Defining the normal range of fractional exhaled nitric oxide in children: one size does not fit all

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Shareable abstract (@ERSpublications)
As height increases rapidly and substantially during childhood, a single F_{ENO} cut-off for asthma diagnosis may not be appropriate in children. A validated height-adjusted F_{ENO} centile chart can be easily and effectively used in the asthma diagnostic process. https://bit.ly/3nQs2PS

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Abstract

Background The normal range of fractional exhaled nitric oxide (F_{ENO}) is influenced by demographic factors. However, single, fixed cut-off values are used for clinical interpretation in children despite rapid growth. We aimed to define the normal range of F_{ENO} during childhood and evaluate its utility in a diagnostic setting.

Method F_{ENO} percentile charts were developed using data from nonasthmatic children in a population-based birth cohort (Manchester Asthma and Allergy Study). Children were skin prick tested, F_{ENO} measured at the ages of 8, 11, 13–16 and 18 years and clinical information collected. This chart was externally validated in the Study of Eczema and Asthma to Observe the Influence of Nutrition (SEATON) cohort before being prospectively tested in symptomatic, treatment-naïve patients with suspected asthma in a diagnostic setting (Rapid Access Diagnostics for Asthma study).

Results Height, weight, body mass index and age were predictive of F_{ENO} in univariate analysis using 1220 F_{ENO} measurements. Only height remained significant after adjustment in the overall, nonatopic and atopic populations, and was included in the predictive equations for 50th, 75th 90th and 98th percentiles. The proposed percentile lines corresponded to the 57th (95% CI 53rd–61st), 80th (76th–83rd), 90th (87th–92nd) and 98th (96th–99th) percentiles in the SEATON cohort (660 measurements). When tested in 73 symptomatic treatment-naïve children and young adults (median (interquartile range) age: 11 (8–14) years), an F_{ENO} >90th percentile gave a 96% specificity and positive predictive value of 97%, identifying 59% of children who were subsequently diagnosed with asthma after extensive testing.

Conclusion We developed a height-based F_{ENO} percentile chart which quantifies the probability of asthma in symptomatic children and merits further validation towards clinical implementation.

Introduction

The rate of asthma misdiagnosis is substantial, with up to one third of patients being over-treated, whilst others go undiagnosed [1, 2]. The use of fractional exhaled nitric oxide (F_{ENO}) is recommended to facilitate the diagnosis of asthma in clinical practice guidelines, where it is used as a dichotomous outcome ("positive" or "negative"). However, the cut-off values are inconsistent between guidelines; in the National Institute for Health and Care Excellence (NICE) guidance [3] the paediatric threshold is 35 ppb whereas in the European Respiratory Society (ERS) guidance it is 25 ppb [4]. There is a significant overlap in F_{ENO} levels between healthy and asthmatic populations, and the range is wide [5]. Whilst a single diagnostic

cut-off value may be easy for clinicians to implement in practice, it is clear that the clinical probability of asthma increases with increasing F_{ENO} levels above the recommended cut-off [4, 5], and collapsing this continuous variable into a dichotomous outcome loses a large amount of information [6]. The normal ranges of F_{ENO} are influenced by factors such as sex, age, height, ethnicity and allergic sensitisation [3, 7–12]. Moreover, pubertal growth spurts influence F_{ENO} trajectory, potentially further affecting the diagnostic accuracy within this age group when a single fixed cut-off value is used [13]. Currently, none of these factors are considered when F_{ENO} levels are interpreted in practice [3, 4, 14]. Other tests used in asthma diagnosis, such as spirometry, are interpreted in relation to predicted values calculated based on individual patient characteristics (age, height, sex and ethnicity) and automatically calculated by the measuring device.

The aim of the current study was to develop individualised normal ranges of F_{ENO} in growing children and to evaluate its utility in symptomatic, treatment-naïve children who have suspected asthma.

Methods

To define what constitutes the upper limit of normal for F_{ENO}, we developed an F_{ENO} percentile chart within nonasthmatic children using data from the Manchester Asthma and Allergy Study (MAAS) [15]. We externally validated the percentile chart in the Study of Eczema and Asthma to Observe the Influence of Nutrition (SEATON) cohort [16], before testing in a diagnostic setting (Rapid Access Diagnostics for Asthma (RADicA) study) [17].

