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Cohort profile: the vitamin A and D and nitric oxide (AD-ON) observational cohort on lung development and symptoms in premature and mature children in North Zealand, Denmark

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ABSTRACT

Purpose The risk of developing asthma-like symptoms and asthma in childhood is influenced by genetics, environmental exposures, prenatal and early postnatal events, and their interactions. The cohort name refers to vitamins A and D, and nitric oxide (NO) spelt backwards and this cohort profile paper aims to present the data collection and aim of the cohort.

The overall aim when establishing this cohort was to investigate if childhood lung function can be traced back to early neonatal lung function and fractional exhaled NO (FeNO) and investigate prenatal and postnatal risk factors including maternal and neonatal vitamin A and D levels in preterm and term born children.

Participants One thousand five hundred women and their babies born at Nordsjaellands Hospital in Denmark from 2013 to 2014 were included in the AD-ON research biobank prior to birth.

Neonates from the AD-ON research biobank, admitted to the Neonatal Intensive Care Unit at Nordsjaellands Hospital, were included in the AD-ON neonatal cohort. The neonatal cohort consisted of 149 neonates hereof 63 preterm and 86 term born. The children in the cohort have been invited to follow-up visits at age 1 and 6 years.

Findings to date Published data from this cohort includes a validated and clinically applicable method to measure FeNO in neonates. We found an age-specific pattern of association between respiratory symptoms at age 1 and neonatal FeNO in preterm children. Moreover, we found that the respiratory symptoms risk was associated with postnatal factors (Respiratory Syncytial Virus infection and parental smoking) in preterm infants and prenatal factors (parental asthma and maternal infection during pregnancy) in term born infants.

Future plans In the future, the children will be examined continuously with 3-year to 5-year intervals until the age of 18. Lung function, allergy tests, environmental exposure measurements and questionnaires will be collected at each follow-up visit.

Strengths and limitations of this study

- The strengths of this cohort are the inclusion of both preterm and term born children, allowing for comparison between the groups.
- The prospective and longitudinal fractional exhaled nitric oxide (FeNO) and tidal breath measurements and the method validation on neonatal FeNO with a low subject-specific-prediction variance and high success rate of the method in neonates give us reliable and robust neonatal baseline data for this cohort.
- The data collected from this cohort include detailed prenatal and postnatal data from medical records, questionnaires and clinical observations by a trained physician.
- · Limitations include the relatively small cohort and the risk of losing attendance at follow-up visits due to long intervals between visits and, in the case of the 6-year follow-up, the ongoing COVID-19 pandemic. This effect is expectedly minimised in the years to come.

INTRODUCTION

The overall aim of this cohort is to illuminate lung function development and risk factors for later respiratory diseases in children born preterm and at term. We hypothesised that the adult lung function can be traced back to early neonatal lung function and neonatal fractional exhaled nitric oxide (FeNO) and that prenatal and postnatal risk factors including but not exclusively maternal and neonatal vitamin A and D levels are predictive of future lung function and respiratory morbidity.

Respiratory diseases such as asthma, pneumonia and bronchiolitis are the most common reasons for hospitalisation in the paediatric population.[1] The risk of developing asthma-like symptoms and asthma is influenced by the interaction of genetic disposition, environmental exposures and prenatal and early postnatal events.[2 3] It is known today that

the lung function you acquire in childhood predicts the adult lung function and, accordingly, the potential risk of developing chronic obstructive lung disease.[4]

An increasing number of extreme and early preterm infants survive today due to advanced prenatal and antenatal treatment. However, less research has focused on the long-term pulmonary outcomes of the largest preterm group, the moderate-to-late preterm born children.[5 6] Children born preterm (before 37 weeks of gestation) have a lower lung function and a higher incidence of airway diseases than children born at term during infancy, childhood and adulthood; however, findings are conflicting when examining moderate-to-late preterm born children.[7–11]

Nitric oxide (NO) has multiple physiological functions in the inflammatory processes, angiogenesis and neonatal lung development.[12] The role of NO in lung development is still being explored. NO is produced in the cells by different types of NO synthetases (NOS). NO is the main vasodilator in the transition from fetal to postnatal breathing.[13] During fetal development, the expression of endothelial NOS increases in lung tissue.[14] It has been proposed that this increase in NOS expression may enhance angiogenesis and respiratory function in the postnatal period.[14] The use of exhaled NO (eNO) has expanded in pulmonology in recent years, both as a diagnostic tool and as an indicator of treatment response.[15–18] However, the use of neonatal eNO shows conflicting results and validated applicable methods for measuring eNO in neonates are lacking.[18 19]

