Catch-up Alveolarization in Ex-Preterm Children

Evidence from ^3He Magnetic Resonance

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Rationale: Histologic data from fatal cases suggest that extreme prematurity results in persisting alveolar damage. However, there is new evidence that human alveolarization might continue throughout childhood and could contribute to alveolar repair.

Objectives: To examine whether alveolar damage in extreme-preterm survivors persists into late childhood, we compared alveolar dimensions between schoolchildren born term and preterm, using hyperpolarized helium-3 magnetic resonance.

Methods: We recruited schoolchildren aged 10–14 years stratified by gestational age at birth (weeks) to four groups: (1) term-born (37–42 wk; n = 61); (2) mild preterm (32–36 wk; n = 21); (3) extreme preterm (<32 wk, not oxygen dependent at 4 wk; n = 19); and (4) extreme preterm with chronic lung disease (<32 wk and oxygen dependent beyond 4 wk; n = 18). We measured lung function using spirometry and plethysmography. Apparent diffusion coefficient, a surrogate for average alveolar dimensions, was measured by helium-3 magnetic resonance.

Measurements and Main Results: The two extreme preterm groups had a lower FEV₁ ($P = 0.017$) compared with term-born and mild preterm children. Apparent diffusion coefficient was 0.092 cm²/second (95% confidence interval, 0.089–0.095) in the term group. Corresponding values were 0.096 (0.091–0.101), 0.090 (0.085–0.095), and 0.089 (0.083–0.094) in the mild preterm and two extreme preterm groups, respectively, implying comparable alveolar dimensions across all groups. Results did not change after controlling for anthropometric variables and potential confounders.

Conclusions: Alveolar size at school age was similar in survivors of extreme prematurity and term-born children. Because extreme preterm

AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

It is thought that children born extremely preterm have deranged alveolar structure (manifesting as simpler, larger, and fewer alveoli) caused by persistence of alveolar damage sustained in early life.

What This Study Adds to the Field

We provide evidence of normalization of alveolar dimensions in this group, possibly caused by catch-up alveolarization.

birth is associated with deranged alveolar structure in infancy, the most likely explanation for our finding is catch-up alveolarization.

Keywords: alveolar structure; lung acinus; bronchopulmonary dysplasia; neonatal chronic lung disease

(Received in original form October 14, 2012; accepted in final form February 18, 2013)

Supported by the Wellcome Trust, London (081367/B/06/Z); Marie Curie Actions 7th Framework Program (M.M.); the Swiss National Science Foundation (3200B0-122341) (B.D.S.); and Asthma UK (07/048) (B.D.S.).

Author Contributions: M.N. was responsible for the practical conduct of the research; was involved in study planning and coordination, preliminary analyses, and interpretation of the data; and wrote the first draft of the manuscript. C.S.B. had overall responsibility for the physiologic measurements. J.O.-B. had overall responsibility for the ^3He magnetic resonance measurements. C.M.D. performed the data analysis including multiple imputation. M.M. set up the protocols for hyperpolarized gas production and collection and for the ^3He magnetic resonance measurements using the RARE sequence. I.B. and R.R.G. performed the ^3He magnetic resonance measurements and analyzed the ^3He data. C.E.K. and B.D.S. were responsible for the selection of subjects and the statistical analysis. M.S., J.O.-B., C.S.B., and C.E.K. planned the study. All authors contributed to the different drafts of the manuscript and approved the final version. M.S. had the overall responsibility for the project and major input into study design, analysis, and manuscript. M.S. acts as the guarantor.

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This article has an online supplement, which is accessible from this issue's table of contents at www.atsjournals.org

Am J Respir Crit Care Med Vol 187, Iss. 10, pp 1104–1109, May 15, 2013

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Originally Published in Press as DOI: 10.1164/rccm.201210-1850OC on March 14, 2013

Internet address: www.atsjournals.org

Advances in preterm care have led to increased survival of extremely premature babies, with increasing numbers reaching adulthood. This has shifted the focus of research from survival toward long-term sequelae of prematurity (1, 2). Infants born extremely preterm are known to have arrested alveolar development, manifesting as fewer and larger alveoli (3, 4). School-age and adult survivors of extreme preterm birth are known to have long-term respiratory problems, particularly decreased forced expiratory volumes and increased residual lung volumes, suggesting airway damage (5, 6). There are insufficient data regarding structure and development of the periphery of the lung in long-term survivors of preterm birth because studies on this cohort have relied on traditional lung function tests that only provide an overall estimate of function.

