From neonatal lung function to lung function and respiratory morbidity at 6-year follow-up

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Abstract

Background: Lung function is traceable from infancy to adulthood. Only a few studies have examined lung function from birth to childhood longitudinally in children born moderate to late preterm. We aimed to investigate how prematurity and lung function in the neonatal period are related to lung function and respiratory morbidity at age 6 in former moderate to late preterm children compared with children born at term.

Methods: Lung function was measured in a cohort of moderately to late preterm (n = 48) and term-born (n = 53) infants in the neonatal period by FeNO, and tidal breathing flow-volume loops (TBFVL) and at age 6 (n = 52) by spirometry, whole-body plethysmograph and impulse oscillation combined with a respiratory symptom questionnaire.

Results: Moderate to late preterm children had a higher TPEF/TE ratio neonatally (42.6% vs. 33.7%, p = 0.02) and a lower % predicted orced expiratory volume in the first second at age 6 (94.4% vs. 101.9%, p = 0.01) compared to term-born children. We found a significant association between the variability of neonatal tidal volume and effective airway resistance at age 6 (β = −0.34, p = 0.03). No association between neonatal FeNO or TBFVL and respiratory morbidity at 6-year follow-up was shown.

Conclusion: Children born moderate to late preterm had lower lung function at age 6 than term-born children. We did not find evidence for the use of neonatal tidal breathing parameters as a predictor for subsequent respiratory morbidity or lung function, however sample size was small.

KEYWORDS
asthma and early wheeze, neonatal pulmonary medicine, pulmonary function testing

1 | INTRODUCTION

The development and growth of the lungs start in fetal life and continues through childhood.¹ The risk of developing respiratory diseases, such as asthma, and lower lung function in young adults is influenced by fetal and early postnatal events and environmental exposures in childhood.²⁻⁴ We know that a reduced maximal plateau for Forced Expiratory Volume in the first second (FEV₁) attained in the third decade of life might determine the development of early-onset chronic respiratory diseases, even without a subsequent accelerated decline in FEV₁.⁵ Studies have shown that impaired lung function in infancy is associated with respiratory symptoms and low
lung function through childhood and adulthood. Furthermore, low lung function in adulthood is a risk factor for overall mortality.

Children born extremely and very preterm (Gestational age (GA) <28 weeks and 28–31 weeks +6 days, respectively) are at an increased risk for impaired lung function and respiratory morbidity in childhood and adulthood. Increased interest is directed towards investigating the long-term consequences of preterm birth, such as the risk of asthma and COPD in adulthood. However, less research has focused on the long-term respiratory outcomes of the largest preterm group, the moderate to late preterm children (GA 32 weeks –36 weeks + 6 days).

Measurement of lung function in neonates and infants, and especially preterm neonates, are complicated and time-consuming. Several studies have used squeeze jackets to perform forced spirometry tests in neonates, which are easily interpreted but have a limited clinical application due to requiring sedation of the newborn. Measurement of tidal breathing flow-volume loops (TBFVL) and FeNO can be performed on a neonate without sedation or discomfort. Reduced ratio of time to peak expiratory flow over total expiratory time (TPEF/TE) in the first week of life has shown association to increased risk of wheezing during infancy and childhood in term-born infants. However, the coefficient of variance (CV) for tidal volume (VT) seems to be better at predicting respiratory symptoms in preterm infants compared to TPEF/TE. FeNO measured at age 5 weeks has shown association to early life wheezing but not to wheeze or asthma in older children.

We aimed to investigate and explore prematurity and neonatal tidal breathing parameters and their association with lung function and respiratory morbidity at age 6 in moderate to late preterm children compared with children born at term. We hypothesized that preterm children would have a lower lung function and increased risk of respiratory morbidity than term-born children and that neonatal tidal breathing parameters would be associated with lung function and respiratory morbidity at age 6.

## 2 METHODS

We conducted a longitudinal cohort follow-up study from the neonatal period to age 6. It encompasses two sub studies since the participants differed in these: Sub study 1 (cross-sectional study) compared neonatal lung function and respiratory morbidity and lung function at age 6 years between preterm and term-born children recruited in the cohort as newborn. Sub study 2 (longitudinal study) used the longitudinal design to explore associations between neonatal lung function and FeNO with respiratory morbidity and lung function at age 6.

