Published in final edited form as:
J Pediatr. 2014 January ; 164(1): 192–195. doi:10.1016/j.jpeds.2013.08.035.

Three-dimensional Reconstruction Identifies Misaligned Pulmonary Veins as Intrapulmonary Shunt Vessels in Alveolar Capillary Dysplasia

Csaba Galambos, MD, PhD[1,2], **Sunder Sims-Lucas, PhD**[3], and **Steven H. Abman, MD**[2,4]

[1]Department of Pathology, University of Colorado School of Medicine and Children's Hospital Colorado, Aurora CO

[2]Pediatric Heart Lung Center, University of Colorado School of Medicine and Children's Hospital Colorado, Aurora CO

[3]Department of Pediatrics, Division of Nephrology, Children's Hospital of Pittsburgh of UPMC, Pittsburgh, PA

[4]Department of Pediatrics, University of Colorado School of Medicine and Children's Hospital Colorado, Aurora CO

Abstract

Alveolar capillary dysplasia with misalignment of pulmonary veins (ACD/MPV) is a lethal neonatal lung disease. Although death is caused by hypoxia, the role of MPV is unknown. Using three-dimensional-reconstruction of ACD/MPV lung tissue, we report that MPV are intrapulmonary shunt vessels and speculate that MPV contributes to poor prognosis.

Keywords

lung development; vascular remodeling; neonatal lung disorders; developmental aspects of the pulmonary circulation; blood flow; hypoxia; persistent pulmonary hypertension of the newborn

Alveolar capillary dysplasia with misalignment of pulmonary veins (ACD/MPV) is a uniformly lethal form of neonatal lung disease associated with severe persistent pulmonary hypertension of the newborn (PPHN; 1). Although inhaled nitric oxide (iNO) can transiently improve oxygenation in ACD/MPV, neonates fail to sustain responsiveness and die shortly after birth (2). Although recent data has shed some light to the underlying genetic

Corresponding author: Csaba Galambos, MD, PhD, Department of Pediatric Pathology and Laboratory Medicine, Children's Hospital Colorado, University School of Medicine of Colorado, 13132 East 16th Avenue, Aurora, CO. 80045, Phone: 720-777-6718; Fax: 720-777-7119;, csaba.galambos@childrenscolorado.org.

The authors declare no conflicts of interest.

No reprints are requested.

abnormality, the pathophysiology of hypoxemia in ACD/MPV is unclear (1,3). Further, no data exist to explain the developmental origin and the potential pathophysiological role of the MPV, a pathognomonic feature of disease (1). Pre-acinar anatomic and functional communications between systemic and pulmonary vascular systems has been previously established in fetal lungs (4), but whether MPV with ACD represents altered growth of intrapulmonary shunt vessels is unknown. To better define the relationships of the vascular components associated with ACD/MPV, we studied lung histology and performed three dimensional reconstruction of lung tissue from infants who died with ACD/MPV. We report histologic evidence that MPV represent a vascular network that connects pulmonary veins with microvasculature surrounding distal airways and arteries. We speculate that intrapulmonary shunt due to abnormal growth of these vessels contributes to profound hypoxemia in ACD/MPV.

Methods

Autopsy tissue from 2 patients who died with ACD/MPV were studied. Data for patient demographic was extracted from the patients' autopsy report. One age-matched patient without apparent lung pathology was also included (control). The lungs were inflated with formalin at room pressure and all lobes were sampled including central and peripheral areas. There was one section per slide. All slides were reviewed. Once a representative area with MPV was chosen we used immunohistochemistry (IHC) and three-dimensional (3D) reconstruction for further analysis. IHC was performed by routine streptavidin-biotin peroxidase methods utilizing the Ventana Benchmark automatic slide stainer. Primary antibodies including CD34 (Ventana Inc, Tuscon, AR, USA) and D2-40 (Dako, Carpinteria, CA, USA) were purchased for use. For 3-D reconstruction, lung tissue from control and ACD patients was serially sectioned at 5mm intervals. Serial stacks were prepared with hematoxylin and eosin stains. To elucidate endothelial cells and lymphatic vessels, one of every 10 sections was stained with CD34, a pan-endothelial marker, and D2 40, a lymphatic specific marker. A total of 36 sections were examined. Sections were placed onto a microscope that was equipped with software to trace and reconstruct the lung vasculature in three dimensions (Stereoinvestigator, Microbright Field, Vermont), as we have previously described (5). Each section was then traced for distinct vascular and bronchial elements with selective colors: pulmonary veins (blue), MPV (yellow), arterial endothelium (red), artery muscle (light blue), lymphatics (pink), and airways (green). The neighboring section was then aligned and traced, and this process continued until the entire stack was completed. The stack of images was then 3-D reconstructed and images and movies were generated.