The short- and long-term outcomes described previously correlate with gestational age (GA) at birth and the presence of chronic lung disease of prematurity (CLD), one of the early pulmonary sequelae of extreme preterm birth (6–8). Most evidence regarding structure and development of periphery of the lung in preterm infants comes from histologic studies, either from animal models (9, 10) or from autopsies of infants who died of CLD (8). However, available histologic data do not extend to children surviving beyond 3 years of age (3, 11, 12), with a sole exception of an 8 year old described by Husain and coworkers (4). The histologic data are necessarily from fatal cases. Animal studies have not assessed preterm survivors beyond a human equivalent of 3 years of age (9). Because human alveolarization was thought to be complete by 3 years of age (13, 14), the histologic data were extrapolated to postulate persistence of acinar damage in preterm and CLD survivors (9, 15).

However, we recently presented new evidence of alveolarization beyond early childhood in humans (16). Many animal studies

support ongoing alveolarization throughout the entire period of lung growth in mammals (17–19). This raises the possibility of recovery from acinar damage in survivors of CLD. The absence of studies investigating acinar structure in extreme preterm survivors is explained by the near impossibility of acquiring appropriate histologic specimen for morphometric analysis. Therefore, noninvasive techniques are essential to test the hypothesis that acinar damage persists in survivors of CLD.

The newly developed noninvasive technique of helium-3 (^3He) magnetic resonance (MR) can be used to determine alveolar structure in humans (20, 21). We used this technique to compare alveolar structure in term-born and preterm-born schoolchildren (including survivors of neonatal CLD). We hypothesized that children born preterm, in particular those who had CLD in infancy, would have fewer and therefore larger alveoli than term-born children. Some of the results of this study have been previously reported in the form of an abstract (22).

METHODS

Subjects

We recruited 119 children aged between 10 and 14 years, stratified into four groups by GA (in weeks) and presence of CLD: (1) term-born (GA 37–42, no neonatal respiratory support; $n = 61$); (2) mild preterm (GA 32–36, neonatal respiratory support <4 wk; $n = 21$); (3) extreme preterm (GA <32 , neonatal respiratory support <4 wk; $n = 19$); and (4) extreme preterm with CLD (GA <32 , neonatal respiratory support >4 wk; $n = 18$).

The term and mild preterm groups were recruited by stratified random sampling from community-based databases: the Leicester Respiratory Cohorts (23) and the Leicester Specialist Community Child Health Services Database. The extreme preterm groups were recruited from the Trent Neonatal Survey, which includes data of all children born between 24 and 32 weeks GA in Leicestershire (24) (*see online supplement for details of recruitment*). We excluded children with coexisting chronic respiratory disorders (e.g., cystic fibrosis, diaphragmatic hernia).

Questionnaires and Measurements

The participants completed validated questionnaires on neonatal history, prior and current respiratory health, treatments, and environmental exposures (25). We performed anthropometric measurements, spirometry, and plethysmography according to standardized guidelines (26, 27). We calculated within-sample z scores for lung function measurements that were adjusted for age, height, sex, and ethnicity. Children with a birth weight below the 10th centile for the gestation (28) were classified as small for GA (SGA).

Alveolar Dimensions

These subjects underwent hyperpolarized ^3He MR. This provides the apparent diffusion coefficient (ADC), a measure of the degree of restriction to the self-diffusion of ^3He molecules within the lungs. We measured ADC in 64 planes parallel to the sagittal plane (*see online supplement*). The mean ADC is a measure of average alveolar dimensions and its standard deviation (SD_{ADC}) is an estimate of the uniformity of ADC within the 64 lung slices. The measured values of ADC and SD_{ADC} were corrected for concentration of helium and relative size of bolus (16).

