Evidence of Pulmonary Vascular Reverse Remodeling After Pulmonary Artery Banding Performed in Early Infancy in Patients With Congenital Heart Defects

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Background: Histomorphometric evidence of the effect of pulmonary artery banding (PAB) in infancy on pulmonary vascular reverse remodeling has not been fully described.

Methods and Results: We retrospectively reviewed 34 patients who underwent serial lung biopsies before and after PAB. Index of pulmonary vascular disease (IPVD) as a measure of the degree of progression of pulmonary arteriopathy significantly decreased after PAB (1.22±0.25 at 1st and 1.13±0.21 at 2nd biopsy, P=0.04). Additionally, $D_{R=100\mu m}$ as an indicator of medial thickness of pulmonary arteries significantly decreased after PAB (15.6±3.7 at 1st and 11.4±2.6 at 2nd biopsy, P<0.0001). Patients were divided into 3 groups by age at PAB: <3 months (Group 1), between 3 and 6 months (Group 2), and >6 months (Group 3). The average second $D_{R=100\mu m}$ of groups 1 and 2 was significantly lower than that of group 3 (11.1±2.2 and 9.8±2.0 vs. 14.9±2.8, respectively; P<0.0001). Additionally, the second IPVD was also significantly lower in groups 1 and 2 than in group 3 (1.1±0.2 and 1.1±0.2 vs. 1.3±0.4, respectively; P=0.02).

Conclusions: Histomorphometric evidence of post-PAB pulmonary vascular reverse remodeling is robust. The magnitude of vascular reversibility is pronounced when PAB is performed before 6 months of age.

Key Words: Congenital heart defects; Pulmonary arteriopathy; Pulmonary artery banding; Reverse remodeling

Pulmonary artery banding (PAB) was introduced in 1952 as a palliative surgical procedure for the treatment of congenital heart diseases with excessive pulmonary blood flow. The technique has been extensively used as part of a staged approach for the surgical correction of congenital heart defects. The procedure is also used for patients who are unable to withstand primary corrective surgery for congenital heart defects because of associated lung pathology and anatomical complexity. PAB is aimed at hemodynamic restoration and reduction of excessive blood flow and pressure in the pulmonary circulation, thus protecting the pulmonary vasculature from irreversible changes that may otherwise lead to severe pulmonary arteriopathy. Reverse remodeling of pulmonary arteriopathy following PAB was once reported by Wagenvoort et al more than 30 years ago; medial hypertrophy and intimal lesions appeared to improve with this treatment. To the best of our knowledge, that report is the only one describing the reversibility of pulmonary artery lesions; however, histomorphometric evidence of such reversibility has not yet been well established. Additionally, the optimal timing for PAB from the viewpoint of reversibility of pulmonary vascular lesions has not been fully described. The aim of this study was to evaluate pulmonary vascular reverse remodeling after PAB using serial lung biopsy specimens and to draw conclusions on the optimal timing for PAB in terms of pulmonary vascular reverse remodeling.

Subjects
Numerous lung biopsy and autopsy specimens have been collected from multiple centers in Japan for diagnosing pulmonary vascular disease (PVD). Out of a total of 1,293 lung biopsy and autopsy specimens collected at our institution between January 2001 and December 2015, 41 patients who had undergone lung biopsies before and after PAB were identified. Of these, 34 patients had a ventricular septal defect (VSD) or atrioventricular septal defect (AVSD) and they were selected as the cohort for the present study. The other 7 patients with complex cardiac anomalies, such as single ventricular morphology, were excluded to evaluate...
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The cross-section of the vessel was hypothetically transformed to a state in which the internal elastic lamina was completely stretched to a circle. In that state, each pulmonary artery’s radius ($R$), the distance from the center of an artery to the midpoint of the media, and medial thickness ($D$) were calculated. $R$ and $D$ were evaluated for more than 15 vessels in each case and plotted on a logarithmic coordinate system. Subsequently, linear regressions of log $R$ and log $D$ were performed, and the $D$ value at $R=100\,\mu\text{m}$ ($D_{R=100}\,\mu\text{m}$) was compared among cases.

