EUROPEAN RESPIRATORY *journal*

FLAGSHIP SCIENTIFIC JOURNAL OF ERS

Early View

Task force report

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Please cite this article as: Högman M, Bowerman C, Chavez L, *et al*. ERS technical standard: Global Lung Function Initiative reference values for exhaled nitric oxide (F_ENO). *Eur Respir J* 2023; in press (https://doi.org/10.1183/13993003.00370-2023).

This manuscript has recently been accepted for publication in the *European Respiratory Journal*. It is published here in its accepted form prior to copyediting and typesetting by our production team. After these production processes are complete and the authors have approved the resulting proofs, the article will move to the latest issue of the ERJ online.

ERS technical standard: Global Lung Function Initiative reference values for exhaled nitric oxide (F_ENO)

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Abstract

Elevated levels of exhaled nitric oxide at a flow of 50 mL/s (F_ENO_{50}) are an important indicator of Th2 airway inflammation and may aid clinicians in the diagnosis and monitoring of asthma. This study aimed to derive Global Lung Function Initiative reference equations and the upper limit of normal for F_ENO_{50}.

Available individual F_ENO_{50} data were collated and harmonised using consensus-derived variables and definitions. Data collected from individuals who met the harmonised definition of "healthy" were analysed using generalised additive models of location shape and scale (GAMLSS) technique.

Data were retrospectively collated from 34,782 individuals from 34 sites in 15 countries, of whom 8,022 met the definition of healthy (19 sites, 11 countries). Overall, height, age and sex only explained 12% of the between-subject variability of F_ENO_{50} (R^2 = 0.12). The addition of NO device was a predictor of F_ENO_{50} and between-subject variability, such that the healthy range of values and the upper limit of normal varied depending on which device was used. The range of F_ENO_{50} values observed in healthy individuals was also very wide, and the heterogeneity was partially explained by the device used. The heterogeneity between sites remained within a sub-set of data where F_ENO_{50} was measured using the same device and a stricter definition of health (n=1,027).

Available F_ENO_{50} data collected from different sites using different protocols and devices was too variable to develop a single all-age reference equation. Further standardisation of NO devices and measurement is required before population reference values might be derived.

Introduction

Nitric oxide (NO) is a ubiquitous intra- and inter-cellular messenger whose synthesis may largely vary due to the complexity of the underlying biological mechanisms regulating the NO synthases (NOS) (1). Acute or chronic inflammatory diseases, including asthma, increase NO synthesis via transcription of the inducible NOS (2). Elevated concentrations of fractional exhaled nitric oxide (F_ENO) are associated with airway inflammation, especially eosinophilic Th2-driven inflammation, and may be useful in diagnosing and monitoring asthma (3, 4). Within clinical guidelines, it is recommended that exhaled nitric oxide, at a flow of 50 mL/s (F_ENO_{50}) (5), is used to detect Th2-driven inflammation, predict inhaled corticosteroid response, assess treatment compliance, select patients with severe asthma for biological treatment, and monitor people with a diagnosis of asthma (6).

Unlike other pulmonary function tests, where results are related to population norms and expressed as percent predicted or z scores, F_ENO_{50} is usually expressed as high cut-off values (6-9). Cut-offs are used since population-based studies of 'healthy' individuals consistently show that the distribution of F_ENO_{50} values is right-skewed, with significant overlap between the distribution in people with stable or controlled asthma. The cut-off values are derived in studies of children and adults with a confirmed diagnosis of asthma and anchored to clinically relevant endpoints such as sputum eosinophil count or response to inhaled corticosteroids. However, several factors influence F_ENO_{50} values, including age, height, sex, smoking, allergen exposure, rhinovirus infections and nitrate intake (6, 10-13). Therefore, using fixed cut-offs that do not consider these non-asthmatic factors may misclassify individuals.

Previous studies have developed reference equations for F_ENO_{50} in single populations and found that the normal upper limit varies with age, height, and biological sex (14). Comparing these reference equations demonstrates considerable differences between the 'upper limit of normal' defined within the published literature. Employing the same methodology which has proven successful for the standardisation of spirometry by the Global Lung Function Initiative (GLI) (15-18), we aimed to develop reference equations for F_ENO_{50} using data from many populations and validate the discriminative ability of the upper limit of normal to differentiate individuals with a confirmed or suspected diagnosis of asthma.

Methods

An application was approved for a European Respiratory Society (ERS) Task Force to develop all-age reference equations for F_ENO_{50}. The Task Force comprised scientists and healthcare professionals with expertise in developing international guidelines, lung physiology, lung function testing, and biostatistics.

A pragmatic review of the literature (see online supplement, Table S1 Medline, Table S2 EMBASE, Table S3 Web of Science, Table S4 Scopus, and Table S5 Cochrane Library) was conducted to identify published studies that included measurement of F_ENO_{50} in healthy individuals and those with confirmed or suspected asthma, COPD or PCD. The authors of studies with at least 50 participants were contacted and invited to share their data with the

Task Force. Invitations were also circulated through international and local respiratory societies to solicit unpublished data.

An online secure data portal (REDCap) (19) was used to capture individual data. In addition to providing NO data, the following mandatory variables were requested sex, age, height, weight, atopy status, and cigarette smoking status (in the last 12 months); individuals with missing values were excluded. All data were pseudo-anonymised before submission and entered into a standard data template; initial data cleaning was performed, and contributors were contacted directly to clarify discrepancies. If centres contributed more than one data point per individual, one measurement was randomly selected. Individual-level data were collected from healthy individuals to define the reference range. Data from individuals with a confirmed or suspected diagnosis of asthma, COPD or PCD were collected to investigate the discriminative ability of the upper limit of normal to differentiate between health and disease. Meta-data describing the study population, NO device, and methodology were also collected. A series of questions (see online supplement, Tables S6 and S7) were asked to verify that submitted data met all acceptability and repeatability criteria outlined in the 2005 American Thoracic Society (ATS)/ERS recommendations (20). Data collected from sites where we could not confirm expiratory flow rates were excluded. A summary of the included sites is presented in the supplement, Table S6.

Healthy individuals were defined as non-smokers within the last year, with no history of self-reported or physician-diagnosed atopy (including eczema, rhinitis, or positive skin prick test/total IgE >110 kU/L) or respiratory disease (e.g. asthma, COPD). Obese individuals were also excluded. In all but one included study, atopy was confirmed using positive skin prick test/IgE levels; this study was excluded from a 'strictly healthy' definition which also excluded overweight and obese individuals and those who had ever smoked. We assumed all under twelve-year-olds were never smokers and were not diagnosed with COPD or PCD. Rhinitis, eczema, sinusitis, chronic bronchitis, and nasal polyps were not mandatory variables; nonetheless, 'healthy' participants with confirmation of any of these were excluded. We assumed that these individuals were healthy if these variables were not reported. Individuals younger than 4 years of age and older than 80 years of age were also excluded. A sensitivity analysis was conducted using the 'strictly healthy' definition. In contrast with the 'healthy' definition, a strictly healthy definition for which individuals fulfilled all criteria for healthy plus the additional criteria that no assumptions were made for any of the mandatory variables, meaning that subjects with unknown smoking status or subjects with an unknown history of ever smoking, or subjects with an unknown history of asthma, COPD, or atopy were not considered as 'strictly healthy´.

Statistical Analysis

The reported F_ENO_{50} values were visualised by plotting sex against height, age, or body mass index (BMI); suspected outliers were confirmed with study sites or against established international cut-offs (e.g., obese individuals were excluded from the healthy population if they had a BMI >30 kg/m^2 in adults, or if BMI centile for age was ≥85th for children) (21). In addition, children with height-for-age or weight-for-age z-scores <-5 or >5 were also

considered outliers and removed (Figure 1). F_ENO_{50} values less than 2 ppb were excluded as not biologically plausible across the 4–80-year age range. Differences between sites and NO devices were first explored using the observed F_ENO_{50} values.

