Functional respiratory imaging provides novel insights into the long-term respiratory sequelae of bronchopulmonary dysplasia
Online supplement

Appendix 1. Detailed methods

a. Study population

Subject recruitment was based on an existing patient cohort at the Antwerp University Hospital included in a study investigating the relationship between histologic chorioamnionitis and early inflammatory variables in preterm infants [1]. Recruitment for this cohort spanned from September 1999 and February 2002. The inclusion criteria were as follows: (1) born at a gestational age < 31 weeks; (2) need for mechanical ventilation starting from day one. Patients who were mechanically ventilated for non-pulmonary reasons (*e.g.* diaphragmatic hernia, congenital malformations, cardiac defects, …) were excluded from the study. Additionally, for the present study, patients with severe mental or physical impairment were excluded since some degree of cooperation is needed for lung function testing and FRI imaging.

b. Data collection

All clinical follow-up data into adolescence, including a medical history (partly assessed through questionnaires), lung function testing and MDCT imaging were obtained during a single clinic visit at the Antwerp University Hospital.

i. Clinical data

The following clinical data were collected: (1) maternal and pregnancy details; (2) neonatal data such as gestational age, birth weight, number of days on ventilator, number of days on oxygen, diagnosis of RDS based on Edwards score [2], diagnosis of BPD and severity, administration of surfactant, administration of steroids. BPD was defined as the need for oxygen > 21% for at least 28 days. Severity was assessed at 36 weeks post-menstrual age or discharge (whichever came first). BPD was categorized as 'mild' if the patient was breathing room air, 'moderate' if there was oxygen need < 30% and as 'severe' if oxygen needs exceeded 30%. Because the percentage of oxygen at any given moment was not recorded in the original study, we could only discriminate BPD patients into 2 groups: mild BPD versus moderate and severe BPD combined.

II. Questionnaires

Questionnaires were used to assess clinical outcome and quality of life at time of inclusion in the present study. These questionnaires comprised 4 domains: (1) family history, including but not limited to pregnancy details, (2) patient history: illnesses, infections, surgeries, hospital admissions,… (3) psychomotor development; (4) current patient characteristics focusing on the respiratory tract, based on the ISAAC-questionnaire and assessing for, amongst other variables, respiratory infections, asthma, atopic constitution, use of inhalers, (nocturnal) cough, wheezing, exercise intolerance,… [3]. The questionnaire was completed in the presence of the adolescent, his or her parents and a trained investigator (MA, NE or MS).

c. Physical examination

Upon their clinic visit, patients received a physical examination (cardiopulmonary auscultation, abdominal auscultation, percussion and palpation). Their current weight, height, blood pressure, Mallampati score and tonsil score were recorded.

d. Lung function testing

Lung function testing comprised conventional spirometry (Jaeger Masterscreen PFT, CareFusion), body box plethysmography (Jaeger Masterscreen Body, CareFusion), determination of the lung clearance index (LCI) via N2-washout (Exhalyzer D with Spiroware software, Eco Medics) and a single breath carbon monoxide diffusion test (Jaeger MasterScreen, CareFusion). All tests were repeated after administration of a bronchodilator (salbutamol). Salbutamol was administered after HRCT with

FRI. Thus, patients first underwent lung function testing before HRCT, were then administered a bronchodilator and subsequently underwent a second round of lung function testing. A positive bronchodilator response was defined as an increase in FEV_1 > 12% after administration of salbutamol.

e. CT imaging

After the first round of lung function testing, but before administration of the bronchodilator, all patients underwent an unenhanced low-dose MDCT (GE VCT Lightspeed 64-slice scanner, GE Healthcare). The scanned area spanned from the upper part of the trachea to the diaphragm. To enable FRI analysis, HRCT images were obtained at 2 specific lung levels: at total lung capacity (TLC) (i.e. at maximal inspiration) and at functional residual capacity (FRC) (*i.e.* after normal expiration). In order to capture a clear image without artefacts, the patient had to perform a breath-hold manoeuvre at TLC and FRC for a few seconds. Breathing was monitored using 'Blue Cherry', technology that provides spirometry information during the scanning process (Geratherm, Germany). The mean total radiation dose was 2.4 mSv. The CT images were reviewed by 5 independent observers who were blinded to the outcome. Three observers (MS, AS, HEA) were experienced radiologists, two observers (KV, EL) did not have a background in radiology but received prior training in CT scoring systems for BPD. CTs were reviewed using the Aukland scoring system, a validated scoring system assessing the presence or absence of the following structural abnormalities: (1) linear or triangular subpleural opacities, (2) mosaic perfusion on inspiration, (3) air trapping, (4) decreased bronchoarterial ratio, (5) bronchiectasis, (6) peribronchial thickening, (7) bullae or blebs, (8) emphysema and (9) collapse or consolidation [4]. A training session for all observers was provided before scoring the patients' images.

