Hypoplasia of the small pulmonary arteries in total anomalous pulmonary venous connection with obstructed pulmonary venous drainage

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Objective: Preoperative pulmonary venous obstruction has been reported to be a risk factor negatively impacting survival in total anomalous pulmonary venous connection. We examined lung tissue from total anomalous pulmonary venous connection patients with pulmonary venous obstruction and demonstrated hypoplasia of small pulmonary arteries to elucidate the mechanism underlying the poor outcome.

Methods: Ten total anomalous pulmonary venous connection patients with preoperative pulmonary venous obstruction between the ages of 2 days and 10 months were studied. As histological parameters, we assessed the size of small pulmonary arteries in relation to the size of accompanying bronchioles to identify small pulmonary artery underdevelopment. Other parameters, such as the radial alveolar count, which reflects alveolar maturity, intimal lesions, lymphangiectasia, and the medial thickness of small pulmonary arteries and small pulmonary veins, were also examined. As a control group, we examined 24 autopsy cases with no congenital heart or pulmonary disease.

Results: When the radius of the accompanying bronchiole was 100 μm, the radius of small pulmonary arteries in the control group was found to enlarge for the first 2 months and then remain stable at approximately 80 μm from 2 to 10 months. In total anomalous pulmonary venous connection with preoperative pulmonary venous obstruction, the radius was significantly lower than in the control (47.0 ± 21.8 μm versus 75.9 ± 9.8 μm, P < .001), and the difference between dead and surviving patients was significant at P < .001 (33.0 ± 14.6 μm versus 68.2 ± 9.2 μm). Examination of the alveoli yielded a radial alveolar count of 4.6 ± 1.5 in the control group and 4.4 ± 0.8 in the total anomalous pulmonary venous connection patients, and the difference was not significant (P = .71).

Conclusions: The small pulmonary arteries of total anomalous pulmonary venous connection patients with preoperative pulmonary venous obstruction were underdeveloped compared with controls but their alveolae were not hypoplastic. These results suggested that the small pulmonary artery hypoplasia may be responsible for the poor outcome of these patients.
otal anomalous pulmonary venous connection (TAPVC) is a rare, life-threatening congenital cardiac anomaly, and without surgical repair, the majority of patients die within the first year of life.\(^1\) There have been remarkable improvements in operative survival over the years and the surgical mortality reported has ranged from 0% to 11%.\(^2,7\) However, postoperative problems such as recurrent pulmonary vein obstruction (PVO), pulmonary hypertensive (PH) crisis, and low cardiac output may still occur, and they can be fatal.\(^3,7\)

TAPVC is classically subdivided into 4 distinct categories according to the anatomy of the drainage of the pulmonary veins, and each category can be subdivided into obstructed and nonobstructed types. Obstructed-type TAPVC is defined as an obstruction of either pulmonary vein, vertical veins, or descending veins at the time of diagnosis and is called “preoperative (primary) PVO” to differentiate it from postoperative (secondary) PVO following repair or intervention. Primary PVO causes elevation of pulmonary artery pressure and usually presents as a neonatal emergency, and it is reported to be a risk factor that negatively impacts early and late survival, even after repair.\(^4,5,7\)

Based on the above, we collected lung specimens taken from obstructed-type TAPVC patients (who account for approximately 30% of all TAPVC patients\(^1,4,7\)) and investigated various histological characteristics to determine the cause of the poor outcome in these patients. We particularly examined them for the presence of hypoplasia of small pulmonary arteries (SPAs; pulmonary arteries with a muscular coat observed in biopsy specimens, with diameters ranging from 100 to 500 \(\mu\)m). Although various types of pulmonary vascular lesions have been reported in TAPVC, few reports have discussed hypoplasia or underdevelopment of SPAs. One reason for this is that there is as yet no clear definition of hypoplasia of SPAs. Noting the fact that bronchioles always accompany SPAs in lung specimens,\(^8,9\) we describe a method of comparing the degree of development of SPA among patients.