### 2.1 Participants

Neonates included in the AD-ON research biobank at birth (n = 1500) and subsequently admitted to the neonatal intensive care unit (NICU) at Nordsjaellands Hospital from September 2013 to September 2014 (n = 179) were asked to participate in the AD-ON neonatal cohort. One-hundred-and-forty-nine neonates, hereof 63 preterm and 86 born at term, were included in the AD-ON neonatal cohort. The neonates were investigated six times (postnatal age 2/3, 5, 7, 14, 21, and 28 days) following a prespecified program during the first month of life and invited for follow-up visits at 1 (09/2014–09/2015) and 6 years of age (available for invitation at age 6, n = 128) (01/2020–07/2022).

In this study we included children from the AD-ON neonatal cohort with GA ≥32 +0 weeks without significant respiratory disease in the neonatal period pragmatically defined as a need for respiratory support on postnatal day 14 (n = 141). Neonates with need for respiratory support for more than 14 days were excluded to make sure the measurements used to predict later lung function were not performed on a neonate with obvious present lung disease.

Figure 1A shows the inclusion and exclusion for children in sub study 1 (cross-sectional study). The inclusion criteria for neonatal data were: Step (1) Children with results from a valid TBFVL or tidal fractional exhaled nitric oxide (FeNO) at postnatal day 14 or later. The inclusion criteria for 6-year data in sub study 1 were: Step (2) Parents answered the questionnaire at six-year follow-up and/or Step (3) Valid lung function test from 6-year follow-up.

Figure 1B shows the inclusion and exclusion for sub study 2 (longitudinal study). The inclusion criteria were: Step (1) children with results from a valid TBFVL or tidal FeNO at postnatal day 14 or later and one of the following: Step (4) Parents answered the questionnaire at six-year follow-up or Step (5) Valid lung function test from 6-year follow-up.

The neonatal data collection and baseline characteristics of the cohort have been described previously in details.

## 2.2 Neonatal tidal breathing measurements

Mixed multiple breath FeNO was measured with a chemiluminescence analyzer online with NO sample gas flow of 110 ml/min (CLD 88sp, Eco Medics AG). FeNO was collected for 60 s, and a mean value of a minimum of two measurements, with a maximum variation of 1 ppb, was calculated. NO output (V’NO) was calculated from the validated FeNO values times the expiratory flow. NO free air was supplied by Denox 88 (Eco Medics AG) with a constant flow of 300 ml/s. The facemasks used (Fisher & Paykel Healthcare size 35–50 mm [XS-M]) had an effective dead space of 5, 8, and 11 ml, respectively.

Simultaneously, TBFVL was analyzed using an ultrasonic flowmeter (CLD 88sp Ecomedics; software Spiroware Client version 3.1.6). All measurements with a maximal variation in the tidal volume of 10% were visually examined, and all curves with stable volume and shape were selected. Only measurements with a minimum of 10 valid flow-volume curves were included.
From these included measurements, mean, standard deviation, and coefficient of variance (CV) for tidal volume (VT), peak expiratory flow (PEF), and TPEF/TE were calculated from the expiratory phase of the breathing cycle. VT and PEF were divided by weight in kilograms at measurement.

### 2.3 Questionnaire at 6-year follow-up

A questionnaire was sent to the parents of all potential participants from the AD-ON neonatal cohort (n = 128). The questionnaire included questions from the standardized ISAAC questionnaires, diagnosis, medication use, hospital visits, and antibiotic use.

### 2.4 Lung function at 6-year follow-up

Spirometry measurements including change after salbutamol inhalation, impulse oscillation technique (IOS) (Vyntus®, IOS, VyaireTM Medical) and body plethysmography (Master Screen Body, Jaeger®) was performed according to American Thoracic Society (ATS) and European Respiratory Society (ERS) guidelines. All variables were analyzed using SentrySuite™ software solution (Vyaire®). For reference spirometry parameters, we used the Global Lung Function Initiative (GLI). Change in percent in Forced mid-expiratory flow (FEF25–75) after salbutamol inhalation was only considered valid if Forced Vital Capacity (FVC) in the same test did not change more than ±5%. IOS parameters (Resistance [R], 5 Hz, R 20 Hz and...
Reactance (X) 5 Hz and airway resistance tests from the whole-body plethysmography (sR$_{TOT}$ and sR$_{EFF}$) are presented as z-scores.$^{26,27}$

