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# Importance of type and degree of IgE sensitisation for defining fractional exhaled nitric oxide reference values

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ABSTRACT

*Background:* Fractional exhaled nitric oxide ($FE_{NO}$) is a marker of type 2 airway inflammation used in clinical practice in asthma. However, reference values are needed to broaden the clinical use of $FE_{NO}$ and this is within the scope of a newly started Global Lung Function Initiative task force. We aim to study $FE_{NO}$ levels with special emphasis on the upper limit of normal (ULN) in relation to the type and degree of IgE sensitisation.

*Methods:* $FE_{NO}$ was measured in 1855 non-smoking, respiratory healthy subjects from the Swedish CArdioPulmonary bioImage Study (SCAPIS). Atopic subjects (n = 424), defined as being IgE-sensitised to aeroallergens (ImmunoCAP Phadiatop™, ≥0.35 PAU/l) were compared to non-atopic subjects (<0.35 PAU/l, n = 1431). Atopic subjects were further characterised according to their grade of IgE sensitisation (IgE antibody tertiles: (T1<1.16, T2 1.16–3.72 and T3 >3.72 PAU/l) and sensitisation to perennial (cat or mite) or seasonal (birch) allergens.

*Results:* Subjects IgE-sensitised to cat or mite had higher $FE_{NO}$ compared to non-atopic subjects ($FE_{NO}$ (ppb): median 20.0 vs. 15.0, and ULN 50.4 vs. 33.0, $p < 0.001$). This was seen to a lesser extent for subjects IgE-sensitised to birch only (median 18.0 vs. 15.0, and ULN 38.0 vs. 33.0, $p = 0.048$). Atopic subjects with a high degree of IgE sensitisation (Phadiatop: >3.72 PAU/l) had the highest $FE_{NO}$ compared to non-atopic subjects (median 20.0 vs. 15.0, and ULN 56.0 vs. 33.0, $p < 0.001$).

*Conclusions:* The type and degree of IgE sensitisation should be considered in generating $FE_{NO}$ reference values.

## 1. Introduction

Fractional exhaled nitric oxide ($FE_{NO}$) is a marker of type 2 airway inflammation [1,2] that is increased in patients with asthma [3] and in patients responding to treatment with inhaled corticosteroids [4]. $FE_{NO}$ is increasingly used in clinical practice due to its non-invasive nature and its place in the current clinical guidelines in assessment of asthma patients [5–7]. The American Thoracic Society (ATS) guidelines (2011) recommend the use of absolute cut-off points when interpreting $FE_{NO}$ levels [8], suggesting that in steroid naïve adults, values of $FE_{NO} < 25$ parts per billion (ppb) should indicate a low likelihood of eosinophilic inflammation and responsiveness to corticosteroid therapy, while levels >50 ppb indicate a greater likelihood of eosinophilic inflammation and responsiveness to corticosteroid therapy in symptomatic patients, leaving a large "grey zone" in between 25 and 50 ppb [8]. However, the use of cut-off points rather than reference values in the interpretation of $FE_{NO}$ was stated as a weak recommendation along with being based on low quality evidence [8], which has not since been updated. Although the fixed cut-off for $FE_{NO}$ has been supported by other studies [9], reported optimal cut-off levels for $FE_{NO}$ to determine an asthma diagnosis have varied and ranged between 10.5 and 64 ppb [9]. Currently a Global Lung Function Initiative (GLI) task force has been initiated to define normal levels for $FE_{NO}$, by using large databases of healthy individuals, in a similar way as for lung function [10].