The generalised additive models of location shape and scale (GAMLSS) technique (22), previously used for other GLI Task Forces, was used to define the reference range of F_ENO_{50} values. Briefly, the GAMLSS technique allows the median value to be summarised ($m\mu$) as a function of multiple explanatory variables (e.g., height, age, sex), the spread of values around the median value to be constant or vary by a function of an explanatory variable, and any departure from a normal distribution (skewness) to be transformed to normal using a Box-Cox transformation. Thus, the resulting model residuals will be normally distributed. Previous GLI reference equations have relied on the BCCG (or Box-Cox Cole and Green family); however, the distribution of the F_ENO_{50} data has a heavy right skew even after the log transformation of F_ENO_{50} values, requiring a more complex model. For F_ENO_{50} values, we used the Box-Cox-*t* (BCT) distribution to allow a fourth parameter (tau) for extreme values. The goodness of fit was assessed by Schwartz Bayesian criteria (SBC), Q-Q plots, and worm plots. Analysis was done using the GAMLSS package in the statistical programme R (R version 4.2.1)

The following explanatory variables were evaluated one at a time and then together (i.e., sex, age, height, weight, and BMI) for each of the four model parameters ($m\mu$, sigma, lambda, tau). The variables significantly associated with F_ENO_{50} were kept in the final model. We did not investigate race and ethnicity as a predictor of F_ENO_{50}, as race and ethnicity are social constructs without a consistent definition globally, and recent statements endorsed by both the ATS and ERS have recommended against its continued use in reference equations (17). We also investigated whether there were differences in the median or upper limit of normal based on the analysing method (chemiluminescence or electrochemical cell). To meet our *a priori* criteria to combine data from multiple sites, the difference between sites (or devices) and the average of all sites combined had to be less than 10 ppb. Similarly, the upper limit of normal from the combined data and each site (or device) had to be less than 10 ppb.

Results

A total of F_ENO_{50} measurements from 34,782 individuals were provided by 34 sites in 15 countries (Figure 1). After exclusions, 8,022 healthy participants (49% female) across 19 sites and 11 countries were used to define the reference range (Table 1). Overall, data were collated across the 4–80-year age range, with relatively fewer observations for individuals between ages 25 and 30 and between 65 and 80 years (Figure 2 A). The distribution of F_ENO_{50} values was right-skewed (Figure 2 B). Of healthy subjects, 3.9% had values above 50 ppb (Table 1), with 3.7% of adults and 7.2% of children (i.e., <18 years) having values greater than 35 ppb. The median F_ENO_{50} varied between sites within the subset of 'healthy' data (Figure 3); in many cases, the average difference in F_ENO_{50} between sites was more than 10 ppb units. We further investigated whether the site differences persisted after accounting for the differences in sex, height, and age between the sites.

Although height, sex, and age were statistically significant predictors of average F_ENO_{50}, the rate of change in F_ENO_{50} with height and age was small (the median F_ENO_{50} increases 0.07 ppb with each year increase in age when holding height and sex constant, Figure 4). In addition to being predictors of average F_ENO_{50}, height and sex were statistically significant predictors of the between-subject variability of F_ENO_{50} (i.e., the spread of values around the median predicted value varied by height and sex). Overall, height, age and sex only explained 12% of the between-subject variability (R^2 = 0.120). The addition of NO device was a predictor of the median F_ENO_{50} and between-subject variability, such that the healthy range of values and the upper limit of normal varied depending on which device was used (Figure 5). Adding a device into the model explained an additional 4% of the variability (R^2=0.164). Including the site in the model instead of device explained an additional 7% of the variability (R^2=0.191). For some devices, the between-subject variability was small (e.g., the coefficient of variation (CV) for F_ENO_{50} in Sievers 280 is 0.51), and there were no observations with F_ENO_{50} values outside the upper limit of normal (e.g., NIOX VERO, NIOX FLEX). Whereas for other devices, the between-subject variability was twice as big (e.g., the coefficient of variation (CV) for F_ENO_{50} in Medisoft is 1.05), such that a larger proportion of healthy individuals would fall outside the upper limit of normal (Figure 5). Consequently, it was not possible to define a single reference equation for F_ENO_{50} that can be used across all devices.

We further explored differences between sites in a subset of data (n= 4,254 from 7 sites) that used the same device (NIOX MINO®). Within this subset, we observed heterogeneity between the sites in terms of the F_ENO_{50} and the between-subject variability (Figure 6), even after adjusting for differences in height, sex, and age between participants in each site.

We further analysed a subset of data meeting our strictly healthy definition (n=1,027), such that individuals were included if no assumptions were made about the inclusion criteria. This excluded one of the largest datasets where atopy status was self-reported and not confirmed with skin prick test or IgE levels. The spread of residuals was still wide (Figure 7).

Discussion

Measured values of F_ENO_{50} in healthy individuals from different devices across 19 sites vary between individuals. The variability between sites and devices precludes the meaningful collation of data from defining a reference range. Even when limiting the analysis to sites that used the same device, heterogeneity in the observed data remained such that it was not appropriate to develop a reference range. Standardisation of F_ENO_{50} measurements made using different devices and at different sites is required before robust population reference values might be derived.

This study applied an established methodology, as recommended in a systematic review, and supported by ERS, to determine population reference values for F_ENO_{50}, and data from 8,022 healthy individuals were obtained from nations around the world across the age range of 4-80 years. We believe this work has collected F_ENO_{50} measurements from the largest number of individuals using numerous devices from sites around the world. Therefore, these findings have important implications for ongoing and future research. The heterogeneity of

F_ENO_{50} measurements between devices and centres is large, and the use of existing reference equations or cut-offs derived from a single study or single device (14, 23, 24) should be applied cautiously in other populations and with other devices.

In the collated dataset, we observed that the distribution of F_ENO_{50} in healthy individuals is skewed to the right. Although it was methodologically possible to apply the GAMLSS technique to derive reference equations for this type of data, the heterogeneity of F_ENO_{50} data between centres and device types meant it was not methodologically useful to develop a single reference range and upper limit of normal. Forcing a single reference equation would result in some centres under-identifying elevated F_ENO_{50} in individuals, while other centres over-identified elevated F_ENO_{50} and would not improve existing site-specific equations. Even within the strictly healthy definition (n=1,027), the differences between centres and devices persisted, suggesting that factors other than an individual's health status contribute to differences in F_ENO_{50} values between sites. These findings suggest that unmeasured factors such as measurement protocols, population characteristics, or even individual-level factors influence the NO measurement. It is also possible that the smaller sample size used in the 'strictly healthy definition' also introduced sampling variability.

Although it is possible to address differences between devices using device-specific reference equations, substantial heterogeneity remains between centres measuring F_ENO_{50} using the same device, meaning that adjustment for the device would not provide sufficiently accurate normative data. Further, some devices are no longer commercially available, and in many cases, the number of observations was too small to derive specific equations for all devices.

Establishing reference equations for F_ENO_{50} may help clinicians to diagnose and manage chronic respiratory conditions. Unlike other pulmonary function parameters with lower and upper limits of normal (25), low levels of F_ENO_{50} do not necessarily imply underlying respiratory disorders, as background synthesis of NO is required for optimal bronchial and pulmonary vascular tone (26, 27). Elevated F_ENO_{50} is associated with conditions such as asthma and COPD but also atopy without respiratory symptoms. As a result, determining the upper limits of normal for F_ENO_{50} and other exhaled NO parameters has always been challenging (4, 24, 28), especially for respiratory specialists interested in chronic inflammatory airway diseases (29-31). The fact that biological pathways resulting in NO synthesis cross-link with those of many key molecules of Th2 inflammation in asthma (2) has made F_ENO_{50}, together with eosinophils, two major biomarkers in asthma and other Th2-related inflammatory diseases (32-34). Interestingly many international guidelines, including the one published by the ATS in 2011 (4) and the recent ERS guidelines for the diagnosis of asthma in adults (35), have set 50 ppb as the optimal cut-off supportive of a diagnosis of asthma. Results from the present study show that less than 4% of healthy subjects worldwide have F_ENO_{50} higher than 50 ppb (Table 1), and 7% of children higher than 35 ppb. Results from Figure 2 C are in line with current major international asthma guidelines.

It is well established that F_ENO_{50} measurements are not interchangeable between different devices (36). Further, differences in measurement protocol (e.g., single exhalation vs three exhalations and lack of flow registrations) may contribute to the observed differences in the

GLI dataset. Until these differences are mitigated through standardised NO devices and measurement protocols, it is unlikely that a reference equation can be derived for clinical applications applicable across different centres, whether they use the same device or not.

