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Morphometric Analysis of the Lung in Prolonged Bronchopulmonary Dysplasia

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Summary

Morphometric analysis of the lungs and heart of a male infant who died at 33 months of age of bronchopulmonary dysplasia after prematurity and respiratory distress syndrome and its treatment is presented. Alveolar internal surface area was 8.4 m^2 compared to 15.3–27.8 for age-matched controls. The number of alveoli was 19×10^6 ($123.3–172.5 \times 10^6$ for controls); however, the mean small airway diameter, 0.44 mm was normal, and small airway abnormalities were minimal. There was marked muscular hypertrophy of the right ventricle.

Speculation

Severe neonatal lung injury, with persistent oxygen dependency throughout infancy might result in inhibition or marked slowing of lung growth. Because development of conducting airways is completed early during intrauterine life and because most alveolar development takes place postnatally, one might expect disturbances in alveolar growth to overshadow residual airway injury in survivors of bronchopulmonary dysplasia.

The growth and development of the lungs follow a continuous course, beginning with conception and finishing when somatic growth ceases. Conducting airways are fully formed by the sixteenth wk of gestational age, whereas multiplication of alveoli continues throughout gestation, infancy, and early childhood (21).

In the premature infants with mild hyaline membrane disease, pulmonary function tests after the first year of life seem to indicate that such infants subsequently progress toward normal lung development (4, 11, 19, 23). On the other hand, little is known of the nature and degree of "catch-up" lung growth during early childhood in premature infants who are survivors of severe respiratory distress syndrome and its sequel, bronchopulmonary dysplasia (BPD). In this group of older infants with BPD, pulmonary function studies during the first year of life (4, 19) have shown marked abnormalities, including hypoxemia, increased lung volume, maldistribution of ventilation, and increased airway resistance. These findings have been attributed mostly to the presence of interstitial edema (13, 22) and/or increased bronchiolar secretions (3, 18). Obliterative airway disease and simplified alveoli with interstitial fibrosis have been described in infants with BPD, (3, 16, 18), but, to our knowledge, quantitative anatomic studies have not been done.

MATERIALS AND METHODS

Case report. A 33-month-old male child died of pulmonary insufficiency and congestive heart failure secondary to cor pulmonale. The patient was a 30-wk gestation infant born to a mother whose pregnancy had been uncomplicated except for unexplained early onset of labor. The family history revealed childhood asthma in the patient's mother and asthma in an older brother. After a spontaneous cry and Apgars of 7 and 8 at 1 and 5 min, the child required continuous positive airway pressure (CPAP) with 100% oxygen mask-bag because of bradycardia and cyanosis. He was admitted to the intensive care nursery and had a gestational age of 31 wk by Dubowitz criteria (weight, 1500 g; length, 41 cm; and head circumference, 27.5 cm). Despite CPAP and 100% inspired oxygen the child developed immediate and severe respiratory distress with apneic spells. At 35 min of age he was intubated and mechanically ventilated with an inspired oxygen concentration (FIO_2) of 80% and positive end-expiratory pressure (PEEP) of 8 cm H_2O. The peak pressure remained between 40–50 cm of H_2O during the first wk; the oxygen requirement diminished rapidly to 60% at 12 h and 40% at 6 days, the PEEP being 6. The child was extubated on day 10 and maintained on 35% oxygen. By 2 wk of age, the occurrence of severe apneic episodes prevented further weaning off supplemental oxygen. Subsequent problems over the next 12 months included: staphylococcal and pseudomonas pneumonias, chronic cor pulmonale (mild right ventricular hypertrophy documented by echocardiogram at the age of 4 months), culture-proven pertussis (at 9 months of age, which lead to respiratory failure and a short period of artificial ventilation), and failure to thrive despite adequate oral caloric intake.

His radiologic changes are summarized as follows: at 2 h of age, bilateral "ground glass" appearance of the lung fields compatible with respiratory distress syndrome, improving at day 7, followed by recurrent shifting infiltrates and atelectasis; at day 42, cystic changes in both lungs compatible with severe BPD, associated with right upper lobe atelectasis. These findings persisted, over the following year with progressive hyperlucency of the left upper lobe, and atelectasis of the right middle lobe. He finally was discharged at 13 months of age on supplemental oxygen given by nasal catheter.

During the second and third years of life, he was hospitalized 9 more times for numerous episodes of pneumonia, sometimes associated with respiratory failure and CO_2 retention up to a $PaCO_2$ of 100 mm Hg. A virus, Echo 4, was isolated only once, although repeated viral cultures were done. From 23–28 months of age, the patient required supplemental oxygen at night only. At 33 months he required mechanical ventilation for several wk; terminally, he developed a cardiac arrhythmia. The patient had been on several medications intermittently since birth, including oral and inhaled bronchodilators, diuretics, antibiotics, and cardiotonic drugs; he also was on salt and fluid restrictions. During the last few months of life, corticosteroids were given to control progressive and severe reactive airways disease. He died at age 1014 days (33 months), having spent only 491 days outside the hospital.