$FE_{NO}$ levels are known to be influenced by a number of additional

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**Abbreviation list**

| | |
|---|---|
| $FE_{NO}$ | Fractional exhaled nitric oxide |
| ATS | American Thoracic Society |
| ppb | parts per billion |
| GLI | Global Lung Function Initiative |
| ULN | upper limit of normal |
| IgE | Immunoglobulin E |
| SCAPIS | Swedish CArdioPulmonary bioImage Study |
| CVD | cardiovascular disease |
| COPD | chronic obstructive pulmonary disease |
| $FEV_1$ | Forced expiratory volume in 1 s |
| FVC | Forced vital capacity |
| ERS | European Respiratory Society |
| NO | Nitric oxide |
| LLN | lower limit of normal |
| (PAU/l) | Phadia arbitrary units per litre |
| ANOVA | Analysis of variance |

factors that are necessary to take into account for accurate interpretation [11]. Active smoking is well known to reduce $FE_{NO}$ levels [12–14], whereas increasing age [13,15], male gender [15–18], increasing height [13,15,17,18], and presence of atopy [13,15,17–19] are associated with increased levels of $FE_{NO}$. Recent evidence suggests that in adults over the age of 50 years, age and sex should be taken into account when interpreting values of $FE_{NO}$ [20]. Additionally, in non-smokers, it has been suggested that individual reference values based on reference equations should be used rather than fixed cut-offs, due to the upper limit of normal (ULN) obtained from reference equations being significantly influenced by age, sex, height and presence of atopy [15].

Immunoglobulin E (IgE) sensitisation is a factor known to influence $FE_{NO}$, and this association has been found independently of the presence of asthma [21]. A study in 2017 by Torén et al. [15], presented coefficient estimates of reference equations for $FE_{NO}$ separately for those with atopy, defined as IgE sensitisation towards a mix of aeroallergens, and for those without atopy as it was found that the ULN for individuals differed by 13 ppb if they were atopic compared to non-atopic [15]. However, no special emphasis has been put on the type or degree of IgE sensitisation.

The aim of the present study was to explore the effects of the degree and type of IgE sensitisation (including both seasonal and perennial allergens) on levels of $FE_{NO}$, in particular the ULN, and to establish whether both degree and type of IgE sensitisation should be considered when proposing reference values for $FE_{NO}$.

## 2. Method

### 2.1. Study population

Swedish CArdioPulmonary bioImage Study (SCAPIS) is a national, multi-centred population-based study of randomly selected men and women aged 50–64 years. The study was formed through a collaboration between six Swedish universities (Gothenburg, Linköping, Malmö/Lund, Stockholm, Umeå and Uppsala) for a joint national effort to prevent cardiovascular disease (CVD) and chronic obstructive pulmonary disease (COPD) by improving risk prediction of cardiopulmonary conditions using updated knowledge relevant to today's pattern of risk factors [22]. Nationally, 30154 men and women participated and from the Uppsala cohort 5036 subjects were recruited between October 2015 and June 2018 (participation rate of 46.7%). Study participants took part in a comprehensive questionnaire, blood sampling, extensive physical examinations, lung function tests and imaging as previously described [22]. The study was approved by the Regional Ethical Review Board in Umeå (2010-228-31 M), and the analyses of the SCAPIS study were approved by the Regional Ethical Review Board in Uppsala (2018–272). The study participants gave their written and informed consent.

$FE_{NO}$ measurements were performed as an add-on to the core SCAPIS protocol in Uppsala, and were done during the entire study inclusion period with the exception of the period from December 2016 to April 2017 due to staffing issues.

Of the total SCAPIS Uppsala cohort (n = 5036 subjects), 1081 subjects were excluded with missing information on $FE_{NO}$. A further 1928 subjects were excluded that were not considered respiratory healthy, either due to "any respiratory disease", "any respiratory symptoms" (see next paragraph for definitions) or abnormal lung function (Forced expiratory volume in 1 s ($FEV_1$)/forced vital capacity (FVC) < lower limit of normal according to Brisman et al. [23]). Furthermore, subjects with missing information on smoking status were also excluded (n = 27). For the analyses assessing $FE_{NO}$ according to IgE sensitisation, a further 30 subjects were excluded with missing information on atopy. Fig. 1 illustrates the flow of subjects through the study.

"Any respiratory disease" was defined as reporting a diagnosis of COPD, chronic bronchitis, emphysema, asthma, tuberculosis or reporting a diagnosis of any other respiratory disease on the questionnaire.