Analysis

We first compared ADC and SD_{ADC} among the four groups of children in the entire sample using linear regression. We controlled for age, sex, height, and ethnicity (basic model). Then, we adjusted for potential confounders (adjusted model): SGA; history of prenatal and postnatal exposure to environmental tobacco smoke (ETS); treatment with inhaled or systemic steroids (before and after age 3 yr); and crowding (persons per room, an indicator of socioeconomic status). Although

ADC is known to increase slightly with increasing FRC (i.e., lung growth) (16), we decided not to adjust for FRC in our models because the preterm groups had higher probability of air trapping. However, models including FRC were performed for comparative purposes. For 20 children, information was missing on one or more potential confounders. Therefore, we repeated the analyses using multiple imputation (29).

Similarly, we analyzed the association of ADC and SD_{ADC} with risk factors in the subsample of extreme preterms. Two adjusted models were performed, controlling separately and collectively for the following risk factors: SGA, number of days ventilated, on continuous positive airway pressure (CPAP), or on oxygen therapy.

The entire analysis was repeated with an alternative definition for CLD: children who were born at less than 32 weeks gestation who were oxygen dependent at 36 weeks post-menstrual age (*see online supplement*). We used Stata 11.2 for analysis (Stata Corporation, Austin, TX).

RESULTS

Characteristics of the Study Population

The study participants had a mean (SD) age of 11.85 (1.01) years and 60 (50.4%) were boys (Table 1). Ninety-two (77.3%) were of white and 25 (21.1%) of South Asian ethnicity; children with mixed Asian-white ethnicity were classified as South Asian if mother was South Asian. Two children belonged to other ethnic groups. The results did not change when these two children were excluded from the analysis. Compared with mild preterm and term-born children, extreme preterms with and without CLD tended to be slightly younger and shorter, and were more likely to have received corticosteroid therapy (inhaled or systemic) before age 3 (Table 1). Extreme preterms with CLD had also received more corticosteroids after age 3 and were less likely to have a South Asian mother (Table 1). Thirty-two percent of children were exposed to ETS at some point (antenatally or postnatally). ETS exposure tended to be slightly higher in the extreme preterm groups (Table 1). Crowding index was similar among the groups.

Characteristics of the Extreme Preterm Groups

Compared with extreme preterms without CLD, those with CLD had lower birth weight; received more surfactant; needed longer respiratory support (including more ventilation days, more days on CPAP, and more days on supplemental oxygen); and had a longer duration of hospitalization (Table 2). A high proportion of children were exposed antenatally to corticosteroids in both the extreme preterm subgroups.

Lung Function Tests

The extreme preterm groups had lower FEV_1 z scores than the term and mild preterm groups (Table 3). Z scores of FVC were similar among the groups. The extreme preterms with CLD had higher FRC z score than the other groups (Table 3).

Alveolar Dimensions

Apparent diffusion coefficient. The ADC of ^3He was very similar across the four groups. There was no evidence for an association between ADC and either preterm birth or presence of CLD after controlling for age, sex, height, and ethnicity (basic model) (Table 4, Figure 1). Furthermore, there was no trend for ADC across the groups (P value for trend = 0.409). The results remained similar in the model additionally adjusted for prenatal and postnatal ETS exposure, treatment with corticosteroids, crowding, and SGA (adjusted model) (Table 4). Inclusion of FRC in the model did not change the results: there was no statistically significant difference among the ADCs of the four

TABLE 1. CHARACTERISTICS OF THE STUDY PARTICIPANTS BY GESTATIONAL AGE AND PRESENCE OF CLD (N = 119)

