Hypoplasia of the Small Pulmonary Arteries in Hypoplastic Left Heart Syndrome With Restrictive Atrial Septal Defect

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Background—Restrictive atrial septal defect (ASD) (including intact atrial septum [IAS]) has been reported to be a risk factor that negatively impacts survival in hypoplastic left heart syndrome (HLHS). Although lymphangiectasia and “arterialization” of the veins of the lung in HLHS with restrictive ASD have been reported, they cannot fully explain the high mortality. We have introduced a new method of evaluating the development of the pulmonary vasculature in histological sections and used it to assess patients’ lungs. We tested the hypothesis that the small pulmonary arteries (SPA), which are pulmonary arteries in a histological section whose radii are ∼25 μm to 250 μm, in HLHS with restrictive ASD are hypoplastic, but that the alveoli are not, to elucidate the mechanism underlying the poor outcome of these patients.

Methods and Results—Fourteen HLHS patients between 1 day and 40 days of age were studied. In 8 cases, the ASD was restrictive [R(+) group], and in the other 6 cases it was not [R(−) group]. Specimens from 12 autopsies of cases with no congenital heart or pulmonary disease were examined as a control group (C group). As a novel histological parameter, we assessed the size of SPA in relation to the size of accompanying bronchioles to identify SPA underdevelopment. To evaluate the development of alveoli and interstitial tissue, radial alveolar counts (RAC), which reflect alveolar maturity and complexity, were also performed. Statistical comparisons between groups were made by analysis of covariance with age as a covariant factor. When the radius of the accompanying bronchiolo was 100 μm, the radius of the SPA was 34.0±10.8 μm in the R(+) group, and significantly lower than the 46.6±8.5 μm in R(−) group (P=0.0022) and 70.5±8.4 μm in the C group (P<0.0001). The RAC was in 3.5±0.9 in the R(+) group, 3.4±0.6 in the R(−) group, and 3.7±0.9 in the C group (no significant differences between groups).

Conclusion—The SPA in HLHS with restrictive ASD were underdeveloped compared with the SPA in HLHS with nonrestrictive ASD and the controls, but their alveoli were not hypoplastic. Based on these results, it is speculated that SPA hypoplasia may be responsible for the poor outcome of HLHS with restrictive ASD. (Circulation. 2004;110[suppl II]:II-139–II-146.)

Key Words: hypoplastic left heart syndrome ■ restrictive atrial septal defect ■ lung pathology ■ hypoplastic pulmonary artery

The staged reconstructive operation by the Norwood procedure has markedly improved operative survival in hypoplastic left heart syndrome (HLHS) over the years,¹ and an early study identified significant tricuspid valve regurgitation as the only physiological factor that negatively affected early outcome.² However, recent experience has suggested that the presence of a restrictive atrial septal defect (ASD) or intact atrial septum (IAS) without a decompression pathway negatively affects the survival of infants who have undergone cardiac transplantation or Norwood palliation for HLHS.³,⁴ Although the reason for the poor outcome in these patients even after cardiac transplantation has been speculated to be pulmonary pathology, such as increased medial thickness of the pulmonary arteries and veins or lymphangiectasia,⁵–⁷ the mechanism has yet to be clearly identified.

Based on the aforementioned, to identify the mechanism, we collected lung specimens taken from HLHS cases with and without restrictive ASD and investigated various histological characteristics to determine the cause of the poor outcome of patients with restrictive ASD. To complement the various morphological analyses of the lungs in HLHS in previous studies, we used a novel technique described in our study in total anomalous pulmonary venous connection (TAPVC)⁸ to investigate the intrapulmonary arterial system. More specifically, we were interested in identifying underdevelopment of small pulmonary arteries (SPA), which are
pulmonary arteries with muscular coats observed in biopsy specimens; their radii ranges from 25 µm to 250 µm. Although various types of pulmonary vascular lesions have been reported in HLHS with restrictive ASD, few have reported hypoplasia or underdevelopment of pulmonary arteries. One reason for this is that there had been no clear definition of SPA hypoplasia. We therefore turned our attention to the bronchiole that always accompany SPA and compared their diameters (Figure 1 and Figure 2A and 2B)

The purpose of this study was to evaluate the pulmonary vascular morphology of neonates with HLHS and to determine whether a restrictive atrial septal defect is associated with more severe pulmonary vascular changes. We hypothesized that HLHS patients with restrictive ASD have more severely hypoplastic small pulmonary arteries.