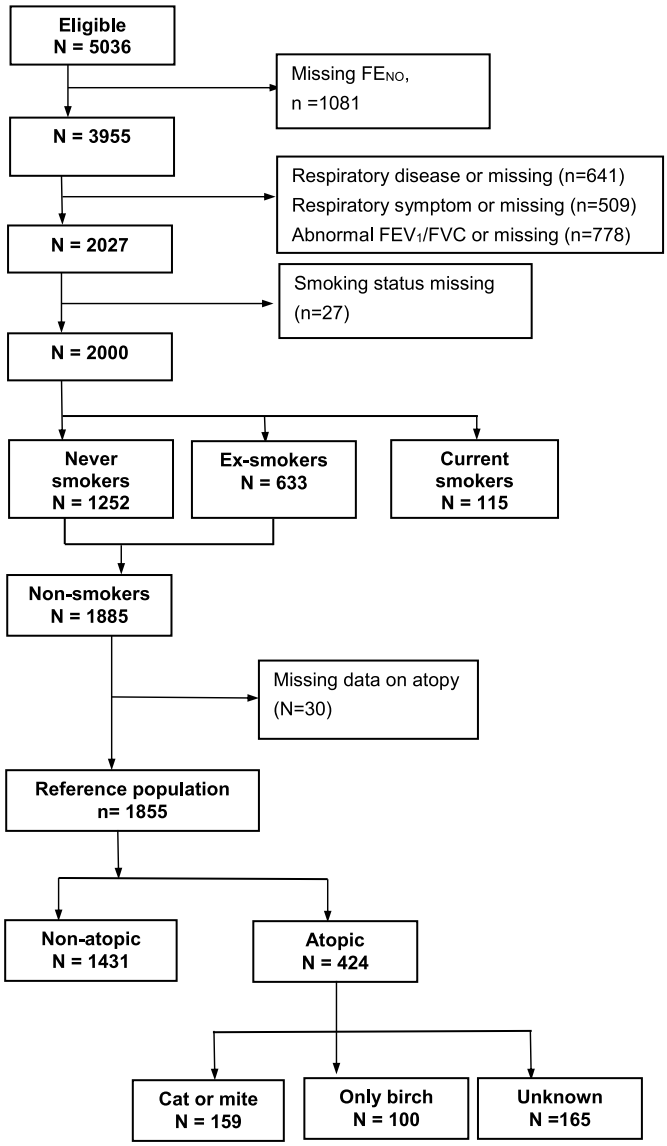


**Fig. 1.** Flow of subjects through the study.

"Any respiratory symptoms" was defined as any of the following: 1) a cough without a cold; 2) coughing up phlegm or chest phlegm without a cold; 3) a wheeze or whistling.

### 2.2. Height and weight

Height was measured without footwear to the nearest centimetre. Weight was measured without footwear and in light indoor clothing on a digital scale.

### 2.3. Smoking status

Smoking status was obtained from questionnaire responses. Subjects were classified as never smokers, former smokers or current smokers according to their response.

### 2.4. $FE_{NO}$ measurements

$FE_{NO}$ measurements were performed by means of an electrochemical sensor using NIOX Vero® (previously Aerocrine, currently Circassia, Oxford, UK), according to ATS/European Respiratory Society (ERS) standards [24] with exception for performing a single measurement [25]. Subjects were asked to empty their lungs and then fill the lungs with nitric oxide (NO)-free air after placing their mouth on the mouthpiece of the analyser. With the mouth still on the mouthpiece, they were then asked to breathe out at a constant flow of 50 ml/s for 10 seconds. All $FE_{NO}$ measurements were done in the morning and subjects were fasting as fasting blood samples were taken before the $FE_{NO}$ measurements.

### 2.5. Lung function measurements

Dynamic spirometry (Jaeger MasterScreen™ PFT; Carefusion, Hoechberg, Germany) was performed both pre-bronchodilation and 15 min after bronchodilation using 400 μg salbutamol, with subjects in the sitting position and wearing a nose clip. $FEV_1$ and FVC were obtained according to the ATS and ERS standards [26,27]. Reference equations by Brisman et al. [23] were used to determine the lower limit of normal (LLN) for the ratio of $FEV_1$/FVC and for %predicted values of $FEV_1$ and FVC.