	Term-Born (n = 61)	Mild Preterm (n = 21)	Extreme Preterm without CLD (n = 19)	Extreme Preterm with CLD (n = 18)	P Value
Age, yr*	12.0 (1.2)	12.2 (0.9)	11.3 (0.6)	11.6 (0.6)	0.016
Height, cm*	151.7 (10.5)	154.6 (7.0)	148.2 (5.8)	148.2 (7.6)	0.068
Weight, kg*	45.2 (10.5)	47.4 (15.4)	42.3 (9.7)	43.1 (12.1)	0.49
South Asian, n (%)	15 (24.6)	4 (16.0)	5 (26.3)	1 (5.9)	0.36
Gestational age, wk*	39.6 (1.4)	35.2 (1.2)	29.7 (1.3)	28.0 (2.0)	<0.001
SGA, n (%)	1 (1.7)	0 (0)	3 (16.7)	4 (23.5)	0.004
Crowding, persons per room* [†]	0.79 (0.50)	0.81 (0.25)	0.91 (0.38)	0.76 (0.30)	0.032
ETS during pregnancy, n (%)	6 (9.8)	1 (7.7)	4 (20.0)	2 (11.8)	0.48
ETS exposure at 0–3 yr, n (%)	14 (23.0)	6 (17.7)	9 (45.0)	5 (29.4)	0.313
ETS exposure after 3 yr, n (%)	16 (26.2)	6 (17.7)	6 (30.0)	6 (35.3)	0.88
Steroids, 0–3 yr, n (%) [‡]	3 (4.9)	0 (0)	4 (21.1)	6 (37.5)	0.001
Steroids, after 3 yr, n (%) [‡]	6 (9.8)	2 (11.8)	2 (10.0)	7 (43.8)	0.012
Steroids, current, n (%) [‡]	4 (6.9)	2 (18.2)	2 (10.0)	3 (18.7)	0.53
Current wheeze, n (%)	7 (11.5)	1 (8.3)	2 (10.5)	2 (11.8)	0.841

Definition of abbreviations: CLD = chronic lung disease of prematurity; ETS = environmental tobacco smoke; SGA = small for gestational age.

* Mean (SD).

[†] Crowding index: calculated as mean number of inhabitants (≥ 2 yr) per room in the residence of the subject (excluding kitchen, bathroom, and toilets).

[‡] Steroids: inhaled or oral corticosteroids for any disease (given mainly for reactive lung disease [asthma] in this group). One child who had multiple doses of oral corticosteroids for severe asthma was excluded from analysis.

groups (not shown). The model using multiple imputation gave similar results (see Table E1 in the online supplement). ADC was lower in males and increased with height (see Table E2).

We investigated the association between ADC and factors related to neonatal intensive care in the subgroup of extreme preterm children with and without CLD (see Table E3). There was no association between ADC and the following risk factors: ventilation days, CPAP days, days on oxygen therapy, and being born SGA, whether taken individually or collectively (see Table E3). These results did not change on multiple imputation. The results remained similar when analyzed using an alternative definition of CLD (oxygen dependency at 36 wk post-menstrual age) (see Table E4).

Within-subject standard deviation of the ADC. In the basic model we found evidence for an association between preterm type and SD_{ADC} , an assessment of the uniformity of ADC within the lung. Compared with term-born children, SD_{ADC} was 0.003 cm²/second (95% confidence interval [CI], –0.006 to 0.000; $P = 0.025$) lower in extreme preterms without CLD, but 0.003 cm²/second (95% CI, 0.000–0.006; $P = 0.048$) higher in

extreme preterms with CLD. Results remained similar in the adjusted model (Table 4). When we used the alternative definition of CLD, the differences in SD_{ADC} among the groups remained substantially the same, but were no longer statistically significant (see Table E4).

DISCUSSION

This is the first fully reported study assessing alveolar dimensions in school-age survivors of extreme prematurity and CLD compared with term-born and mild preterm children, using a noninvasive measurement technique (³HeMR). In common with others, we found evidence for abnormal airway function in extreme preterm and CLD survivors. In contrast, alveolar dimensions were similar in these children compared with term-born and mild preterm children. Moreover, alveolar dimensions were not related to risk factors for CLD. This indicates that in survivors of extreme preterm birth, deranged alveolar development found in early childhood in fatal cases may be compensated within the first decade of life in survivors, probably by later alveolarization.

