antibody (1:400; monoclonal mouse anti-human CD31, Clone JC/70A, DAKO). The slides were incubated with secondary antibody (1:100; rabbit anti-mouse horseradish peroxidase [HRP], S23) for 30 minutes. Incubation for 30 minutes with the tertiary antibody (1:100; goat anti-rabbit HRP, S20) followed. For Caldesmon, sections were heated at 95°C for 52 minutes with Tris-EDTA buffer for antigen retrieval. Slides were incubated for 32 minutes at 36°C with primary antibody (1:800; monoclonal mouse anti-human Caldesmon, Clone h-CD, DAKO). Finally, all sections were stained with diaminobenzidine for 10 minutes and counterstained with hematoxylin. The CD31-stained sections were used for assessment of endothelial integrity. The number of CD31-stained sections analyzed for each group was: Fontan Group A, $n = 4$; Control Group A, $n = 3$; Fontan Group B, $n = 6$; and Control Group B, $n = 4$. Twenty intra-acinar pulmonary vessels (<100 μm) in each control and 40 in each patient were randomly chosen. Disruption of endothelial integrity was defined as an incomplete or absent circumferential CD31 staining on the luminal side of the vessel, which was expressed as a percentage per total of analyzed vessels.