Prominent Intrapulmonary Bronchopulmonary Anastomoses and Abnormal Lung Development in Infants and Children with Down Syndrome

Douglas Bush, MD[1,2], Steven H. Abman, MD[1,2], and Csaba Galambos, MD, PhD[1,3]

Objectives To determine the frequency of histologic features of impaired lung vascular and alveolar development and to identify the presence of intrapulmonary bronchopulmonary anastomoses (IBA) in infants and children who died with Down syndrome.

Study design A retrospective review of autopsy reports and lung histology from 13 children with Down syndrome (ages: 0-8 years) was performed. Histologic features of abnormal lung development were identified and semiquantified, including the presence of IBA. Three-dimensional reconstructions of IBA were also performed. Comparisons were made with 4 age-matched patients without Down syndrome with congenital heart defects who underwent autopsies during this time period.

Results Of the 13 subjects with Down syndrome, 69% died from cardiac events, 77% had a congenital heart defect, and 46% had a clinical diagnosis of pulmonary hypertension. Lung histology from all subjects with Down syndrome demonstrated alveolar simplification, and 92% had signs of persistence of a double capillary network in the distal lung. The lungs from the subjects with Down syndrome frequently had features of pulmonary arterial hypertensive remodeling (85%), and prominent bronchial vessels and IBA were observed in all subjects with Down syndrome. These features were more frequent in subjects with Down syndrome compared with control subjects.

Conclusions Children with Down syndrome who died of cardiopulmonary diseases often have histologic evidence of impaired lung alveolar and vascular development, including the presence of prominent IBA and pulmonary hypertension. We speculate that children with Down syndrome are at risk for reduced lung surface area and recruitment of IBA, which may worsen gas exchange in subjects with Down syndrome. *(J Pediatr 2017;180:156-62).*

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Down syndrome, or trisomy 21, is characterized by an increased risk for diverse cardiopulmonary diseases that contribute to chronic hypoxemia and early development of pulmonary hypertension (PH).[1-4] PH in Down syndrome is more common and more severe than PH in children without Down syndrome with similar cardiopulmonary diseases, including structural heart defects and obstructive sleep apnea.[5-8] However, mechanisms contributing to the increased risk for PH in patients with Down syndrome remain unknown.

Past studies of children with Down syndrome have reported abnormalities of lung vascular and alveolar development with histology demonstrating features of vascular immaturity, including persistence of double capillary networks, reduced secondary septa, and fewer, more dilated alveoli.[9-11] In laboratory models, early abnormalities of pulmonary vascular development contribute to high risk for PH,[12] which can be induced experimentally by treatment with antiangiogenesis agents in rodents.[13] Early disruption of angiogenesis also impairs distal airspace development[13-15] and is considered important in the pathogenesis of developmental lung diseases.[16-18] Importantly, potent antiangiogenic agents are expressed on chromosome 21, including endostatin, beta-amyloid peptide, and Down syndrome critical region 1, which have led to the hypothesis that early overexpression of potent antiangiogenic factors may disrupt vascular development in the Down syndrome lung contributing to abnormal lung development.[19]

ACD	Alveolar capillary dysplasia
BPD	Bronchopulmonary dysplasia
CDH	Congenital diaphragmatic hernia
CHDs	Congenital heart defects
IBA	Intrapulmonary bronchopulmonary anastomoses
PH	Pulmonary hypertension
3D	3-dimensional

From the [1]The Pediatric Heart Lung Center, University of Colorado School of Medicine and Children's Hospital Colorado, Aurora, CO; [2]Department of Pediatrics, The Section of Pulmonary Medicine, University of Colorado School of Medicine and Children's Hospital Colorado, Aurora, CO; and [3]Department of Pathology, University of Colorado School of Medicine and Children's Hospital Colorado, Aurora, CO

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In addition, recent studies of children dying with PH and diverse causes of developmental lung diseases, including bronchopulmonary dysplasia (BPD),[20] congenital diaphragmatic hernia (CDH),[21] alveolar capillary dysplasia (ACD),[22] and meconium aspiration syndrome,[23] have evidence of prominent intrapulmonary bronchopulmonary anastomoses (IBAs), or precapillary shunts contributing to chronic hypoxemia. The presence of IBA in these disorders may contribute to chronic hypoxemia and lead to early development of PH.[21] It is not known whether IBA can be identified in subjects with Down syndrome who died with severe PH and related abnormalities.