TABLE 2. CLINICAL CHARACTERISTICS OF THE EXTREME PRETERM CHILDREN BY PRESENCE OF CLD

	Extreme Preterm without CLD (n = 19)*	Extreme Preterm with CLD (n = 18)*	P Values for Difference
Antenatal steroids, n (%)	17 (94.4)	16 (94.1)	1.0
Spontaneous prelabor rupture of membranes, n (%)	2 (11.8)	4 (23.5)	0.66
Fetal distress, n (%)	8 (47.1)	5 (29.4)	0.48
Birthweight centile, median (IQR) [†]	63.4 (34–78)	36.0 (20–56)	0.038
Apgar score at 5 min, median (IQR) [†]	9 (8–10)	9 (8–10)	0.86
Surfactant administered, n (%)	8 (47.1)	14 (82.4)	0.07
Mechanical ventilation, d, median (IQR), range [†]	0.0 (0–2), 0–8	7.0 (5–19), 0–44	<0.001
CPAP, d, median (IQR), range [†]	1.0 (0–2), 0–12	7.0 (0–21), 0–53	0.49
O ₂ therapy alone, d, median (IQR), range [†]	2.0 (0–4), 0–14	30.0 (7–46), 0–115	0.015
Duration of any respiratory support, [‡] d, median (IQR), range [†]	4.0 (2–8), 1–24	62 (38–73), 29–172	<0.001
Total length of stay, d, median (IQR), range [†]	41.0 (28–62), 20–92	83.0 (69–112), 45–249	0.005

Definition of abbreviations: CLD = chronic lung disease of prematurity; IQR = interquartile range; CPAP = continuous positive airway pressure.

* Some of the neonatal data were not available for all the children.

[†] N = 17 for both groups.

[‡] Total duration of ventilation + CPAP + oxygen.

TABLE 3. LUNG FUNCTION PARAMETERS (N = 119)

	Term-born (n = 61)	Mild Preterm (n = 21)	Extreme Preterm without CLD (n = 19)	Extreme Preterm with CLD (n = 18)	P Value
FEV ₁ * (z score)	0.15 (0.91)	0.31 (0.87)	−0.37 (1.23)	−0.51 (0.98)	0.017
FRC* (z score)	−0.04 (1.20)	0.05 (0.74)	−0.08 (0.67)	0.17 (0.88)	0.75
FVC* (z score)	0.04 (1.02)	0.25 (0.81)	−0.16 (1.07)	−0.28 (1.09)	0.39
RV* (z score)	−0.15 (1.00)	0.22 (0.88)	−0.03 (0.85)	0.24 (1.29)	0.35
TLC* (z score)	−0.02 (1.12)	0.28 (0.78)	−0.11 (0.87)	−0.15 (0.98)	0.39

Definition of abbreviation: RV = residual volume.

*z scores were calculated within sample, adjusting for age, sex, height, and ethnicity. Numbers in parenthesis represent standard deviation.

This interpretation of our results depends on the assumption that ADC measured by ³HeMR is a valid proxy for alveolar dimensions. Many studies have compared histologic measures of alveolar dimensions against ADC in animal (30, 31) and human (32, 33) lungs. All confirmed the validity of ADC as a noninvasive tool for assessing peripheral airspace dimensions. Moreover, in our previous study of healthy children (16), we assessed the change of ADC with lung inflation in a group of volunteers and found that ADC changed by 0.41% (95% CI, 0.31–0.52%) for every 1% inflation of alveoli. This confirms that ADC is an effective measure of alveolar dimensions. This study has ample statistical power (90.5% statistical power of detecting a 20% increase in alveolar volume between the preterm and the term groups with α of 95) (see online supplement). Finally, our ³HeMR measurements were highly repeatable, with a within-subject coefficient of variation of ADC of 3.1%, similar to the value reported by Altes and coworkers (34). We therefore conclude that our technique could have reliably detected structurally and clinically significant differences in alveolar dimensions among the groups if they existed (35).

Alveolar structure in survivors of extreme preterm birth and CLD has been inadequately studied in the past. This is partly because such studies depend on morphometric histologic techniques that can only be performed on autopsy specimens. The current hypothesis regarding alveolar structure in preterm survivors is based on morphometric studies on children up to 3 years of age (4, 8). To our knowledge, there has been only one human patient with CLD whose lung has been analyzed histologically after 3 years of age (4). The consensus from these histologic studies is that there is delay in acinar and alveolar development in preterm survivors up to 3 years of age. Taken together with the theory that human

alveolarization is complete at 3 years, it was assumed that de-ranged alveolar structure would be a lifelong feature in preterm survivors (9, 15).

This conclusion can now be challenged for two reasons. First, histologic data are necessarily limited to the fatal, severe cases of preterm CLD and therefore cannot be easily generalized. The preterm baboon model of CLD was designed to resolve this problem. It (9, 36) showed that alveolar hypoplasia was the predominant feature of “new CLD” (use of prenatal steroids and postnatal surfactant combined with “gentle ventilation”). Despite this pioneering work, there were no data on long-term survivors, because the maximum survival reported is 8 months, a human developmental equivalent of 3 years.