Materials and Methods

Fourteen cases of HLHS in infants ranging from 2 days to 40 days of age (mean age, 14.4 ±14.4 days) at the time of death (13 cases) or biopsy (1 case) between 1994 and 2003 were studied after obtaining informed consent from the infants' relatives. We retrospectively reviewed the echocardiography database and classified the patients into 2 groups according to the status of their atrial septum. Based on a previous study, a criterion for inclusion in the restrictive ASD [R(+) group] was having a mean spectral Doppler gradient of 8 mm Hg or more (a velocity of more than ~1.5 m/s in velocity) from the left atrium to the right atrium across the atrial septum at the time of birth. Two cases with intact atrial septum were included in R(-) group. The criterion for inclusion in the nonrestrictive ASD [R(−) group] was a mean gradient of <8 mm Hg. According to disease type, there were 9 MA/AA cases, 3 MS/AA cases, and 2 MS/AS cases. The clinical profiles and outcomes of the patients are summarized in the Table. The size of the ASD was determined from the maximal width of the color jet flow on the left atrial side of the communication on the subcostal long-axis and short-axis views. Tricuspid regurgitation was assessed by color flow mapping in the apical 4-chamber view with the ratio of color flow jet area to right atrial area used to determine its magnitude (none, grade 0; area ratio <0.2, grade 1; area ratio 0.2 to 0.4, grade 2; and area ratio >0.4, grade 3).

As a control group (C group), we examined tissue from 12 autopsy cases with no congenital heart or pulmonary disease and in which death occurred between 1 and 50 days of age (mean age, 14.0 ±15.2 days). All control group infants had been born full-term. Five of them had died of infection, 2 as a result of accidents, 3 from brain hemorrhage, 1 from a tumor, and 1 from a metabolic disorder.

Tissue Preparation

One block of tissue from each lung was fixed in 10% formalin, and paraffin histological sections were prepared. To prevent the influence of overextension or underextension of SPA by fluid pressure, the tissue was not injected with any fluid under pressure during fixation. Thirty step sections at 50-µm intervals were prepared in each case. Elastica–Goldner staining was performed, and the following were assessed: (1) severity of intimal lesions; (2) radius and medial thickness of SPA and small pulmonary veins (SPV), which are pulmonary veins with a muscular coat observed in biopsy specimens; their radius range is ~25 µm to 250 µm; (3) radius of bronchioles, and the radius of SPA accompanying the bronchioles (supernumeral arteries12,13 that did not run parallel to the bronchioles were excluded); (4) radial alveolar counts (RAC), which reflects lung maturity; and (5) the presence of lymphangiectasia. Slides of pathology specimens were qualitatively and quantitatively examined by the investigators blinded to the status of the atrial septum.

Measurements

The medial thickness (Dₐ) and radius (Rₐ) of the SPA and SPV were measured by a previously reported morphohistometric method. All SPA were scanned with a digitizing camera (Olympus PDMC/I/
OL), and the images were fed into a Macintosh computer (PowerMac G4). All morphohistological measurements below were performed using National Institutes of Health image version 1.53 software. Assuming uniform thickness, $D_A$ and $R_A$ were calculated from the length of the internal elastic membrane ($L_{iA}$) and the area of the media ($S_A$) by means of the equation (Figure 3A):

$$D_A = \left[ L_{iA}^2 + 4 \pi S_A \right]^{1/2} = L_{iA} / 2 \pi$$

$$R_A = S_A / \left[ L_{iA}^2 + 4 \pi S_A \right]^{1/2}$$

The values of $R_A$ and $D_A$ were obtained from at least 20 cases from each case and plotted on a logarithmic coordinate system. Linear regressions of these 2 radii were performed, and the SPA radius when the radius of the accompanying bronchiole was 100 µm was calculated, so that the radii of SPA in relation to the accompanying bronchiole could be compared among cases. Only transversely cut airways and SPA were used for these measurements. To avoid the influence of branching, we excluded pairs in which the arterial and bronchial branching occurred in different planes (in such situations, a single bronchus might be accompanied by 2 arteries).