Representative histological findings with elastica-Masson staining of small pulmonary arteries with diameters of approximately $400\,\mu\text{m}$ at the 1st and 2nd biopsies are shown in Figure 1. On the 1st biopsy, medial hypertrophy is identifiable (Figure 1A), but the 2nd biopsy shows thinning of the medial muscular layer, and intimal lesions are not seen (Figure 1B). The correlation between radius and medial thickness of the small pulmonary arteries measured in the 1st and 2nd biopsies from the same case is described in Figure 1C. Examples of small pulmonary arteries (diameter $400\,\mu\text{m}$) at (A) the 1st and (B) the 2nd biopsy in a representative case. (C) Correlation between the radius ($R$) and medial thickness ($D$) of multiple small pulmonary arteries on a logarithmic coordinate system as assessed by separate series of histomorphometry analysis of the 1st and 2nd biopsy derived from the same case. Representative values of medial thickness ($D$ value) at $R=100\,\mu\text{m}$ ($D_{R=100}\,\mu\text{m}$) were calculated on linear regression of $R$ and $D$ values for comparative analysis.

Tissue Preparation
Lung tissue was obtained from a lobe of the lung, fixed in 10% formalin, and paraffin-embedded sections were prepared. In each case, 30 semi-serial histological sections at 50-$\mu\text{m}$ intervals (each 3-$\mu\text{m}$ thick) were prepared as previously described, and elastica-Masson staining performed.

Measurements
The medial thickness of the small pulmonary arteries was measured using a computerized method proposed by Yamaki and Tezuka. The cross-section of the vessel was hypothetically transformed to a state in which the internal elastic lamina was completely stretched to a circle. In that state, each pulmonary artery’s radius ($R$), the distance from the center of an artery to the midpoint of the media, and medial thickness ($D$) were calculated. $R$ and $D$ were evaluated for more than 15 vessels in each case and plotted on a logarithmic coordinate system. Subsequently, linear regressions of log $R$ and log $D$ were performed, and the $D$ value at $R=100\,\mu\text{m}$ ($D_{R=100}\,\mu\text{m}$) was compared among cases.

The requirement for informed consent was waived by the review board.

Figure 1. Examples of small pulmonary arteries (diameter $400\,\mu\text{m}$) at (A) the 1st and (B) the 2nd biopsy in a representative case. (C) Correlation between the radius ($R$) and medial thickness ($D$) of multiple small pulmonary arteries on a logarithmic coordinate system as assessed by separate series of histomorphometry analysis of the 1st and 2nd biopsy derived from the same case. Representative values of medial thickness ($D$ value) at $R=100\,\mu\text{m}$ ($D_{R=100}\,\mu\text{m}$) were calculated on linear regression of $R$ and $D$ values for comparative analysis.
To evaluate the influence of age at PAB on pulmonary vascular remodeling, we divided the patients into 3 groups based on the timing of PAB and compared the measurements among the groups. Currently, PAB is often performed before 6 months of age, and therefore, we first divided the patients at 6 months of age, and the younger patients were further subdivided at 3 months of age. The groups were categorized as follows: group 1 (n=17): <3 months of age; group 2 (n=11): 3–6 months of age; and group 3 (n=6): >6 months of age. All measurements were performed by an observer who was unaware of the patient grouping.

In order to evaluate the risk factors for irreversible PVD post-PAB, we divided the patients into 2 groups with and without irreversible PVD, which was defined as follows: intimal fibrous thickening, or >grade 3 of the Heath-Edwards classification. There were 6 patients with irreversible PVD at 2nd biopsy. We compared the baseline variables between the 2 groups.

### Statistical Analysis
Data are expressed as mean±standard deviation, median (range), or as frequency (percentage). The histomorphometric values before and after PAB were compared using paired t-test. Intergroup differences were assessed by one-way analysis of variance with repeated measures (ANOVA), followed by post-hoc Tukey test. To compare associations between variables were assessed on linear regression analysis and coefficients for the observed correlations calculated. P<0.05 was considered statistically significant. JMP software (SAS, Cary, NC, USA) was used for data analysis.

### Results

#### Patients’ Characteristics
The median age at first biopsy was 2.8 months (range, 1–156 months); the median interval time between the 1st and 2nd biopsies was 10.5 months (range, 1–68 months; Table 1). Clinically, 23 patients (67.7%) were diagnosed with VSD, and 11 patients (32.3%) were diagnosed with AVSD. In addition, 23 patients (67.7%) had Down syndrome. Hemodynamic data obtained at pre-PAB catheterization...
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in 17 patients revealed pulmonary hypertension in all of them. Systolic pulmonary arterial blood pressure (PAP) was 70.5±14.9 mmHg; the pulmonary to systemic blood pressure ratio was 0.9±0.1, and the pulmonary vascular resistance (PVR) index was 5.3±1.6 Wood units/m².