Results

Lung histology was studied in 2 infants who died with ACD/MPV. Both patients were born at term and presented with severe respiratory failure and pulmonary hypertension, requiring mechanical ventilation. Age at death was 2 and 20 days; both infants died of respiratory failure and severe hypoxemia. Reconstruction of lungs from one age-matched control patient was performed as a non-ACD control; the infant died of acute cardiovascular arrest of unknown cause at 3 days of life (born at 39 weeks of gestation).

Histologic examination showed the typical microscopic features of ACD/MPV, as characterized by underdeveloped acinus, thick pulmonary arteries, misaligned pulmonary veins and dysplastic and sparse capillaries within the interstitium. No venous outflow abnormalities were noted. The MPV were seen in all sections and their location varied; MPV were more commonly present between the pulmonary arteries and airways, but they were occasionally noted lateral to the pulmonary artery or the airway (Figure 1). MPV appeared to connect pulmonary veins located in the septum with the area surrounding the broncho-arterial bundle (Figures 1 and 2 and Video 1; Video available at www.jpeds.com). The appearance and location of pulmonary veins in the distal lung was strikingly different from the lungs of normal, age-matched controls. In the normal lung (Figure 2, A), the pulmonary veins lie remotely from bronchi and bronchioles, in contrast to the intimate association between airways and pulmonary arteries. MPV have thin walls and lack discernable elastic laminae. Immunostaining with D2-40, a specific marker for lymphatic endothelium, clearly distinguish MPV from lymphatic vessels neighboring the broncho-arterial bundles (Figure 1, B).

We traced the course of MPV through the combination of serial sectioning, histology, immunohistochemistry (including anti-CD34 and D2-40 antibodies) and high precision computerized 3D reconstruction. These vessels run in the lobular periphery, where pulmonary veins are typically located, and travel towards and then contact the microvascular plexus surrounding pulmonary arteries (vasa vasorum) and airways (bronchial vascular plexus) (Figure 2 and Video 1). In the age-matched control lung, 3D reconstruction failed to identify any shunt vessels between pulmonary veins and the microvascular plexuses of pulmonary arteries and bronchi (Figure 2, A, and Video 2; Video available at www.jpeds.com).

Discussion

Mechanisms contributing to severe pulmonary hypertension with profound hypoxemia in ACD/MPV are incompletely understood. In addition to extra-pulmonary right to left shunting due to PPHN physiology, we report that aberrant vascular growth and the persistence of prominent vascular connections between MPV and pulmonary arteries likely provides a source of intrapulmonary shunt in ACD/MPV.

Past studies suggest that distinct anatomical pathways or intrapulmonary shunt vessels (IPSV) exist in the normal fetus (4). These pre-acinar vascular anastomoses have been named “broncho-pulmonary” or “pulmo-bronchial” vessels, depending on their location in the lung (4). These IPSV are normally present in fetal lung but the persistence or prominence of IPSV after birth is considered pathological (4). Whether misaligned pulmonary veins represent marked enlargement of normal fetal IPSV is uncertain, but one could speculate that the prominence of these vessels reflect altered fetal hemodynamics due to severe underdevelopment and obstructive remodeling of distal pulmonary arteries. Based on standard histology, Cullinane et al suggested that the “misaligned pulmonary veins” may be bronchial veins (6). Cardiac catheterization studies have found severe pulmonary hypertension with marked pruning of the pulmonary arterial tree with normal pulmonary venous return and a markedly decreased or absent capillary blush phase in ACD cases

(2,7,8). Pulmonary hypertension in ACD is likely due to decreased vascular growth, high vascular tone and marked smooth muscle cell hyperplasia. The lack of blush phase and rapid filling of the pulmonary veins during angiography suggests decreased flow through an abnormal arterial microcirculation and subsequent shunt through the “misaligned” pulmonary veins. (2,7,8)