FeNO and tidal breathing parameters can be measured simultaneously and without risk in non-sedated neonates, making it clinically applicable from the first days of life. Previous studies have examined FeNO and tidal breathing parameters in early preterm (gestational age (GA) <32 weeks) neonates during the first days of life.[20–23] Few studies have examined FeNO and neonatal lung function in moderate-to-late preterm infants from the first days of life. To our knowledge, none of these studies have longitudinal data in infancy and childhood. However, longitudinal data from 1-month-old in term born infants have been presented earlier.[18 24]

The transplacental transfer of vitamins occurs primarily during the last trimester. Therefore, preterm neonates generally have low levels of vitamins and minerals.[25 26] Furthermore, vitamin A consumption is exceptionally high during the last trimester and shortly after birth due to its role in lung maturation.[26] Vitamin D has been shown to increase surfactant production in the rat fetus.[27] It is involved in the interaction between epithelium and mesenchyme during lung growth and inhibits smooth muscle proliferation in the airways.[28] A low level of vitamin D during pregnancy is associated with recurrent wheeze in early childhood.[29 30]

The name of this cohort refers to vitamins A and D plus nitric oxide (NO) spelt backwards. When the cohort was first established, the primary aim was to assess and validate the feasibility of clinical measurement of FeNO in neonates in an unselected group of preterm and term born infants. We measured lung function and FeNO longitudinally and collected biological samples (ie, blood) from birth into childhood in preterm and term born children. We started by developing a validated method for neonatal FeNO measurement and in our further work on the cohort we are using these data to explore the association between neonatal measurements as well as risk factors, including genes, and prenatal, perinatal and postnatal events, environmental exposure, and their interactions with lung function and respiratory morbidity later in life. By doing this, we expect to identify biomarkers and to evaluate the role of vitamins A and D for subsequent respiratory symptoms and lung disease, as well as illuminating lung function development in moderate-to-late preterm and term born children.

This paper describes the recruitment, inclusion and data collection in the AD-ON research biobank and AD-ON neonatal cohort.

COHORT DESCRIPTION

Recruitment and inclusion in the cohort

All women (n=2700) who planned to give birth at Nordsjaellands Hospital, Denmark, from September 2013 to September 2014 were invited to participate in the AD-ON research biobank. One thousand five hundred infants and mothers were included prior to birth.

The parents of infants included in the AD-ON research biobank and subsequently admitted to the neonatal intensive care unit (NICU) at Nordsjaellands Hospital, Denmark, were invited to participate in AD-ON neonatal cohort.

Criteria for inclusion in the AD-ON neonatal cohort were: inclusion in the AD-ON research biobank, admission to the NICU within the first 7 days of life, at least one parent able to read and understand Danish, and an informed consent form signed by both parents. Exclusion criteria were known congenital pulmonary or heart disease.

The sample size for the AD-ON neonatal cohort was based on data from earlier studies where neonatal FeNO was used to differentiate between healthy and sick neonates. Due to a lack in previous research investigating the long-term predictive value of neonatal FeNO when the cohort was established, we chose to use data from a study investigating differences in neonatal FeNO between healthy neonates and neonates who developed bronchopulmonary dysplasia (BPD).[31] We sought to show a difference in FeNO of 3.1 ppb (SD 2.0) (minimal relevant difference=1.55 ppb)[31] between groups with the power of 80% and a significance level of 0.05. The calculation was performed on the most relevant groups and extrapolated to the other groups. The sample size was calculated using a two-sample t-test in R software (V.3.5.2; https://www.r-project.org/), resulting in a sample size in each group of N=8 neonates. The groups were defined as (1) GA <28 weeks, (2) GA 28–32 weeks, (3) GA 32–34 weeks, (4) GA

35–37 weeks and (5) GA >37 weeks and each subgroup further into two groups (healthy/disease), making it a total of 10 groups.

Additionally, in the literature, it is recommended to include 12 patients in each subgroup for method validations and pilot studies.[32–34] We, therefore, chose to include 120 neonates (N=12× 10 subgroups) plus 30 neonates (due to an expected dropout rate of 20%), which added up to 150 infants.

Due to experience from previous years, we expected that approximately 10% of the newborns would be admitted to the NICU and, therefore, included 1500 mother–infant pairs in the AD-ON research biobank.

Furthermore, we expected a higher incidence of respiratory symptoms in childhood in the term born children in our cohort compared with the background population (due to the inclusion criteria being admission to the NICU) were the prevalence of asthma symptoms at age 5 is approximately 20%.[35] Earlier studies have found that the difference in prevalence of wheeze or asthma between preterm and term born children are between 5% and 20%.[9 36 37] To be able to show a difference in the prevalence of respiratory symptoms in childhood between preterm and term born children with a power of 80% and significance level of 0.05 we needed 62 children in each group (preterm vs term).