### 2.6. IgE antibody measurements

Atopy was assessed by measuring IgE antibodies in serum using ImmunoCAP Phadiatop™ (Phadia AB/Thermo Fisher Scientific, Uppsala, Sweden). The Phadiatop assay includes a mix of common perennial and seasonal aeroallergens, and the IgE antibody values are reported as Phadia arbitrary units per litre (PAU/l). Subjects with IgE levels ≥0.35 PAU/l were regarded as atopic [28]. The atopic subjects were classified as low, medium or highly IgE-sensitised by grouping their Phadiatop results in tertiles (degree of sensitisation [29,30]). Further characterization of the atopic subjects was done by measuring serum IgE antibodies to cat dander, house dust mite (*Dermatophagoides pteronyssinus)* and birch pollen, using ImmunoCAP™ Specific IgE (Phadia AB/Thermo Fisher Scientific). A value of ≥0.35 $kU_A/l$ was defined as a positive sensitisation to the specific allergen [31]. The atopic subjects were grouped according to their sensitisation to i) perennial allergen (cat and/or mite) or ii) seasonal allergen (birch only, i.e. no co-sensitisation to cat or mite).

### 2.7. Statistical analysis

Statistical analyses were performed using R (R Core Team, Version 4.0.2). Median values of $FE_{NO}$ (ppb) along with 95th percentile cut-offs (i.e. ULN) were obtained using quantile regression models, which unlike linear regression do not require any distributional assumptions [32]. In respiratory healthy subjects, an initial analysis, stratified according to smoking groups, was carried out in men and women separately (n = 2000).

All further analyses were carried out on respiratory healthy, non-smoking subjects (never and ex-smokers). We analysed median and ULN for $FE_{NO}$ in relation to age, presence of atopy and degree of IgE sensitisation, as well as positive sensitisation to individual allergens. Regarding the latter, subjects were divided into type of allergen that tested positive and compared to non-atopic subjects (those who tested negative on the aeroallergen mix test): i) cat or mite positive or ii) Only birch positive (cat or mite negative).

Since $FE_{NO}$ was not normally distributed, a non-parametric test was used, (two-tailed Mann-Whitney *U* test) for calculating the p-values for comparison of median $FE_{NO}$ levels in atopic and non-atopic subjects. P-values were adjusted for multiple testing according to the Benjamini-Hochberg method. Analysis of variance (ANOVA) was used to compare the median $FE_{NO}$ levels in current smokers to never and ex-smokers.

## 3. Results

General baseline characteristics and lung function are presented in Table 1 for i) the total SCAPIS Uppsala cohort (n = 5036), ii) SCAPIS Uppsala cohort with valid $FE_{NO}$ measurements (n = 3955) iii) subjects with missing $FE_{NO}$ from SCAPIS Uppsala (n = 1081) and iv) the reference population in this study (valid $FE_{NO}$ measurements, respiratory healthy subjects with information on atopy, n = 1855).

Comparing subjects that did have $FE_{NO}$ measurements with those excluded due to no $FE_{NO}$ measurements, there were no differences