**Materials and Methods**

Ten infants with obstructed-type TAPVC, ranging in age from 2 days to 10 months (mean age, 92.9 days) at the time of biopsy or autopsy, between 1996 and 2002, were studied. The study was approved by the institutional review board, and informed consent was obtained from the infants’ relatives. Excluded from analysis were infants with associated complex lesions, such as single ventricle or hypoplastic left ventricle. All patients had preoperative PVO, which is considered to be present when (1) the gradient between the pulmonary artery wedge and mean left atrial pressure is >5 mm Hg by cardiac catheterization; (2) there is angiographic evidence or intraoperative observation of a localized or segmental reduction in pulmonary vein (or vertical vein or descending vein) diameter by >50%; or (3) there is a mean gradient of >5 mm Hg across the narrowed pulmonary vein as measured by Doppler echocardiography.\(^6\) Biopsies were performed during TAPVC repair in 5 patients. The lung tissue in the 5 other patients was obtained at autopsy. All biopsy and autopsy tissue was taken from the lobe in which the pulmonary blood drainage was obstructed. Breakdown by disease type was as follows: supracardiac type (Daring’s classification), 3; cardiac type, 2; infracardiac type, 5; and mixed type, 0. The clinical profiles and outcomes of these patients are summarized in Table 1.

As a control group, we examined tissues from 24 autopsy cases with no congenital heart or pulmonary disease in which death occurred from 1 day to 10 months of age (mean age: 80.5 days). All control group infants had been born full-term. Ten of the infants had died from infection, 5 from accidents, 3 from brain hemorrhage, 3 from tumors, 2 from metabolic disorders, and 1 from intussusception.

**Tissue Preparation**

One block from each lung was fixed in 10% formalin, and paraffin histologic sections were prepared. In each case, 30 step-sections at 50-\(\mu\)m intervals were prepared. Elastica-Goldner staining was performed.\(^10\) We assessed the following: (1) severity of intimal lesions; (2) radius and medial thickness of SPAs and small pulmonary veins (SPVs; pulmonary veins with a muscular coat observed in biopsy specimens, with diameters ranging from approximately 100 to 500 \(\mu\)m); (3) radius of bronchioles and the radius of SPAs accompanying the bronchioles (supernumeral arteries\(^9\) that did not run parallel with the bronchioles were excluded); (4) RAC, which reflects lung maturity; and (5) the presence of lymphangiectasia.

**Measurements**

The medial thickness (\(D_A\)) and radius (\(R_A\)) of SPAs and SPVs were measured using a previously reported morphohistometric method.\(^3,11\) All SPAs were scanned with a digitizing camera (Olympus PDMC/OL; Olympus, Tokyo, Japan), and the images were fed into a Macintosh computer (PowerMac G4; Apple Japan Inc, Tokyo, Japan). All the morphohistological measurements below were performed using NIH image version 1.53 software (National Institutes of Health, Bethesda, Md). Assuming uniform thickness, \(D_A\) and \(R_A\) were calculated from the length of internal elastic membrane (\(L_A\)) and the area of the media (\(S_A\)) by using the following equation (Figure 1, A):

\[
D_A = \frac{(L_A^2 + 4\pi S_A)^{1/2} - L_A}{2\pi}
\]

\[
R_A = S_A/(L_A^2 + 4\pi S_A)^{1/2} - L_A
\]

The values of \(R_A\) and \(D_A\) were obtained from at least 20 samples from each patient and plotted on a logarithmic coordinate system. Linear regressions of \(R_A\) and \(D_A\) were performed, and the \(D_A\) value at \(R = 100 \, \mu\)m (\(D_{R=100\mu m}\)) was calculated so that medial thickness could be compared.

The radius of bronchioles (\(R_Q\)) accompanying preacinar SPAs was measured by modifying the technique of Berend and colleagues.\(^12\) Because bronchioles are sometimes distorted and contracted in prepared specimens, the radius was calculated in our study in the state in which the internal basement membrane was extended in the form of a circle to avoid errors resulting from distortion or contraction. The length of the basement membrane of
the bronchiole (L_B) was measured (Figure 1, A), and R_B was calculated by using the following equation:

\[ R_B = \frac{L_B}{2\pi} \]

The values of R_A and R_B were obtained in at least 20 pairs from each tissue and plotted on a logarithmic coordinate system (Figure 2). Linear regressions of these 2 radii were performed, and the SPA radius at an accompanying bronchiolar radius of 100 \( \mu \)m was calculated so that the radius of SPA in relation to that of the accompanying bronchiole could be compared among patients. Only transversely cut airways and SPAs were used for the measurements above. In addition, to avoid the influence of ramifications, we excluded pairs in which arterial and bronchial ramifications occurred in different planes (in such situations, a single bronchus might be accompanied by 2 arteries).

