Altered Relaxation Times in Magnetic Resonance Imaging Indicate Bronchopulmonary Dysplasia: Supplementary Material

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S1 – S4 Patients and Methods

S1 Study population

Approval by the local Ethics Committee was obtained prior to the initiation of the study and parents provided written informed parental consent for all study infants (Munich #195-07; Giessen #135/12). Study registration was performed at the German Registry for Clinical Studies (DRKS00004600).

Preterm infants with or without later development of BPD and a gestational age (GA) of ≤32 weeks born at the Perinatal Center of the Ludwig-Maximilians-University, Campus Grosshadern (exploration cohort, n=40) were prospectively included in the study. Imaging markers established in this cohort were then confirmed in a second, independent cohort recruited at the Perinatal Center of the Justus-Liebig-University, Giessen (confirmation cohort, n=21); see Table S1 for patient characteristics.

Exclusion criteria were severe congenital malformations (e.g. hypoplastic left-heart syndrome, severe hypoplasia of the lungs or congenital diaphragmatic hernia), chromosomal abnormalities (e.g. trisomy 13 or 18), inborn errors of metabolism, and decision for palliative therapy directly after birth.

The following definitions were applied: BPD was defined according to Jobe and Bancalari [1] and graded as follows: mild (oxygen supplementation at 28 days

postnatally), moderate (need for < 30% oxygen at 36 weeks PMA or discharge, whichever comes first), or severe (need for ≥ 30% oxygen and/or ventilator support, i.e. positive pressure ventilation (PPV) or nasal continuous positive airway pressure (NCPAP)) at 36 weeks PMA or discharge, whichever comes first) BPD. Days with ventilator support were recorded as endotracheal (invasive) mechanical ventilation, nasal intermittent mandatory ventilation or nasal intermittent positive pressure ventilation and/or nasal continuous positive airway pressure in days. A course of antenatal corticosteroids was recorded as "complete" if two doses of betamethasone were given >24 hours before birth with the last dose administered no later than 7 days before birth. Chorioamnionitis was defined as the presence of inflammatory alterations of the chorionic plate at histologic examination or signs of infection in both mother and infant [2]. Intrauterine growth restriction was defined as birth weight below the 10th percentile. Postnatally, diagnosis and severity of RDS (respiratory distress syndrome) was scored on anterior-posterior (a.-p.) chest radiographs according to Couchard et al [3] by a consensus panel of two experienced neonatologists and an experienced radiologist with over 15 years of experience and blinded to the clinical information of the patients. Systemic infections were diagnosed according to Sherman et al. [4] with one or more clinical and laboratory signs of infection.

After discharged, patients were subject to home monitoring (getemed, Potsdam, Germany) in case of frequent desaturations < 85% due to short apnoeic episodes or periodic breathing requiring tactile stimulation or oxygen therapy. Home monitoring was discontinued when breathing pattern normalized and apnoeic episodes did not result in frequent oxygen saturation levels below 85%.

S2 Pulmonary MR imaging

MR imaging protocol. Pulmonary MR imaging measurements were performed at 36 weeks GA (35.4 weeks median GA) in non-sedated, spontaneously breathing infants (fiO2 0.21) without administration of intravenous contrast and positioned in a supine position while swaddled in a vacuum mattress after feeding (exploration cohort). A size-adapted number of coil elements from a 32-channel spine array coil, an 18-channel flexible body array coil and a 20-channel head-and-neck array coil was used in a 3-Tesla whole-body MR scanner (Magnetom Skyra, Siemens Healthineers, Erlangen, Germany). Hearing protection was performed by the use of MiniMuffs® (Neonatal Noise Attenuators, Natus Medical Incorporated, Seattle, USA).

MR imaging measurements in the second cohort (confirmation cohort) followed the same imaging protocol under light sedation with chloral hydrate (30-40 mg/kg administered orally).

The protocol included pulse sequences for the assessment of morphology, volume, and of quantitative relaxation parameters of the lung. In detail, the following pulse sequences were used: i) T2-weighted single-shot fast-spin-echo (ssFSE) sequences in coronal, axial, and sagittal orientation; spatial resolution 1.9×1.3×4.0 mm^3, 20 slices with a field of view (FOV) of 340×255 mm^2. The echo time (TE) was 57 ms; the acquisitions were ECG-triggered with a minimum repetition time (TR) of 2 RR intervals; 2 signal averages were acquired. ECG triggering was performed to provide images with lower level of motion artifacts around the heart. ii) Single-slice T2-mapping and T1-mapping using ssFSE sequences (T2-mapping: TR = 2000 ms, TE = 26, 41, 61, 92 ms; T1-mapping: TR/TE = 3000 ms/26 ms, inversion time (TI) for slice-selective inversion = 25, 150, 400, 800, 1600, 2600 ms and one acquisition without inversion pulse) in coronal orientation (8 averages); spatial resolution: 2.3×2.3×20.0 mm^3. For T1 and T2 mapping, data from 8 acquisitions were averaged, thereby mitigating the

impact of respiratory or cardiac motion. FOV: 300×300 mm²; the 20 mm slab was positioned in a representative central lung area capturing a substantial fraction of the lungs in the anterior-posterior direction. The total acquisition time of the three T2-weighted ssFSE sequences was approximately 5 minutes (depending on the heart rate of the infant); the total acquisition time of the T2 and T1 mapping sequences was also approximately 2 and 3 minutes, respectively.