2.5 | Statistical analysis

Due to the study's design being a follow-up study, the sample size was opportunistic and reliant on the attendance rate at follow-up. We expected a follow-up rate of 70%, allowing for approximately 100 participants equally divided between preterm and term-born. Using data from previous studies, we estimated to detect a difference in FEV$_1$ of 0.5 z-scores between preterm and term-born children with a power of 70%–90%.$^{28,29}$

Sub study 1: The student's t-test and the Kruskal–Wallis test were used to compare continuous variables (normally and not normally distributed, respectively) mean (SD) or median values (IQR). The $\chi^2$ test was used for categorical variables. For binary outcomes, the relative risk for preterm children was calculated from two by two tables.

Sub study 2: The association between neonatal tidal breath parameters (VT, PEF, T$_{PEF}$/T$_{E}$, CV for VT, FeNO, and V'NO) and lung function parameters at 6-year follow-up (% predicted FEV$_1$, FVC, FEV$_1$/FVC, FEF$_{25-75}$%, reversibility of FEV$_1$ and FEF$_{25-75}$, R 5 Hz, R 20 Hz, The difference in resistance between 5 and 20 Hz (RDIFF5–20), SRTOT and SREFF) were examined with multiple linear regressions and for binary outcomes (respiratory morbidity) logistic regression, adjusted for parental asthma, parental smoking, prematurity and days of Continuous positive airway pressure (CPAP) treatment neonatally. We also performed unadjusted subgroups analysis for preterm and term-born children respectively. Moreover, we performed post hoc analysis investigating the association between FeNO and V'NO measured on postnatal day 2–7 and subsequent...
respiratory morbidity and lung function at age 6 (data not presented in this paper). Due to the small sample size, a strong association was needed to find any evidence for association, therefore, we choose to consider a $p < 0.05$ to be significant. This decision was made to ensure we did not overlook potential important associations.

All statistical analyses were performed using R-project software (Version 4.0.3).

2.6 | Ethics

All participants’ parents and/or guardians signed an informed consent form before entering the AD-ON neonatal cohort and again at the 6-year follow-up. This study was approved by the Copenhagen Capital Region Ethics Committee (H-4-2012-091 and H-19011004) and the regional data protection agency (P-2019-251).

3 | RESULTS

One-hundred-and-one infants had valid TBFVL or FeNO on day 14 or later (Step 1). Out of the 101 included at Step 1, 78 had a valid TBFVL, and 99 had a valid FeNO (Figure 1B). One-hundred-and-twenty-eight children were invited to 6-year follow-up. The parents of 66 children answered the questionnaire (Step 2), and 53 attended 6-year follow-up (Figure 1A). At 6-year follow-up, one child had invalid lung function tests and missing data, and this participant was excluded from further analysis.

In sub study 1 (cross sectional study) differences between preterm and term-born children (included at Step 1) showed that preterm neonates needed CPAP for longer, had a lower VT, but not when corrected for weight, and a higher $T_{PEF}/T_0$ compared to the infants born at term (Table 1). Differences between preterm and term-born children at 6-year follow-up (included at Step 2 and 3), showed that preterm born children were lower in weight and height than those born at term (Table 2). Moreover, preterm children had a significantly lower % predicted FEV$_1$ compared to children born at term (Table 2). We found no significant association between CPAP treatment for more than 24 h and % predicted FEV$_1$ ($\beta = 2.5$, CI 95% = $-4.9, p = 0.45$) or evidence for interaction between prematurity and CPAP ($\beta = 2.1$, CI 95% = $-12.16, p = 0.76$). The prevalence of ever having a wheezing episode was not different between preterm and term-born children in our cohort (47% and 50%, respectively) (Supporting Information: Table E1). During the past 12 months, wheezing episodes during exercise and night-time cough was more common among preterm born children (16% vs. 10% and 29% vs. 13%, respectively), but the difference did not reach significance (Supporting Information: Table E1). We found no significantly increased risk of respiratory morbidity at age 6 in moderate to late preterm compared to our cohort’s term-born children, however there was a tendency towards increased risk for some of the outcomes; disturbed sleep due to wheeze $RR = 3.0$ (CI 95%, 0.9–13.8), wheeze during exercise $RR = 1.7$ (CI 95%, 0.4–6.4) and night-time cough $RR = 2.3$ (CI 95%, 0.8–6.8) (Figure 2).