ACD is the most frequent condition that is linked with MPV; however, MPV has also been associated with other conditions. Similar histologic lesions are noted in the clinical setting of congenital venous obstruction and a related animal model (9). One potential explanation for the presence of MPV is that high venous pressure may dilate broncho-pulmonary anastomotic pathways (8). In ACD, radiologic evidence suggests that MPV are bronchial veins and may function as “decompressing pathways” (8). Bronchial veins drain lobular, segmental and peripheral bronchi and bronchioles and return blood into pulmonary veins (10). Histologically, the MPV appear as veins and connect with pulmonary veins, further suggesting that these vessels are bronchial veins. In ACD the elevated pulmonary vascular resistance (PVR) is due to markedly thickened pulmonary arterial wall structure, decreased arterial density and high vascular tone elevate pulmonary vascular resistance (PVR). High PVR could subsequently dilate or enhance growth of pre-capillary shunt pathways that direct blood flow away from alveoli via a pulmonary artery to bronchial and pulmonary vein anastomotic pathways. The consequence of increased flow through these shunt pathways would be marked reductions of blood flow to the alveoli, further exacerbating hypoxemia due to the right to left extrapulmonary shunting due to PPHN physiology.

In conclusion, histologic and 3D reconstruction show that in ACD, MPVs represent intrapulmonary shunt vessels that are most likely anastomotic bronchial veins due to their location and histologic and immuno-staining characteristics. We speculate that persistence of these prominent vascular pathways contributes to the pathophysiology of ACD/MPV via intrapulmonary right-to-left shunting in ACD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Supported by the National Institutes of Health (HL085703 and HL68702 [both to S.A.]).

List of abbreviations

3-D	three-dimensional
ACD/MPV	alveolar capillary dysplasia with misalignment of pulmonary veins
IHC	immunohistochemistry
iNO	inhaled nitric oxide
IPSV	intrapulmonary shunt vessels

MPV	misalignment of pulmonary veins
PPHN	persistent pulmonary hypertension of the newborn
PA	pulmonary artery
PVR	pulmonary vascular resistance
PV	pulmonary vein

References

1. Bishop NB, Stankiewicz P, Steinhorn RH. Alveolar capillary dysplasia. Am J Respir Crit Care Med. 2011; 184:172–9. [PubMed: 21471096]
2. Parker TA, Ivy DD, Kinsella JP, Torielli F, Ruyle SZ, Thilo EH, et al. Combined therapy with inhaled nitric oxide and intravenous prostacyclin in an infant with alveolar-capillary dysplasia. Am J Respir Crit Care Med. 1997; 155:743–6. [PubMed: 9032222]
3. Stankiewicz P, Sen P, Bhatt SS, Storer M, Xia Z, Bejjani BA, et al. Genomic and genic deletions of the FOX gene cluster on 16q24.1 and inactivating mutations of FOXF1 cause alveolar capillary dysplasia and other malformations. Am J Hum Genet. 2009; 84:780–91. [PubMed: 19500772]
4. McMullan DM, Hanley FL, Cohen GA, Portman MA, Riemer RK. Pulmonary arteriovenous shunting in the normal fetal lung. J Am Coll Cardiol. 2004; 44:1497–500. [PubMed: 15464334]
5. Sims-Lucas S. Analysis of 3D branching pattern: hematoxylin and eosin method. Methods in molecular biology. 2012; 886:73–86. [PubMed: 22639252]
6. Cullinane C, Cox P, Silver M. Persistent pulmonary hypertension of the newborn due to alveolar capillary dysplasia. Pediatr Pathol. 1992; 12:499. [PubMed: 1409149]
7. Hintz SR, Vincent JA, Pitlick PT, Fineman JR, Steinhorn RH, Kim GE, et al. Alveolar capillary dysplasia: diagnostic potential for cardiac catheterization. J Perinatol. 1999; 19(6 Pt 1):441–6. [PubMed: 10685275]
8. Eulmesekian P, Cutz E, Parvez B, Bohn D, Adatia I. Alveolar capillary dysplasia: a six-year single center experience. J Perinat Med. 2005; 33(4):347–52. [PubMed: 16207122]
9. Becker AE, Becker MJ, Edwards JE. Dilated bronchial veins within pulmonary parenchyma. Observations in congenital pulmonary venous obstruction. Arch Pathol. 1971; 91:256–60. [PubMed: 5541694]
10. Matthews AW, Buchanan R. A case of pulmonary veno-occlusive disease and a new bronchoscopic sign. Respir Med. 1990; 84:503–5. [PubMed: 2274690]