Thus, we hypothesize that children dying with Down syndrome will have histologic evidence of immature pulmonary vascular and alveolar development and prominent IBA. Further, we hypothesize these features are more prevalent in patients with Down syndrome compared with age and congenital heart defect-matched control patients without Down syndrome.

Methods

This study was approved by the University of Colorado Institutional Review Board through the Decedent Research Certification Program. Study subjects were included after a retrospective review of the post-mortem autopsy database available through the Children's Hospital Colorado Department of Pathology. This database is inclusive of all autopsies that occurred at Children's Hospital Colorado from the years 1999-2016 and is searchable by diagnoses, clinical descriptors, and comorbid conditions. As such, this study was not blinded. Two study populations were identified, all less than 8 years of age at time of death: (1) patients with Down syndrome with and without congenital heart defects (CHDs) and (2) age-matched patients without Down syndrome who had CHD similar to those in the Down syndrome group (without Down syndrome). Patients over 8 years of age, and patients who died with other known genetic syndromes or known developmental lung disorders were excluded from study. Developmental lung disorders include CDH, BPD, ACD, surfactant genetic abnormalities, lung hypoplasia, and other diseases. The presence and type of CHD, clinical diagnosis of PH, and cause of death were recorded from case records.

Lung Histology

Lung histology from previously prepared autopsy slides, generally stained with hematoxylin and eosin, were reviewed by the primary investigator (D.B.) and senior author (C.G.), a board certified pediatric pathologist. Poorly inflated lungs or specimens that were difficult to interpret because of autolysis were excluded from the study. When available, a best representative slide from the left and right lungs were identified to determine relative frequency of lung histologic features within a given patient. The presence of simplified alveoli, double capillary network, pulmonary arterial hypertensive remodeling, prominent bronchial vessels, arterialization of veins, and prominent IBA were determined. Simplified alveoli were defined as large, dilated distal airspaces that reflected reduced secondary septa formation.[24] The presence of double capillary networks was defined as the finding of 2 capillary layers adjacent to one another separated by a central sheet of pulmonary interstitium.[24] Pulmonary arterial hypertensive remodeling was defined as an increased medial thickness compared with external vessel diameter in small pulmonary arteries.[25] Prominent bronchial vessels were defined by the presence of dilated or congested vessels surrounding small airways or in the pleura. Arterialization of the pulmonary veins was defined as the abnormal muscularization of the pulmonary veins. Finally, the presence of IBA was defined by identification of open vascular connection between pulmonary and bronchial vessels in the distal lung parenchyma, which include pulmonary artery to bronchial artery and bronchial vein to pulmonary vein connections. If discordance between the 2 investigators occurred, repeat evaluations, discussions, and agreement determined final assessment.

We applied a semiquantitative scale to further describe the relative frequency of histologic features within each specimen. Scoring was performed by the primary investigator (D.B.). A score of 0 was given when the relevant features was not observed; a score of 1 was given if the feature was rarely observed (less than 25% of images obtained at a magnification of ×10); a score of 2 was given if the feature was commonly observed (26%-75%), and if the feature was readily present throughout the lung tissue (76%-100%), a score of 3 was given. The following features were scored: simplified alveoli, double capillary networks, hypertensive remodeling, prominence of bronchial vessels, and arterialization of the pulmonary veins. Average scores between the group with Down syndrome and control group were then compared.

Three-Dimensional Modeling

Serial sections of areas containing IBAs were saved in the tagged image file format. Utilizing the 3-dimensional (3D) rendering software Free-D (Philippe Andrey, Versailles, France), serial histologic sections were stacked and registered to one another through defined structures as previously described.[26] Through the creation of "models" of histologic structures, a 3D image is rendered. This technique was used to further clarify the presence and connections of IBAs.

Results

From review of an autopsy database, we identified 25 patients with Down syndrome who died, 13 of which fulfilled inclusion criteria for this study. Of the 12 patients excluded, 4 were premature infants with BPD, 6 died outside of the designated age range, and 2 did not have lung histology available for review. We identified 171 patients without Down syndrome who died with CHD during this time period. Of these, 49 had known congenital syndromes; 65 had CHD different from the cohort with Down syndrome; 37 had known lung developmental abnormalities (24 with BPD, 12 with CDH, 1 with pleural effusions because of hydrops fetalis); and 16 were outside of the designated age range or did not have available lung histology for review. Four age- and CHD-matched controls without Down syndrome meeting inclusion and exclu-