The second challenge is to the assumption that human alveolarization is complete at 3 years. Recent evidence from animal studies using newer morphometric techniques has shown alveolarization through adolescence and repair of alveolar damage after pneumonectomy (17, 18). We have applied ³HeMR to healthy children and young people aged between 7 and 20 years, and showed results suggesting strongly that alveolarization extends through adolescence in humans (16). Neoalveolarization has been reported after pneumonectomy in an adult human (37). This, combined with our current data, makes it likely that extreme preterm survivors have catch-up alveolarization.

Given the high rates of antenatal steroid and surfactant use in our cohort of survivors of CLD, it is highly likely that these children had new CLD as described by Bancalari and Claure (38). Many previous studies have noted the persistence of obstructive airway disease in new survivors of CLD (6, 7, 39), similar to the results from our cohort.

TABLE 4. ALVEOLAR DIMENSIONS AND INTRASUBJECT SPREAD OF ALVEOLAR DIMENSIONS (ADC AND SD_{ADC}) ESTIMATED BY ³HE MAGNETIC RESONANCE

	ADC			SD _{ADC}		
	Mean (CI)	Difference (CI)	P Value	Mean (CI)	Difference (CI)	P Value
Basic model*						
Term	0.092 (0.089 to 0.095)	—	—	0.007 (0.005 to 0.008)	—	—
Mild	0.096 (0.091 to 0.101)	0.004 (−0.002 to 0.010)	0.160	0.007 (0.005 to 0.009)	0.001 (−0.002 to 0.003)	0.664
Extreme	0.090 (0.085 to 0.095)	−0.002 (−0.008 to 0.004)	0.514	0.003 (0.001 to 0.006)	−0.003 (−0.006 to −0.001)	0.018
CLD	0.089 (0.083 to 0.094)	−0.003 (−0.010 to 0.003)	0.301	0.010 (0.008 to 0.013)	0.003 (0.000 to 0.006)	0.022
Adjusted model†						
Term	0.093 (0.089 to 0.096)	—	—	0.007 (0.005 to 0.008)	—	—
Mild	0.093 (0.088 to 0.099)	0.001 (−0.005 to 0.007)	0.795	0.007 (0.004 to 0.010)	0.000 (−0.003 to 0.003)	0.865
Extreme	0.091 (0.085 to 0.097)	−0.001 (−0.008 to 0.005)	0.668	0.003 (0.000 to 0.006)	−0.004 (−0.007 to −0.000)	0.036
CLD	0.089 (0.083 to 0.095)	−0.003 (−0.010 to 0.004)	0.328	0.010 (0.007 to 0.014)	0.004 (0.000 to 0.007)	0.038

Definition of abbreviations: ADC = apparent diffusion coefficient; CI = 95% confidence interval; CLD = chronic lung disease of prematurity.

Group names: Term = term born; Mild = mild preterm; Extreme = extreme preterm without CLD; CLD = extreme preterm with CLD.

Linear regression adjusted for anthropometric data (basic model) and additional confounders (adjusted model).

*Basic model: adjusted for age, sex, height, and ethnicity (n = 119).

† Adjusted model: adjusted, in addition, for environmental tobacco smoke exposure (before and after 3 yr); steroids treatment (before and after 3 yr); and crowding (number of persons per room, indicator of socioeconomic status) (n = 98).

Figure 1. Apparent diffusion coefficient by degree of prematurity and presence of chronic lung disease (CLD). Term = gestational age 37–42 weeks, no neonatal respiratory support. Mild = gestational age 32–36 weeks, neonatal respiratory support for less than 4 weeks. Extreme = gestational age less than 32 weeks, neonatal respiratory support for less than 4 weeks. CLD = gestational age less than 32 weeks with neonatal respiratory support for more than 4 weeks.

Only one previous study has looked at alveolar structure in preterm survivors using $^3\text{HeMR}$, with results being reported only in abstract form (40–42). They reported a higher ADC in survivors of CLD compared with term-born children, but they included a wide age range (5–18 yr) and did not have independent measures of lung volumes. Furthermore, there was a trend toward normalization of ADC in preterm survivors from age 5–15 years.