f. Functional Respiratory Imaging

HRCT images were imported into Mimics, a commercial FDA-approved medical image processing software package (Materialise, Leuven, Belgium; Food and Drug Administration, K073468; CE certificate, BE 05/1191 CE01). This software package converts HRCT images into patient-specific, three-dimensional computer models of the lung structures.
The FRI process includes segmentation of the lung volumes at FRC and TLC from the HRCT images by using a HU threshold of [-1024; -400]. In addition, the fissures that separate the individual lung lobes are identified. By using the fissure lines as cutting planes, the individual lobe volumes can be determined. The airway tree, *i.e.* intraluminal air, could be segmented down to bronchi with a diameter of about 1–2 mm. Beyond this point, the HRCT resolution is insufficient to distinguish alveolar and intraluminal air. A typical airway model includes 5–10 generations, depending mainly on the disease state of the individual patient.
Functionality is added to the static segmented images by applying computational fluid dynamics methods to characterize airway resistance. The airway models are converted into a computational grid in order to solve the Navier-Stokes flow equations numerically, using commercial software packages (Ansys Inc., Canonsburg, PA, USA). Resistance was defined as the total pressure drop over an airway divided by the flow rate through that airway. Air trapping can be determined through segmentation based on Hounsfield unit (HU) thresholds of [-1024; -850] performed on the FRC scan. Blood vessels density and emphysema score can be extracted the same way but from the TLC scan, using HU thresholds of [-600; 600] and [-1024; -950], respectively.

g. Statistical analyses

Statistical analyses were computed in SPSS version 24 (IBM Corporation, USA). Normality was assessed based on graphical representation of the data as well as the Kolmogorov-Smirnov test. The independent t-test and Mann-Whitney U test were used to compare categorical and continuous variables as deemed appropriate after normality assessment. When appropriate, the chi-square or Fisher exact tests were used to compare 2 categorical variables. Spearman correlation coefficients were calculated to assess the relation between 2 continuous variables. Logistic regression was used for building prediction models with a categorical dependent variable, linear regression for continuous dependent variables. Intraclass correlation coefficients (ICCs) were computed to evaluate interobserver variability of the Aukland CT scores. For all analyses, $p \leqslant 0.05$ was considered statistically significant.

Appendix 2. Supplementary results

a. CT imaging

ICCs for the Aukland CT score were calculated for the following groups: (1) all 5 observers combined, (2) radiologists (MS, AS, HEA) and (3) non-radiologists (KV, EL). Interobserver variability (MS, AS, HEA, KV, EL) of total CT score was good (ICC=0.8). ICCs for the items of the Aukland CT score are shown in Table 3 and Appendix 2. Overall, there were no differences in ICCs between radiologists and non-radiologists. Therefore, the mean of all observers' scores was obtained and used in all subsequent analyses. We did not observe any significant difference in total Aukland CT score or one of the assessed parameters between both groups. There was a trend towards more emphysema in the BPD group (p = 0.08). Results are shown in Table 4. In addition, when comparing CT scores for children with and without respiratory symptoms (wheezing, nocturnal cough, dyspnea, exercise intolerance) and for children with and without a physician-made asthma diagnosis, no significant differences in structural abnormalities on CT were observed.