RAC were made according to the description of Emery and Mithal, with the modifications of Cooney and Thurbuck. A perpendicular line was drawn from the center of a respiratory bronchiole lined by epithelium in one part of the wall back to the nearest, definite connective tissue septum, and the number of alveoli that the line passed through was counted. The procedure measures the number of airways in the secondary lobe and is suitable for material available in a retrospective study. The mean RAC in each case was calculated from all suitable areas in all available sections. Only uninfated lungs were used for the assessment in our study group.

The severity of the intimal lesions was evaluated by using the Heath–Edwards classification and the index of pulmonary vascular disease as described in our previous report. The index of pulmonary vascular disease was determined by classifying the pulmonary vascular intimal lesions of the SPA into 4 grades, with a rating of 1 to 4 assigned to each artery, and the mean rating was calculated for each case. The ratings were based on the following pathologic findings: 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 of the slides we had prepared for the presence or absence of lymphangiectasia. Lymphangiectasia is a condition in which lymph vessels are excessively dilated, giving rise to clusters of grossly distended lymph vessels in tissue. If markedly dilated lymphatics having a thin endothelial cell lining were observed within most of the interlobular septa and beneath the visceral pleural surface, then lymphangiectasia was concluded to be present.
demographic data for the patients in the R(+) group and R(−) group are shown in the Table. No significant differences between them were noted with regard to gestational age, sex, body weight, age at death, age at the time of Norwood procedure, aortic root diameter, or degree of tricuspid regurgitation.

The causes of death in the R(+) group were extensive hypoxia resulting in preoperative death in 2 cases (balloon

Atrial septostomy [BAS] had been performed in 1 of them, but no intervention had been performed in the other), severe hypoxia despite reconstruction of BTS or RV-PA conduit in 5 cases, and severe bleeding causing hemodynamic instability in 1 case. Transthoracic or transesophageal or direct echocardiography was performed in every case in the R(+) group and the absence of obstruction of the shunt because of technical problems and incomplete ASD enlargement was confirmed. The causes of death in the R(−) group, however, were low-output syndrome secondary to heart failure in 2 cases, severe hypoxia and hemodynamic instability in 1 case, multiple organ failure in 1 case, sepsis in 1 case, and performance of a biopsy at the time of the Norwood operation in 1 case. Based on the Sat O2 values, there were no cases of persistent pulmonary high flow.

When histomorphometric parameters were calculated by linear regressions (D, radius and SPA radius at an accompanying bronchiole with a radius of 100 μm), P<0.05 in all cases.

Radius of SPA Accompanying a Bronchiole Having a Radius of 100 μm
The radius of the SPA was 34.0±10.8 μm in the R(+) group and 46.6±8.5 μm in the R(−) group, and the values in both groups were significantly smaller than in the C group (70.5±8.4 μm, both P<0.0001). The difference between the R(+) group and R(−) group was also significant (P=0.0022; Figure 4). The smallest radius was 22.5 μm in a patient with an intact atrial septum without any clear decompression pathway who died at 2 days of age from severe hypoxia and severe pulmonary hypertension that failed to respond to therapy. According to our previous study, the radius of the SPA of normal infants is ~65 μm at birth, increases during the first 2 months, and then remains stable at ~80 μm from 2 to 10 months. All the radii in the HLHS patients in the present study were less than the 5% to 95% confidence limits, and the largest radius was 58.7 μm in a 40-day-old girl in the R(−) group.

RAC
The RAC was 3.7±0.9 in the control group. The RAC in the R(+) group and the R(−) group were 3.6±0.9 μm and 3.4±0.6 μm, respectively, and not significantly different from the value in the C group (P=0.35 and P=0.31, respectively). The difference between the 2 HLHS groups was not significant. The RAC in all HLHS cases was within the 5% to 95% confidence limits of the control group (Figure 5).

Intimal Lesions
All index of pulmonary vascular disease values for the intimal lesions in the HLHS cases were 1.0, and Heath–Edwards classification was grade 1. Because all of the subjects were infants younger than 40 days of age, intimal lesions seemed to not have yet developed.

Lymphangiectasia
No lymphangiectasia was detected in the C group, but it was present in 6 (75%) of 8 cases in the R(+) group and in 3 (50%) of the 6 cases in the R(−) group (group C versus R(+)}
group, \( P < 0.01 \); group C versus R(−) group, \( P < 0.01 \); R(+) group versus R(−) group, \( P = 0.19 \), NS. Interstitial emphysema was observed in all of the cases in which lymphangiectasia was present.