Limitations

The analysis reported here is limited to datasets shared with the GLI Task Force and may not be fully representative of all populations and all devices. Although a literature search was conducted and all corresponding authors were contacted, some centres declined, were unable to gain appropriate approvals to share data or had not collected the mandatory variables. Further, during the conduct of this Task Force, stricter General Data Protection Regulation (GDPR) rules were established, which further limited the sharing of data from some regions of the world. We do not believe that the differences between devices and sites would have been reduced by including data from more sites.

We could not verify the specific methodology for F_ENO_{50} measurement used by each site, only what was reported in the meta-data (see supplement). Therefore, we cannot be sure how much inter-site differences between F_ENO values are attributable to methodological differences. A further limitation is that one dataset (NHANES) contributed the largest proportion of data (approximately 1/3 of the dataset) and only included self-reported atopy. Therefore, our findings may be influenced by a single study.

Conclusions

Due to heterogeneity in F_ENO_{50} values between sites and NO devices, it was not possible to develop a single all-age reference equation for F_ENO_{50} by collating data collected in healthy individuals. Further standardisation of F_ENO_{50} measurement and NO devices is required before population reference values can be derived.

Table 1 Summary of data included in final model by site.

Country	n	Age range, yrs	Device	% Female	% Over-weight*	Median F_ENO_{50}, ppb	IQR F_ENO_{50} ppb	F_ENO_{50} % above 50 ppb
Kazakhstan	350	20 - 47	NObreath~	16	42	19.0	12.0 – 28.0	3.1
Netherlands	311	8 - 10	NIOX§	51	12	9.5	6.8 – 13.1	0.6
Netherlands	123	19 - 61	NIOX MINO~	15	51	17.0	12.0 - 23.0	4.1
Netherlands	637	13 - 14	NIOX MINO~	55	9	13.0	10.0 - 19.0	4.9
Netherlands	86	4 - 5	Other#,§	41	16	8.7	6.4 - 11.3	0.0
New Zealand	86	28 - 76	NIOX§	56	52	17.5	15.0 - 25.5	3.5
Paraguay	95	20 - 79	NObreath~	51	54	15.0	11.0 - 19.0	0.0
Portugal	359	7 - 11	NObreath~	48	19	10.0	6.0 - 16.0	2.8
South Africa	455	15 - 72	NIOX MINO~	37	39	15.0	10.0 - 21.0	2.9
South Korea	136	4 - 7	NIOX MINO~	63	10	8.0	7.0 - 11.0	0.0
South Korea	61	26 - 77	NIOX VERO~	89	31	14.0	10.0 - 18.0	1.6
Sweden	1197	25 - 76	NIOX§	52	47	16.3	12.1 - 22.3	1.7
Sweden	69	11 - 31	NIOX FLEX§	55	20	10.8	8.2 - 13.9	4.3
Sweden	115	30 - 54	Sievers 280§	43	37	18.0	12.9 - 25.9	2.6
UK	265	11 - 13	NIOX§	55	14	8.4	6.6 - 11.3	3.4
UK	357	14 - 20	NIOX MINO~	50	15	14.0	10.0 - 20.0	3.6
UK	212	14	NIOX MINO~	50	18	13.0	10.0 - 18.0	2.4
USA	2334	12 - 80	NIOX MINO~	52	46	14.0	9.5 - 21.0	4.2
Vietnam	774	4 - 79	Medisoft~	51	17	13.3	7.8 - 27.0	10.9
11 countries	8022	4 - 80	8 devices	49	33	14	9.2 - 21.0	3.9

IQR= Interquartile Range; UK = United Kingdom; USA = United States of America.

*% overweight limited to those who were not already excluded for being above the WHO criteria for obesity.

One site collected F_ENO_{50} on two different devices (Sievers 280 and Eco Physics CLD 700) but did not specify which observations were made on which device.

§ Chemiluminescence sensor

~Electrochemical sensor

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Figure captions

Figure 1 Flow Diagram of Exclusions.

Figure 2 A) Histogram showing the number of healthy individuals where F_ENO_{50} concentrations were analysed, stratified by age and sex. B) Histogram showing the distribution of F_ENO_{50} values in healthy individuals, with stratification by sex. C) Scatter plot comparing histogram of F_ENO_{50} by age and sex.

Figure 3 Box and whisker plot (median and interquartile range contained within the box) showing F_ENO_{50} values by the different sites situated in 11 countries.

Figure 4 Distribution of F_ENO_{50} by age, lines represent median, 5^{th} and 95^{th} centile in A) Females, B) Males.

Figure 5 Box and whisker plot (median and interquartile range contained within the box) of the residual values (z-scores) from the best fitting F_ENO_{50} model without NO devices included as a covariate showing considerable range within and between devices. In a well-fitting model, median residuals should approximate to zero, and all values should be within the range of ±2 z-scores. 'Other' includes two types of chemiluminescence devices (Sievers 280 and Eco Physics CLD 700) used at the same site but without verifying which measurements were made on.

Figure 6 Comparison of residual values across sites using the same F_ENO_{50} device (NIOX MINO). In a well-fitting model, residuals should centre around 0 and be within the range of ±2 z-scores. In this subset, there was considerable heterogeneity in both the site-specific median and the range of residuals between the sites.

Figure 7 Model residuals (Z-scores) by NO device in a strictly healthy subset of data. In a well-fitting model, residuals should centre around 0 and be within the range of ±2 z-scores.

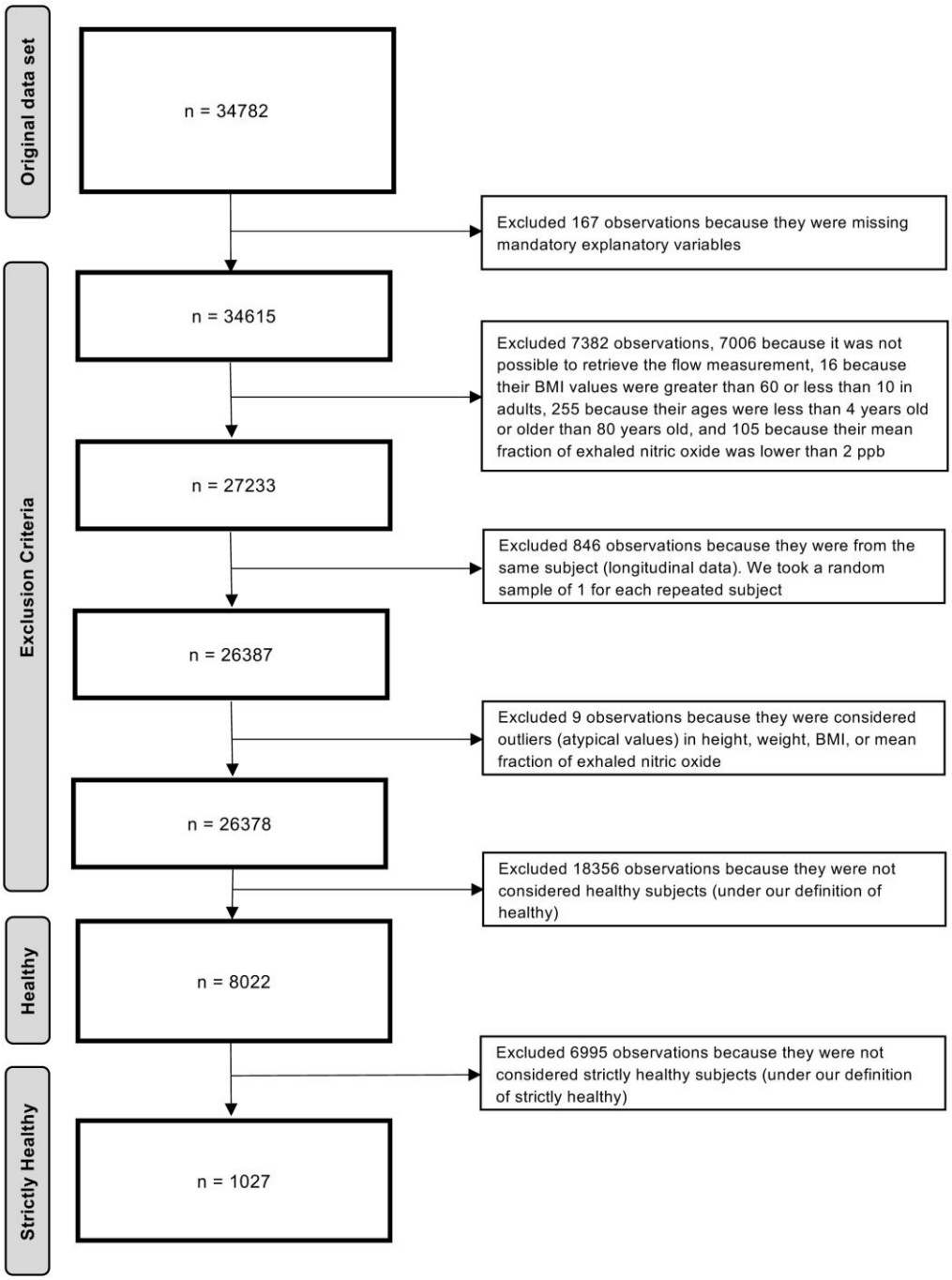
Original data set
n = 34782
Excluded 167 observations because they were missing mandatory explanatory variables
Exclusion Criteria
n = 34615
Excluded 7382 observations, 7006 because it was not possible to retrieve the flow measurement, 16 because their BMI values were greater than 60 or less than 10 in adults, 255 because their ages were less than 4 years old or older than 80 years old, and 105 because their mean fraction of exhaled nitric oxide was lower than 2 ppb
n = 27233
Excluded 846 observations because they were from the same subject (longitudinal data). We took a random sample of 1 for each repeated subject
n = 26387
Excluded 9 observations because they were considered outliers (atypical values) in height, weight, BMI, or mean fraction of exhaled nitric oxide
n = 26378
Excluded 18356 observations because they were not considered healthy subjects (under our definition of healthy)
Healthy
n = 8022
Excluded 6995 observations because they were not considered strictly healthy subjects (under our definition of strictly healthy)
Strictly Healthy
n = 1027