Population 1: chart development cohort (MAAS)

MAAS is a population-based birth cohort and described in detail elsewhere [15]. In brief, children were recruited before birth and followed prospectively. The predominant ethnic group was white British. We analysed data from validated, interviewer-administered questionnaires on parentally reported (age: 8, 11, 13–16 years) or self-reported (age: 18 years) symptoms, physician-diagnosed diseases and asthma medication. Pubertal status was measured at the ages of 11 and 13–16 years (Tanner scale [18, 19]). Allergic sensitisation to common aeroallergens was ascertained using skin prick tests (SPTs).

F_{ENO} was measured at each follow-up clinic using either a chemiluminescence analyser (NIOX, Aerocrine, Sweden) or an electrochemical analyser (NIOX Mino, Aerocrine, Sweden), changed on 4 May 2012 (devices gave comparable results in previous studies [20, 21]). We calculated the predicted 50th, 75th, 90th and 98th percentiles in nonasthmatic children (defined as no self-reported wheeze over the past 12 months, no history of physician diagnosed asthma and no asthma medication use) (Table E1).

Population 2: chart validation cohort (SEATON)

To externally validate the percentile chart, we analysed data collected from children who attended follow-up clinics at the ages of 10 and 15 years within the SEATON birth cohort [16] (Table E1). Validated questionnaires were parentally administered at the age of 10 years and self-administered at 15 years. Children were skin prick tested for common inhaled allergens and F_{ENO} (NIOX chemiluminescence analyser at 10 years old and NIOX MINO (both Aerocrine, Sweden) at 15 years old) were measured. The percentages of children above and below the corresponding proposed percentile lines were calculated.

Population 3: symptomatic and treatment-naïve patients (RADicA)

We prospectively tested our newly proposed percentiles of F_{ENO} for the probability of asthma in symptomatic children and young adults in the RADicA study (ISRCTN 11676160; www.radica.org.uk) described in detail elsewhere [17]. Briefly, symptomatic (wheeze, shortness of breath, chest tightness or cough), treatment-naïve patients with a clinician suspicion of asthma were recruited from primary care. Clinical history, examination and asthma diagnostic tests were performed before a trial of inhaled corticosteroids (ICS) was given. Symptom control was measured using an asthma control questionnaire (ACQ) [22]. The diagnostic tests and ACQ were repeated after 8 weeks of treatment before a diagnosis was established following an expert panel objective evidence review (EPOER) comprising a minimum of two senior asthma physicians (Table E2).

RADicA study procedures

Diagnostic procedures are described in detail elsewhere [17]. Briefly, F_{ENO} was measured using NIOX VERO (Circassia, UK) in accordance with international guidelines [23]. Spirometry was measured (JAEGER™ Vyntus™ PNEUMO, Vyaire medical, USA) before and after bronchodilator use; reversibility was calculated as percentage change of forced expiratory volume in 1 s 15 min after administration of 400 µg of salbutamol *via* a large volume spacer. SPTs for common inhalant allergens were performed and

blood eosinophil levels measured. Peak expiratory flow variability was measured using an eMini Wright digital flow meter (Clement Clarke Ltd. Harlow, UK). Methacholine (Vyaire medical, USA) and mannitol (Osmohale, Pharmaxis Pharmaceuticals Limited, Ireland) bronchial challenges were performed (Table E2).

All study protocols were approved by the local research ethics committee, all parents gave written informed consent and children gave assent.

Statistical analysis

Data were analysed using paired or unpaired Student t-tests for parametric data and the Mann–Whitney U test for nonparametric data, as appropriate. As F_{ENO} is log-normally distributed, the geometric mean was presented. For cross-sectional data, correlations between the absolute F_{ENO} levels and other associated variables were calculated using Spearman's rank test; for longitudinal data, the correlation with the repeated measurement function was used. For model development, longitudinal data were analysed using mixed-effect quantile regressions for the predictions of percentile equations using the absolute F_{ENO} levels (without log transformation) for ease of clinical interpretation. Quantile regression models do not assume normal data distributions and have been previously used to model F_{ENO} percentiles [7, 24]. Bootstrapping with 1000 iterations was used to calculate the 95% confidence interval. Within the RADicA dataset, the diagnostic probability of F_{ENO} above each percentile line was evaluated using sensitivity, specificity, positive and negative predictive values and positive (+LR, sensitivity/(1-specificity)) and negative likelihood ratios (−LR, (1-sensitivity)/specificity) [6]. All analyses were performed using RStudio (version 1.4.1106) and R (version 4.1.1).