MR image analysis. To ensure equal conditions for pulmonary imaging analysis, a consensus panel ruled out significant atelectasis, air leaks or pleural effusion before processing the respective images for further analysis. For T2- and T1-mapping analysis, the lung was manually virtually segmented into four lung quadrants (upper left, upper right, lower left, and lower right quadrants) using the software "Osirix MD" (version 6.5) [5]. T2 and T1 relaxation time values were calculated by non-linear exponential fitting and evaluated for these four lung quadrants and for the entire lung in order to consider regional differences and total lung changes. Free-breathing average total lung volume was measured by manual lung segmentation in axial and coronal acquisitions with the editor tool in the open-source software "3D Slicer" (version 4.3.1 r22599) [6]; left and right main bronchi were excluded; further exclusion of airways was limited by the small size of the segmented lungs. To reduce the influence of measurement errors, the arithmetic means of the two volumes derived from axial and coronal images was used (sagittal slices were not analysed due to the lack of discernibility between tissues next to the mediastinum).

S3 Infant Lung Function Testing (ILFT) in preterm infants at 36 weeks GA

ILFT protocol. ILFT was performed at 36 weeks GA and standardized according to the recommendations of the American Thoracic Society and European Respiratory Society [7 8]. All infants were spontaneously breathing room air under light sedation

(chloral hydrate, 30-40 mg/kg; orally) and had been free of respiratory infection for more than three weeks, presenting with normal blood pH values; optional inhalation therapy was terminated three days prior to the study for the purpose of pulmonary function testing. A highly standardized and optimized measurement setting minimized intrinsic limitations of lung function measurements using body plethysmography in infants with 2 kg bodyweight. Oxygen saturation levels (SpO_2; Masimo Radical-7 pulse oximeter, Irvine, CA) and heart rate were monitored.

Lung function measurements were recorded during relaxed quiet sleep in supine position using the Jaeger MasterScreen BabyBody device (v4.6; CareFusion, San Diego, CA; Rendell-Baker Soucek facemask (size 0 or 1; Rüsch UK Ltd., High Wycombe, UK), sealed with a rim of therapeutic putty. Measurements included tidal breathing analysis, passive respiratory mechanics and functional residual capacity (FRC_p). FRC_p was measured as described previously with the infant making respiratory efforts against a closed shutter not using the raised volume technique [8 9]. Total respiratory compliance (C_{rs}), was assessed in single occlusion technique (SOT) using five to eight regular tidal breaths to establish a stable end-expiratory level (EEL) before activating the balloon shutter. FRC_p, C_{rs} and tidal volume (TV) were normalized to body weight (kg).

ILFT analysis. The following quality criteria were applied[10]: For C_{rs} and FRC_p a minimum of five technically satisfactory measurements were obtained and the mean of 3–5 valid measurements was calculated. The 'within subject within-occasion coefficient of variability (CV)' was reported as [SD/mean] x 100.

S4 Statistical analyses

Missing values were imputed by sampling from a normal distribution with the sample mean and standard deviation of the observed values for each variable, in which the

covariance structure was taken into account for the highly correlated MRI variables. A logistic Group Lasso model [11] with alpha optimization was used to identify MR imaging and lung function variables best explaining the disease outcome. For all variables used in the Grouped Lasso model a maximum of 19.6 percent missing values had to be considered. The percentage of missing values was mainly impacted by the availability of lung function data. Missing values of the imaging data were imputed considering the covariance structure because of the highly correlated MRI variables. Missing values for the lung function data was imputed by sampling from a normal distribution with the sample mean and standard deviation of the observed values for each variable.

To model binary disease outcomes, BPD was dichotomized in two different ways: mild, moderate and severe BPD vs. no BPD; moderate/severe BPD vs. no/mild BPD.

A Grouped Lasso Logistic model was used to estimate the impact of different variables on the outcome BPD; independent variables included gestational age, gender, birth weight, antenatal corticosteroids, early onset infection and imaging data. To account for confounder effects, the clinical variables were included in the statistical model reflecting the panel of known risk factors for disease development [12,13-15].