**Table 1**
Baseline characteristics.

| | General population n = 5036 | $FE_{NO}$ population n = 3955 | $FE_{NO}$ missing n = 1081) | Reference population n = 1855 |
|---|---|---|---|---|
| Age (years) | 57.6 (4.4) | 57.6 (4.4) | 57.6 (4.4) | 57.5 (4.4) |
| Sex (% Female) | 51.3 | 50.6 | 53.9 | 49.2 |
| Height (cm) | 172.5 (9.7) | 172.8 (9.7) | 171.4 (9.7)* | 173.4 (9.7) |
| Weight (kg) | 80.7 (15.7) | 80.9 (15.6) | 80.1 (16.0) | 80.5 (15.5) |
| BMI ($kg/m^2$) | 27.0 (4.4) | 27.0 (4.3) | 27.2 (4.5) | 26.7 (4.2) |
| Smoking status (%) | | | | |
| Never smokers | 58.8 | 58.3 | 60.3 | 64.4 |
| Former smokers | 32.2 | 33.5 | 27.5 | 33.6 |
| Current smokers | 9.0 | 8.2 | 12.1* | 0 |
| $FEV_1$ (% predicted) | 96.7 (14.1) | 96.9 (14.1) | 94.5 (13.6)* | 100.1(12.3) |
| FVC (% predicted) | 100.2 (13.1) | 100.4 (13.0) | 98.2 (13.3)* | 101.0 (12.3) |
| $FEV_1$/FVC (% predicted) | 96.6 (8.2) | 96.6 (8.2) | 96.4 (7.9) | 99.2 (5.7) |
| Atopy (%) | 25.7 | 25.7 | 26.0 | 22.9 |
| $FE_{NO}$ (ppb)† | 15.9 (15.6, 16.2) | 15.9 (15.6, 16.2) | NA | 16.1 (15.8, 16.5) |

Values are mean (±SD) unless otherwise stated. † Geometric mean (95% CI). % predicted values for spirometry by Brisman et al. [23] *denotes statistical significance between the $FE_{NO}$ population and the population with missing $FE_{NO}$ data. **General population**: Total SCAPIS Uppsala cohort. **$FE_{NO}$ population:** SCAPIS Uppsala subjects with valid $FE_{NO}$ measurements **Reference population**: Non-smoking, respiratory healthy SCAPIS Uppsala subjects with valid $FE_{NO}$ measurements and information on atopy status.

regarding gender, age, BMI, $FEV_1$/FVC, proportion with atopy or proportion reporting asthma. However, those that had $FE_{NO}$ measurements were slightly taller, a smaller proportion of them were current smokers, and the mean $FEV_1$ (%predicted) and FVC (%predicted) values were slightly greater. Median $FE_{NO}$ values with ULN (95th percentiles) according to smoking status for men and women in the total population are presented in Table 2.

In never smokers and former smokers, $FE_{NO}$ levels were significantly higher in men than in women ($p < 0.001$). In both men and women, $FE_{NO}$ levels were similar in never and former smokers and were therefore examined as one group of "non-smokers" for further analyses. Similar to previous findings [33], current smokers had significantly lower $FE_{NO}$ levels than never and former smokers in both men and women ($p < 0.001$). Median values of $FE_{NO}$ in non-smokers by three age groups are presented for men and women in Table 3.

$FE_{NO}$ levels were again higher in men than in women for all three age groups ($p < 0.001$ for all age groups), with a small increase seen with age. Table 4 shows $FE_{NO}$ (ppb) according to type of allergen sensitisation and grade of sensitisation (tertiles of IgE antibody levels) in atopic subjects compared to non-atopic subjects.

Atopic subjects had significantly higher $FE_{NO}$ levels than non-atopic subjects (p-value <0.001) and the ULN (95th percentile) for these subjects was also higher (46.0 vs 33.0 ppb). This pattern was seen in both men and women, although the differences were larger in men. Looking at IgE sensitisation to specific allergens, subjects sensitised to cat or mite allergens had significantly higher median and ULN $FE_{NO}$ levels compared to non-atopic subjects ($p < 0.001$). Median values of $FE_{NO}$ were borderline significantly higher in subjects who were positive for only birch compared to the non-atopic group, with smaller differences in the ULN of $FE_{NO}$ compared to non-atopic subjects. The degree of IgE sensitisation was studied in relation to median $FE_{NO}$ and ULN in atopic subjects and is also presented in Table 4. There was an association between the grade of sensitisation and the median and ULN $FE_{NO}$, starting with significantly higher $FE_{NO}$ levels from the 2nd tertile of the Phadiatop results (IgE levels >1.16 PAU/l). Atopic subjects with the highest grade of sensitisation (IgE levels >3.72 PAU/l) also had the highest $FE_{NO}$ levels compared to non-atopic subjects (median 20.0 vs 15.0 ppb, and ULN 56.0 vs 33.0 ppb, $p < 0.001$).