Figure 1

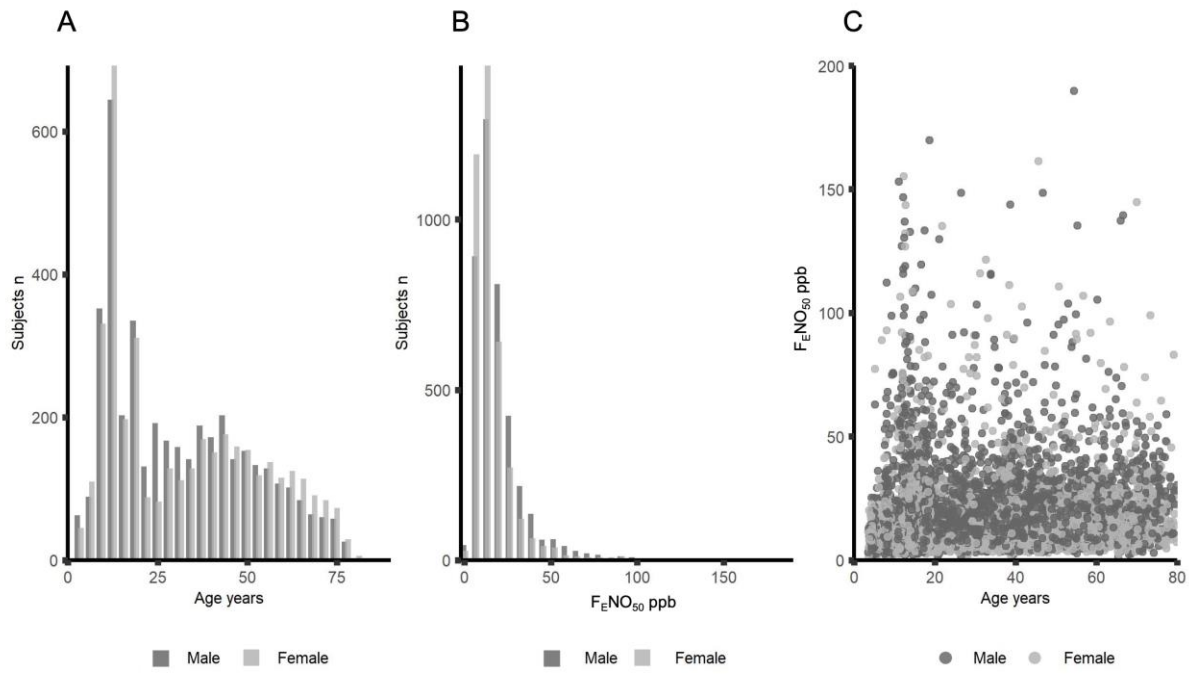

Figure 2

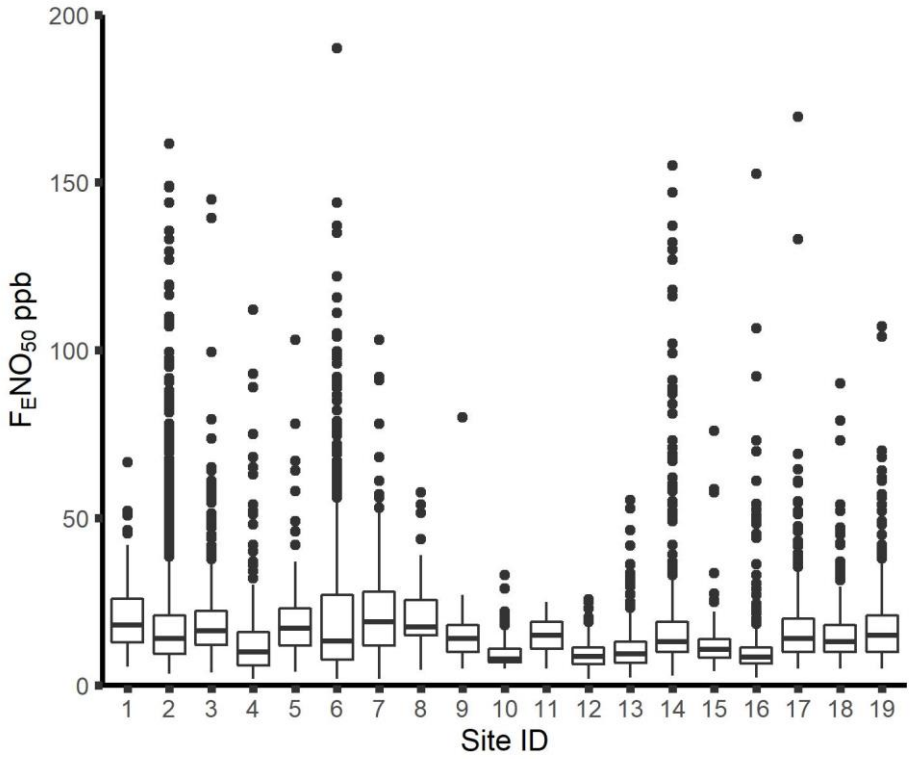
200
150
100
50
0
F_ENO_{50} ppb
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
Site ID