One hundred seventy-nine of the 1500 neonates included in the AD-ON research biobank were admitted to NICU within the first 7 days of life. Of these, 149 fulfilled the inclusion criteria and were included in the AD-ON neonatal cohort. However, the included infants did not distribute into GA groups as expected in the sample size calculation. Therefore, the cohort has been divided into only two subgroups (preterm and term born) in most analysis.

Visits and follow-up

Neonates in the AD-ON neonatal cohort were scheduled for visits at postnatal days 2–3, 5, 7, 14, 21 and 28 in 2013–2014. Preterm infants had an extra visit at GA 36 weeks. The cohort was invited to a follow-up visit in 2014–2015 at the age of 1 year. Of the 149 neonates included, 146 were available for follow-up. A questionnaire was collected from 135 families; 112 families attended a clinical follow-up visit (figure 1). The cohort was invited to a second follow-up visit at age 6–7 years in years 2019–2021. Data from 6-year follow-up are yet to be published. Hereafter, the plan is to continuously follow the children with 3-year to 5-year intervals until age 18. At each follow-up, lung function testing, allergy tests, questionnaires on respiratory symptoms and general health, as well as samples of blood, urine, stool and hair to investigate long-term environmental exposures, will be collected.

Data collection

AD-ON research biobank cohort

Blood samples were collected from the mother and the umbilical cord by needle puncture (covered to prevent a reaction with light) immediately after birth and separated after centrifuging as serum, EDTA plasma and buffy coat. All samples were frozen at −80 °C within 4 hours for quality reasons. Plasma calcium was included in the routine blood samples at NICU and immediately analysed; results were entered into the database. The frozen samples are kept anonymously in the research biobank until analysis.

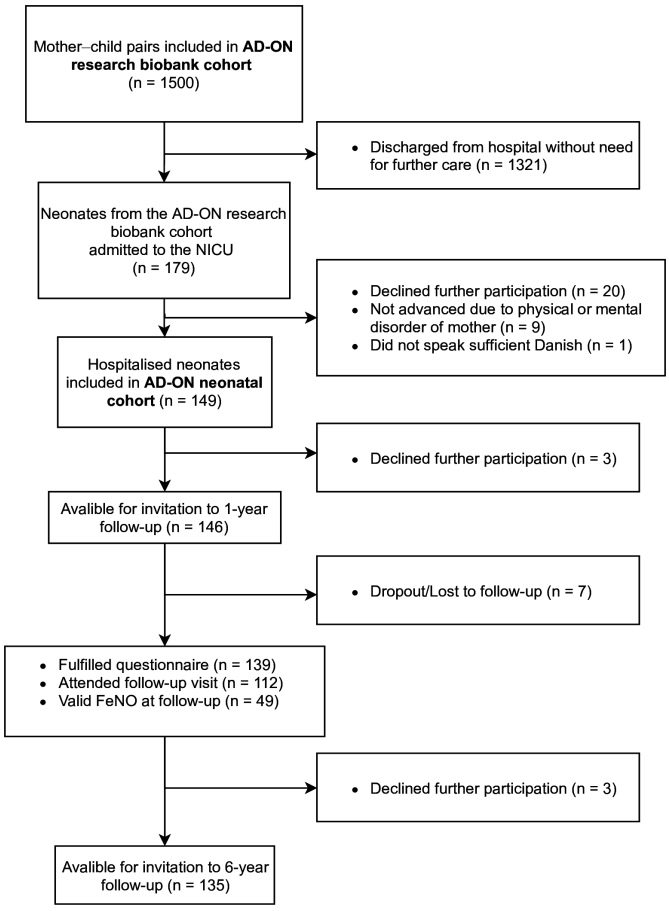

Figure 1 Flow chart showing the inclusion and exclusion of participants in the AD-ON neonatal cohort from birth to 6 years of age. FeNO, fractional exhaled nitric oxide; NICU, neonatal intensive care unit.

AD-ON neonatal cohort

Baseline data

At the NICU unit, medical history and medical records of mothers and neonates were reviewed. Detailed information on maternal health status, ultrasound findings during pregnancy, fetal growth data and diagnoses were collected and entered in the database. Parents were also interviewed systematically to gather further information on health, social status and lifestyle.