Results

Chart development (MAAS)

Baseline characteristics

From the MAAS, 840 children had one or more F_{ENO} reading during follow-up, totalling 1954 measurements (Tables E3–4, Figures E1–2). After exclusions (Figure E2) 1474 F_{ENO} measurements were available for further analysis, of which 254 were from children with asthma.

F_{ENO} levels were significantly higher in children with current wheeze, physician-diagnosed asthma, current asthma medication use, SPT sensitisation and current hay fever (Table E5). Whilst the F_{ENO} levels of atopic nonasthmatic and asthmatic children were substantially higher than those in healthy nonatopic children, there was significant overlap between the groups (Figures E3 and E4).

F_{ENO} measurements from children with current wheeze, history of doctor-diagnosed asthma or current asthma medication use were excluded from the model development. Asymptomatic nonasthmatic children who had complete datasets were included in the development of the F_{ENO} percentile chart (table 1).

Among children without asthma, F_{ENO} increased with age in nonatopic and atopic children (table 2). Age, height, weight and body mass index were correlated with F_{ENO} in children with and without atopy (Table E6). Boys had higher F_{ENO} than girls beyond the age of 13–16 years, coinciding with the age when boys outgrow girls in height (Tables E7–8, Figures E5). Tanner scales at the ages of 11 and 13–16 years were associated with increased F_{ENO} in longitudinal univariate analysis, but this was no longer significant after adjustment for height (Tables E9–10).

TABLE 1 Demographic data for included nonasthmatic children in the Manchester Asthma and Allergy Study cohort.

	Age 8 years (n=275)	Age 11 years (n=387)	Age 13–16 years (n=309)	Age 18 years (n=249)
Sex, male, n (%)	135 (49.1)	175 (45.2)	147 (47.6)	109 (43.8)
Age (years), mean (SD), (range)	8.0 (±0.1) (7.1–8.6)	11.5 (±0.5) (10.0–12.8)	16.1 (±0.5) (14.2–17)	19.4 (±0.7) (18.0–21.8)
Ethnicity, white, n (%)	266 (97.8)	370 (97.4)	292 (96.7)	233 (97.1)
Height (cm), mean (SD), (range)	128 (±5.0) (116–145)	149 (±7.2) (130–170)	170.1 (±8.6) (150–196)	171.7 (±9.5) (151–198)
Weight (kg), mean (SD), (range)	28 (±5.1) (20–51)	42.7 (±9.9) (23.6–91.4)	63.3 (±11.6) (42.3–116)	69.6 (±13.5) (46.3–138)
Body mass index (kg·m^{-2}), mean (SD), (range)	16.9 (±2.2) (13.1–26.3)	19.1 (±3.3) (12.9–40.8)	21.9 (±3.4) (16.2–37.4)	23.5 (±4.0) (16.6–41.3)
SPT sensitisation, n (%)	64 (23.3)	93 (24)	141 (45.6)	114 (45.8)
Current eczema (self-reported), n (%)	36 (13.2)	59 (15.6)	28 (9.1)	28 (11.3)
Current hay fever (self-reported) n(%)	30 (11.4%)	71 (18.8%)	97 (32.4%)	90 (36.1%)

SPT: skin prick test.

Model development for F_{ENO} percentiles

To establish the percentile lines of F_{ENO} in children without asthma (regardless of atopic status), univariate analysis (including age, height, weight and sex as predictors) for the 50th, 75th, 90th and 98th percentiles were performed. In the quantile multivariate backwards regression model, predictors with $p<0.05$ in the univariate analysis (sex for the 50th percentile, height, weight and age) were included, but only height remained significant and was included in the final model (Table E11, table 3, figure 1). Log transformation of F_{ENO} did not affect the results. The ERS (25 ppb) and NICE (35 ppb) cut-off values intercept the 90th percentile line at the heights of 127 cm and 158 cm, respectively.