Figure 3

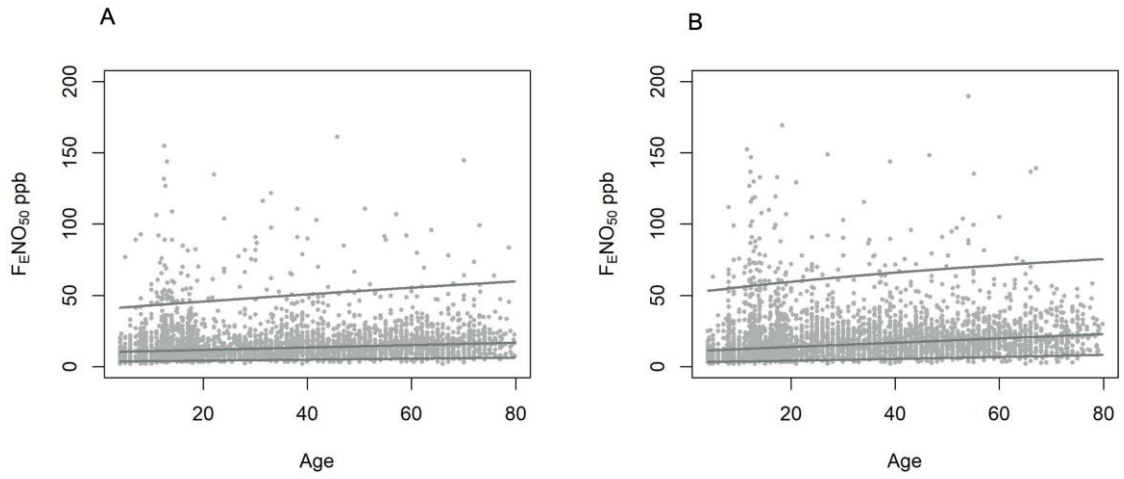

Figure 4

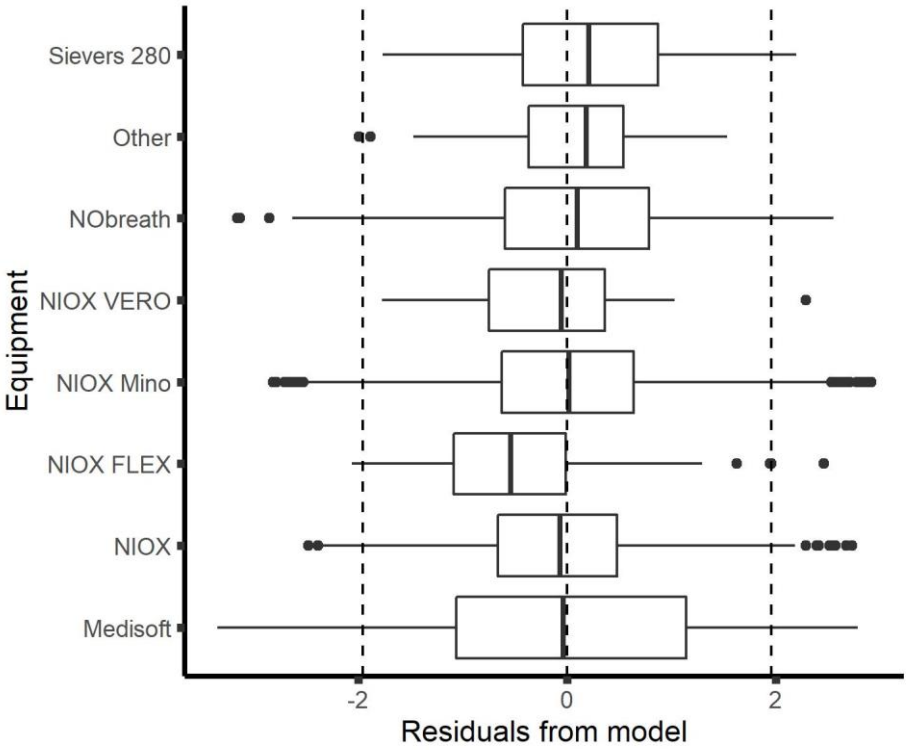
Sievers 280
Other
NObreath
NIOX VERO
NIOX Mino
NIOX FLEX
NIOX
Medisoft
Equipment
-2
0
2
Residuals from model

Figure 5

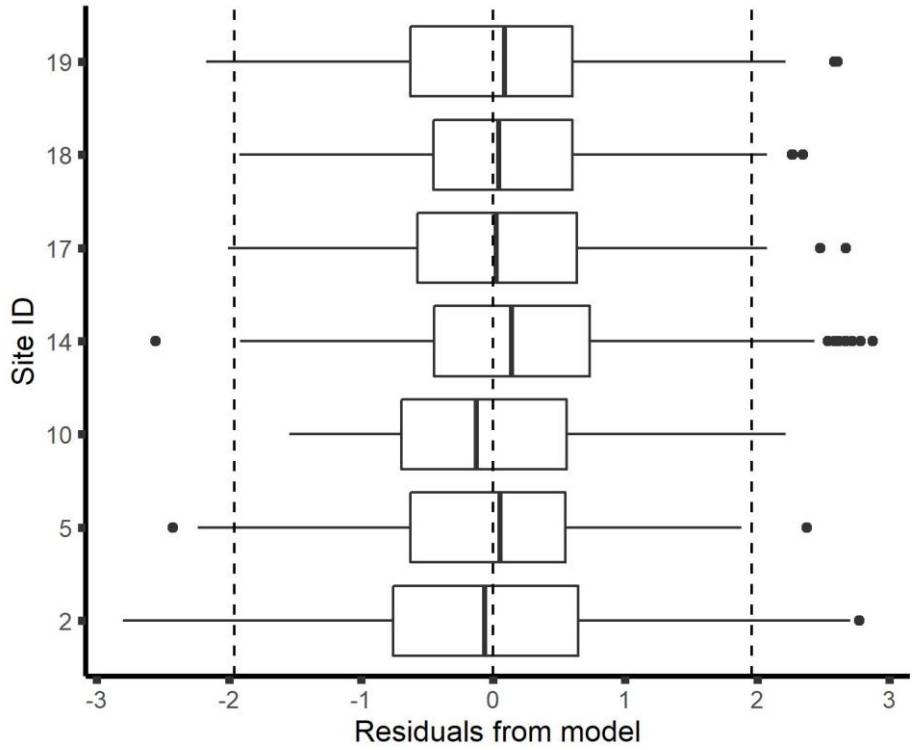

Figure 6

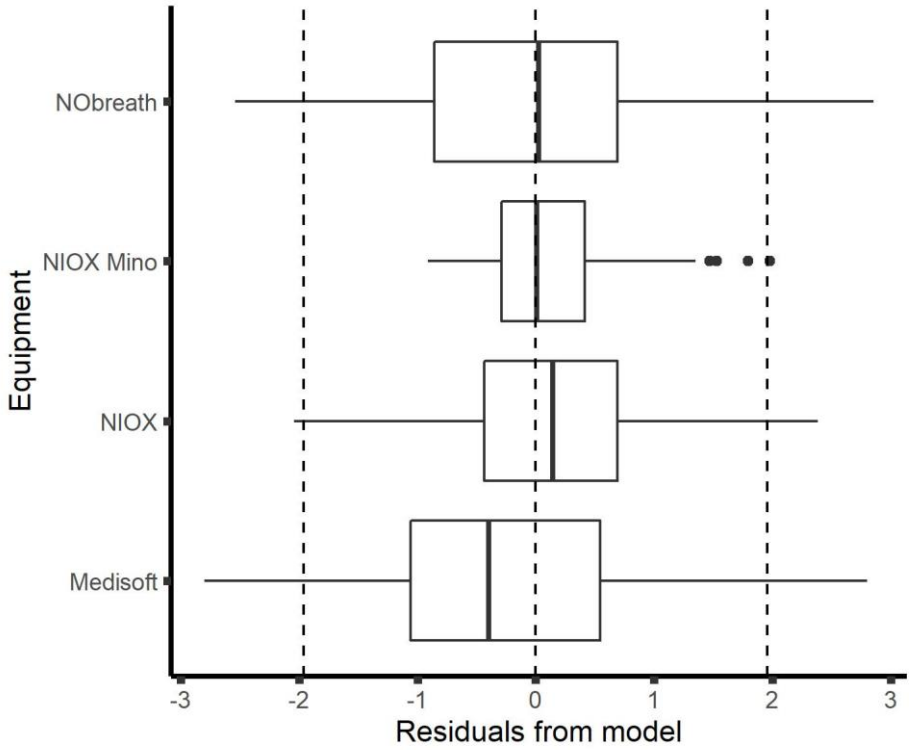
NObreath
NIOX Mino
NIOX
Medisoft
Equipment
-3
-2
-1
0
1
2
3
Residuals from model

Figure 7

ONLINE SUPPLEMENTARY MATERIAL

Systematic literature search

The systematic literature search was done by an information specialist from the University of Zurich, Switzerland. The search strategy was developed and validated using 20 published research articles on exhaled nitric oxide (F_ENO_{50}) and nasal nitric oxide in health and respiratory disease (i.e., chronic obstructive pulmonary disease, asthma, primary ciliary dyskinesia, chronic cough). We excluded animal studies, review articles, conference proceedings, editorials, and book chapters and restricted the search to publications from 2005 onwards, when the latest American Thoracic Society/European Respiratory Society Statement on the measurement of F_ENO and nasal nitric oxide was published.[1] The detailed literature search strategy of the different databases (I.e., Medline, EMBASE, Web of Science, Scopus, Cochrane Library) is given in Tables S1-S5.

Table S1 Search strategy – Medline.