b. Functional respiratory imaging

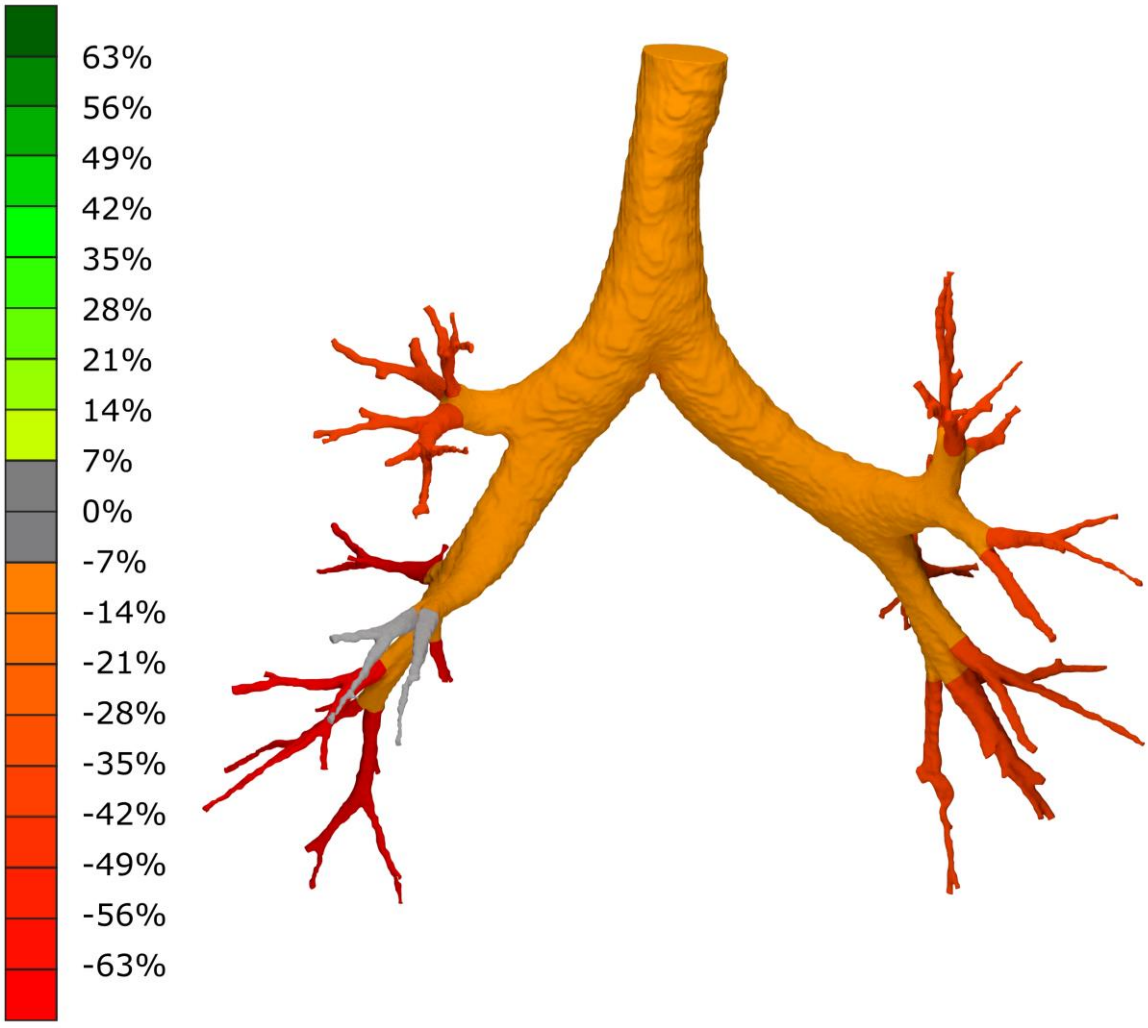

Figure E1. Functional respiratory imaging: visualisation of specific airway volumes. Specific airway volume is defined as the airway volume divided by the corresponding lobe volume. In this visualization, specific airway volumes of a representative BPD patient are compared to a representative preterm control patient. Thus, this image shows smaller specific airway volumes in the BPD patient compared to the preterm control (negative % values).