We could not find any correlation between respiratory symptom score, defined as the sum of positive answers to the ISAAC questionnaire, and FEV$_1$ at 6-year follow-up (Pearson’s correlation coefficient $= -0.07$, $p = 0.67$).

The proportion of preterm children was significantly higher in the group that attended the 6-year follow-up than in the dropout and lost to follow-up group ($p = 0.001$) (Supporting Information: Table E2). As a result, the group that attended 6-year follow-up also had significantly lower birth weight ($p = 0.04$) and needed supplemental oxygen for longer ($p = 0.006$) than the dropouts. Children who attended the 6-year follow-up had a significantly lower neonatal PEF and V’NO compared to dropouts (Supporting Information: Table E3).

In sub study 2 (longitudinal study) the number of participants with paired valid measurements from both neonatal and at 6-year follow-up is shown in Figure 1B and Supporting Information: Table E4.

The multiple linear regression, adjusted for parental asthma status, parental smoking, days of CPAP treatment neonatally and prematurity, failed to find a significant association between neonatal VT$_{CV}$ and $s_R^{EFF}$ z-score ($\beta = -0.34$ CI 95% = $-0.6--0.03$, $p = 0.03$) and between neonatal PEF/kg and $R_{DFF}5–20$ ($\beta = 0.01$ CI 95% = $0.001–0.01$, $p = 0.01$). No other significant association between lung function parameters at 6 years and the neonatal measures of TBFVL and FeNO were found (Supporting Information: Table E5A–D).

In analyses stratified by prematurity we found a negative association between an increase in VT$_{CV}$ and reversibility in $FEF_{25–75}$ after salbutamol inhalation ($\beta = -5.93$, CI 95% = $-11.5–0.4$, $p = 0.04$) in preterm children. In term-born children we found that neonatal FeNO was negatively associated with the FEV$_1$/FVC ratio ($\beta = -1.1$, CI 95% = $-2.1--0.2$, $p = 0.03$) and neonatal V’NO was positively associated with a change in FEV$_1$ after salbutamol ($\beta = 0.3$, CI 95% = $0.06–0.6$, $p = 0.02$) at 6-year follow-up. Furthermore, VT (kg) and VT$_{CV}$ was negatively associated with % predicted FEV$_1$ and $s_R^{EFF}$ respectively ($\beta = -1.0$, CI 95% = $-1.6--0.4$, $p = 0.007$ and $\beta = -0.3$, CI 95% = $-0.6--0.002$, $p = 0.05$, respectively) and $T_{PEF}/T_0$ was positively associated to $s_R^{EFF}$ and $s_R^{TOT}$ ($\beta = 0.1$, CI 95% = $0.05–0.1$, $p = <0.001$ and $\beta = 0.1$, CI 95% = $0.04–0.1$, $p = 0.001$, respectively).

We could not find any significant associations between neonatal tidal breathing parameters and 6-year respiratory morbidity, including subgroup analysis for preterm and term-born infants (Supporting Information: Table E6).

Post hoc analysis investigating the association between neonatal FeNO and V’NO measured on postnatal day 2–7, and subsequent respiratory morbidity and lung function at age 6 showed a positive association between FeNO and reversibility $FEF_{25}$ in % after salbutamol inhalation ($\beta = 1.29$, CI 95% = $0.6–2.0$, $p = 0.002$) in preterm children (all data not shown).
<table>
<thead>
<tr>
<th>TABLE 1 Baseline characteristics, demographics, and neonatal data of infants born 2013–2014 fulfilling the inclusion criteria Step 1 (Figure 1A) in sub study 1</th>
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<td><strong>Birth weight</strong></td>
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<td><strong>SGA</strong></td>
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<td><strong>AGA</strong></td>
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<td><strong>LGA</strong></td>
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<td>Female</td>
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<td><strong>GA (days)</strong></td>
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<td>Cesarean</td>
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<td><strong>CPAP (hours)</strong></td>
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<td><strong>Oxygen (hours)</strong></td>
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<tr>
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<td><strong>VT (ml)</strong></td>
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<td><strong>VT (ml/kg)</strong></td>
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<td><strong>VT cv</strong></td>
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<td><strong>PEF (ml/s/kg)</strong></td>
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<td><strong>TPEF/TE (%)</strong></td>
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<td><strong>RR TBFVL (min⁻¹)</strong></td>
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<td><strong>Age FeNO (days)</strong></td>
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<tr>
<td><strong>FeNO (ppb)</strong></td>
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<td><strong>V NO (nL/min)</strong></td>
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<tr>
<td><strong>Missing FeNO data</strong></td>
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</table>