The Grouped Lasso Logistic model was repeated using lung function and imaging data as independent variables. Quality of the models was assessed by leave-one-out cross validation for unbiased validation avoiding overoptimistic AUC for model validation.

Model validation was realized in an independent study cohort calculating ROC and AUC by comparing predicted results with true output.

Confirmation of the results was obtained using logistic regression (no vs. mild/moderate/severe BPD) under consideration of the confounders GA, gender, steroids and early infection. Testing for a potential difference in T2/T1 relaxation times regarding the need for home monitoring was performed using a Two Sample t-test.

S5 Results

Quantification of structural changes in BPD lungs by advanced MRI

40 preterm infants were included in the exploration cohort (mean age at imaging 35.4 weeks GA, n=21 female). 21 infants were included in the independent validation cohort (mean age at imaging 37.7 weeks GA, n=14 female) (Table S1).

By regularized linear modelling, we identified quantitative MR imaging parameters associated with the diagnosis of BPD in premature infants near term. As correlation plot analysis of regional values, i.e. upper right, upper left, lower right, lower left lung, for T1 and T2 relaxation times indicated their clustering, presentation of the results refers to the entire group of T1 and T2 values, respectively.

Model coefficients for the logistic imaging model are summarized in Table 1 in the main document. The estimates for the model parameter coefficients express the strength of the imaging variables to identify the outcome variable BPD. To provide more information about the precision of our estimates we performed a non-parametric bootstrap procedure with B=100 replicates and calculated 95% percentile bootstrap intervals (Figures S4, S6 and S7).

Furthermore, we included 95% confidence intervals for the coefficients of the imaging and the clinical regression model (Figures S8 and S9).

Logistic regression analysis with the count variables ‘days of oxygen’ or ‘days of mechanical ventilation (MV)’ (birth to discharge) confirmed the results obtained by nominal variable analysis in the grouped Lasso Logistic model (Figures S2 and S3).

Comparison of T1 and T2 relaxation times between the exploration and the confirmation cohort showed no statistical differences.

Infants with the need for home monitoring had significantly higher T2 relaxation times compared to those discharged without home monitoring (p=0.04), indicating more

severe and persistent lung disease in infants with increased T2 relaxation times (Figure S5).

Image analysis was done considering potential regional differences reflecting the characteristic inhomogeneity of the disease. Total values represent results obtained for the entire lung (Figure S1).

Legend Figures S2-S4 and S6-S9

beta coefficients: quantify the strength of the variables (clinical, imaging and lung function) identifying the outcome variable BPD; steroids (yes)=antenatal steroids; T2 total=total value of T2 relaxation time; T1 total=total value of T1 relaxation time; T1 (upper right), T1 (lower right), T1 (upper left) and T1 (lower left): for mapping analysis the lung was manually virtually segmented into four lung quadrants; FRC_p_pre=functional residual capacity; CrsSO.kg_pre=compliance of total respiratory system per kilogram (single occlusion); TV.kg=tidal volume per kilogram

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Table S1. Patient characteristics

	Exploration cohort	Confirmation cohort
n	40	21
Gestational age (weeks PMA)	27.7 (24.1-30.6)	25.6 (24.4-29.6)
Birth weight (g)	925 (415-1770)	810 (340-1470)
Gender (female/male)	21/19	14/7
pH, umbilical artery	7.34 (6.95-7.47)	7.34 (7.01-7.48)
Antenatal corticosteroids	36 (90%)	20 (95.2%)
Chorioamnionitis	19 (47.5%)	15 (71.4%)
Early onset infection	9 (22.5%)	4 (19%)
RDS ≥ grade 3	11 (27.5%)	3 (14.3%)
Days of mechanical ventilation	36.5 (0-78)	24 (2-74)
- Endotracheal mechanical ventilation (n/days)	6 (0-34)	2 (0-32)
- Pharyngeal ventilation/CPAP (n/days)	33 (0-55)	18 (2-56)
PDA	20 (50.0%)	15 (71.4%)
Postnatal corticosteroids	18 (45.0%)	1 (4.8%)
ROP ≥ grade 3	2 (5.0%)	6 (28.6%)
IVH ≥ grade 3	1 (2.5%)	3 (14.3%)
ICU stay (days)	60 (30-127)	36 (5-93)
BPD		
- None	20 (50.0%)	4 (19%)
- Mild	12 (30.0%)	9 (42.9)
- Moderate	4 (10.0%)	2 (9.5%)
- Severe	4 (10.0%)	6 (28.6%)

Data are given as median and range or number and percent of total in group. PMA, post-menstrual age; RDS, respiratory distress syndrome; PDA, patent ductus arteriosus; CPAP, continuous positive airway pressure; ROP, retinopathy of prematurity; IVH, intraventricular haemorrhage; ICU, intensive care unit; BPD, Bronchopulmonary Dysplasia.