RACs were made according to the description of Emery and Mithal\textsuperscript{13} with the modifications of Cooney and Thurbeck.\textsuperscript{14,15} This procedure measures the number of airways in the secondary lobule and is suitable for material available in a retrospective study. The mean RAC in each patient was calculated from all suitable areas in all available sections. Only uninflated lungs were used for the assessment in our study group.

The severity of the intimal lesions was evaluated by using the Heath-Edwards (HE) classification\textsuperscript{16} and the index of pulmonary vascular disease (IPVD) as described in our previous report.\textsuperscript{11} The IPVD was determined by classifying the pulmonary vascular intimal lesions of SPAs into 4 grades, with ratings of 1 to 4 assigned to each tissue and the mean rating was calculated for each patient. The ratings were based on the following pathologic findings: A rating of 1: no intimal lesions; 2: cellular proliferation of the intima; 3: fibrous thickening of the intima; and 4: destruction of the media.

We also examined all the slides we had prepared for the presence of lymphangiectasia. Lymphangiectasia is a condition in which lymph vessels are excessively dilated, giving rise to clusters of grossly distended lymph vessels in tissue.\textsuperscript{17}

**Statistics**

All values are reported as means ± standard deviation. Because the standard value calculated from the 24 control patients varied with age, statistical comparisons among groups were made by analysis of covariance using age as a covariant factor. The regression equations derived from the correlation between age and histologic parameters are used to describe their relationship, and 5% and 95% confidence limits were calculated. A \( P \) value of less than .05 was considered significant. All statistical analyses were performed using Statview version 5.0J software.

**Results**

**Radii of SPAs When Accompanying Bronchiolar Radius = 100 \( \mu \)m**

The radius of SPA was approximately 60 \( \mu \)m at birth, increased for the first 2 months, and then remained stable at about 80 \( \mu \)m from 2 to 10 months. The radius of SPA in TAPVC patients with preoperative PVO was 47.0 ± 21.8 \( \mu \)m, and significantly smaller than in the controls (75.9 ± 9.8 \( \mu \)m, \( P < .001 \)). The difference between the 2 surviving patients was significant at \( P < .001 \) (33.0 ± 14.6 \( \mu \)m versus 68.2 ± 9.2 \( \mu \)m; Figure 3). The smallest radius was in patient 4, who underwent emergency surgery at 2 days of age because of severe hypoxia and developed severe PH that failed to respond to any therapy before death at 0 postoperative day (Figure 2, B).

**RAC**

The RAC was 4.6 ± 1.5 in the control group, and it tended to increase with age. In the TAPVC patients, the RAC was 4.4 ± 0.8 and not significantly different from the value in the control group (\( P = .71 \)). The difference between the 2 TAPVC groups (dead versus surviving, 4.1 ± 0.8 versus 4.9 ± 0.8; \( P = .19 \)) was not significant. The RAC in all TAPVC patients was within the 5% to 95% confidence limits of the control group (Figure 4).

**Intimal Lesions**

The results for intimal lesions and lymphangiectasia are shown in Table 1. All the IPVD values for intimal lesions in

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**TABLE 1. Patient characteristics**