Baseline data include: maternal ethnicity, maternal age, weight gain in pregnancy, maternal blood pressure, maternal diabetes, parental asthma, allergy or/and eczema, parental smoking, parental alcohol use, social group and postal code of parents at delivery, maternal infections during pregnancy, hyperemesis during pregnancy, intrauterine infections, chorioamnionitis, preeclampsia,

maternal medication use, rupture of membranes, colour of amniotic fluid, amount of amniotic fluid (normal/polyhydramnion/oligohydramnion/no amniotic fluid), antenatal steroid, antibiotics during labour, sex, GA, day and time of birth, delivery mode, birth weight, small for gestational age at birth, birth length, head circumference at birth, single/multiple pregnancy, APGAR score at 1 and 5 min,[38] age of APGAR=10, age at first breath, resuscitation needed at birth, FiO_2 in the delivery room, continuous positive airway pressure (CPAP) at birth, intubation, umbilical cord blood sample (base excess, pH and pCO_2), placenta weight, placenta pathology, hours to first meconium, respiratory frequency and symptoms at admission to NICU, fontanelle size (normal or large), capillary blood samples 2 hours after birth (pH, pCO_2, pO_2, bicarbonate, blood glucose, base excess).

Clinical data and data from medical records

A trained physician performed a complete physical examination on all infants in the cohort at every visit. During admission at the NICU, daily respiratory symptoms, clinical and vital parameters were collected prospectively to the database. Two different research assistants double checked all data entries.

Anthropometric parameters include weight, height and head circumference. Weight was measured with SECA model 376 on neonates and at 1-year follow-up and 6-year follow-up with SECA model 769. The results were recorded in kg with two decimals. In neonates and at 1-year follow-up, the length was measured with SECA model 207, and at 6-year follow-up, height was measured with SECA model 216. Head circumference was measured with a measuring tape three times, and the highest value was recorded in cm with one decimal.

Blood pressure, pulse and oxygen saturation were measured with Sure Signs vs2+ (ViCare Medical) at the 6-year follow-up.

Clinical neonatal data include CPAP treatment (start and stop dates and maximum pressure), treatment with caffeine citrate, treatment with surfactant (and any medication in relation to this administration), neonatal infection (including date of infection, core temperature, and results of bacterial culture), respiratory symptoms during admission, antibiotic treatment, postnatal corticosteroid treatment, jaundice (including plasma bilirubin level and treatment), need for parenteral nutrition, the start of enteral nutrition, age when daily nutritional need were met enterally (formula or breast milk), vitamin supplements, diagnose of BPD, ICD-10 diagnosis at discharge, days of hospitalisation after birth, GA at discharge, number of hours with a need for supplemental oxygen (including maximum FiO_2 given).

Any other examination or treatment clinically indicated during admission at the NICU was registered, including results of ultrasonic investigation of the brain, echocardiography, examining retinopathy of prematurity (only neonates born before 32 weeks of gestation), and blood transfusion.

Data on hospital admissions, ICD-10 diagnoses, given medicine and vaccinations[39] were after permission by the parents, collected from the medical records.

Questionnaires

At 1-year follow-up, parents answered a questionnaire on the child's general health, focusing on respiratory and allergy symptoms, airway infections, use of asthma medication (inhaled beta2agonist, inhaled corticosteroid (ICS), leukotriene receptor antagonist), antibiotic treatment during the first year of life and hospital admissions.

At 6-year follow-up, questionnaires on the child's general health focused on respiratory and allergy symptoms, medication, and airway infections, as well as standardised questionnaires (International Study of Asthma and Allergy in Childhood (ISAAC) and Asthma Control Index), were distributed.[40 41]

Biological samples and biobanking

In addition to the umbilical cord blood collected, all neonates in the AD-ON neonatal cohort had blood samples drawn at postnatal days 2–3, 21 and at 1-year follow-up. Preterm neonates also had a blood sample drawn at GA 36 weeks. All these blood samples were stored in a biobank at −80 °C as described above.

At 6-year follow-up, we collected stool, urine and blood samples for biobank storage or immediate analysis. Stool samples were stored fresh-frozen at −20 °C to ensure future microbiome analysis and, after that, moved to −80 °C freezers.[42] Urine samples were stored at −80 °C without prior handling. Some of the blood was analysed for IgE and eosinophil count. Buffy coat and EDTA plasma were stored in the biobank at −80 °C.

When collecting blood samples from neonates, infants and children, it is recommended not to exceed 10% of the expected total blood volume.[43] The amount of blood collected in this study is far below this.

Hair samples collected are stored in sealed packaging at room temperature.