**Medial Thickness of SPA**

The medial thickness of the SPA in the C group ranged from 5.6 to 13.1 \( \mu m \) (7.6±2.3 \( \mu m \)). The medial hypertrophy regressed during the first month after birth and remained almost stable at \( \approx 6 \) to 8 \( \mu m \). By contrast, the medial thickness of the SPA in the HLHS cases was significantly greater than in the controls (15±3.5 \( \mu m \) and 12±2.8 \( \mu m \), \( P < 0.0001 \) and \( P = 0.021 \), respectively). The SPA in the R(+) group tended to be thicker than in the R(−) group, but the difference was not statistically significant (\( P = 0.075 \); Figure 6A). However, the number of cases was limited, and if there had been a large number, then the difference may have been significant.

**Medial Thickness of SPV**

The medial thickness of the SPV in the C group was 0.7 to 3.7 \( \mu m \) (1.7±0.90 \( \mu m \)) and remained constant at \( \approx 2 \) to 3 \( \mu m \) after birth. The medial thickness of the SPV in the HLHS cases was significantly greater than in the C group [R(+) group, 6.5±2.6 \( \mu m \), \( P < 0.0001 \); R(−) group, 4.1±1.6 \( \mu m \), \( P = 0.011 \)]. There was a slight difference between the R(+) group and the R(−) group (\( P = 0.019 \); Figure 6B).

**Discussion**

Previous morphological analyses of the pulmonary vasculature of patients with HLHS have repeatedly demonstrated abnormalities of the pulmonary vascular bed.\(^{19-21}\) The abnormalities have generally been described as affecting the pulmonary arteries and consisting of increased arterial medial thickness with muscle extension to the smaller respiratory bronchioles. Early observations suggested more severe abnormalities consisting of “intralobular pulmonary artery tortuosity” and “pulmonary vein elastic hyperplasia” in patients with closure of the foramen ovale.\(^{19}\) Some have suggested that the cause of the pulmonary hypertension in HLHS with restrictive ASD is the increased medial thickness of the intrapulmonary arteries; however, because medial thickening of the pulmonary arteries and veins is also seen to some
extent in HLHS without restrictive ASD, it is unclear that the cause of pulmonary hypertension is attributable to the medial thickness of the pulmonary arteries alone.

Our results showed much smaller SPA compared with the accompanying bronchioles in the HLHS with restrictive ASD cases. In the C group, the radius of the SPA accompanying bronchioles having a radius of 100 μm was ~65 μm at birth and gradually increased to 80 μm by 2 months of age, whereas the smallest diameter in the HLHS with restrictive ASD cases was 22.5 μm. Although the SPA in HLHS without restrictive ASD were also smaller than in the controls, the difference in SPA size in HLHS with restrictive ASD and HLHS without restrictive ASD was statistically significant.

No reports had ever clearly described hypoplasia of the pulmonary arteries in HLHS patients, and one reason for this is that there had been no quantitative method of clearly identifying the hypoplasia of small pulmonary arteries. Several published reports have discussed the problem of underdevelopment of the pulmonary vasculature or reduction in the number of lung vessels, but SPA counts have been questioned, because other studies had shown the number of SPA was either not decreased or even increased. Mooi and Wagenvoort have stated that the controversy regarding the underdevelopment or disappearance of pulmonary vessels to a large extent centers on methodology. To avoid the drawbacks of previous methods, we devised a novel method of measuring the degree of hypoplasia in small pulmonary arteries in histological sections and used it in our study of TAPVC patients.