Histologic and Histomorphometric Changes Between the 2 Biopsy Examinations

The DR=100μm of all patients for the 1st and 2nd biopsies are shown in Figure 2A. The DR=100μm for the 2nd biopsy was significantly smaller than that for the 1st biopsy according to a paired t-test (15.6±3.7 at 1st biopsy and 11.4±2.6 at 2nd biopsy, P<0.0001). In addition, the intimal lesion (IPVD score) at 1st biopsy (1.13±0.21) was significantly less than that at the 2nd biopsy (1.22±0.25, P=0.04; Figure 2B). The correlation of the distal systolic PAP measured during the catheterization performed immediately after PAB with the medial thickness of the small pulmonary arteries at the 2nd biopsy is shown in Figure 3 (n=25). A decrease in the post-PAB PAP was associated with thinning of the medial muscular layer of the small pulmonary arteries (r=0.60).

The results of group comparisons are shown in Table 2, Figure 4 and Figure 5. The time interval between the 1st and 2nd biopsies tended to be longer in group 2 and group 3 compared with group 1. The first DR=100μm in group 1 was significantly greater than that in groups 2 and 3 (17.4±3.8, 13.8±2.8* and 13.9±2.8, respectively, P<0.05 vs. Group 1).

Table 2. Variables Used for Group Assessments

<table>
<thead>
<tr>
<th></th>
<th>Group 1 (n=17)</th>
<th>Group 2 (n=11)</th>
<th>Group 3 (n=6)</th>
<th>P value</th>
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<tr>
<td>Age at 1st biopsy (months)</td>
<td>1.6±0.6</td>
<td>3.5±0.8</td>
<td>61.8±69.5</td>
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<td>Age at second biopsy (months)</td>
<td>9.6±5.4</td>
<td>22.0±18.5</td>
<td>84.2±71.1</td>
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<td>Interval time (months)</td>
<td>8.0±5.3</td>
<td>19.0±18.2</td>
<td>22.3±22.9</td>
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<td>AVSD</td>
<td>5 (29)</td>
<td>6 (55)</td>
<td>0 (0)</td>
<td>0.07</td>
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<tr>
<td>Trisomy 21</td>
<td>13 (76)</td>
<td>7 (64)</td>
<td>3 (50)</td>
<td>0.46</td>
</tr>
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<td>sPAP at 1st biopsy</td>
<td>73.0±11.4</td>
<td>62.3±16.9</td>
<td>79.4±10.5</td>
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</tr>
<tr>
<td>sPAP at 2nd biopsy</td>
<td>35.7±20.4</td>
<td>33.7±13.4</td>
<td>51.3±16.8</td>
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<tr>
<td>mPAP at 1st biopsy</td>
<td>45.0±9.3</td>
<td>41.0±12.3</td>
<td>54.4±8.4</td>
<td>0.12</td>
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<tr>
<td>mPAP at 2nd biopsy</td>
<td>23.0±13.7</td>
<td>22.8±12.7</td>
<td>31.7±11.1</td>
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<td>PVR at 1st biopsy</td>
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<td>4.9±0.6</td>
<td>5.8±0.7</td>
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<tr>
<td>PVR at 2nd biopsy</td>
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<td>DR=100μm at 1st biopsy</td>
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<tr>
<td>DR=100μm at 2nd biopsy</td>
<td>11.1±2.2</td>
<td>9.8±2.0</td>
<td>14.9±1.4**</td>
<td>&lt;0.0001</td>
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<td>2nd/1st DR=100μm ratio</td>
<td>0.7±0.2</td>
<td>0.7±0.2</td>
<td>1.1±0.2**</td>
<td>0.0004</td>
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<td>H-E grade at 1st biopsy</td>
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<td>2 (18)</td>
<td>1 (17)</td>
<td>0.33</td>
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<tr>
<td>1</td>
<td>6 (35)</td>
<td>6 (55)</td>
<td>2 (33)</td>
<td>1.00</td>
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<tr>
<td>2</td>
<td>3 (18)</td>
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<td>3 (50)</td>
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<tr>
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<td>0 (0)</td>
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<tr>
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<td>2 (12)</td>
<td>1 (9)</td>
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<tr>
<td>4–6</td>
<td>0 (0)</td>
<td>0 (0)</td>
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<tr>
<td>IPVD at 1st biopsy</td>
<td>1.1±0.2</td>
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<td>1.3±0.3</td>
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<tr>
<td>IPVD at 2nd biopsy</td>
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<td>1.1±0.2</td>
<td>1.3±0.4**</td>
<td>0.02</td>
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<tr>
<td>2nd/1st IPVD</td>
<td>1.0±0.1</td>
<td>0.8±0.1</td>
<td>1.1±0.1**</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Data are presented as mean±SD, or n (%). *P<0.05 vs. Group 1, **P<0.01 vs. Group 1, †P<0.05 vs. Group 2, ‡P<0.01 vs. Group 2. H-E, Heath-Edwards classification; IPVD, index of pulmonary vascular disease; mPAP, mean pulmonary arterial pressure; PVR, pulmonary vascular resistance; sPAP, systolic pulmonary arterial pressure.
be larger in groups 2 and 3 compared with group 1 (1.3±0.3, 1.3±0.3, and 1.1±0.2, \( P=0.09 \)); however, IPVD in group 2 was significantly reduced after PAB (1.34±0.27 at 1st biopsy and 1.05±0.08 at 2nd biopsy, \( P=0.003 \)). Therefore, the IPVD at 2nd biopsy was significantly larger only in group 3 compared with the other 2 groups (Figure 5B).