As F_{ENO} is also associated with atopic status, we used the same model to develop the percentile equations for nonatopic and atopic populations separately (Tables E12–E16, Figure E6).

External validation (SEATON cohort)

External validation of the percentiles developed from the MAAS was performed within the SEATON cohort where F_{ENO} measurements were available in 419 children at the age of 10 years and 462 children at 15 years, of which 372 (88.8%) and 379 (82.0%), respectively, had information to determine if they had current asthma or not. Of these, 332 (89.2%) at 10 years and 328 (86.5%) at 15 years were asymptomatic nonasthmatics (regardless of atopy) and were included in the analysis (Table E16). The MAAS percentile lines correlated well with the SEATON percentile lines (table 4, Table E17).

Using the F_{ENO} percentile chart for asthma diagnosis in symptomatic children (RADicA study)

Of 214 symptomatic and treatment-naïve patients referred to the RADicA study from primary care for possible asthma, 73 (median (interquartile range) age: 11 (8–14) years, 47.9% male, 54.8% white) were aged 22 years or younger (age-matching for chart development cohort), had a definitive diagnostic outcome (51 had asthma, 22 did not have asthma) and a measurement of F_{ENO} before treatment was initiated (Table E18). Data from these participants were used to assess sensitivity and specificity of F_{ENO} percentile charts for asthma diagnosis. Children with an unclassified diagnostic outcome were excluded from the analysis. The distribution of F_{ENO} in relation to height, atopic and asthmatic status is shown in Figure E7.

As expected, sensitivity decreased with increasing F_{ENO} percentiles whilst the specificity improved (table 5). Where F_{ENO} levels fell above the 90th percentile, 97% were diagnosed with asthma, with a +LR of 13.1, corresponding to a large increase in post-test probability after taking into account asthma prevalence [6]. For individuals with F_{ENO} above the 98th percentile (accounting for over a third of patients who were subsequently diagnosed with asthma), asthma diagnosis can be confidently made (100% specificity). The negative likelihood ratios for all percentiles were poor, indicating insufficient power to exclude asthma.

Using charts stratified by atopic status, for symptomatic and nonatopic children, an F_{ENO} >98th percentile gave a 100% specificity and 21% sensitivity; in symptomatic and atopic children, an F_{ENO} level of more

TABLE 2 The distribution of fractional exhaled nitric oxide (F_{ENO}) in nonatopic and atopic children without asthma across age groups.

Follow-up clinics	F_{ENO} geometric mean (geometric SD) (ppb)	5th and 95th percentile (ppb)
Healthy nonatopic children		
8 years (n=211)	8.7 (1.6)	4.6–16.4
11 years (n=294)	8.9 (1.6)	4.7–20
13–16 years (n=168)	13.7 (1.6)	7–32
18 years (n=135)	12.1 (1.6)	6–27
Atopic children without asthma		
8 years (n=64)	15.3 (2.3)	5.3–57.9
11 years (n=93)	17.3 (2.4)	4.3–70.0
13–16 years (n=141)	23.3 (2.2)	7–90
18 years (n=114)	19.7 (2.1)	7–77

As F_{ENO} is not normally distributed, the geometric mean and the 5th and 95th percentiles are presented in the table. SD: standard deviation

TABLE 3 Intercepts and regression coefficient for children without current asthma (regardless of atopic status) for 50th, 75th, 90th and 98th percentiles.

Percentile	RC	RC bootstrap (95% CI)	Intercept	Intercept bootstrap (95% CI)	Equations
50th	0.18	(0.12–0.23)	−15.56	(−23.21–−7.45)	0.18×height (cm)−15.56
75th	0.22	(0.16–0.27)	−15.56	(−23.21–−7.45)	0.22×height (cm)−15.56
90th	0.32	(0.25–0.38)	−15.56	(−23.21–−7.45)	0.32×height (cm)−15.56
98th	0.57	(0.49–0.66)	−15.56	(−23.21–−7.45)	0.57×height (cm)−15.56

CI: confidence interval; RC: regression coefficient.

than the 90th percentile on the atopic percentile chart gave a sensitivity of 46% with 100% specificity (Table E19).