Methods. At autopsy the lungs were inflated and fixed endobronchially with 10% neutral buffered formalin for 24 h at 25 cm H_2O pressure. The lungs from three children of similar age with normal physical development and no history of lung disease were used as controls and handled similarly. Informed, written consent was obtained from the childrens' parents. Lung volumes were measured by water displacement. Lungs were sectioned parasagittally at 1-cm intervals and 10 random sections were taken from

each lung, using a template. The sectioned lung surfaces were point-counted grossly to derive the volumetric % of parenchyma, airways, vessels, and interstitium. Shrinkage of microscopic sections occurring with processing was corrected, but the trivial shrinkage from fresh to fixed lung was not corrected (6). Mean linear intercept (Lm), that is, average distance between alveolar walls, and internal surface area (ISA) in all cases were determined by standard methods (7, 20). Specific internal surface area (ISA_{spec}), or internal surface area divided by parenchymal volume, was also calculated (6). The number of alveoli was determined in each case (2). Small airways were defined as those airways measuring 2 mm or less in internal diameter and containing no glands, cartilage, or alveoli in their walls. Microscopic slides were projected at approximately ×100 magnification to measure the diameters of small airways. The mean diameter of small airways, number of small airways per square cm of lung tissue, % of small airways less than 0.35 mm, and histographic distribution of % of these small airways were determined. The formalin-fixed heart was dissected to evaluate hypertrophy (8). The weights of the free walls of the right and left ventricle and of the septum were determined and compared to established values (10).

RESULTS

At the autopsy the child appeared very small (8 kg, 79 cm in crown-heel length) for his chronologic age. Major pathologic findings were limited to the heart and lungs. Externally, the lungs showed areas of hyperinflation, with adjacent areas of relative atelectasis. Fissuration was abnormal; the left lung had four apparent lobes, and the right lung, five apparent lobes. The bronchial tree to the level of segmental bronchi had the usual pattern of branching. The main pulmonary artery and its major branches were dilated and thick-walled. The heart weighed 115 g. The right atrium and right ventricle showed marked dilatation and hypertrophy.

Microscopic examination showed uniform inflation of the pulmonary parenchyma except for parts of the left upper lobe and left lower lobe. Alveolar walls showed severe hyperemia of capillaries. The alveolar walls varied from normal to being moderately thickened by fibrous tissue with scattered infiltrates of lymphocytes and large mononuclear cells. Hyperplasia of alveolar lining cells was seen focally. In some areas, alveoli were well-formed, with numerous protruding alveolar septa, and were of a size comparable to that of age-matched controls (see below). In other areas alveoli were much larger and had few protruding alveolar septa (Fig. 1). Cartilagenous bronchi had a normal appearance. The majority of small airways were unremarkable or showed mild fibrosis of the contiguous interstitium. Occasional small airways in areas of atelectasis were narrowed by fibrosis with minimal chronic inflammation but the vast majority of the bronchioles appeared normal. Muscular pulmonary arteries showed marked medial thickening and duplication of elastic laminae. Some of these arteries showed development of the subintimal and longitudinal smooth muscle. Subintimal fibrosis was not seen. Small muscular arteries extended to the acinar level accompanying respiratory bronchioles and alveolar ducts.

Compared to the three age-matched controls, the child with BPD had a markedly reduced number of alveoli (19.0×10^6 compared to $123.3–213.8 \times 10^6$ for the controls) with a greatly elevated mean linear intercept, (Table 1), implying increased alveolar diameter. ISA and ISA_{spec} were reduced by approximately one-half; thus, the lung consisted of enlarged, simplified and fewer