c. Integrating clinical outcomes, lung function and lung structure

	FRC_siRaw _central	FRC_siRaw _distal	FRC_siRaw _total	TLC_siRaw _central	TLC_siRaw _distal	TLC_siRaw _total
FEV_1	-0.272	-0,133	-0,177	-0,336	-0,346	-0,372
	0.103	0.433	0,295	0,042*	0,036*	0,023*
ΔFEV_1	0,142	0,421	0,433	0,503	0,594	0,570
	0,402	0,009*	0,007*	0,002*	< 0,001*	<0,001*
FVC	-0,091	0,299	0,262	0,103	0,183	0,176
	0,593	0,073	0,117	0,544	0,280	0,297
FEV_1/FVC	-0,188	-0,509	-0,504	-0,455	-0,691	-0,666
	0,266	0,001*	0,001*	0,005*	< 0,001*	<0,001*
MEF50	-0,226	-0,385	-0,401	-0,471	-0,578	-0,626
	0,179	0,019*	0,014*	0,003*	< 0,001*	< 0,001*
MEF25	-0,268	-0,303	-0,325	-0,468	-0,534	-0,575
	0,108	0,069	0,049*	0,004*	0,001*	< 0,001*
MEF75-25	-0,276	-0,261	-0,287	-0,473	-0,469	-0,524
	0,098	0,119	0,086	0,003*	0,003*	0,001*
Specific airway resistance	0,175	0,416	0,422	0,527	0,583	0,582
	0,301	0,011*	0,009*	0,001*	< 0,001*	<0,001*

Table E1. Correlations between spirometry and FRI airway resistance analysis. Spearman or Pearson correlation coefficients and respective p-values are shown. Significant values are indicated with an asterisk. Spirometry parameters are expressed as % predicted, except for MEFs (l/s) and specific airway resistance (kPas). FRI airway resistance parameters are expressed in kPas.

	FRC_air trapping_total	FRC_air trapping_lower	FRC_air trapping_upper
RV	0,663	0,650	0,615
	< 0,001*	< 0,001*	< 0,001*
FRC	0,685	0,715	0,618
	< 0,001*	< 0,001*	< 0,001*
TLC	0,582	0,622	0,554
	< 0,001*	< 0,001*	< 0,001*

Table E2. Correlations between body plethysmography lung volume measurements and FRI air trapping analysis. Spearman correlations and respective p-values are shown. Significant values are indicated with an asterisk. Lung volume parameters are expressed as % predicted; FRI air trapping parameters are expressed in % of air trapping compared to total lung volume, lower lobes and upper lobes respectively.

Appendix 3. Questionnaire

Patients and their parents were asked to complete the following questionnaire before the study clinic visit. Note: original questionnaires were distributed in Dutch.

A. Family history – to be completed by the parents

Please provide the details listed below.

1. Concerning the mother of the patient
    a. Age at the time of giving birth to the patient
    b. Ethnicity
    c. Medical history
2. Concerning the father of the patient
    a. Age at the time of giving birth to the patient
    b. Ethnicity
    c. Medical history
3. Other children
    a. Were there other pregnancies before the pregnancy with this patient?
    b. Were other children born preterm, and if yes, how many?
    c. How many children are currently alive, and do they have a significant medical history?
    d. Are there abortions in the mother's history?
        i. Number of abortions
        ii. Spontaneous abortions and number?
        iii. Therapeutic abortions and number?
    e. Are there deceased children?
        i. Before birth
        ii. At birth
        iii. After birth? At which age?

B. Pregnancy details – to be completed by the mother of the patient

1. Did the pregnancy occur spontaneously or after medical intervention?
2. Single pregnancy or multiples?
3. Did you have a regular follow-up during this pregnancy?
4. Was any form of prenatal diagnostics performed?
    a. No
    b. Chorion villus sampling
    c. Cordocentesis
    d. Amniocentesis
5. Did you smoke during pregnancy? If yes, how many cigarettes per day?
6. Did you drink coffee during pregnancy? If yes, how many cups per day?
7. Did you drink alcohol during pregnancy? If yes, how many units per day?
8. Pregnancy details. Please indicate if any of the following events occurred during pregnancy, and if yes, when/how long.
    a. Vaginal bleeding
    b. Pre-existing hypertension (diagnosed before pregnancy)
    c. Hypertension
    d. Pre-existing diabetes (diagnosed before pregnancy)
    e. Cardiovascular disease
    f. Renal disease
    g. Neurological disease
    h. Respiratory disease
    i. Iso immunisation
    j. Other disease
    k. Growth retardation of the foetus
    l. Preterm contractions
    m. Congenital malformations in the foetus
    n. Hospitalisation before delivery
    o. Chorioamnionitis

p. Pre-eclampsia
q. Medication during pregnancy, in particular one of the following
    i. Tocolysis
    ii. Corticoids
    iii. Antihypertensive medication
    iv. Antibiotics
    v. Anti-epileptics
    vi. Narcotics
    vii. Other medication