Date of search: March 28th, 2019

#1	((exhal* adj3 ("nitric oxide" or "NO")).ti,ab. or ((FENO or "FE(NO)").ti,ab. and ('respiratory tract disease'/exp or (air or airway* or breath* or respir*).ti,ab.))) not (animals not humans).sh.
#2	limit 1 to yr="2005 -Current"
#3	limit 2 to (comment or editorial or festschrift or letter or published erratum or "review")
#4	2 not 3
#5	exp Reference Values/ or healthy.ti,ab. or normal.ti,ab. or ((control or reference) adj3 (value* or range*)).ti,ab.
#6	exp Asthma/ or ((asthma or lung) adj1 allerg*).ti,ab.
#7	exp Pulmonary Disease, Chronic Obstructive/ or (chronic adj3 obstruct* adj3 (airway or bronch* or lung or pulmonary or respiratory)).ti,ab. or (COAD or copd).ti,ab.
#8	exp Ciliary Motility Disorders/ or (ciliary adj3 (immotility or disorder* or dyskinesia)).ti,ab. Or (cilia adj3 (immot* or immob*)).ti,ab. or ciliosta*.ti,ab. Or ciliopath*.ti,ab.
#9	((chronic or persist*) adj3 cough*).ti,ab.
#10	Or/5-9
#11	4 and 10
#12	(((nasal or nose) adj3 ("nitric oxide" or "NO")) or (nno and (nasal or nose or ciliary))).ti,ab. not (animals not humans).sh.
#13	limit 12 to yr="2005 -Current"
#14	limit 13 to (comment or editorial or festschrift or letter or published erratum or "review")
#15	13 not 14
#16	5 or 8
#17	15 and 16

Table S2 Search strategy – EMBASE.

Date of search: March 29th, 2019

#1	('fractional exhaled nitric oxide'/exp OR ((exhal* NEAR/3 ('nitric oxide' OR 'no')):ti,ab) OR ((feno:ti,ab OR 'fe(no)':ti,ab) AND ('respiratory tract disease'/exp OR air:ti,ab OR airway*:ti,ab OR breath*:ti,ab OR respir*:ti,ab))) NOT [conference abstract]/lim AND [2005-2019]/py NOT ([animals]/lim NOT [humans]/lim) NOT ('chapter'/it OR 'conference paper'/it OR 'conference review'/it OR 'editorial'/it OR 'erratum'/it OR 'letter'/it OR 'note'/it OR 'review'/it OR 'short survey'/it)
#2	'normal human'/exp OR 'reference value'/de OR 'normal value'/exp OR healthy:ti,ab OR normal:ti,ab OR (((control OR reference) NEAR/3 (value* OR range*)):ti,ab)
#3	'asthma'/exp OR asthma:ti,ab OR ((lung NEAR/1 allerg*):ti,ab)
#4	'chronic obstructive lung disease'/exp OR ((chronic NEAR/3 obstruct* NEAR/3 (airway OR bronch* OR lung OR pulmonary OR respiratory)):ti,ab) OR coad:ti,ab OR copd:ti,ab
#5	'ciliary dyskinesia'/exp OR ((ciliary NEAR/3 (immotility OR disorder* OR dyskinesia)):ti,ab) OR ((cilia NEAR/3 (immot* OR immob*)):ti,ab) OR ciliosta*:ti,ab OR ciliopath*:ti,ab
#6	'chronic cough'/exp OR (((chronic OR persist*) NEAR/3 cough*):ti,ab)
#7	#2 OR #3 OR #4 OR #5 OR #6
#8	#1 AND #7
#9	l358468454 OR l368054883 OR l52901374 OR l623611973 OR l626288800
#10	#8 AND #9
#11	((((nasal OR nose) NEAR/3 ('nitric oxide' OR 'no')):ti,ab) OR (nno:ti,ab AND (nasal:ti,ab OR nose:ti,ab OR ciliary:ti,ab))) NOT [conference abstract]/lim AND [2005-2019]/py NOT ([animals]/lim NOT [humans]/lim) NOT ('chapter'/it OR 'conference paper'/it OR 'conference review'/it OR 'editorial'/it OR 'erratum'/it OR 'letter'/it OR 'note'/it OR 'review'/it OR 'short survey'/it)
#12	#2 OR #5
#13	#11 AND #12
#14	l622453439 OR l626304845 OR l613500249 OR l372107713 OR l359980552
#15	#13 AND #14

Table S3 Search strategy – Web of Science.

Date of search: March 29th, 2019

#1	TS=(exhal* NEAR/3 ("nitric oxide" OR "NO")) OR TS=((FENO OR "FE(NO)") AND (air OR airway* OR breath* OR respir*))
#2	TS=(healthy OR normal) OR TS=((control OR reference) NEAR/3 (value* OR range*))

#3	TS=(asthma) OR TS=(lung NEAR/1 allerg*)
#4	TS=(chronic NEAR/3 obstruct* NEAR/3 (airway OR bronch* OR lung OR pulmonary OR respiratory)) OR TS=(COAD or copd)
#5	TS=(ciliary NEAR/3 (immotility OR disorder* OR dyskinesia)) OR TS=(cilia NEAR/3 (immot* OR immob*)) OR TS=(ciliosta* OR ciliopath*)
#6	TS=((chronic OR persist*) NEAR/3 cough*)
#7	#6 OR #5 OR #4 OR #3 OR #2
#8	#7 AND #1
#9	#7 AND #1 Refined by: [excluding] DOCUMENT TYPES: (EDITORIAL MATERIAL OR PROCEEDINGS PAPER OR CORRECTION OR REVIEW OR LETTER OR BOOK CHAPTER OR MEETING ABSTRACT)
#10	TS=((nasal OR nose) NEAR/3 ("nitric oxide" OR "NO")) OR TS=(nno AND (nasal OR nose OR ciliary))
#11	#5 OR #2
#12	#11 AND #10
#13	#11 AND #10 Refined by: [excluding] DOCUMENT TYPES: (MEETING ABSTRACT OR BOOK CHAPTER OR EDITORIAL MATERIAL OR REVIEW OR PROCEEDINGS PAPER OR LETTER)

Table S4 Search strategy – Scopus.

Date of search: March 29th, 2019

#1	TITLE-ABS-KEY (exhal* W/3 ("nitric oxide" OR "NO")) OR TITLE-ABS-KEY ((feno OR "FE(NO)") AND (air OR airway* OR breath* OR respir*))
#2	TITLE-ABS-KEY (healthy OR normal) OR TITLE-ABS-KEY ((control OR reference) W/3 (value* OR range*))
#3	TITLE-ABS-KEY (asthma) OR TITLE-ABS-KEY (lung W/1 allerg*)
#4	TITLE-ABS-KEY (chronic W/3 obstruct* W/3 (airway OR bronch* OR lung OR pulmonary OR respiratory)) OR TITLE-ABS-KEY (coad OR copd)
#5	TITLE-ABS-KEY (ciliary W/3 (immotility OR disorder* OR dyskinesia)) OR TITLEABS- KEY (cilia W/3 (immot* OR immob*)) OR TITLE-ABS-KEY (ciliosta* OR ciliopath*)
#6	TITLE-ABS-KEY ((chronic OR persist*) W/3 cough*)
#7	(TITLE-ABS-KEY (healthy OR normal) OR TITLE-ABS-KEY ((control OR reference) W/3 (value* OR range*))) OR (TITLE-ABS-KEY (asthma) OR TITLE-ABSKEY (lung W/1 allerg*)) OR (TITLE-ABS-KEY (chronic W/3 obstruct* W/3 (airway OR bronch* OR lung OR pulmonary OR respiratory)) OR TITLE-ABS-KEY (coad OR copd)) OR (TITLE-ABS-KEY (ciliary W/3 (immotility OR disorder* OR dyskinesia)) OR TITLE ABS-KEY (cilia W/3 (immot* OR immob*)) OR TITLE-ABSKEY (ciliosta* OR ciliopath*)) OR (TITLE-ABS KEY ((chronic OR persist*) W/3 cough*))
#8	(TITLE-ABS-KEY (exhal* W/3 ("nitric oxide" OR "NO")) OR TITLE-ABS-KEY ((feno OR "FE(NO)") AND (air OR airway* OR breath* OR respir*))) AND ((TITLEABS- KEY (healthy OR normal) OR TITLE-ABS-KEY ((control OR reference) W/3 (value* OR range*))) OR (TITLE-ABS-KEY (asthma) OR TITLE-ABS-KEY (lung W/1 allerg*)) OR (TITLE-ABS-KEY (chronic W/3 obstruct* W/3 (airway OR bronch* OR lung OR pulmonary OR respiratory)) OR TITLE-ABS-KEY (coad OR copd)) OR (TITLE-ABS-KEY (ciliary W/3 (immotility OR disorder* OR dyskinesia))