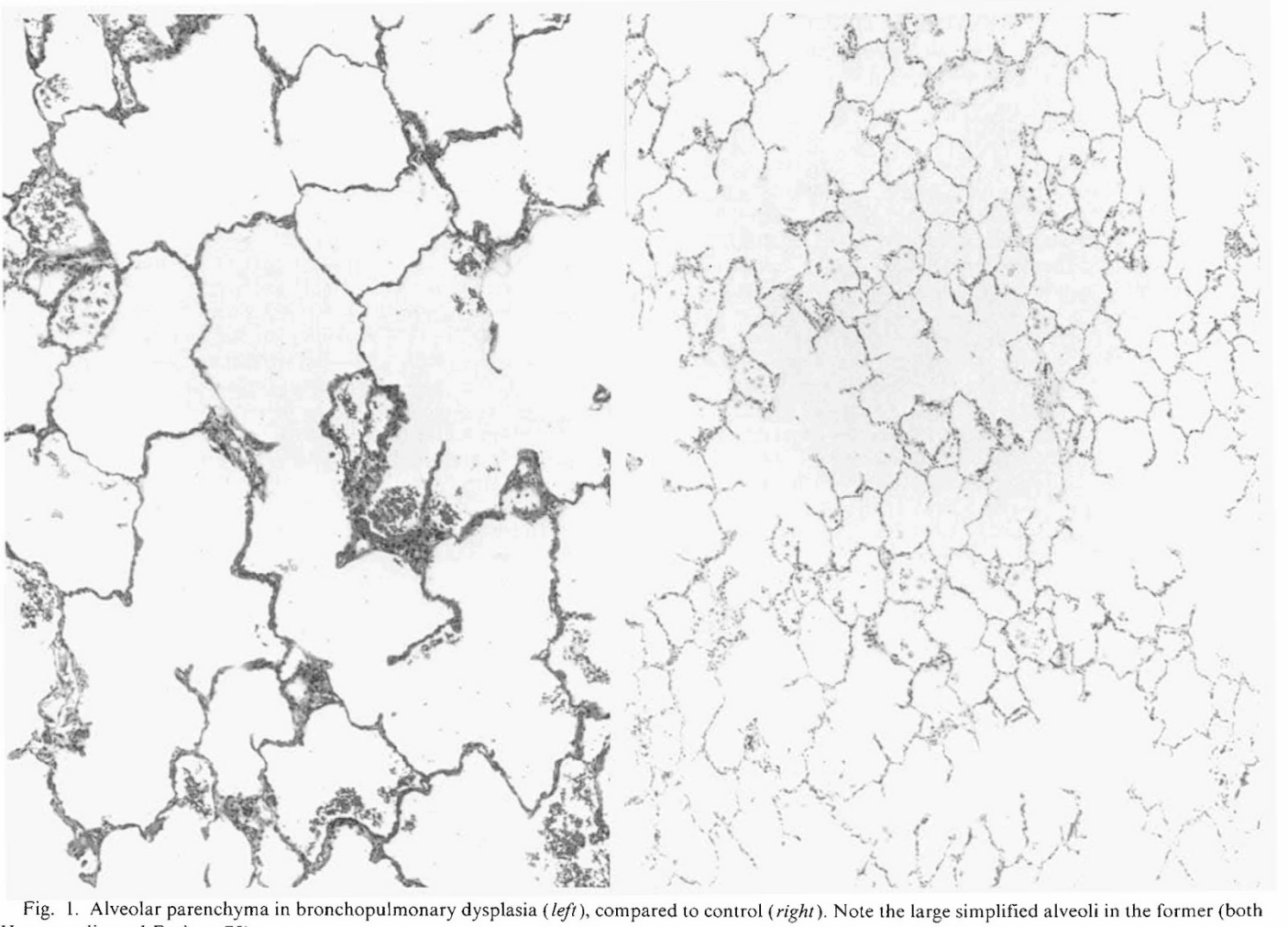

Fig. 1. Alveolar parenchyma in bronchopulmonary dysplasia (*left*), compared to control (*right*). Note the large simplified alveoli in the former (both Hematoxylin and Eosin, ×75).

Table 1. *Alveolar size, number, and surface area*[1]

Age, sex, diagnosis	Lm (μ)	ISA (m^2)	ISA_{spec} (m^2/liter)	Number of alveoli ($\times 10^6$)
34 mo, M, Bronchopulmonary dysplasia	284	8.4	13.0	19.0
34 mo, F, Control	171	15.3	19.1	123.3
26 mo, M, Control	120	27.8	29.0	213.8
41 mo, F, Control	149	17.8	23.9	172.5

[1] Lm, mean linear intercept; ISA, internal surface area; M, male; and F, female.

Table 2. *Small airway (SA) size and number*

Age, sex, diagnosis	Average diameter SA (mm)	% SA <0.35 mm	Number SA/cm^2 tissue
34 mo, M, Bronchopulmonary dysplasia	0.44 ± 0.26[1]	40	2.4
34 mo, F, Control	0.44 ± 0.16	30	2.1
26 mo, M, Control	0.34 ± 0.18	69	4.1
41 mo, F, Control	0.43 ± 0.25	43	6.0

[1] ± standard deviation.

alveoli, resulting in decreased internal surface area. The morphometric data on small airways (Table 2) revealed an average diameter of 0.44 ± 0.26 (similar to the controls); their number (2.4 per cm^2 of tissue) was also within the normal range. There was no histologic evidence of obliterated airways. The % of small airways less than 0.35 mm was 40%, within the range seen in controls. A small airway histogram showed 10% of the small airways in the 0–0.2 mm size range, which was a higher % than for the two older children but comparable to the younger control child.