We additionally assessed the group of atopic subjects without positive IgE tests for cat, mite or birch (n = 165), and found they had significantly higher median and ULN $FE_{NO}$ compared to non-atopic subjects. However, this association was seen only in men (Table 4).

No relationship was found between $FE_{NO}$ and allergic rhinitis in the atopic or non-atopic subject groups (Supplement Tables S1 and S2).

## 4. Discussion

The main finding of this study is that both the type and degree of IgE sensitisation strongly influences levels of $FE_{NO}$, in particular the ULN, and therefore should be considered when proposing $FE_{NO}$ reference equations. IgE sensitisation to perennial allergens or a high grade of sensitisation (i.e. high IgE antibody levels) to common aeroallergens is related to increased ULN of $FE_{NO}$ close to the recommended ATS cut-off for high likelihood of airways inflammation.

Although, there have been studies assessing the effect of atopy on $FE_{NO}$, the ULN of $FE_{NO}$ in relation to the degree and type of allergic sensitisation has not been explored. A recent study of Torén et al. presented coefficient estimates for reference equations for $FE_{NO}$, stratified according to the presence of atopy, as it was found the ULN for individuals with IgE sensitisation to a range of aeroallergens was significantly higher than those without any IgE sensitisation [15]. A large scale study from China also found atopic status (as defined by a positive skin prick test) and serum IgE level along with blood eosinophil count to be independently associated with $FE_{NO}$ levels [34]. Reference equations were therefore recommended that took into account atopic status along with age, sex and height. Taller, older men who were also atopic had an ULN of $FE_{NO}$ in the range indicating presence of eosinophilic inflammation according to ATS guidelines [34]. However, these studies did not look further into which type of allergens the subjects were sensitised to; as not only presence, but degree and type of sensitisation may affect $FE_{NO}$ [21,35]. Moreover, both these studies used a chemiluminescence analyser for $FE_{NO}$ that is used to limited extent nowadays and considering that the $FE_{NO}$ results might differ from the technologies clinically

**Table 2**
$FE_{NO}$ (ppb) results of respiratory healthy subjects (n = 2000) according to smoking habits.

| Smoking | Women | | | Men | | | |
|---|---|---|---|---|---|---|---|
| | n | Median | ULN | n | Median | ULN | p-value |
| Never | 567 | 15.0 | 32.0 | 685 | 18.0 | 38.0 | <0.001 |
| Former | 361 | 14.0 | 30.0 | 272 | 18.0 | 41.0 | <0.001 |
| Current | 62 | 10.0[a] | 28.9 | 53 | 12.0[a] | 34.4 | 0.083 |
| All | 990 | 14.0 | 31.0 | 1010 | 18.0 | 39.0 | <0.001 |

ULN= Upper limit of normal, 95th percentile.
[a] p<0.001 compared to never-smokers and ex-smokers, ANOVA study for $FE_{NO}$ quantiles regression model fits. P-values adjusted according to Benjamini-Hochberg method.

**Table 3**
$FE_{NO}$ (ppb) results of non-smokers, (never and former smokers, n = 1885), according to age class and sex.

| Age class (years) | Women n = 928 | | | Men n = 957 | | | |
|---|---|---|---|---|---|---|---|
| | n | Median | ULN | n | Median | ULN | p-value |
| 50–54 | 294 | 13.5 | 29.0 | 324 | 17.0 | 35.9 | <0.001 |
| 55–59 | 311 | 15.0 | 31.5 | 315 | 18.0 | 40.3 | <0.001 |
| 60–65 | 323 | 16.0 | 31.9 | 318 | 19.0 | 39.2 | <0.001 |

P-values adjusted according to Benjamini-Hochberg method.
ULN= Upper limit of normal, 95th percentile.