It seems reasonable to assume that underdevelopment of the SPA results in increased pulmonary vascular resistance and limits pulmonary blood flow. If the limitation of the pulmonary blood flow is appropriate, it benefits patients with functional hypoplastic left heart syndrome by limiting excessive flow. A critically restrictive ASD, however, will cause increasing hypoxemia and death. Pulmonary vascular maldevelopment has significant implications in HLHS, because the current palliative interventions require low pulmonary resistance to ensure adequate pulmonary blood flow via aortopulmonary or right ventricular pulmonary artery shunts (ie, Norwood procedure), and, ultimately, a cavopulmonary connection. Heart transplantation also remains a potential treat-
ment modality, but the outcome has not been satisfactory, and its success is optimized by normal pulmonary vasculature, which prevents post-transplantation right ventricular failure. The results of our analysis of the pathology imply that pulmonary vascular abnormalities that pose a significant physiological risk to later cavopulmonary connections or transplantation are present in essentially all patients with HLHS and a restrictive ASD. Day et al histologically studied the pulmonary vessels in biopsy specimens from 20 newborns (mainly HLHS cases) awaiting heart transplantation by nitrogen therapy. They found potentially irreversible pulmonary vascular disease in 1 HLHS case with restrictive ASD after performing BAS. This seems to suggest that HLHS with restrictive ASD is complicated by early development of pulmonary vascular lesions, and their development may be related to the hypoplasia of SPA.

The pathogenesis of the reduced size of the SPA is not understood. Some studies have shown that banding a pulmonary artery in newborn sheep leads to the development of fewer and smaller intra-acinar arteries than normal, and the SPA of patients with pulmonary atresia are said to be fewer than normal. Our previous study also demonstrated hypoplasia in TAPVC with preoperative pulmonary venous obstruction, implying that the hypoplasia of the SPA may be related to restrictive pulmonary venous return in the fetal period. Although blood flow via the pulmonary circulation has been described to be minimal, accounting for 3% to 7% of the total right ventricular output, it represents flow that returns to the left atrium. In patients with mitral atresia or severe mitral stenosis, decompression of this return of flow to the left atrium must occur via the foramen ovale. The absence of an adequate-size foramen ovale is likely to result in left atrial hypertension in utero that is transmitted to the pulmonary venous structures, producing secondary pulmonary venous hypertension, and may reduce pulmonary arterial flow. Few articles have described the pulmonary flow in patients with HLHS with restrictive ASD (IAS). Figure 7 is a Doppler ultrasonogram of a patient with HLHS with IAS at term. It showed that the flow velocity in the main right pulmonary artery was decreased, despite normal flow velocity in the pulmonary trunk. Although further study is needed, the flows in these patients are decreased, and normal growth of pulmonary vascular bed is prevented.

With regard to other histomorphometric parameters, 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 lung maturity. George et al reported 10 cases of congenital diaphragmatic hernia in infants who died in the immediate perinatal period, and the RAC was reduced in every case, possibly because of persistent lung compression during the fetal period. In our study, however, the RAC was not significantly reduced in the HLHS cases, and all values were within the 5% to 95% confidence limits of the control curve. The mechanism of the vascular hypoplasia in HLHS is different from the mechanism of the alveolar immaturity. Lymphangiectasia was previously claimed to be the main cause of the poor outcome of HLHS with restrictive ASD cases, but there was no significant difference between the HLHS cases with restrictive ASD and the HLHS cases without restrictive ASD, suggesting that lymphangiectasia may not necessarily be related to restrictive ASD.

It remains unknown how long it takes for the hypoplastic small pulmonary arteries to grow sufficiently. Moreover, because of the lack of available material, our knowledge is almost completely limited to autopsy cases, and the limitations imposed by hypoplasia of SPA on survival after the Norwood operation are unclear. According to our previous study, the SPA of normal infants grow gradually over several months. If patients with hypoplastic SPA receive appropriate (not excessive) pulmonary flow, then the SPA should grow gradually without developing plexogenic arteriopathy, and the second or third stage Fontan-type operation can be performed successfully.

There were several limitations to this study. It was a retrospective study, and the number of cases was limited. The series of patients does not represent a random sample of HLHS patients during the study period. In addition, because biopsy cannot be approved for ethical reasons, all of the lungs we studied were taken from autopsy cases after operations except in 1 case. Although the patients were examined by echocardiography for possible technical failures, there is a possibility that inappropriate surgical repair (ie, excessive or insufficient pulmonary blood flow, inadequate surgical septectomy) had an influence on postnatal changes in SPA and SPV. However, our results suggest that SPA hypoplasia is present in cases of HLHS with restrictive ASD. This, combined with the finding by others that restrictive ASD is a negative factor for survival, leads us to speculate that restrictive ASD is related to the poor outcome of these patients, although further study is needed to support our findings.

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