Predictive value of exhaled nitric oxide in healthy infants for asthma at school age

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Take home message: Exhaled nitric oxide fraction measured in newborns before exposure to environmental factors is not associated with school age asthma development

Exhaled nitric oxide fraction (FeNO) is a non-invasive biomarker that is elevated in subjects with asthma and allergic diseases [1]. Nitric oxide (NO) is produced by three NO synthase (NOS) enzymes: neuronal (nNOS), endothelial (eNOS) and inducible (iNOS), all present in the human lung [2]. The increase of FeNO in asthmatic patients has mainly been attributed to an activation of iNOS, mediated through proinflammatory cytokines in the airways [3].

Early monitoring of airway inflammation assessed by elevated FeNO provides information on asthma evolvement and helps to identify subjects at risk. Few studies have investigated the association between FeNO during early childhood and asthma at school age. In preschoolchildren with recurrent respiratory symptoms, those with higher FeNO were at an increased risk for asthma at 6 years [4]. In a selected cohort of infants with eczema, increased FeNO prior to any wheezing episodes was associated with an increased risk for asthma at 5 years [5]. Notably, in these studies, FeNO was measured in high-risk children that were already exposed to environmental factors known to modify FeNO levels [6-8].

The value of FeNO after birth as predictive for later symptoms before relevant exposure to environmental factors has been prospectively investigated in only two studies [9, 10]. Latzin *et al.* reported that infants born to atopic mothers had increased FeNO prior to respiratory symptoms, and that this association was enhanced in mothers who smoked [9]. In infants born to asthmatic mothers, Chawes *et al.* showed that increased FeNO was associated with recurrent wheezing episodes during the first year of life, but not thereafter [10].

Taken together, while there is cumulative evidence that elevated FeNO in high-risk children *after* a possible impact by environmental exposures is associated with later asthma [4, 5], it is unknown if FeNO after birth, *prior* to a possible influence by postnatal environmental exposures and first respiratory symptoms is associated with asthma. Given that environmental factors are known to induce NOS activity and modify FeNO levels [6, 7, 11, 12], we hypothesised that FeNO measured after birth, and before relevant exposure to these factors, is not associated with asthma at school age.

The aim of this prospective cohort study was to investigate if FeNO levels after birth in unselected newborns are associated with asthma or atopy at school age.

This prospective birth cohort study comprised of unselected, healthy, term-born infants recruited in the region of Bern, Switzerland [13]. At 5 weeks of age, FeNO from multiple breaths during natural sleep was measured, as previously described [9, 14], with a rapid response chemiluminescence analyser (CLD 77; EcoMedics, Duernten, Switzerland; Analysis software: WBreath version 3.28.0.0, ndd, Zurich, Switzerland).

At 6 years, asthma was assessed by study physicians with questions adapted from the International Study of Asthma and Allergies in Childhood (ISAAC) [15], defined as a history of wheezing within the 12 months prior to follow-up. Study physicians assessed atopy, defined as allergic asthma, allergic rhinitis, atopic dermatitis or positive skin prick test (SPT). An SPT, including 7 common allergens, was determined positive in the case of hives bigger than positive control histamine in any of the tested allergens [13]. We assessed risk factors for asthma or atopy of the child: parental asthma was defined as self-reported or doctor-diagnosed asthma; parental atopic disease was defined as allergic asthma, hay fever, or eczema by history.

The Governmental Ethics Committee of the Canton of Bern, Switzerland approved the study and informed written consent was obtained at enrolment.

Logistic regression was used for analysis of an association between FeNO at birth and asthma, atopy and positive SPT at school age. Adjustment for confounders (e.g. parental asthma and atopy) and nonconfounding factors (minute ventilation, known to modify FeNO levels [16]) was done. Linear regression was used for the analysis of an association between parental variables and FeNO at birth. Results were reported as odds ratio (OR) or difference in FeNO (ppb) with 95% confidence intervals (95% CI). Data were analysed with STATA® 13 (STATA Corporation, College Station TX). Patient characteristics for those with and without follow-up were compared using Mann-Whitney U and Chi-squared tests.

We measured FeNO in n=278 infants with n=44 (16%) being excluded for technical reasons, resulting in n=234 subjects. Of those n=26 (12%) were lost to follow-up, resulting in n=208 final study participants. Demographics, exposure to risk factors and FeNO levels did not differ between subjects followed-up and those lost to follow-up (data not shown). For the entire group, neither maternal atopy, nor maternal smoking was associated with postnatal FeNO. Smoking during pregnancy was only associated with decreased FeNO levels in infants of non-atopic mothers (-2.91 ppb, 95% CI -5.76 – -0.048). These findings were similar to previously published data [16], although the fraction of smoking mothers was lower in this study (9% *versus* 13% in [16]). At 5 weeks, mean FeNO was 13.9 ppb, (range 1.8–32.9 ppb). Parents of n=43 (20%) had asthma and n=126 (61%) were atopic; n=19 (10%) mothers smoked during pregnancy. Among 6-year olds (range 5–7 years), n=31 (15%) had asthma with n=13 (6%) allergic asthma cases, and none of the children used corticosteroids. There were n=62 (30%) atopics, and in n=164 an SPT was completed with n=26 (19%) being positive.