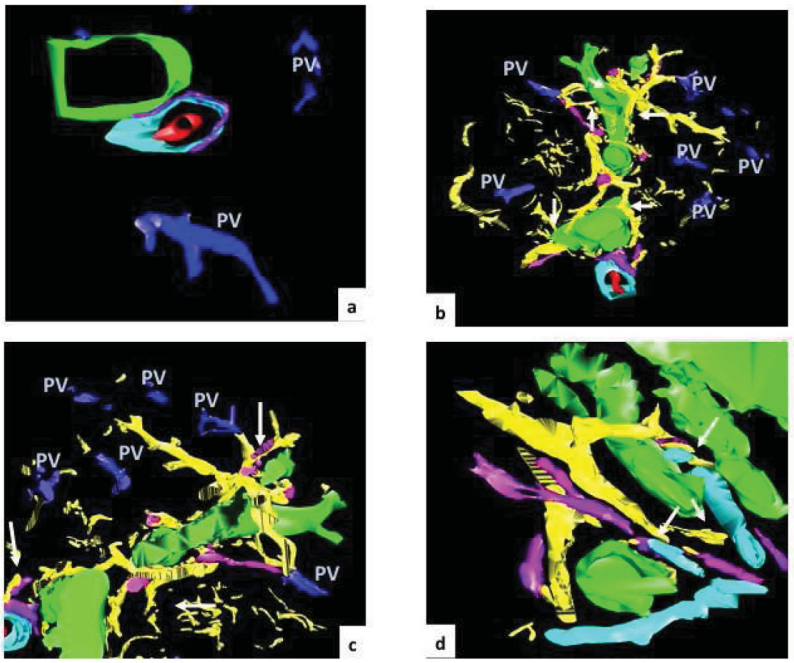

Figure 1.

Lung histology from a young infant who died of alveolar capillary dysplasia with misalignment of pulmonary veins (ACD/MPV). Hematoxillin and eosin (H&E) stained sections show the typical histologic features of ACD/MPV including large, thin walled vascular channels (MPV) surrounding the broncho-arterial bundle (PA: pulmonary artery; B: airways). Thickened muscular wall of pulmonary arteries (PA) is evident. Serial step sections stained with H&E (a) compared with sections stained with lymphatic endothelium specific immunomarker D2-40 (b) highlights scattered lymphatic channels (L) near the broncho-arterial bundles, but not the MPV confirming that MPVs are not of lymphatic in origin. MPV appears to connect with the pulmonary veins (PV) located in the primary septum (dashed outline, c, d).

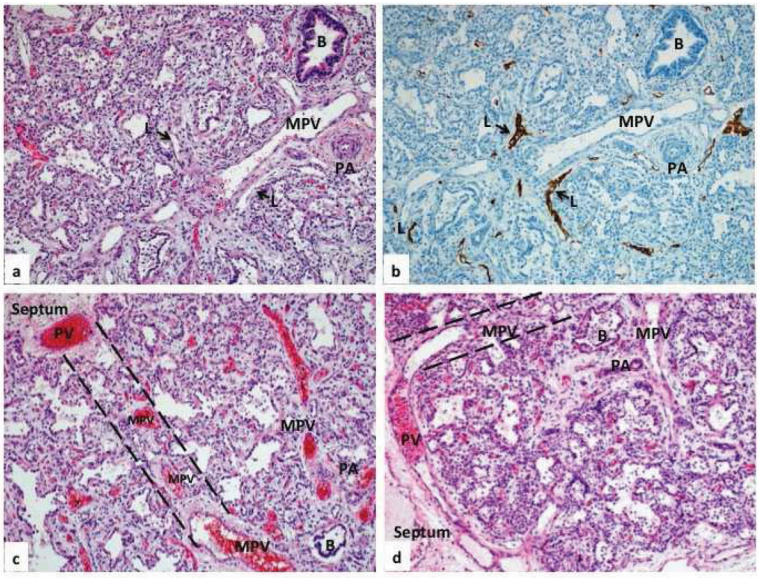

Figure 2.

Three-dimensional reconstruction of microscopic images from ACD lungs. The pulmonary artery and airway run side-by side with few lymphatics and the pulmonary veins (PV) are clearly separated in the age-matched control (a; pulmonary artery: red; arterial muscular wall: aqua, MPV/shunt vessels: yellow, pulmonary veins: blue; airway: green; lymphatic: pink;). In contrast, extensive vascular network is present surrounding the broncho-arterial bundles of ACD lungs (b–d). These vascular channels appear to course toward the vessels located within the interlobular septa (b) and make contact with the microvessels surrounding the pulmonary arteries and airways (white arrows; b–d).