Abbreviations: CPAP, Continuous positive airway pressure; GA, Gestational age; TBFVL, tidal breathing flow-volume loops; VT, tidal volume.

aSmall for gestational age at birth (<−2 SD of expected(48)).
bAppropriate for gestational age at birth (−2 SD–2 SD of expected(48)).
cLarge for gestational age at birth (>2 SD of expected(48)).
dGestational age.
eContinuous positive airway pressure.
fOne or both parents smoke indoors or outdoors at 6-year follow-up.
gTidal breath flow-volume loops.
hTidal volume.
iCoefficient of variance.
jPeak expiratory flow.
kTime to peak expiratory flow divided by total expiratory time.
lRespiratory rate.
mFractional exhaled nitric oxide.
nFeNO × flow.
<table>
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<tr>
<th></th>
<th>Preterm (n = 30)</th>
<th>Term (n = 22)</th>
<th>Total (n = 52)</th>
<th>p Value</th>
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<td>6.5 [6.4, 6.8]</td>
<td>6.5 [6.3, 6.8]</td>
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<td>Mean (SD)</td>
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<td>25.7 (3.6)</td>
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<td><strong>Height (cm)</strong></td>
<td>Mean (SD)</td>
<td>121 (7.2)</td>
<td>124.6 (4.6)</td>
<td>122.5 (6.4)</td>
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<tr>
<td>Male, N (%)</td>
<td>16 (53.3)</td>
<td>10 (45.5)</td>
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<td>Female, N (%)</td>
<td>14 (46.7)</td>
<td>12 (54.5)</td>
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<td><strong>FEV1% of predicted</strong></td>
<td>Mean (SD)</td>
<td>94.4 (10.2)</td>
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<td>Mean (SD)</td>
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<td><strong>FEV1/FVC % of predicted</strong></td>
<td>Mean (SD)</td>
<td>96.1 (6.8)</td>
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<td><strong>FEF25%-75% of predicted</strong></td>
<td>Mean (SD)</td>
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<td>98.2 (18.5)</td>
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<td>Mean (SD)</td>
<td>6.6 (8)</td>
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<td><strong>FEF25-75 change in % after salbutamol</strong></td>
<td>Mean (SD)</td>
<td>24.9 (26.7)</td>
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<td><strong>R 5 Hz (z-score)</strong></td>
<td>Median [IQR]</td>
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<td><strong>R 20 Hz (z-score)</strong></td>
<td>Median [IQR]</td>
<td>-0.9 [-1.3, -0.3]</td>
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<td><strong>X 5 Hz (z-score)</strong></td>
<td>Median [IQR]</td>
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<td><strong>R DIFF5–20</strong></td>
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<td><strong>sREFF (z-scores)</strong></td>
<td>Median [IQR]</td>
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Abbreviation: FEV1, forced expiratory volume in the first second.

*a For forced expiratory volume in the first second.

*b For forced expiratory volume in the first second.

*c For forced vital capacity.

*d For forced mid-expiratory flow.

*e Resistance at 5 Hz.

*f Resistance at 20 Hz.

*g Reactance at 5 Hz.

*h The difference in resistance between 5 and 20 Hz.

*i Specific effective resistance.

+j Total specific resistance.
4 | DISCUSSION

This follow-up study aimed to investigate the association between preterm birth, neonatal FeNO and TBFVL with lung function and respiratory morbidity at age 6. The most important positive finding in this study was that moderately and late preterm children have lower % predicted FEV₁ at age 6 compared with term-born children. However, no evidence was found for the association between neonatal and 6-year lung function.

In sub study 1 (cross sectional study) we found that preterm infants have a lower VT than term-born. This is expected due to their smaller size and the difference disappeared when correcting for weight at measurement.