FeNO measurements

Online measurements of mixed tidal FeNO were performed on infants placed in their cribs or their parent's arms in a supine position during natural (often postprandial) sleep. Measurements were performed with Ecomedics CLD 88sp according to American Thoracic Society (ATS)/European Respiratory Society (ERS) standards.[44] The FeNO analysis was performed at 60%–80% of the expired volume of each selected breath. The computer software automatically included breaths only if they fell within 10% variation of the mean tidal volume. The machine's minimal tidal volume was 10 mL, and the time delay of the flow was 0.65 s. A DENOX 88 device provided NO free air. The infants breathed through a silicone facemask without septum (Fisher&Paykel Healthcare size 35–50 mm (XS-M)) covering both mouth and nose. The effective dead space of the mask was 5, 8 and 11 mL, respectively. Every measurement was performed

over 60 s at least three times to obtain three measurements with maximal FeNO deviance of 1 ppb.[45]

Measurements were performed on postnatal days 3, 5, 7, 14, 21 and 28, and again at 1-year follow-up. At the 6-year follow-up, FeNO was measured by single breath exhalation with Ecomedics CLD 88sp according to the ERS and the ATS guidelines.[44]

Calibration and maintenance of the device were performed following the guidelines of the manufacturer.

Lung function measurements

Tidal flow-volume parameters were measured simultaneously with FeNO measurements using the Ecomedics CLD 88sp (software Spiroware Client V.3.1.6) on days 3, 5, 7, 14, 21, 28, and at 1 year. All measurements with a minimum of 10 consecutive flow-volume curves with a maximal variation in the tidal volume of 10% were visually examined. All curves with stable volume and shape were selected by visual examination, and from these curves, mean, SD and coefficient of variance were calculated for each measurement.[46 47]

At 6-year follow-up, multiple lung function tests were performed such as spirometry with reversibility for salbutamol (performed on Jæger IM PRO), resistance measurements by body plethysmograph (performed on Jaeger Master Screen Body), resistance and reactance measurements by impulse oscillation technique (performed on Jæger IOS, Vyntus) and diffusion capacity for carbon monoxide (performed on Jaeger SB Diffusion Real-Time adaptor for Master Screen Body). All measurements were performed using the SentrySuite software solution from Vyaire and according to the ERS and ATS standards.[47–49] Calibration and maintenance of the devices were performed according to the manufacture's guidelines.

Cardiac measurements

At the 6-year follow-up, all participants were offered an ECG and transthoracic echocardiography to examine for pulmonary hypertension and structural cardiac abnormalities.[50] Transthoracic echocardiograms were performed in accordance with recommendations from the American Society of Echocardiography.[51]

All echocardiographic parameters collected are presented in online supplementary table E1 and calculations in online supplementary table E2. A trained paediatric cardiologist performed the echocardiography using the EPIQ 5 ultrasound system (Philips). All images and measurements were by blinded method double checked by a senior paediatric cardiologist to ensure validity.

Skin prick testing (SPT)

Children and their biological parents were all offered an SPT for the positive and negative control, and the most common inhalation allergens (grass, birch, mugwort, house dust mites, mould fungi, dog, cat, and horse (all allergen extracts were purchased at ALK- Abelló A/S, Horsholm, Denmark)) at 6-year follow-up. The SPT was performed on clean and healthy skin, and it was made sure no medication that could affect the result had been taken before the SPT. The SPT was considered positive if the reaction size was larger than 3 mm (two measurements of size added and divided by 2).

For an overview of the data collection, see table 1.

Outcomes at follow-up

The outcomes at 1-year follow-up were troublesome respiratory symptoms (TRS) and asthmatic bronchitis (AB) during the first year of life. TRS was defined as wheeze or cough minimum twice a week for more than 2 months continuously and/or wheeze or night-time cough for a shorter period (2 weeks) more than three times a week during periods without respiratory infections. AB was defined as respiratory wheeze and cough in association with an airway infection. AB was the expression and diagnosis used in Denmark in 2013–2015 and corresponds to the more commonly used episodic viral wheeze.[52]

At the 6-year follow-up, the primary outcomes were doctor-diagnosed asthma or wheeze during the previous year.

At both follow-up visits, we defined the use of short-acting inhaled beta-agonist and ICS during the first year of life as surrogate measures for respiratory symptoms.

As secondary outcomes, we looked at hospitalisations due to respiratory problems, pneumonia treated with antibiotics, and atopic sensitisations defined as a positive skin prick test and/or positive IgE.

Participant and patient involvement

The study participants and their parents did not directly influence the study design and baseline data collection. However, the participants have shown a great interest in the project and willingness to answer questionnaires and attend follow-up visits.

Precautions concerning the COVID-19 pandemic in 2020

While we were completing the 6-year follow-up, the world was hit by the COVID-19 pandemic. This affected the follow-up, and many visits had to be postponed. For those visits that could take place, the setting was altered to ensure both children's, parents' and investigators' health and well-being. All necessary precautions were taken with appropriate social distancing, use of protective equipment and close personal contact was kept to a minimum, following regional recommendations at the time to minimise the risk of viral transmission. All children and parents should not show any signs of possible cold or fever at the time of the visit, and children also had to present a negative PCR COVID-19 test that was no older than 48 hours. During the pandemic, only one parent could accompany the child at the visit due to regional recommendations.