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Gestational age (wk)</th>
<th>Age at operation (d)</th>
<th>BW at operation (kg)</th>
<th>Anatomic type</th>
<th>Biopsy or autopsy</th>
<th>HE</th>
<th>IPVD</th>
<th>Lym.</th>
<th>Outcome and cause of death</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>36</td>
<td>12</td>
<td>2.9</td>
<td>TAPVCI(1 b)</td>
<td>autopsy</td>
<td>1</td>
<td>1.0</td>
<td>+</td>
<td>Dead at 0 POD (PHC, AVF)</td>
</tr>
<tr>
<td>2</td>
<td>40</td>
<td>18</td>
<td>2.6</td>
<td>TAPVC(II)</td>
<td>autopsy</td>
<td>1</td>
<td>1.0</td>
<td>+</td>
<td>Dead at 5 POD (PHC)</td>
</tr>
<tr>
<td>4</td>
<td>36</td>
<td>2</td>
<td>3</td>
<td>TAPVC(II b)</td>
<td>autopsy</td>
<td>1</td>
<td>1.0</td>
<td>–</td>
<td>Dead at 0 POD (PHC, AVF)</td>
</tr>
<tr>
<td>5</td>
<td>40</td>
<td>14</td>
<td>3.1</td>
<td>TAPVC(II b)</td>
<td>autopsy</td>
<td>1</td>
<td>1.0</td>
<td>+</td>
<td>Dead at 14 POD (PHP)</td>
</tr>
<tr>
<td>6</td>
<td>39</td>
<td>45</td>
<td>3.3</td>
<td>TAPVC(III)</td>
<td>Biopsy (left lung)</td>
<td>2</td>
<td>1.2</td>
<td>+</td>
<td>Dead at 30 POD (PPH, PVS)</td>
</tr>
<tr>
<td>9</td>
<td>38</td>
<td>284</td>
<td>6.2</td>
<td>TAPVC(III)</td>
<td>Biopsy (left lung)</td>
<td>3</td>
<td>1.8</td>
<td>–</td>
<td>Dead at 28 POD (PPH, PVS)</td>
</tr>
<tr>
<td>3</td>
<td>40</td>
<td>43</td>
<td>3.1</td>
<td>TAPVC(III)</td>
<td>Biopsy (left lung)</td>
<td>1</td>
<td>1.0</td>
<td>–</td>
<td>Alive (5 y follow-up)</td>
</tr>
<tr>
<td>7</td>
<td>38</td>
<td>301</td>
<td>7.8</td>
<td>TAPVC(III)</td>
<td>Biopsy (left lung)</td>
<td>3</td>
<td>1.7</td>
<td>–</td>
<td>Alive (5 y follow-up)</td>
</tr>
<tr>
<td>8</td>
<td>27</td>
<td>180</td>
<td>4.3</td>
<td>TAPVC(II)</td>
<td>Biopsy (right lung)</td>
<td>3</td>
<td>1.8</td>
<td>–</td>
<td>Alive (6 y follow-up)</td>
</tr>
<tr>
<td>10</td>
<td>36</td>
<td>30</td>
<td>3.2</td>
<td>TAPVC(II)</td>
<td>Biopsy (right lung)</td>
<td>1</td>
<td>1.2</td>
<td>+</td>
<td>Alive (4 y follow-up)</td>
</tr>
</tbody>
</table>

**Notes:** 1) TAPVC, Pulmonary hypertension crisis; AVF, acute ventilatory failure; PPH, persistent pulmonary hypertension; HE, Edwards classification; IPVD, intrapulmonary vascular disease score; Lym., lymphangiectasia.

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the control patients were 1.0, whereas the mean IPVD score in TAPVC with PVO was 1.3 ± 0.4 (significantly higher than in the control patients at $P < .001$). In 5 of the 10 patients, the scores were 1.0, which means that there were no intimal lesions in the SPAs. Three infants whose IPVD scores were greater than 1.0 survived the operation. The difference between the dead and surviving patients was not significant (1.2 ± 0.3 versus 1.4 ± 0.4; $P = .059$). According to HE classification, there were 6 grade 1 patients, 1 grade 2 patient, 3 grade 3 patients, and no patients at grade 4 or more. It is noteworthy that 2 of the patients with HE grade 3 survived.

**Lymphangiectasia**

Lymphangiectasia was not detected in any of the control patients but was present in 4 of the 6 dead TAPVC patients (66.6%) and in 1 of the 4 surviving TAPVC patients (25%) (control versus TAPVC patients, $P < .01$; dead versus surviving patients, $P = .19$, NS). Interstitial emphysema was seen in all of the patients with lymphangiectasia.