	OR TITLE-ABS-KEY (cilia W/3 (immot* OR immob*)) OR TITLE-ABSKEY (ciliosta* OR ciliopath*)) OR (TITLE-ABS-KEY ((chronic OR persist*) W/3 cough*)))
#9	KEY (ciliosta* OR ciliopath*)) OR (TITLE-ABS-KEY ((chronic OR persist*) W/3 cough*))) AND (LIMIT-TO (PUBYEAR , 2019) OR LIMIT-TO (PUBYEAR , 2018) OR LIMIT-TO (PUBYEAR , 2017) OR LIMIT-TO (PUBYEAR , 2016) OR LIMITTO (PUBYEAR , 2015) OR LIMIT-TO (PUBYEAR , 2014) OR LIMIT-TO (PUBYEAR ,2013) OR LIMIT-TO (PUBYEAR , 2012) OR LIMIT-TO (PUBYEAR , 2011) OR LIMITTO (PUBYEAR , 2010) OR LIMIT-TO (PUBYEAR , 2009) OR LIMIT-TO (PUBYEAR , 2008) OR LIMIT-TO (PUBYEAR , 2007) OR LIMIT-TO (PUBYEAR , 2006) OR LIMITTO (PUBYEAR , 2005))
#10	(TITLE-ABS-KEY (exhal* W/3 ("nitric oxide" OR "NO")) OR TITLE-ABS-KEY ((feno OR "FE(NO)") AND (air OR airway* OR breath* OR respir*))) AND ((TITLEABS- KEY (healthy OR normal) OR TITLE-ABS-KEY ((control OR reference) W/3 (value* OR range*))) OR (TITLE-ABS-KEY (asthma) OR TITLE-ABS-KEY (lung W/1 allerg*)) OR (TITLE-ABS-KEY (chronic W/3 obstruct* W/3 (airway OR bronch* OR lung OR pulmonary OR respiratory)) OR TITLE-ABS-KEY (coad OR copd)) OR (TITLE-ABS-KEY (ciliary W/3 (immotility OR disorder* OR dyskinesia)) OR TITLE-ABS-KEY (cilia W/3 (immot* OR immob*)) OR TITLE-ABSKEY (ciliosta* OR ciliopath*)) OR (TITLE-ABS-KEY ((chronic OR persist*) W/3 cough*))) AND (LIMIT-TO (PUBYEAR , 2019) OR LIMIT-TO (PUBYEAR , 2018) OR LIMIT-TO (PUBYEAR , 2017) OR LIMIT-TO (PUBYEAR , 2016) OR LIMITTO (PUBYEAR , 2015) OR LIMIT-TO (PUBYEAR , 2014) OR LIMIT-TO (PUBYEAR , 2013) OR LIMIT-TO (PUBYEAR , 2012) OR LIMIT-TO (PUBYEAR , 2011) OR LIMITTO (PUBYEAR , 2010) OR LIMIT-TO (PUBYEAR , 2009) OR LIMIT-TO (PUBYEAR , 2008) OR LIMIT-TO (PUBYEAR , 2007) OR LIMIT-TO (PUBYEAR , 2006) OR LIMITTO (PUBYEAR , 2005)) AND (EXCLUDE (DOCTYPE , "re") OR EXCLUDE (DOCTYPE , "le") OR EXCLUDE (DOCTYPE , "cp") OR EXCLUDE (DOCTYPE , "ed") OR EXCLUDE (DOCTYPE , "no") OR EXCLUDE (DOCTYPE , "ch") OR EXCLUDE (DOCTYPE , "sh") OR EXCLUDE (DOCTYPE , "er"))
#11	TITLE-ABS-KEY ((nasal OR nose) W/3 ("nitric oxide" OR "NO")) OR TITLE-ABSKEY (nno AND (nasal OR nose OR ciliary))
#12	(TITLE-ABS-KEY ((nasal OR nose) W/3 ("nitric oxide" OR "NO")) OR TITLE-ABSKEY (nno AND (nasal OR nose OR ciliary))) AND ((TITLE-ABS-KEY (healthy OR normal) OR TITLE-ABS-KEY ((control OR reference) W/3 (value* OR range*))) OR (TITLE-ABS-KEY (ciliary W/3 (immotility OR disorder* OR dyskinesia)) OR TITLE-ABS-KEY (cilia W/3 (immot* OR immob*)) OR TITLE-ABS- (TITLE-ABS-KEY (healthy OR normal) OR TITLE-ABS-KEY ((control OR reference) W/3 (value* OR range*))) OR (TITLE-ABS-KEY (ciliary W/3 (immotility OR disorder* OR dyskinesia)) OR TITLE-ABS-KEY (cilia W/3 (immot* OR immob*)) OR TITLE-ABS-KEY (ciliosta* OR ciliopath*))
#13	(TITLE-ABS-KEY ((nasal OR nose) W/3 ("nitric oxide" OR "NO")) OR TITLE-ABSKEY (nno AND (nasal OR nose OR ciliary))) AND ((TITLE-ABS-KEY (healthy OR normal) OR TITLE-ABS-KEY ((control OR reference) W/3 (value* OR range*))) OR (TITLE-ABS-KEY (ciliary W/3 (immotility OR disorder* OR dyskinesia)) OR TITLE-ABS-KEY (cilia W/3 (immot* OR immob*)) OR TITLE-ABSKEY (ciliosta* OR ciliopath*))) AND (LIMIT-TO (PUBYEAR , 2019) OR LIMITTO (PUBYEAR , 2018) OR LIMIT-TO (PUBYEAR , 2017) OR LIMIT-TO (PUBYEAR , 2016) OR LIMIT-TO (PUBYEAR , 2015) OR LIMIT-TO (PUBYEAR , 2014) OR LIMITTO (PUBYEAR , 2013) OR LIMIT-TO (PUBYEAR , 2012) OR LIMIT-TO (PUBYEAR , 2011) OR LIMIT-TO (PUBYEAR , 2010) OR LIMIT-TO (PUBYEAR , 2009) OR LIMITTO (PUBYEAR , 2008) OR LIMIT-TO (PUBYEAR , 2007) OR LIMIT-TO (PUBYEAR , 2006) OR LIMIT-TO (PUBYEAR , 2005))

#14	(TITLE-ABS-KEY ((nasal OR nose) W/3 ("nitric oxide" OR "NO")) OR TITLE-ABSKEY (nno AND (nasal OR nose OR ciliary))) AND ((TITLE-ABS-KEY (healthy OR normal) OR TITLE-ABS-KEY ((control OR reference) W/3 (value* OR range*))) OR (TITLE-ABS-KEY (ciliary W/3 (immotility OR disorder* OR dyskinesia)) OR TITLE-ABS-KEY (cilia W/3 (immot* OR immob*)) OR TITLE-ABSKEY (ciliosta* OR ciliopath*))) AND (LIMIT-TO (PUBYEAR , 2019) OR LIMITTO (PUBYEAR , 2018) OR LIMIT-TO (PUBYEAR , 2017) OR LIMIT-TO (PUBYEAR , 2016) OR LIMIT-TO (PUBYEAR , 2015) OR LIMIT-TO (PUBYEAR , 2014) OR LIMITTO (PUBYEAR , 2013) OR LIMIT-TO (PUBYEAR , 2012) OR LIMIT-TO (PUBYEAR , 2011) OR LIMIT-TO (PUBYEAR , 2010) OR LIMIT-TO (PUBYEAR , 2009) OR LIMITTO (PUBYEAR , 2008) OR LIMIT-TO (PUBYEAR , 2007) OR LIMIT-TO (PUBYEAR , 2006) OR LIMIT-TO (PUBYEAR , 2005)) AND (EXCLUDE (DOCTYPE , "re") OR EXCLUDE (DOCTYPE , "cp") OR EXCLUDE (DOCTYPE , "ch") OR EXCLUDE (DOCTYPE , "le") OR EXCLUDE (DOCTYPE , "ed") OR EXCLUDE (DOCTYPE , "no"))