Cohort characteristics

More than 50% of all newborn babies at Nordsjaellands Hospital were enrolled in the AD-ON research biobank cohort during the 12-month inclusion period. Approximately 10% of the neonates in the AD-ON research biobank cohort were admitted to the NICU. The primary

Table 1 Overview of data collection into 6 years of age in the AD-ON neonatal cohort

	The first month of life	1-year follow-up	6-year follow-up
Baseline data			
Baseline data*	X		
Clinical data†	X		
Data from patient record (disease and diagnosis)	X	X	X
Lung function measurements			
Body plethysmography			X
Spirometry (flow-volume) with reversibility test			X
Impuls oscillometri system (IOS)			X
Diffusion capacity for CO			X
Tidal breathing lung function	X	X	X
Tidal breathing exhaled NO	X	X	X
Single breath exhaled NO			X
Allergy tests			
Skin Prick Test children			X
Skin Prick Test biological parents			X
Total IgE			X
Specific IgE			X
Eosinophil count			X
Cardiopulmonary measurements			
Transthoracic echocardiography	X+		X
ECG	X+		X
Biobank samples			
Blood to biobank	X	X	X
Stool to biobank			X
Urine to biobank			X
Hair sample to biobank			X
Vital parameters and anthropometric			
Weight	X	X	X
Height	X	X	X
Head circumference	X	X	X
Blood pressure, oxygen saturation, heart rate	X	X	X
Height parents			X
Questionnaires			
General health questionnaire		X	X
ISAAC questionnaire			X
Asthma Control Index (only children with asthma diagnosis)			X

*Baseline data include: maternal ethnicity, maternal age, weight gain in pregnancy, maternal blood pressure, maternal diabetes, parental asthma, allergy or/and eczema, parental smoking, parental alcohol use, social group and postal code of parents at delivery, maternal infections during pregnancy, hyperemesis during pregnancy, intrauterine infections, chorioamnionitis, preeclampsia, maternal medication use, rupture of membranes, colour of amniotic fluid, amount of amniotic fluid (normal/polyhydramnion/oligohydramnion/no amniotic fluid), antenatal steroid, antibiotics during labour, sex, gestational age, day and time of birth, delivery mode, birth weight, small for gestational age at birth, birth length, head circumference at birth, single/multiple pregnancy, APGAR score at 1 and 5 min, age of APGAR=10, age at first breath, resuscitation needed at birth, FiO_2 in the delivery room, continuous positive airway pressure (CPAP) at birth, intubation, umbilical cord blood sample (base excess, pH and pCO_2), placenta weight, placenta pathology, hours to first meconium, respiratory frequency and symptoms at admission to NICU, fontanelle size (normal or large), capillary blood samples 2 hours after birth (pH, pCO_2, pO_2, bicarbonate, blood glucose, base excess).
†Clinical data from the admission at NICU were collected prospectively from the medical charts and include CPAP treatment (start and stop dates and pressure), treatment with caffeine citrate, treatment with surfactant (and any medication in relation to the administration), neonatal infection (including date of infection, core temperature and results of bacterial culture), respiratory symptoms during admission, antibiotic treatment, postnatal corticosteroid treatment, jaundice (including plasma bilirubin level and treatment), need for parenteral nutrition, the start of enteral nutrition, age when daily nutritional need fulfilled enterally (formula or breast milk), vitamin supplements, diagnose of bronchopulmonary dysplasia, ICD-10 diagnosis at discharge, days of hospitalisation after birth, gestational age at discharge, number of hours with a need for supplemental oxygen (including maximum FiO_2 given).
‡Only if the clinician with treatment responsibility found it clinically indicated during the admission at the NICU.
NICU, neonatal intensive care unit; NO, nitric oxide.

Table 2 Cohort characteristics at inclusion in AD-ON neonatal cohort, N (%)