**Table 4**
$FE_{NO}$ (ppb) according to type of allergen sensitisation and grade of sensitisation in atopic subjects compared to non-atopic subjects.

| Subjects | n | Median | ULN | p-value |
|---|---|---|---|---|
| ***All*** | | | | |
| Non-atopic subjects | 1431 | 15.0 | 33.0 | Reference |
| Atopic subjects | 424 | 18.0 | 46.0 | <0.001 |
| Sensitised to cat or mite | 159 | 20.0 | 50.4 | <0.001 |
| Sensitised only to birch | 100 | 18.0 | 38.0 | 0.048 |
| Sensitised only to other allergens[a] | 165 | 18.0 | 44.2 | 0.003 |
| Phadiatop 1st tertile (<1.16 PAU/l) | 141 | 17.0 | 35.0 | 0.204 |
| Phadiatop 2nd tertile (1.16–3.72 PAU/l) | 142 | 18.5 | 38.0 | <0.001 |
| Phadiatop 3rd tertile (>3.72 PAU/l) | 141 | 20.0 | 56.0 | <0.001 |
| ***Females*** | | | | |
| Non-atopic subjects | 752 | 14.0 | 30.0 | Reference |
| Atopic subjects | 161 | 16.0 | 37.0 | 0.041 |
| Sensitised to cat or mite | 63 | 17.0 | 49.9 | 0.030 |
| Sensitised only to birch | 39 | 17.0 | 36.1 | 0.108 |
| Sensitised only to other allergens[a] | 59 | 14.0 | 30.0 | 0.911 |
| Phadiatop 1st tertile (<0.97 PAU/l) | 54 | 12.0 | 34.4 | 0.484 |
| Phadiatop 2nd tertile (0.97–3.06 PAU/l) | 53 | 17.0 | 41.2 | 0.022 |
| Phadiatop 3rd tertile (>3.06 PAU/l) | 54 | 16.5 | 43.3 | 0.032 |
| ***Males*** | | | | |
| Non-atopic subjects | 679 | 18.0 | 35.0 | Reference |
| Atopic subjects | 263 | 20.0 | 46.9 | <0.001 |
| Sensitised to cat or mite | 96 | 22.0 | 48.0 | <0.001 |
| Sensitised only to birch | 61 | 18.0 | 40.0 | 0.641 |
| Sensitised only to other allergens[a] | 106 | 19.5 | 49.8 | 0.009 |
| Phadiatop 1st tertile (<1.32 PAU/l) | 88 | 18.0 | 37.0 | 0.211 |
| Phadiatop 2nd tertile (1.32–4.28 PAU/l) | 87 | 20.0 | 36.7 | 0.012 |
| Phadiatop 3rd tertile (>4.28 PAU/l) | 88 | 22.0 | 58.3 | <0.001 |

[a] Group defined as sensitisation to Phadiatop, but not to cat/mite or birch allergens. ULN= Upper limit of normal, 95th percentile.

used today [36,37], we think that the results presented in the current study are more generalizable to the current devices used in the clinics. Previously, Olin et al. [38] also proposed reference equations for $FE_{NO}$, and although they found higher $FE_{NO}$ levels in atopic subjects (defined as subjects having a positive Phadiatop result), they recommended the use of reference values including both atopic and non-atopic subjects for practicality in clinical practice.

Degree and type of IgE sensitisation affects the estimated ULN. Our results are consistent with previous studies that have found the degree of IgE sensitisation to influence $FE_{NO}$ [21,39]. One of these studies found the degree of allergic sensitisation to be related to an increase of airway concentration and diffusion of NO [21]. They also found sensitisation to cat allergens to have the highest association to exhaled NO levels. Our findings are also consistent with this, as we also found perennial allergens (cat or mite) to have the greatest impact on exhaled $FE_{NO}$ and we additionally were able to find the ULN of $FE_{NO}$ in subjects sensitised to perennial allergens to be in the range recommended by ATS as high indication of steroid responsive airway inflammation. This was not found as strongly for IgE sensitisation to birch pollen, the most important seasonal allergen(s) in Sweden [40]. Over a third of the atopic subjects were not sensitised to mite, cat or birch and therefore classified as "unknown sensitisation". Grass pollen was included as part of the aeroallergen mix and due to the high prevalence of timothy grass sensitised individuals in Sweden it is likely that it was the largest group within the unknown sensitisation subgroup [41]. The median levels of $FE_{NO}$ in this group were significantly higher than non-atopic subjects.