Discussion

Using two population-based birth cohorts, we have confirmed that height is the key independent predictor of F_{ENO} in childhood. As height increases rapidly and substantially during childhood, a single cut-off for asthma diagnosis may not be appropriate in this age group. To define the normal ranges, we developed and validated a height-adjusted percentile chart for F_{ENO}, covering childhood and adolescence. Within a prospective, symptomatic and untreated cohort of children and young adults who had undergone a detailed diagnostic work up to confirm or refute an asthma diagnosis, we demonstrated that the use of height-adjusted F_{ENO} percentile charts can be effectively used in the asthma diagnostic process.

The percentile chart may provide more information than the current dichotomous approach and may be more clinically useful, allowing the identification of individuals with F_{ENO} measurements that are well above the norm for their height. For example, at a height of 125 cm, an F_{ENO} value of 25 ppb would be at the 90th percentile, giving a high probability of asthma (+LR of 13), whereas at a height of 185 cm the same value would fall below the 75th percentile with a poor diagnostic value (+LR of 3). We postulate that those with asthma-like symptoms and a very high F_{ENO} for height (*i.e.* >98th percentile) could be confidently diagnosed with asthma. In contrast, for individuals who have only a moderately elevated F_{ENO} for their height, the probability of an asthma diagnosis is lower and appropriate clinical decisions regarding further diagnostic tests should be made. Whilst the interpretation of spirometry is moving away from a single standard for all [25], the height-adjusted centile charts also provide individualised ways to define

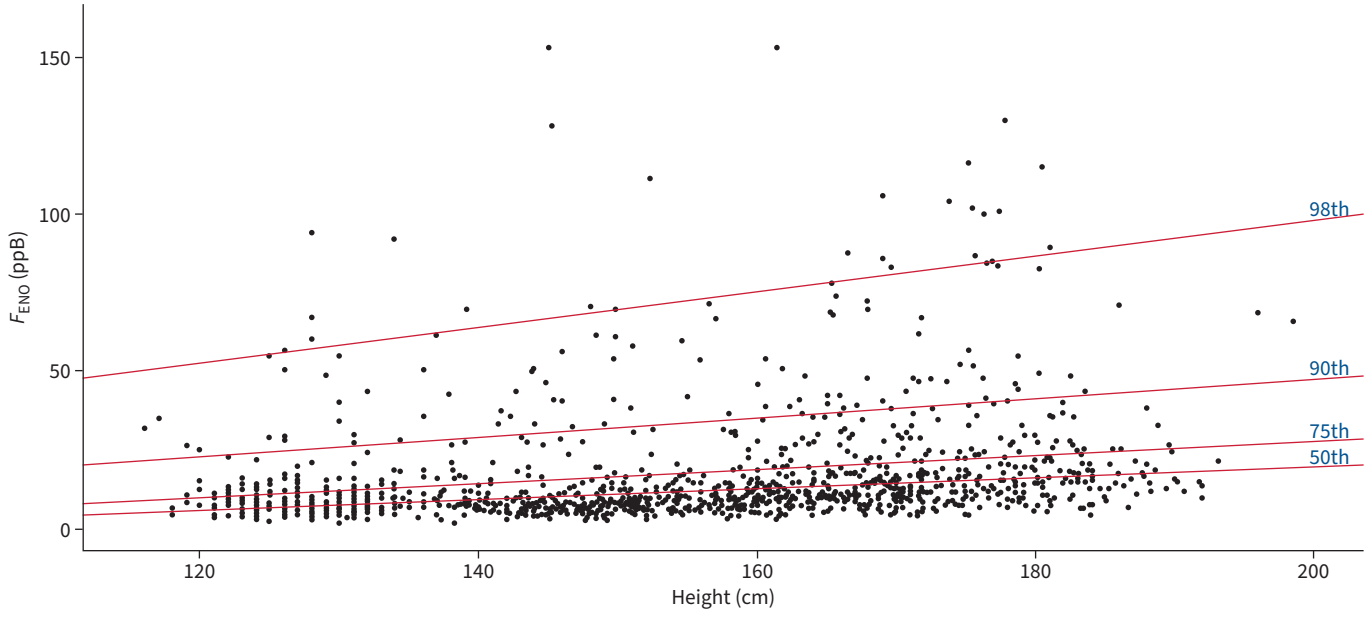

FIGURE 1 Percentile chart for nonasthmatic children and adolescents.