Table S5 Search strategy - Cochrane Library

Date of search: March 29th, 2019

#1	(exhal* NEAR/3 ("nitric oxide" OR "NO")):ti,ab,kw OR ((FENO OR "FE(NO)") AND (air OR airway* OR breath* OR respir*)):ti,ab,kw
#2	healthy:ti,ab,kw OR normal:ti,ab,kw OR ((control OR reference) NEAR/3 (value* OR range*)):ti,ab,kw
#3	(asthma OR lung NEAR/1 allerg*):ti,ab,kw
#4	(chronic NEAR/3 obstruct* NEAR/3 (airway OR bronch* OR lung OR pulmonary OR respiratory)):ti,ab,kw OR (COAD or copd):ti,ab,kw
#5	(ciliary NEAR/3 (immotility OR disorder* OR dyskinesia)):ti,ab,kw OR (cilia NEAR/3 (immot* OR immob*)):ti,ab,kw OR ciliosta*:ti,ab,kw OR ciliopath*:ti,ab,kw
#6	((chronic OR persist*) NEAR/3 cough*):ti,ab,kw
#7	#2 OR #3 OR #4 OR #5 OR #6
#8	((nasal OR nose) NEAR/3 ("nitric oxide" OR "NO")):ti,ab,kw OR (nno AND (nasal OR nose OR
#9	#2 OR #5
#10	#9 AND #10

Table S6 Characteristics of submitted F_ENO_{50} data included in the final analysis by site.

Country	N	Type of Device	Analyser	ATS (2005) Quality Control Met?	Flow Rate Achieved	Quality Control	Calibration
Sweden	122	Chemiluminescence	Sievers 280	Yes	Software	NA	Measurement of ambient NO, daily calibration using zero gas
USA	3,531	Electrochemical Cell	NIOX Mino	Yes	Software Guided	2 within 10%	Daily Biological control, measurement of ambient NO, daily calibration using zero gas
Sweden	1,367	Chemiluminescence	NIOX	Yes	NA	NA	Weekly calibration using zero gas
Portugal	457	Electrochemical Cell	NObreath	Inspired air not free of NO.	Software	3 within 10%	None
Netherlands	137	Electrochemical Cell	NIOX Mino	Only single measure reported. Not instructed to refrain from smoking/eating/exercise	Software Guided	Not specified	None

				for 1h prior to test. FENO measured after other PFTs.			
Viet Nam	853	Electrochemical Cell	Medisoft	Nose clip used.	Operator Guided	2 within 5%	Weekly biological control, daily calibration using zero gas
Kazakhstan	355	Electrochemical Cell	NObreath	Yes	Software Guided	2 within 10%	Measurement of ambient NO, daily records of temperature
New Zealand	104	Chemiluminescence	NIOX	Yes	Operator Guided	NA	Daily records of temperature
Italy	25	Electrochemical Cell	Medisoft	Yes	Software Guided	3 within 10%	Weekly biological control, measurement of ambient NO, daily records of temperature
South Korea	66	Electrochemical Cell	NIOX VERO	Only single measure reported. F_ENO measured after	Software Guided	Not specified	None

				other PFTs.			
South Korea	154	Electrochemical Cell	NIOX Mino	Unknown collection standard	Operator Guided	3 within 10%	Daily biological control
Paraguay	97	Electrochemical Cell	NObreath	Yes	Software Guided	2 within 10%	Weekly biological control
Netherlands	97	Chemiluminescence	Sievers 280 and Ecophysics CLD 700	Yes	Operator Guided	NA	Daily calibration using zero gas
Netherlands	327	Chemiluminescence	NIOX	Not instructed to refrain from smoking/eating/exercise for 1h prior to test. F_ENO measured after other PFTs.	Software Guided	NA	Biweekly calibration using zero gas
Netherlands	656	Electrochemical Cell	NIOX Mino	Only single measure reported.	Software Guided	Not specified	None
France	2	Electrochemical Cell	NIOX Vero	Only single measure reported.	Software Guided	Not specified	Weekly biological control
Sweden	70	Chemiluminescence	NIOX FLEX	Yes	Software Guided	NA	Biweekly calibration

							using zero gas
UK	284	Chemiluminescence	NIOX	Yes	Software Guided	NA	Manufacturer Recommended
UK	385	Electrochemical Cell	NIOX Mino	Yes	Software Guided	2 within 10%	Manufacturer Recommended
UK	232	Electrochemical Cell	NIOX Mino	Yes	Software Guided	2 within 10%	Manufacturer Recommended
South Africa	282	Electrochemical Cell	NIOX Mino	Yes	Software Guided	2 within 10%	Measurement of ambient NO, daily record of temperature
South Africa	86	Electrochemical Cell	NIOX Mino	Yes	Software Guided	2 within 10%	Measurement of ambient NO, daily record of temperature
South Africa	78	Electrochemical Cell	NIOX Mino	Yes	Software Guided	2 within 10%	Measurement of ambient NO, daily record of temperature
South Africa	180	Electrochemical Cell	NIOX Mino	Yes	Software Guided	2 within 10%	Measurement of ambient NO, daily record of temperature

South Africa	33	Electrochemical Cell	NIOX Mino	Yes	Software Guided	2 within 10%	Measurement of ambient NO, daily record of temperature

Table S7 Meta-data collected from each site.

Have your data been published?		If yes, please upload a PDF of the most relevant publication
Analyser	Chemiluminescence	Eco Medics CLD 88 Eco Medics CLD 77 EndoNO (Seres, France) Logan LR 2000 Logan LR 2500 NA623N (Kimoto, Japan) NIOX NIOX FLEX Sievers 240 Sievers 280 Other
	Electrochemical cell	NIOX Mino NIOX VERO NObreath (Bedfont) Medisoft FeNO+N-6008, HypAir Fenom Pro (Vyaire) Vivatmo (COSMED) Other
What software was used?		
Equipment and Protocol Details		
Was data collection method was used?		Online Offline Don't know
Was the inspired air free from nitric oxide?		Yes No Don't know
Did subjects exhale to RV before measurement		Yes No Don't know
Did subjects inhale to TLC before measurement		Yes No

	Don't know
What was the exhalation time?	≥6 s <6 s Don't know
Was the measurement taken over a 3 s plateau?	Yes No Don't know
What was the flow rate? (Select all that apply)	50 mL/s +/-10% 100 mL/s 150 mL/s 200 mL/s 250 mL/s 300 mL/s 350 mL/s Other Don't know
How was flow rate achieved?	Software guided Operator guided Other Don't know
Were measurements outside 5-20 cm of H_2O excluded	Yes No Don't know
How many measurements are reported?	Average of at least 2 measurements Single measurement Don't know
What calibration techniques were conducted? (Select all that apply)	Biological control – daily Biological control – weekly Measurement of ambient NO at testing location Daily calibration using zero gas Daily calibration using zero gas and standard NO concentration Daily records of temperature, barometric pressure, humidity Other
If other, please specify:	
What Quality Control was applied?	3 measurements within 10% 2 measurements within 10% 2 measurements within 5% Other
Study Protocol: Subject details	
Did subjects refrain from smoking in the last hour?	Yes No Don't know
Did subjects refrain from eating in the last hour?	Yes No Don't know
Did subjects refrain from exercise in the last hour?	Yes

	No Don't know
Was F_ENO measured before spirometry or other lung function tests?	Yes No Don't know
Was a nose clip used during tests?	Yes No Don't know
Were subjects using steroids for non-respiratory conditions included in the study sample?	Yes No Don't know
Asthma Studies	
Did your study include individuals with confirmed asthma?	Yes No
For those with confirmed asthma, how was asthma defined?	Doctor Confirmed Questionnaire Objective measure Combination Other Don't know
If an objective measure was used, please specify:	
Atopy Studies	
Did your study include individuals with confirmed atopy?	Yes No
For those with confirmed atopy, how was atopy defined?	Sensitivity to aeroallergens Skin prick test RAST Other Don't know
COPD studies	
Did your study include individuals with confirmed COPD?	Yes No
For those with confirmed COPD, how was COPD defined?	Doctor confirmed Questionnaire LLN $FEV_1/FVC < 0.7$ Other Objective Measure Don't know
If other method was used, please specify:	
Nasal NO	
Does your laboratory sample nasal NO? (Please note this is for future reference and will not be collected through this task force)	Yes No
What technique(s) do you use to sample nasal NO?	Restricted exhalation method Continuous nasal expiration Inflation of a balloon in the posterior nasopharynx Other