9. Concerning the delivery
    a. Vaginal delivery or C section?
    b. Gestational age at delivery

C. Neonatal events – to be completed by the parents

1. Biometry
    a. Birth weight
    b. Length at birth
    c. Head circumference at birth
2. APGAR scores
    a. At 1 minute
    b. At 5 minutes
3. Please indicate if any of the following occurred during the neonatal period
    a. Asphyxia
    b. Respiratory system
        i. Hyaline membrane disease
        ii. Transient tachypnea of the neonate
        iii. Pneumonia
        iv. Mechanical ventilation (intubation). If yes, how long?
        v. CPAP (intubation). If yes, how long?
        vi. Oxygen therapy (intubation). If yes, how long?
        vii. Surfactant therapy
    c. Neurological system
        i. Brain bleeding
        ii. Leukomalacia
        iii. Convulsions
        iv. Apnea
    d. Cardiovascular system
        i. Patent ductus arteriosus
        ii. Other
    e. Infections
    f. Metabolic problems or diseases
        i. Hypoglycaemia
        ii. Hypocalcaemia
    g. Intra uterine growth retardation
    h. Gastrointestinal system
        i. Necrotising enterocolitis
        ii. Feeding difficulties and/or problems
    i. Congenital abnormalities
        i. Spina bifida
        ii. Hydrocephalus
        iii. (palato)schisis
4. How was the psychomotor development of your child during the following periods?
    - Pre school
    - Primary school
    - High school
5. Was your child immunised? If not or not entirely, please indicate which vaccinations your child did not receive.
6. Did your child suffer from serious diseases or medical problems beyond the neonatal period?
7. Did your child undergo surgical procedures beyond the neonatal period?

D. Current health assessment – to be completed by the parents and patient together

1. Did your child suffer from frequent respiratory infections in the past 2 years?
2. Did your child ever have pneumonia? If yes, when?
3. Does your child have asthma? If yes, when was this diagnosed?
4. Did your child have asthma symptoms in the past 12 months?
5. Did your child present with wheezing during the past 12 months?
   If yes, how often did your child present with wheezing during the past 12 months?
6. Did your child use medication for wheezing during the past 12 months?
7. Does your child regularly use nebulizers? If yes, what is the medication regimen?
8. Did your child wake up during the night due to wheezing during the past 12 months?
   a. Not at all
   b. Less than once a week
   c. More than once a week
9. Did your child experience shortness of breath at night during the past 12 months?
   a. Not at all
   b. Sometimes
   c. Often
   d. All the time
10. Did your child experience wheezing during physical activity during the past 12 months?
11. Did your child present with a dry cough at night (without concurrent respiratory infection at that moment) during the past 12 months?
12. Did your child cough daily for at least 4 consecutive weeks during the past 12 months?
13. Did your child use medication for chronic cough during the past 12 months? If yes, which medication?
14. Does your child snore?
15. Is your child allergic? If yes, was this confirmed with objective testing?
16. Does your child have nasal obstruction?
17. Did your child present with sneezing, a runny or blocked nose (without concurrent respiratory infection at that moment) during the past 12 months?
18. Did your child have symptoms of hay fever during the past 12 months?
19. Did your child use medication for hay fever or nasal symptoms during the past 12 months?
20. Did your child have an itching skin rash (coming and going) for at least 6 months during the past 12 months?
21. Did your child have eczema during the past 12 months?
22. Did your child use medication to treat itching skin rash during the past 12 months?
23. Does your child suffer from significant fatigue?
24. Does your child have learning difficulties?
25. Does your child have heart problems?
26. Does your child smoke?
27. Is your child exposed to second-hand smoking in the home environment?
28. Does your child currently present with symptoms that were not yet addressed in this questionnaire?
29. Does your child currently use medication that was not yet mentioned above?

References

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