The findings from our study provide insights into how information on IgE sensitisation can be incorporated into reference equations by defining meaningful cut-off values of IgE. An IgE level >3.72 PAU/l, as measured in the ImmunoCAP Phadiatop assay, was found to be associated with an ULN of $FE_{NO}$ in the range that indicates a strong likelihood of eosinophilic inflammation (ULN $FE_{NO}$, 56.0 ppb). Assessing IgE sensitisation together with $FE_{NO}$ measurement in clinical practice may increase the likelihood of detecting those with clinically relevant eosinophilic inflammation.

### 4.1. Study limitations

In observational studies of this nature the issue of selection bias must be addressed. Healthier subjects are more likely to participate in such studies and therefore a difference in lifestyle characteristics may exist in those who agreed to take part vs those who did not. However, in this study we aimed for our reference population to be relatively healthy (respiratory healthy and non-smokers) therefore any potential selection bias in SCAPIS Uppsala is unlikely to have affected results to any large extent. There is additionally an issue of generalisability of our findings to other age groups. Our cohort consisted of subjects in a narrow older age range, which is a limitation to the study as prevalence of allergic sensitisation and additionally its effect on $FE_{NO}$ is found to vary by age and therefore it may be difficult to generalise our results to a younger population. In this study, only one measurement of $FE_{NO}$ was taken on which the results are based. This has obvious drawbacks, and the reliability of repeated successful measurements would have been an advantage. We do however use a measurement device that is more widely used and therefore our results are likely to represent those that can be more widely replicated. This study was limited by the cross-sectional nature of the study design. Further studies should assess the clinical significance of having a high degree of allergic sensitisation along with an increased ULN of $FE_{NO}$. Additional studies should also investigate if IgE antibody assessment along with $FE_{NO}$ measurement provides additional diagnostic information in asthma.

### 4.2. Conclusion

IgE sensitisation to perennial allergens and a higher degree of IgE sensitisation (i.e. higher IgE antibody levels to aeroallergens) results in higher $FE_{NO}$ levels than in non-atopic subjects. The degree and type of IgE sensitisation should additionally be included in the generation of reference values of $FE_{NO}$. We support the use of individual reference values where type of allergic sensitisation should be accounted together with the grade of IgE sensitisation.

## CRediT authorship contribution statement

**Suneela Zaigham:** Methodology, Writing – original draft, preparation, Writing – review & editing, Visualization. **Xingwu Zhou:** Methodology, Formal analysis, Data curation, Software. **Magnus Molin:** Writing – review & editing, Resources. **Anders Sjölander:** Writing – review & editing, Resources. **Robert Movérare:** Writing – review & editing, Resources. **Christer Janson:** Writing – review & editing, Funding acquisition, Resources. **Andrei Malinovschi:** Conceptualization, Methodology, Writing – original draft, Preparation, Writing – review & editing, Supervision, Funding acquisition, Resources.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.rmed.2021.106621.

## Author Contributions

SZ, XZ, MM, AS, RM, CJ and AM participated in the study design, interpretation of the data, drafting the manuscript and approved the final version of the manuscript. XZ performed the statistical analysis. AM and SZ take responsibility for the integrity of the work as a whole, from inception to published article.

## Declaration of interests

AS, RM and MM are employed by Thermo Fisher Scientific. SZ, CJ, AM and XZ have no conflicts of interest to declare.

## Patient consent

Study participants gave their written and informed consent.

## Data Availability

Data can be made available to researchers upon request, subject to a review of secrecy and approved ethical application. More information can be found at http://scapis.org/

## Ethical Statement

The study was approved by the Regional Ethical Review Board in Umeå (2010-228-31 M), and the analyses of the SCAPIS study were approved by the Regional Ethical Review Board in Uppsala (2018–272).

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