With regard to the irreversible PVD that we defined as Heath-Edwards classification grade 3 severity, such lesions were observed in 9 patients (3 patients in each group) at the 1st biopsy (Table 2). Of these, 5 patients (2 in group 1, 2 in group 2, 1 in group 3) exhibited diminished irreversible PVD at the 2nd biopsy, but the remaining 4 patients retained
irreversible PVD. On the other hand, irreversible PVD was observed at 2nd biopsy in 2 patients among the 25 patients who did not have irreversible PVD at 1st biopsy. One patient with VSD associated with Down syndrome underwent PAB at 10 months of age, and his mean PAP remained >30 mmHg. Another Down syndrome patient with AVSD went PAB at 10 months of age, and his mean PAP remained high despite PAB. The chromosomal anomaly of trisomy 21 was not observed at 2nd biopsy in 2 patients among the 25 patients who did not have irreversible PVD at 1st biopsy. One patient with VSD associated with Down syndrome underwent PAB at 10 months of age, and his mean PAP remained >30 mmHg. Another Down syndrome patient with AVSD went PAB at 10 months of age, and his mean PAP remained high despite PAB.

### Risk Analysis of Irreversible PVD

The group comparisons for the risk analysis of irreversible PVD are shown in Table 3. The patients with irreversible PVD tended to be older. Additionally, high baseline PAP and IPVD at 1st biopsy were significant risk factors for irreversible PVD at post-PAB. The chromosomal anomaly of trisomy 21 and the diagnosis of AVSD were not identified as risk factors for irreversible PVD.

### Discussion

In the present study, we characterized reverse remodeling of small pulmonary arteries induced by PAB using serial lung biopsies, obtained before and after PAB in 34 patients with either VSD or AVSD. In addition, we demonstrated a positive correlation between post-PAB PAP and medial thickness on the 2nd biopsy. Moreover, the benefit of PAB in inducing reverse remodeling of small pulmonary arteries appeared to be pronounced in patients who underwent PAB at a younger age, which has been proved for the first time. In recent years, PAB is less often performed than before because intracardiac repair can be performed safely at a younger age; however, this procedure remains the surgical option of choice for a subgroup of patients with congenital heart disease associated with seemingly advanced pulmonary hypertension. Additionally even in several recent reports PAB was performed at various ages, and the optimal timing of PAB was not been fully elucidated. We believe that the results of our study corroborate the fundamental histologic basis for possible reverse remodeling of PVD by PAB.