**Medial Thicknesses of SPAs**

In the control group, medial thicknesses ranged from 4.2 to 13.1 μm (7.4 ± 2.2 μm), and in relationship to age, hypertrophy of the media was regressed for the first month after
birth and remained almost stable at approximately 6 to 8 μm. By contrast, the medial thickness of the SPAs in the 10 TAPVC patients with preoperative PVO was 8.7 to 16.9 μm (11.7 ± 2.5 μm, significantly thicker at \( P < .001 \)). The difference in medial thickness of the SPAs between the dead patients and surviving patients was not significant (12.2 ± 2.8 μm versus 11.1 ± 2.3 μm; \( P = .60 \)). There was no clear age difference between the TAPVC groups (Figure 5, A).

**Medial Thickness of SPVs**

The medial thickness of SPVs in the control group was 0.7 to 4.0 μm (2.2 ± 1.0 μm) and remained constant at approximately 2 to 3 μm after birth. Medial thickness of the SPVs in the TAPVC patients was 1.1 to 7.8 μm (4.5 ± 2.3 μm), significantly greater than in the control group (\( P = .001 \)). There was a slight difference between the dead and surviving patients with medial thickness in the surviving patients

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**Figure 2.** There was a significant correlation between bronchiolar radii and the radii of accompanying small pulmonary arteries on a logarithmic coordinate system. The radius of small pulmonary arteries at a bronchiolar radius of 100 μm was calculated based on linear regression and used for comparative analysis.

**Figure 3.** The radii of small pulmonary arteries at a bronchiolar radius of 100 μm are plotted against age in 24 control patients and 10 TAPVC patients. The logarithmic curve fit with 5% and 95% confidence limits is also shown. A significant difference between control patients and TAPVC with preoperative PVO patients was found, and the radii in the deceased patients were significantly smaller than in the surviving patients.
being thicker than that in the dead patients (6.4 ± 1.3 μm versus 3.3 ± 1.8 μm; \( P = .039 \); Figure 5, B).

**Discussion and Conclusion**

In spite of the great improvements in preventing pulmonary hypertension, almost all deaths after repair of TAPVC are associated with eventual pulmonary hypertensive events. Persistent PH or PH crisis after repair of other congenital heart anomaly has been reported to be caused by plexogenic arteriopathy, especially by irreversible intimal lesions. However, as previously reported, the intimal lesion in TAPVC is not particularly severe, and the pathogenesis of PH events related to poor outcomes does not appear to be solely plexogenic arteriopathy.

Several published reports have discussed the problem of underdevelopment of the pulmonary vasculature or of reduction in the number of lung vessels. This problem was first reported in primary plexogenic arteriopathy and subsequently in other types of congenital heart disease. However, counting SPAs has been questioned, because the number of SPAs was demonstrated not to be decreased or to even be increased in other studies. Mooi and Wagen-voort stated that the controversy regarding underdevelopment or disappearance of lung vessels centers to a large extent on methodology. One reason, they say, is that almost all histological studies in which a decrease in the number of lung vessels has been reported were based on lungs subjected to vessel filling at a pressure of 100 cm of water. However, such high pressures produce unpredictable results and may sometimes cause such excessive dilatation of blood vessels that small vessels become unidentifiable and, hence, are not counted. Another reason is the size of the field of lung tissue used for quantitative analysis being relatively small, as pulmonary arteries are not distributed evenly throughout the lung tissue. To eliminate these influences, in our study (1) only uninflated lungs not injected under pressure were used, and (2) we calculated the ratio between SPAs and accompanying bronchiole in at least 20 pairs to compare patients and demonstrate hypoplasia.

The relationship between bronchial diameter and arterial diameter in normal human lungs was first reported by Be-mo, and colleagues in 1979. They measured 8 lungs from 4 adult patients without lung disease and established an average ratio between internal bronchial and external arterial diameter of 0.62. However, they included the adventitia in the latter diameter, which is rather unusual and produces variable results. Moreover, bronchioles contract after death, and the degree of constriction needs to be corrected for. We therefore measured the length of the basement membrane and estimated the diameter of bronchioles in a state of complete extension.