FeNO at birth was not associated with asthma, atopy, or positive SPT at school age. Per one ppb increase in FeNO, the simple and adjusted OR (95% CI) for asthma were 0.99 (95% CI 0.92–1.07) and 0.97 (95% CI 0.89–1.06); for atopy 0.99 (95% CI 0.94–1.05) and 0.99 (95% CI 0.94–1.06); and for positive SPT 0.95 (95% CI 0.88–1.03) and 0.95 (95% CI 0.87–1.03) (table 1), respectively.

Our study is the first to investigate the association between FeNO at birth and asthma at school age in unselected infants. It was previously shown that the association of elevated FeNO after birth with respiratory symptoms is restricted to infancy [10]. In this study, we found supporting evidence for this finding since FeNO after birth was not associated with diagnosis of school age asthma.

Based on this finding, and on previous studies in selected high-risk populations, we propose two different models on FeNO metabolism in early infancy (figure 1):

Model 1: Elevated FeNO is an expression of an intrinsic mechanism, determined by pre-and early postnatal risk factors. In this model, FeNO levels during infancy would then not be altered by environmental factors, and FeNO measured at birth could serve as a predictor for later asthma and atopy.

Model 2: Environmental factors (e.g. infections, air pollution) are needed to induce iNOS [6, 7, 11] which then results in elevated FeNO. In this hypothesis, we would expect no association between FeNO at birth (measured before environmental exposures) and school age outcomes. FeNO may then only serve as a phenotype specific biomarker in infants after the first activation of the environmentally or genetically induced iNOS.

We speculate that the second, rather than the first, model better explains NO metabolism after birth, since studies measuring FeNO *after* environmental exposures found an association between FeNO with asthma development, while our study, measuring FeNO *before* environmental exposures, did not find any association.

Measurements were performed using a face mask, which is the only available technique for measuring FeNO in infants at this time. This introduces the possibility that FeNO from the upper airways contributes to the overall FeNO measured. However, we believe this potential contribution is unlikely due to the fact that the nasal sinuses of infants are not developed.

Asthma prediction with FeNO might further be hampered by the physiological variability of FeNO levels *per se*, by the intersubject variability of up to 50% [17], and influenced by

different measurement techniques. In school-aged children, comparison of FeNO measured from single *versus* multiple breaths resulted in higher FeNO values using the latter technique [18]. Multiple-breath FeNO measurement technique is, at this time, the only one available for infants. The present study is limited by the low number of asthmatics (n=31) and the questionnaire-based assessment for diagnosis, which could lead to possible misclassification. In general, the cohort reflects the epidemiological situation in Switzerland, with a low prevalence of mild to moderate asthmatics. Coincidentally, however, in our study sample only mild intermittent asthmatics (without corticosteroid use in the last 12 months) were included. However, this study was conducted in a prospective unselected cohort, representing the general population. In contrast to previous studies in high-risk populations, we measured FeNO at a single point in time after birth, excluding possible age- or time dependent effects on FeNO levels.

The interpretation of FeNO levels, its predictive value and its modifiers, are age dependent [4, 5, 7, 9-11]. Postnatal FeNO metabolism seems to be modified by various environmental factors. On a cellular level, maternal tobacco smoke modified NOS activity in fetal vascular bed in newborns [12]. Consistent with this observation, postnatal FeNO is modified by prenatal tobacco smoke exposure in offspring [16, 17], interestingly enough, in an interaction with maternal atopy [16]. In contrast to the pre- and early postnatal situation, infancy and preschool age seems to be critical for further gene-environment interactions through exposures other than smoking and maternal atopy and their impact upon NO metabolism [4, 5].

In summary, we show that postnatal FeNO measured in unselected healthy newborns is not associated with asthma diagnosis at school age. We speculate that NO metabolism may play a role in the pathophysiology of childhood asthma and atopy only after exposure to environmental factors at preschool age. To confirm that environmental exposures indeed modify NOS expression during infancy, frequent longitudinal assessment of FeNO levels and

NOS expression would be necessary. Our findings should encourage further research on factors impacting upon NO metabolism during infancy that can serve as targets for new preventive strategies on childhood asthma development.

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Conflict of interest: The authors declare no conflicts of interests.