Variable		Preterm (n=63)	Term (n=86)	Total (n=149)	P value
Sex	Male	36 (57.1)	48 (55.8)	84 (56.4)	
	Female	27 (42.9)	38 (44.2)	65 (43.6)	>0.99
Delivery mode	Vaginal delivery	30 (47.6)	55 (64.0)	85 (57.0)	
	Caesarean section	33 (52.4)	31 (36.0)	64 (43.0)	0.07
Birth weight (kg)	Mean (SD)	2.165.1 (516.4)	3.374 (686.1)	2.862.8 (860.9)	<0.001
Gestational age in days	Mean (SD)	239 (15)	276.1 (9.8)	260.4 (22.1)	<0.001
Twins		17 (27.0)	12 (14.0)	29 (19.5)	0.08
SGA*		8 (12.7)	10 (11.6)	18 (12.1)	0.47
Parental asthma		17 (27.0)	14 (16.3)	31 (20.8)	0.17
Smoking during pregnancy		1 (1.6)	7 (8.1)	8 (5.4)	0.17
Social group parents†	1	15 (24.2)	23 (26.7)	38 (25.7)	
	2	14 (22.6)	25 (29.1)	39 (26.4)	
	3	12 (19.4)	20 (23.3)	32 (21.6)	
	4	16 (25.8)	15 (17.4)	31 (20.9)	
	5	5 (8.1)	3 (3.5)	8 (5.4)	0.48
	Missing	1	0	1	

*Small for gestational age (SGA) at birth (< −2 SD of expected[58]).
†Social group parents (1) academics, self-employed in a large company, highest officials/white-collar workers, (2) persons with a medium-to-long academic education, self-employed in a medium to a large company, and high officials/white-collar workers, (3) self-employed in a smaller company and intermediate officials/white-collar workers, (4) subordinate official/white-collar workers and skilled professionals/blue-collar workers; and (5) unskilled/blue-collar workers.

reason for admission at the NICU was transient tachypnea of the newborn or respiratory distress in 48 (32 %), prematurity in 37 (25 %), small for GA in 16 (11 %), hypoglycaemia in 7 (5 %), sepsis in 6 (4 %), asphyxia in 6 (4%), jaundice in 6 (4%), weight loss in 2 (1%), seizures in 1 (0.5 %) and other reasons that required observation at the NICU in 20 neonates (13%).

In the AD-ON neonatal cohort, including 149 neonates, 86 were term born (GA ≥37+0), 55 moderate to late preterm (GA 32+6–36+6), and 8 were born very preterm (GA 28–32+6), for demographics and characteristics, see table 2.

At 1 year follow-up, questionnaires were collected from 139 infants, and 112 infants attended the follow-up visit (table 3). Thirty-two infants had TRS during the first year of life, and 25 were diagnosed with AB (table 4).

Findings to date

Published data on this cohort include a validated and clinically applicable method to measure FeNO and V'NO (NO × flow) in preterm and term born neonates.[45] With this method, we also showed that measuring tidal FeNO in respiratory unstable infants can be performed during a short (minutes) pause from CPAP treatment.[53] The study design and cohort allowed us to construct longitudinal neonatal tidal FeNO and V'NO reference charts for preterm and term born neonates.[54] Other key findings include the change of longitudinal FeNO values with postnatal age. Prematurity altered the FeNO curve pattern compared with the pattern of term born infants.[45] Based on data from this cohort, we have identified and suggested that prenatal, neonatal and environmental modifiers of FeNO and V'NO are age dependent and could depend on the antenatal or postnatal time of exposure.

Moreover, we found that postnatal age at measurement had a significant effect on the association between FeNO and respiratory symptoms at 1 year in moderate preterm infants (GA 32–34 weeks) (p=0.02) but not in late preterm (GA 35–37 weeks) or term born infants (GA >37 weeks) (p=0·50 and 0.29, respectively). A high FeNO on postnatal day 3 was associated with a low risk of TRS compared with a high FeNO on postnatal day 14, which was associated with a high risk of TRS in moderate preterm infants.[55] Preterm born infants did not have significantly more respiratory symptoms compared with term born infants at 1-year follow-up.[55] Additionally, we found that TRS risk was associated with postnatal factors (RSV infection with OR 33.9, 95% CI 4.2 to 794.4, p=0.005 and parental smoking with OR 7.4, 95% CI 2.1 to 30.5, p=0.003) in preterm born infants and prenatal factors (parental asthma with OR 4.9, 95% CI 1.3 to 18.3, p=0.02, and maternal infection during pregnancy with OR 3.7, 95% CI 1.1 to 12.8, p=0.03) in term born infants.[55] Noteworthy, parental asthma did not influence the TRS risk at 1 year old in preterm infants in our cohort.