TABLE 4 Proposed fractional exhaled nitric oxide (F_{ENO}) percentile developed from the Manchester Asthma and Allergy Study (MAAS) cohort correlated well with percentile within nonasthmatic children within the Study of Eczema and Asthma To Observe the influence of Nutrition (SEATON) cohort.

MAAS F_{ENO} percentile in nonasthmatic children	Number of SEATON participants with F_{ENO} above MAAS-defined percentile, n (% (95%CI))	Equivalent percentile in nonasthmatic children from the SEATON cohort (95% CI)
50th	282/660 (43 (39–47)	57th (53rd–61st)
75th	134/660 (20 17–24)	80th (76th–83rd)
90th	67/660 (10 8–13)	90th (87th–92nd)
98th	15/660 (2 1–4)	98th (96th–99th)

what is a "normal" F_{ENO}, especially during adolescence. Notably, it will particularly benefit children with heights towards the lower and upper extremes, ensuring that the diagnostic process is inclusive for those who are much shorter or taller than average.

As the atopic status of most children is not known at the time of measuring the F_{ENO}, we have developed a percentile chart for all children regardless of atopic status. We also developed separate charts adjusted for atopic status and note that they are quite different, highlighting the importance of assessing atopic status in F_{ENO} interpretation. Atopic and symptomatic children have increased F_{ENO} (94% of those with F_{ENO} above 98th percentile line on the atopic/nonatopic combined chart were atopic in the RADicA cohort), consistent with previous reports [26, 27]. The nonatopic asthma phenotype is uncommon in children, accounting for 27% in our symptomatic cohort. Whilst the role of F_{ENO} in nonatopic children is less clear [28], we have found that F_{ENO} may still be useful for risk stratification in this group in diagnostic settings. However, it is important to note that the sample size was small (n=14) and future research is needed to further elucidate the role of F_{ENO} in such groups of children.

Although we acknowledge not all children can master F_{ENO} measurements, spirometry tests can also be challenging, and many struggle to perform forced expiratory manoeuvres to residual volume, resulting in variable quality and difficult interpretation. Using nonaerosol-generating procedures (such as F_{ENO}, SPT or immunoglobulin E) to facilitate asthma diagnosis may be beneficial during a pandemic (such as coronavirus disease 2019) when access to procedures with aerosol-generating potential is limited and thus may avoid delays in commencing treatment in some children [17]. However, we acknowledge the role of spirometry-based tests in demonstrating airflow obstruction and variability over time and following bronchodilator therapy, as well as during bronchial challenge testing, and spirometry remains an essential part of the asthma diagnostic armamentarium. Nevertheless, the current study provides useful information to facilitate future work in the determination of the diagnostic algorithm, taking into account different clinical probabilities and health-economic scenarios.

Many studies evaluating the diagnostic efficiency of F_{ENO} were limited by heterogeneity of the children and adult participants, use of ICS and potential selection bias of the study populations [27–31]. We note that no studies to date have assessed the diagnostic efficiency of using height-adjusted values.

Like other measures of pulmonary function, F_{ENO} is influenced by demographic factors in both healthy and asthmatic children (particularly height [8, 11, 32]), and it seems something of an anomaly that clinicians use height- (as well as age- and sex-) adjusted values for spirometry, with results usually presented as % predicted (as well as the absolute value), but this has not been the case for F_{ENO}. The association between height and F_{ENO} may be particularly important in childhood, with a steep growth

TABLE 5 Asthma risk stratification based on percentile cut-off.