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TABLE 1 Association between exhaled nitric oxide in newborns and subsequent diagnoses of asthma, atopy and positive prick test in school-aged children

	Exposure fraction of exhaled nitric oxide					
	Simple[§] model			Adjusted[£] model		
Outcome	OR	(95% CI)	p-value	OR	(95% CI)	p-value
Asthma[+]	0.99	(0.92–1.07)	0.970	0.97	(0.89–1.06)	0.586
Atopy[$]	0.99	(0.94–1.05)	0.976	0.99	(0.94–1.06)	0.971
Prick positive[#]	0.95	(0.88–1.03)	0.285	0.95	(0.87–1.03)	0.256

Odds ratios (OR) and 95% confidence intervals (CI) per one ppb increase in exhaled nitric oxide fraction (FeNO) were determined by logistic regression. [+]: defined as a history of wheezing within the 12 months prior to follow-up. [$]: defined if one of the following was present: asthma, allergic rhinitis, atopic eczema and positive prick test. [#]: positive in case of hives bigger than positive control histamine in any of the tested allergens. [§]: FeNO was adjusted for minute ventilation [16]. [£]: This model was additionally adjusted for sex, parental asthma, parental atopy and smoking during pregnancy.

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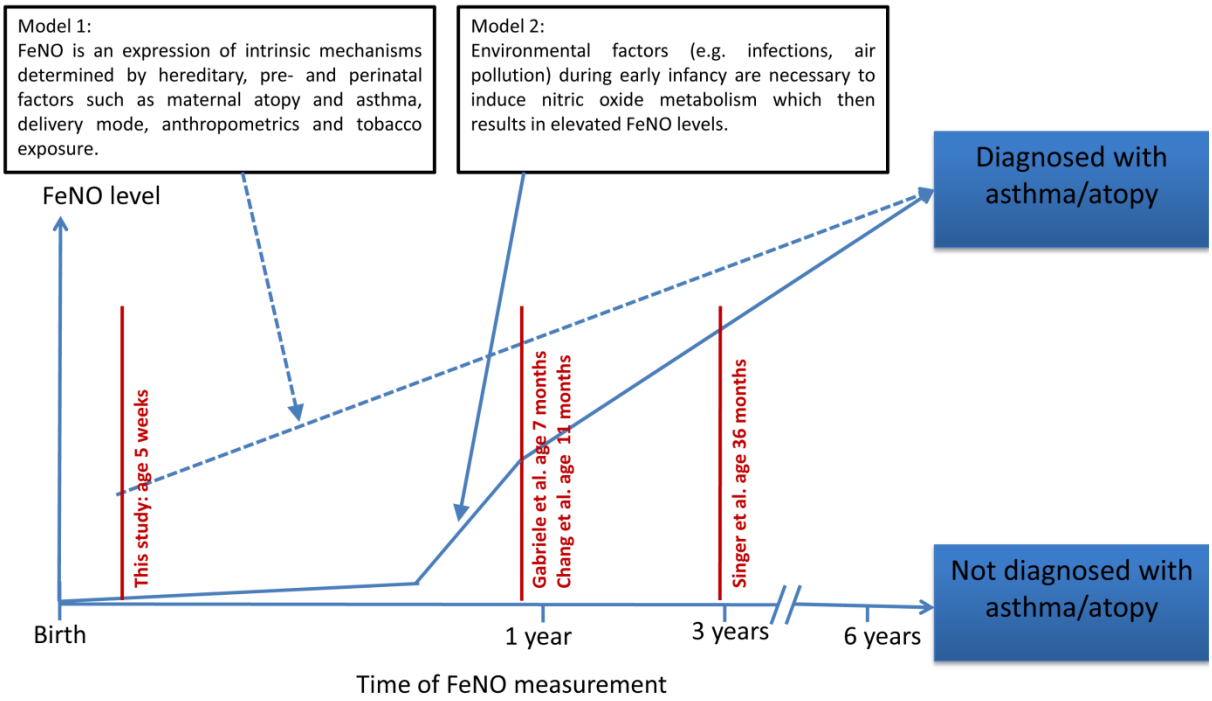

FIGURE 1 We measured fractional exhaled nitric oxide (FeNO) at 5 weeks of age, while previous studies measured FeNO in older children during time ranges. Based on this and previous studies, we propose two hypotheses on FeNO metabolism during infancy:

Model 1 (dashed line): Elevated FeNO is an expression of an intrinsic mechanism, determined by pre-and early postnatal risk factors. In this model, FeNO levels during the first year of life would then not be altered by environmental factors, and FeNO measured at birth could serve as a predictor for asthma/atopy at school age.

Model 2 (continuous line): Environmental factors (e.g. infections, air pollution) are needed to induce nitric oxide synthases (NOS) which then results in elevated FeNO. In this model, we would expect no association between FeNO at birth (measured before environmental exposures) and asthma/atopy at school age. FeNO may then only serve as a phenotype specific biomarker in infants after the first activation of environmentally induced NOS.