Furthermore, we found that preterm infants had a higher T_{PEF}/T_{E} compared to term-born. Earlier studies have found that extremely preterm infants (GA < 28 weeks) have a lower T_{PEF}/T_{E} than term-born infants. A Norwegian study found that extremely preterm infants with bronchopulmonary dysplasia (BPD) had a lower T_{PEF}/T_{E} than healthy term-born infants but there was no difference in T_{PEF}/T_{E} between extremely preterm infants without BPD and healthy term-born infants. However, comparison between studies is difficult since the infants in our cohort had a higher GA and younger postnatal age at measurement than the infants in these previous studies. Moreover, it has been shown that T_{PEF}/T_{E} decreases over the first month of life. Furthermore, the term-born infants in our cohort were admitted to the NICU, indicating that they might be more respiratory challenged than healthy term-born infants and therefore had a lower T_{PEF}/T_{E} than expected.

The difference in % predicted FEV₁ between preterm and term-born children that we found (−7.5% or −0.5 z-scores) was similar to earlier studies. A Swedish study found a difference in FEV₁ in female subjects at age 8 years of −0.36 z-scores between moderate to late preterm and term-born children; however, the difference was nonsignificant in male subjects in their study (−0.06 z-scores). Kotecha et al. found a difference in FEV₁ at age 8–9 years of −0.51 z-scores between children born with GA 33–34 and term-born. Children with low FEV₁ do have an increased risk of reaching a lower level of lung function as young adults and thereby they might have an increased risk of chronic lung diseases with increasing age. Even if the difference in lung function between preterm and term-born children is significant, the preterm children in our cohort still have what is interpreted as clinically normal lung function with a mean % predicted FEV₁ of 94% and FVC of 97% of predicted.

We investigated whether the difference in lung function could be due to the increased need for CPAP treatment in the neonatal period in the moderate to late preterm group; however, this was not the case.

Moreover, in sub study 1 (cross sectional) we found a tendency towards but not significantly increased respiratory morbidity in moderate to late preterm children at age 6 compared to term-born children. However, our small sample size resulted in wide confidence intervals, weakening the results. Previous studies report increased respiratory morbidity in moderate to late preterm children. A Danish register study found that moderate to late preterm children more often had purchased prescribed asthma medication in childhood; however, the increased prevalence seemed to decrease with age. Although our results were nonsignificant, we still find it important to present in accordance with the FAIR-principles (data-sharing) as well as the fact that the point estimates pointed in the same direction for all but one symptom of respiratory morbidity (Figure 2).

An English cohort study followed preterm children with birth weight <2000 g from age 7 to 21. They found, similar to our results, that preterm children had lower lung function at age 7 but not...
increased frequency of wheezing compared to term-born children. At age 21, however, they found that preterm born adults had more respiratory symptoms yet similar lung function levels to term born adults. Further studies on this cohort exposed that preterm-born adults have a reduced gas transfer and cardiac output at rest and recovery but no exercise limitation. An exercise test might provoke symptoms that are otherwise hidden by regulatory behavior by the child, and this would be interesting to investigate. We chose not to perform any exercise or provocation tests at 6-year follow-up due to the children not being very old in our cohort and to limit the number of tests they needed to perform. The spirometry reversibility test was the last test of the day, resulting in more missing values since some of the participants had trouble concentrating enough to perform a valid test.

Respiratory symptom score at age 6 and FEV₁ was not correlated in our cohort. This disagreement between lung function and symptoms has been reported earlier in children with asthma. One single lung function test is not likely to correlate to symptoms, as children with asthma can have a normal FEV₁.

In sub study 2 (longitudinal study) we found that an increased variability of VT neonatally was associated to decreased specific effective airway resistance (sREFF) at age 6. A higher neonatal variability of VT has previously, by Usemann et al. shown to have a protective effect on respiratory disease during the first year of life in preterm children.