Strengths and limitations of this study

The strengths of this cohort are the inclusion of both preterm and term born children, allowing for comparison

Table 3 Characteristics of participants who answered the questionnaire at 1-year follow-up compared with those lost to follow-up or dropped out, N (%)

Variable	Level	Included 1 y FU (n=139)	Dropout/lost to follow-up 1 y FU (n=10)	Total (n=149)	P value
Sex	Male	80 (57.6)	4 (40.0)	84 (56.4)	
	Female	59 (42.4)	6 (60.0)	65 (43.6)	0.45
Delivery mode	Vaginal delivery	80 (57.6)	5 (50.0)	85 (57.0)	
	Caesarean section	59 (42.4)	5 (50.0)	64 (43.0)	0.89
Birth weight (kg)	Mean (SD)	2827.7 (854.1)	3350.5 (847.7)	2862.8 (860.9)	0.06
Gestational age in days	Mean (SD)	260.2 (22)	264.4 (23.6)	260.4 (22.1)	0.56
GA group	Term	80 (57.6)	6 (60.0)	86 (57.7)	
	Preterm	59 (42.4)	4 (40.0)	63 (42.3)	>0.99
Twins		29 (20.9)	0 (0.0)	29 (19.5)	0.23
SGA*		18 (12.9)	0 (0.0)	18 (12.1)	0.44
Parental asthma		31 (22.3)	0 (0.0)	31 (20.8)	0.20
Smoking during pregnancy		8 (5.8)	0 (0.0)	8 (5.4)	0.96
Social group parents†	1	37 (26.8)	1 (10.0)	38 (25.7)	
	2	36 (26.1)	3 (30.0)	39 (26.4)	
	3	29 (21.0)	3 (30.0)	32 (21.6)	
	4	29 (21.0)	2 (20.0)	31 (20.9)	
	5	7 (5.1)	1 (10.0)	8 (5.4)	0.76
	Missing	1	0	1	

*Small for gestational age at birth (< – 2 SD of expected[58]).
†Social group parents (1) academics, self-employed in a large company, highest officials/white-collar workers, (2) persons with a medium-to-long academic education, self-employed in a medium to a large company, and high officials/white-collar workers, (3) self-employed in a smaller company and intermediate officials/white-collar workers, (4) subordinate official/white-collar workers and skilled professionals/blue-collar workers; and (5) unskilled/blue-collar workers.

between the groups. Many similar cohorts have either excluded preterm children or only included preterm children.[56 57]

The prospective and longitudinal FeNO and tidal breath measurements and the method validation on neonatal FeNO with a low subject-specific-prediction variance and high success rate of the method in neonates give us reliable and robust neonatal baseline data for this cohort. In addition, the data include detailed prenatal and postnatal data from medical records, questionnaires,

Table 4 Prevalence of troublesome respiratory symptoms and asthmatic bronchitis and use of asthma inhalation medication at 1-year follow-up, N (%)

	Preterm (n=59)	Term (n=80)	Total (n=139)	P value
TRS*	17 (28.8)	15 (18.8)	32 (23.0)	0.234
AB†	11 (18.6)	14 (17.5)	25 (18.0)	1.000
ICS use 1 y‡	6 (10.2)	5 (6.2)	11 (7.9)	0.597
SABA use 1 y§	13 (22.0)	19 (23.8)	32 (23.0)	0.973
LTRA use 1 y¶	1 (1.7)	0 (0.0)	1 (0.7)	0.878
Pneumonia 1 y**	10 (16.9)	10 (12.7)	20 (14.5)	0.643
Missing data pneumonia 1 y	0	1	1	
Atopic dermatitis	3 (5.1)	8 (10.0)	11 (7.9)	0.4574

*TRS=troublesome respiratory symptoms.
†AB=Asthmatic bronchitis.
‡ICS use 1 y=Use of inhaled corticosteroid during the first year of life.
§SABA use 1 y=Use of short-acting inhaled beta-agonists during the first year of life.
¶LTRA use 1 y=Use of leukotriene receptor antagonist during the first year of life.
**Pneumonia 1 y=Pneumonia treated with antibiotics during the first year of life.

and clinical observations by a trained physician. Moreover, a biobank including plasma, serum, buffy coat, urine and stool samples collected on the cohort from birth to 6 years old exist.

Limitations include the relatively small cohort, especially when dividing into subgroups. Some of the lifestyle variables such as smoking during pregnancy are self-reported, and therefore might be underestimated. This has been addressed by collecting hair and urine samples to evaluate the long-term effect of exposures (eg, cotinine). Finally, the 6-year follow-up was complicated by the ongoing COVID-19 pandemic, which has restrained some participants from attending follow-up visits due to fear of containing coronavirus at the hospital. Still, it is too early to say whether this will impact follow-up attendance since many visits have been postponed until participants felt safe. The risk of losing attendance at follow-up visits due to many years interval between visits is also a limitation in this cohort.

Power analysis were performed before commencing the cohort; however, further analysis and substudies on this cohort were explorative.

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Contributors IMJ, BJS and PA designed the AD-ON birth and neonatal cohort and follow-up at one year. BJS collected the neonatal and 1-year follow-up data. FEMG validated 1-year follow-up data. FEMG, IMJ, LA and KJ planned the data collection at 6-year follow-up. FEMG collected the 6-year follow-up 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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