Our results show that in obstructed-type TAPVC patients, SPAs are reduced in size in comparison with accompanying bronchioles. In the control group, the radius of the SPAs when the accompanying bronchiole had a radius of 100 μm was approximately 60 μm at birth and gradually increased to 80 μm by 2 months of age. By contrast, in the dead patients, the smallest diameter was 14.6 μm and the largest was 33.0 μm. It seems reasonable to assume that underdevelopment of the SPAs results in increased pulmonary vascular resistance despite the presence of only mild intimal lesions. The pressure in the pulmonary arteries then
increases, and the media of SPA hypertrophy proportionately. Under these circumstances, pulmonary hypertension persists even after surgical repair, and PH crisis with right ventricular failure can easily occur in response to minor stimuli.

The pathogenesis of the reduced size of SPAs is not understood. Some studies have shown that banding the pulmonary artery in the newborn pig leads to the development of fewer and smaller intra-acinar arteries than normal,\textsuperscript{26} and the SPAs of patients with pulmonary atresia are said to be smaller than normal.\textsuperscript{27,28} Although blood flow in fetuses with TAPVC and preoperative PVO has not yet been investigated, it can be assumed that pulmonary blood flow is inhibited by the increased pulmonary venous pressure caused by venous obstruction. In this study we were able to obtain tissue from obstructed-type TAPVC patients and normal controls without any PVO. Further investigation, including unobstructed TAPVC patients, is needed to clarify the role of PVO in the pathogenesis of SPA hypoplasia.

The RAC is said to reflect the degree of alveolar hypoplasia or alveolar surface complexity, which is a significant component in hypoplasia of the lung and parallels the lung maturity.\textsuperscript{13,14,29} George and colleagues\textsuperscript{15} reported 10 infants with congenital diaphragmatic hernia who died in the immediate perinatal period; the RAC was reduced in every patient, possibly due to persistent lung compression during

Figure 5. A, Medial thickness of small pulmonary arteries (\(D_R=100\mu m\)), plotted against age in 10 TAPVC patients and 24 control patients. B, Medial thickness of small pulmonary veins (\(D_R=100\mu m\)), plotted against age in the TAPVC and control patients.
the fetal period. In our study, however, the RAC was not significantly reduced in TAPVC patients, and all values were within the 5% to 95% confidence limits of the control curve. The mechanism of the vascular hypoplasia in TAPVC is different from the mechanism of the alveolar immaturity. Lymphangiectasia has been reported in approximately 30% of all TAPVC patients, and in the present study it was found in 50% of the patients. This was probably because our group was limited to obstructed TAPVC patients. In contrast to the rate of 25% in the surviving TAPVC patients, 66% of dead patients showed lymphangiectasia, which may be related to hypoplasia of SPAs and poor outcome.

In the clinical setting, it may be very difficult to detect hypoplasia of SPAs by routine diagnostic procedures. The degree of hypoplasia in which patients can survive operations is unclear. As a possible treatment, patent ASDs may also mitigate PH events in severe hypoplasia. As a means of facilitating catheter access and balloon dilatation for intervention in secondary PVO, electively leaving a small ASD facilitating catheter access and balloon dilatation for intervention in secondary PVO, electively leaving a small ASD at the time of repair might have recently come into favor. This may also facilitate the RL shunt and decrease PA pressure in PH crisis. Once there is no further evidence of PH events or postoperative PVO, the ASD can be closed by transcatheter techniques.

There were limitations to this study. It was a retrospective study, and the number of patients was limited. The series of patients does not represent a random sample of patients who underwent TAPVC repair during the study period. As might be expected, autopsy cases are overrepresented in our series. Because the study group was limited to obstructed-type TAPVC, no comparisons between obstructed- and unobstructed-type TAPVC were made in this study. However, our results suggest that SPA hypoplasia is present at least in obstructed-type TAPVC, and the degree of hypoplasia may be related to an unfavorable outcome. Application of this method may allow other problems associated with poor mortality in congenital heart disease to be solved.

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References