We found various associations between neonatal tidal breathing parameters and lung function at age 6 in the analysis stratified by preterm and term. However, the sample sizes were small in all longitudinal analysis, and these findings need to be replicated in larger cohorts before any conclusions are made. Only 32–45 children had paired valid data from neonatal measurements and 6-year follow-up (Supporting Information: Table E3). This was a smaller sample size than expected, and the linear regression results should be interpreted with caution due to the risk of false-negative findings. Moreover, multiple tests were performed on this data and there is a risk of random significant results being found. However, we choose not to correct for multiple testing in this study, since we consider this part hypothesis generating and exploratory, and significant results need to be replicated in other cohorts before conclusions can be drawn. Earlier studies have found an association between a low TPEF/TE and respiratory symptoms during the first year of life in term-born children and Proietti et al. found a negative association between VT and wheeze during the first year of life in preterm born children. We found no association between neonatal tidal breathing parameters and respiratory morbidity at age 6. Ren et al. examined a large cohort (n = 429) of extremely preterm infants. Similar to our results, they did not find any evidence for a predictive value of tidal breathing parameters in predicting postdischarge respiratory disease during the first year of life.

We have previously shown that the relationship between neonatal FeNO and respiratory symptoms during the first year of life has an age-specific association pattern in moderate to late preterm children. In this previous study, we found that the probability of respiratory symptoms decreases with increasing FeNO values during the first week of life. However, around 14 days postnatally, the pattern was reversed, and the respiratory symptom risk increased with increasing FeNO. We hypothesized that a high level of FeNO on postnatal day three was negatively associated with respiratory morbidity due to NO's role in lung development and therefore interpreted as a surrogate measure for well-developed lungs. The reversed association between high FeNO measured from postnatal day 14 and respiratory morbidity was interpreted as that the high FeNO was due to inflammation in the airways due to external exposures or genetics. Therefore, to investigate whether we could confirm this hypothesis, we chose to perform a post-hoc analysis to examine if high FeNO or VNO measured in the first week of life (postnatal day 2–7) in preterm born children was negatively associated with respiratory symptoms at age 6. This could not be confirmed in this study. However, we showed that a higher FeNO on postnatal days 2–7 in preterm children was associated with increased reversibility of FEV₁.

The strengths of this study are the prospectively and longitudinally collected data and well-characterized cohort. The method for measuring FeNO and validating TBFVL has been validated previously. It should be noted that all neonates had measurements performed on selected chronological days after birth, and age at measurement was not corrected for gestational age at birth. Therefore, a variation in postconceptual age in the preterm group could impact the results since the neonatal measurements are performed during a period when the lungs are undergoing significant development.

Unfortunately, the follow-up turnout was lower than expected, probably due to the ongoing Covid-19 pandemic and a long break between follow-up visits in the cohort. A larger percentage of the preterm children attended the follow-up visit than those born at term. This could be explained by parents of preterm children showing a greater interest in their child's lung health than parents of healthy term-born children. Furthermore, term-born children that did attend follow-up and/or answered the questionnaire might have increased respiratory morbidity than those who did not attend. Therefore, there is a risk of attrition and selection bias. The results presented in this paper should be interpreted with caution due to the biases, low power, and sample size.

This cohort consists of children who were all admitted to the NICU shortly after birth, we endorse other researchers to consider the generalizability of the results presented in this paper to other populations.

5 | CONCLUSIONS

Moderate to late preterm children had lower % predicted FEV₁ at age 6 compared to term-born children. Therefore, the risk of early-onset chronic respiratory diseases in adulthood might be increased in this group. Moreover, we found that a higher neonatal variability of VT was associated to decreased effective airway resistance at age 6,
however the clinical relevance of this is yet to be fully explored. This study did not find evidence for the use of neonatal tidal breathing parameters as a predictor for subsequent respiratory morbidity and decreased lung function, however sample size was small and further studies are needed.

AUTHOR CONTRIBUTIONS
Inger M. Jørgensen designed and planned the AD-ON neonatal cohort. Fanny Edit Maria Goth collected the data at 6-year follow-up. Inger M. Jørgensen, Lone Agertoft and Fanny Edit Maria Goth worked on the hypothesis and study design for this paper. Fanny Edit Maria Goth, Inger M. Jørgensen, Lone Agertoft, Bo M. Hansen, and Kent Green planned the analyses and interpreted the data. All authors contributed to writing and reviewing the paper, have approved it for submission, and agree to be accountable for its content.

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CONFLICT OF INTEREST
The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES


SUPPORTING INFORMATION
Additional supporting information can be found online in the Supporting Information section at the end of this article.

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