Percentile	Sensitivity, % (n)	Specificity, % (n)	PPV, % (n)	NPV, % (n)	+LR[#]	−LR[¶]
>50th	78.4 (40/51)	40.9 (9/22)	75.5 (40/53)	45.0 (9/20)	1.3	0.5
>75th	72.5 (37/51)	77.3 (17/22)	88.1 (37/42)	54.8 (17/31)	3.2	0.4
>90th	58.8 (30/51)	95.5 (21/22)	96.8 (30/31)	50.0 (21/42)	13.1	0.4
>98th	33.3 (17/51)	100 (22/22)	100 (17/17)	39.3 (22/56)	∞	0.7

[#]: +LR: sensitivity/(1-specificity). [¶]: −LR: (1-sensitivity)/specificity. LR: likelihood ratio; NPV: negative predictive value; PPV: positive predictive value.

trajectory occurring between 5 and 16 years old [33]. Indeed, our data suggest that differences in height (>80 cm range in both the MAAS and the SEATON cohorts) markedly influence F_{ENO}, particularly in relation to the higher percentile lines. Consistent with this, GARCIA *et al.* [13] used repeated measures within children going through puberty and reported a substantial increase in F_{ENO} between 8 and 16 years old, with tracking of personalised F_{ENO} measurements, and highlighted the limitations of fixed F_{ENO} reference values. Whilst using a fixed single F_{ENO} cut-off may seem easier for clinicians to implement, technology allowing input of demographic data and automatic calculation of % predicted values (as for spirometry) would streamline the utility of a height-adjusted approach in practice. Furthermore, to meaningfully reduce the misdiagnosis rate in asthma at a population level, it may be that the diagnostic process should occur in "diagnostic hubs" where key tests (including F_{ENO} and assessment for atopy) can be performed before a diagnosis is confirmed or refuted by asthma specialists. Such a healthcare infrastructure should be subjected to future research and health-economics evaluation.

Strength and limitations

To our knowledge, our study is the first to develop and externally validate a height-adjusted percentile chart for F_{ENO} in children. Both MAAS and SEATON are unselected population-based birth cohorts; however, they are predominantly (>95%) of white ethnicity, which although reflecting the local population at the time of recruitment, is not representative of current UK residents. Further external validation in ethnically diverse populations may be necessary.

It is also important to highlight that despite there being a good correlation between the chemiluminescent and electrochemical analysers, the agreement and technical reproducibility vary between the two [34]. As the MAAS and SEATON birth cohorts span >20 years, chemiluminescent analysers were used before electrochemical analysers became available. Furthermore, even among electrochemical analysers, devices produced by different manufacturers demonstrate differences in F_{ENO} levels [35]. Therefore, the types and manufacturers of the analysers used should be taken into consideration in future work.

Moreover, it is well-established that F_{ENO} demonstrates diurnal and day-to-day variabilities in patients with asthma [36]. Whilst diurnal variation may be predictable in some patients, the day-to-day and longer-term variations may be less so. In the current study, we focused on factors which contribute to the predictable longer-term variations of F_{ENO} (e.g. height, weight, age) in growing children, but did not adjust for time-of-the-day factors for diurnal variability and other factors such as allergen exposure or disease severity around the time of testing. We tested the percentile chart in a diagnostic setting using F_{ENO} measurements taken in symptomatic children and young adults before they commenced ICS treatment (RADicA study). The RADicA study participants all underwent extensive assessment and all information was assessed by an expert panel comprising a minimum of two senior asthma physicians (EPOER). An EPOER designation of "asthma" or "not asthma" was used as the gold standard against which the performance of the F_{ENO} percentile chart was assessed. Like many studies evaluating the diagnostic role of F_{ENO} in asthma [28, 30, 37, 38], our symptomatic and untreated patient cohort had high pre-test probability (70%). The performance in probability stratification remains uncertain in children with low pre-test probably where an alternative diagnosis is more likely and therefore should not be extrapolated to this population. The sample size for the symptomatic, untreated patient cohort was limited, particularly in children with extreme heights (in whom the height-adjusted equation is likely to be more advantageous than any unified cut-off values) and therefore it was not possible to compare the diagnostic efficiency of the current approach with established guidelines. It is imperative that our study findings are externally validated in larger cohorts of patients in a diagnostic setting.

Conclusion

We have defined the normal range of F_{ENO} using height-adjusted percentile charts in nonasthmatic children from two UK birth cohorts. By applying the F_{ENO} percentile charts in a diagnostic setting, in symptomatic, treatment-naïve young people with clinician-suspected asthma, we identified one third of those who were subsequently diagnosed with asthma without the need for further tests. Our height-adjusted percentile chart may facilitate the development of a more personalised asthma diagnostic algorithm. Further external validation in a larger cohort of patients in a diagnostic setting is warranted.

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