Numerous studies applied high-resolution computed tomography (HRCT) to survivors of CLD, although only a few have studied new survivors of CLD. Aukland and coworkers (43) studied two cohorts of preterm survivors born between 1982 and 1985, and 1991 and 1992. They performed HRCT in inspiration and expiration and found a decrease in hypoattenuated areas from expiratory CT (26%) to inspiratory CT (14%), compatible with air trapping. In fact, the emphysema score in these subjects was 0% (44). Therefore, it seems likely that hypoattenuation seen in HRCT images of preterm survivors is not caused by deranged alveolar development but rather by air trapping. Van Beek and coworkers (45), in an adult study of chronic obstructive pulmonary disease, suggested that HRCT gives different information from $^3\text{HeMR}$ and that $^3\text{HeMR}$ correlates better to measures of alveolar function, such as diffusing capacity of carbon monoxide (DL_{CO}).

Studies of alveolar function, such as DL_{CO} in preterm survivors, consistently showed decreased DL_{CO} in preterm survivors compared with control subjects (46, 47). DL_{CO} is influenced by the permeability of the alveolocapillary barrier and ventilation-perfusion mismatch, apart from the alveolar surface area. Therefore, DL_{CO} can be abnormal even if alveolar structure has normalized. Such a dichotomy between alveolar structure and function may be more likely to happen in preterm survivors, where the damage occurs during alveologenesis and capillary maturation, than in chronic obstructive pulmonary disease, where damage occurs in mature alveolar-capillary units.

We found that SD_{ADC} was highest in extreme preterms with CLD. It was lower in term-born children and lowest in extreme preterms without CLD. It is plausible that extreme preterm-born infants who do not develop CLD have genetic factors that

confer advantage and this may manifest as relative homogeneity of alveolar dimensions. This unexpected difference in SD_{ADC} remained substantially the same (+0.003 compared with +0.004) when the analysis was done using the alternative definition of CLD. Because the number of subjects with CLD in the latter analysis was only nine, the difference was no longer statistically significant ($P = 0.082$). The relationship of intrasubject standard deviation of ADC with extreme preterm birth requires validation in larger studies. We also found higher values of ADC in girls and we speculate this is because of the earlier onset of puberty in females. The relationship between ADC and pubertal status needs to be assessed in future studies.

The strengths of our study include the availability of a control group of children who had similar characteristics as the study groups. These groups were recruited from subjects randomly selected from population-based databases. All subjects performed lung function tests including plethysmography, which enabled us to correct ADC measurements for relative difference in the helium bolus size and helium concentration. A weakness of our study was that our data were necessarily limited to the group of children who were able to perform lung function tests adequately. This might have excluded children at the most severe end of the spectrum of CLD. Another limitation of this study is that we assumed that our extreme preterm-born children had deranged alveolar structure in infancy. In the absence of noninvasive techniques applicable to infancy and in the presence of good histologic evidence that infants born at this gestation have deranged alveolar structure, this was a reasonable assumption. Finally, we did not assess alveolar function using such techniques as DL_{CO} , because this would have increased the burden of the protocol to an unacceptable extent.

Our findings that alveolar size and, by implication alveolar number, at school-age are normal in childhood survivors of extreme preterm birth with and without CLD support the evidence from our previous study that alveolarization continues through childhood (16). Our results have important implications. First, they alter the understanding of long-term outcome of perinatal lung injury. Second, the results may help in assigning prognosis for survivors of extreme preterm birth. Third, they support research into new therapies to ameliorate CLD, such as vitamin A therapy (48). Although our study does not show precisely when catch-up occurred, it suggests that the window for therapeutic strategies is wider than previously thought.

Author disclosures are available with the text of this article at www.atsjournals.org.

Acknowledgment: The authors thank Prof. David Field and Dr. Bradley Manktelow for allowing access to the records and database of the Trent Neonatal Survey. The authors acknowledge the work of Dr. Kuldeep Panesar, who helped with the performance and analysis of the helium-3 magnetic resonance measurements. They also thank Tony Davis who performed the stratified random selection of subjects from the Leicester Specialist Community Child Health Services Database.

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