The weight of the free wall of the right ventricle was 45.1 g, that of the free wall of the left ventricle, 16.5 g, and that of the interventricular septum 23.3 g, with a total ventricular weight of 84.9 g; thus, the ratio of the left ventricle plus the septum to the right ventricle was 0.99. The predicted total ventricular weight for a 2-year-old male is 29.8 ± 1.30 g, and the predicted ratio of left ventricular wall plus septum to right ventricular wall is 2.75. For a 3-year-old child, these values are 38.3 ± 1.81 g and the ratio is 2.49; thus, marked muscular hypertrophy of the right ventricle was present in this patient.

DISCUSSION

This case emphasizes the lack of alveolar growth that may occur with severe BPD. Alveolar multiplication lagged behind both the growth of the other organs and increases in height and weight. The enlarged appearance of the alveoli is reflected by the increased value for mean linear intercept. The value of 284 μ, in this case is within the size range for adults in this laboratory, namely 265 ± 50 μ (average age, 27 years).

It might have been more appropriate to compare the lung growth of this child with BPD children of same height (13-month-old child) or weight (6-month-old child) than with children of same age, but the number of alveoli determined in this case would still have been markedly reduced, being less than that of a full-term newborn, which is 24×10^6 (9). In the computation of alveolar number, an alveolar shape constant (β) was used, as has been done by others (9) to study alveolar growth. The abnormally large alveoli in this case of BPD may not have the same configuration as a normal alveolus, and thus the value for alveolar number may not be entirely accurate; however, there is no doubt that alveolar number was greatly reduced.

The relatively mild nature of the small airways injury may reflect avoidance of particularly high ventilatory pressures or successful restoration of airways despite continuous exposure to high concentrations of oxygen (16). It was expected that a decreased density of small airways per cm^2 of tissue would be found, but the observed number was close to the lower range of normal. There was no correlation of airways density with body height in the four cases studied. In young children the density of small airways appears quite variable. This may reflect different individual rates of lung growth in apparently normal children.

A recent study (14) has documented a high incidence of (77%) of asthma in the families of children with BPD, as was present in this case. Despite this association and the known occurrence of muscular hypertrophy of bronchial muscle in BPD (3), we did not observe bronchial muscle hypertrophy or any other structural changes that would correlate with the clinically observed bronchospasm. The child reported here had acquired severe clinical hyperreactive airway disease, requiring the use of large amounts of bronchodilators and corticosteroids during the last 6 months of life.

The external appearance of this patient's lungs, namely areas of hyperinflation contrasting with areas of relatively normal size or somewhat collapsed alveoli and abnormal fissuration, have been observed previously in BPD infants during the first year of life (18), but do not appear to have been emphasized. It appears most likely that the abnormal fissures are the result of postnatal asynchronous lung growth, rather than a reflection of intrauterine dysgenesis, the branching of the bronchial tree to the level of the segmental bronchi being normal. The areas of large, simplified alveoli in this case were probably regarded radiographically as cystic areas because of their decreased density.

Oxygen is a well-known pulmonary toxin. Newborn rats and mice exposed to 40% oxygen for 3 wk had smaller lung volumes than controls, although body weights were similar (5, 15, 24). The prolonged exposure of a developing lung to oxygen may constitute a continuous insult despite protective mechanisms (15, 24) and may impede alveolar multiplication during infancy and childhood. This marked reduction in alveoli and consequently in surface area for gas exchange makes the patient oxygen-dependent, may affect his body growth, and may be one of the etiologic factors of his failure to thrive. Injury to the alveolar parenchyma is probably of two types. Hyaline membrane disease and its attendant repair, often associated with fibroproliferative bronchiolitis, may induce hyperinflation, air trapping, and destruction of alveolar walls (1, 12, 18). Oxygen toxicity may lead to destruction of alveolar walls and the capillary bed (16), with inhibition of the development of alveolar dividing septa.

In infants other factors such as radiation exposure may also enhance oxygen toxicity, and this child had a prodigious number of chest roentgenograms (287 films, weighing 4.33 kg). The severe muscular thickening of the pulmonary arteries and of the right side of the heart may also be sequelae of neonatal lung disease followed by continuous enriched oxygen exposure (17). Rendas *et al.* (17) recently have reported morphometric studies on the lungs of a premature infant who died at 10 months of age with failure to thrive and pulmonary hypertension from hypoplasia of the pulmonary vascular bed. The lungs showed a marked decrease in number of alveoli (10×10^6). This value was also less than the expected number for a term newborn. Apparently, the pulmonary vascular disease was sufficient to prevent both alveolar and somatic growth. Unlike the present case, however, there was no history of respiratory distress syndrome. Decreased rates of alveolar growth may be a non-specific response to pulmonary vascular bed injury, even if the alveolar parenchyma is not involved directly.

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