In patients who have a congenital heart defect with left to right shunt, the volume and pressure overload on the pulmonary vascular bed provoke pulmonary arteriopathy in early infancy. In the present study, more than half of the patients had some degree of pulmonary arteriopathy, such as intimal cellular hyperplasia or intimal fibrosis. Several reports have documented complex lesions such as plexiform or dilated lesions and arteritis in the patients with congenital heart defects. However, such lesions were not observed in this cohort, possibly because of the early intervention (surgical palliation or correction of congenital heart disorders) in these patients. The median age at 1st biopsy in this cohort was 2.8 months, which is much lower than reported in earlier studies. The efficacy of corrective surgery, if performed at an early age, in alleviating or reversing pulmonary vascular lesions is well documented. These findings, however, have essentially been based on improvement in clinical hemodynamic data such as PVR and PAP. The histomorphologic reverse remodeling changes have not been fully described. Only 1 report has described the reverse remodeling of small pulmonary arteries by PAB using serial lung biopsy or autopsy specimens. In that study, serial lung biopsy or autopsy specimens were obtained at the time of PAB and at the time of corrective operation in 28 patients. Medial hypertrophy showed a significant tendency to regress, and early intimal lesions such as cellular hyperplasia were found to have been reversed. Those results were similar to the present study; however, the mean age of the patients at PAB in the previous report was considerably higher than in our study population. In addition, severe intimal lesions were identified in several patients and it was difficult to accurately evaluate the medial thickness of the small pulmonary arteries in such cases because the borderline between the media and intima was often unclear, and therefore, it was difficult to precisely assess the difference between the pre- and post-PAB medial thickness.

Medial hypertrophy detected in congenital heart defect patients is considered to be generally a reversible lesion and it invariably improves after corrective surgery or PAB. Additionally, hypoxia-induced medial hypertrophy is also considered to be reversible on restoration of normoxia.

In our study, medial hypertrophy was reversible in most patients, but was not fully achieved in patients aged >6 months at PAB. This finding is different from that in the previous report, which showed reverse remodeling of medial hypertrophy in patients who underwent PAB at over 6 months of age. However, most of the patients in that
study had severe intimal lesions that had progressed in the time between PAB and corrective surgery. Therefore, whether the thinning of media was a regression associated with PAB or whether it represented secondary atrophic change in arteries with severe intimal lesions could not be evaluated.

Cellular intimal hyperplasia and mild intimal fibrosis are also considered to be reversible; however, severe concentric intimal fibrosis and other complex lesions such as plexiform lesions and dilatation lesions are thought to be irreversible.\(^{9,10}\) In the present study, children with VSD or AVSD at 3–6 months of age had slightly more progressed intimal lesions (cellular intimal hyperplasia) than children <3 months of age. However, reverse remodeling of these lesions was observed after PAB. Otherwise, children older than 6 months of age also had a comparable level of severity of intimal lesions. In these children, reverse remodeling of the intimal lesions could not be fully achieved via PAB. Half of the children over 6 months of age had intimal fibrosis of the pulmonary arteries, which was not amenable to remodeling. Therefore, their IPVD scores were similar to those in 3–6-month-old children, although the severity of the intimal lesions was likely to be different. Irreversible pulmonary vascular changes would begin to progress in children aged over 6 months.

We demonstrated a positive correlation between post-PAB systolic PAP and \(D_{50}=100\) \(\mu\)m at 2nd biopsy. However, in some cases, the medial thickness and intimal lesions worsened even with a considerable decrease in the post-PAB PAP, and therefore we could not obtain a cutoff point of PAP as the threshold for reverse remodeling of these lesions. Besides the age at PAB, some factors other than the PAP affect the reverse remodeling of small pulmonary arteries.

### Study Limitations

The retrospective nature of this study and the small sample size are key limitations of our study. Lung specimens were collected from multiple institutions, and therefore the indications for PAB and lung biopsy were different among the institutions. In addition, although observed in a small number of patients, the pulmonary vascular lesions did not regress completely even after PAB, which implies that PAB may not resolve all pulmonary vascular lesions histopathologically. Moreover, therapeutic agents for pulmonary hypertension were used in some cases; however, the influence of these drugs on reverse remodeling of pulmonary arteriopathy could not be evaluated in this study and definitely a topic for future study. Furthermore, the effect of pulmonary artery reverse remodeling on postoperative improvement or reversal of pulmonary hypertension is an aspect that warrants further investigation.

### Conclusion

PAB in VSD and AVSD patients can induce pulmonary vascular reverse remodeling as demonstrated by significant pulmonary artery medial thinning and improvement in intimal lesions. In addition, the magnitude of the reversibility of pulmonary vascular lesions correlated with post-PAB distal PAP and was found to be most pronounced when PAB was performed before 6 months of age.

### Acknowledgment

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### References