Original articles

Measurements of exhaled nitric oxide in healthy subjects age 4 to 17 years

Frederik Buchvald, MD, PhD,[a] Eugenio Baraldi, MD,[b] Silvia Carraro, MD,[b] Benjamin Gaston, MD,[c] Johan De Jongste, MD, PhD,[d] Mariëlle W. H. Pijnenburg, MD,[d] Philip E. Silkoff, MD,[e] and Hans Bisgaard, MD, DMSci[a] *Gentofte, Denmark, Padova, Italy, Charlottesville, Va, Rotterdam, The Netherlands, and Denver, Colo*

Background: Fractional exhaled nitric oxide (FE_{NO}) is used in monitoring of asthma.
Objectives: The aim of this multicenter study was to establish normal values of FE_{NO} and assess feasibility in children with a standardized method and equipment approved for clinical use.
Methods: FE_{NO} was measured in healthy subjects of 4 to 17 years according to American Thoracic Society guidelines (single breath online, exhalation flow 50 mL/s) with a chemiluminescence analyzer (NIOX Exhaled Nitric Oxide Monitoring System, Aerocrine, Sweden) in 3 European and 2 US centers. Each child performed 3 acceptable nitric oxide measurements within 6 attempts and completed an extended International Study of Asthma and Allergy in Children questionnaire.
Results: Measurement of FE_{NO} was attempted in 522 children. Four hundred five children completed the study according to the protocol. Geometric mean FE_{NO} in 405 children was 9.7 ppb, and the upper 95% confidence limit was 25.2 ppb. FE_{NO} increased significantly with age, and higher FE_{NO} was seen in children with self-reported rhinitis/conjunctivitis or hay fever. The success rate was age-dependent and improved from 40% in the children 4 years old to almost 100% from the age of 10 years. The repeatability of 3 approved measurements was 1.6 ppb (95% CI, 1.49-1.64 ppb).
Conclusion: FE_{NO} in healthy children is below 15 to 25 ppb depending on age and self-reported atopy. Measurement of FE_{NO} by NIOX® is simple and safe and has a good repeatability. Feasibility depends on age and may be difficult in the preschool child. (J Allergy Clin Immunol 2005;115:1130-6)

Key words: *Exhaled nitric oxide, children, normal values*

The fractional concentration of nitric oxide (NO) in exhaled air (FE_{NO}) is generally higher in individuals with asthma than in healthy subjects,[1] although reported values of FE_{NO} in healthy subjects vary from 3 ppb to 88 ppb.[2-7] These variations may partly be attributed to different measurement techniques but also to low numbers studied. In addition, confounding factors such as age, sex, and atopic symptoms have not always been taken into account.

Increasing use of FE_{NO} measurement in the diagnosis and monitoring of asthma has urged the need for reference values of FE_{NO} measured with commercially available equipment.

In 1999, guidelines on the measurement of FE_{NO} were issued by the American Thoracic Society (ATS),[8] updated for children in 2001 by an European Respiratory Society/ATS task force.[9] Recently commercial equipment (NIOX, Aerocrine, Sweden) obtained Food and Drug Administration clearance, allowing dissemination of its clinical application.

The primary objective of this study was to establish reference values of FE_{NO} according to international guidelines in children of 4 to 17 years. Secondary objectives were to determine the short-term repeatability of FE_{NO} levels; to investigate the effect of possible confounders (ambient NO level, age, sex, ethnicity, height, weight, and self-reported information on passive smoking, eczema, and atopic symptoms in the children and family); to determine the success rate for the procedure for each age group; and to report any possible discomfort or adverse events during the measurements.

From [a]the Copenhagen Studies of Asthma in Childhood Research Clinic, Copenhagen University Hospital, Gentofte; [b]the Department of Pediatrics, School of Medicine, University of Padova; [c]the University of Virginia; [d]the Erasmus Medical University Center, Sophia Children's Hospital, Rotterdam; and [e]the National Jewish Medical and Research Center, Denver.

Supported by Aerocrine AB, Stockholm, Sweden.

Disclosure of potential conflict of interest: E. Baraldi has served on the Scientific Advisory Board of Aerocrine. B. Gaston has consultant arrangements with SAB and Aerocrine. J. De Jongste has served as a scientific consultant for Aerocrine, Sweden, and has lectured in symposia sponsored by Aerocrine. The Department of Pediactrics, Sophia Children's Hospital Rotterdam, has received research grants from Aerocrine for the current study. H. Bisgaard has within the last 3 years received honoraria for lecture and attendance at pediatric advisory boards for Aerocrine, Altana, AstraZeneca, GlaxoSmithKline, and Merck, and owns a world patent for an inhaler device, but receives no royalty. The COPSAC clinical research unit has in the last 3 years received research grants from the following industry partners: Aerocrine, Merck, GlaxoSmithKline, and AstraZeneca.

Received for publication February 14, 2005; revised March 14, 2005; accepted for publication March 15, 2005.
Available online May 2, 2005.

Reprint requests: Hans Bisgaard, Professor MD, DMSci, Danish Pediatric Asthma Center, Copenhagen University Hospital Gentofte, Niels Andersensvej 79, 2900 Hellerup, Denmark.

0091-6749/$30.00

doi:10.1016/j.jaci.2005.03.020

Abbreviations used
ATS: American Thoracic Society
FE_{NO}: Fractional concentration of exhaled nitric oxide
ISAAC: International Study of Asthma and Allergy in Children
NO: Nitric oxide

METHODS

Study subjects and protocol

Five centers, 3 in Europe (Copenhagen, Denmark, Rotterdam, The Netherlands, and Padova, Italy) and 2 centers in the US (Denver, Colo, and Charlottesville, Va) participated in this open, multicenter study. We planned to recruit 450 children equally distributed between boys and girls and divided into 9 age groups (4, 5, 6, 7, 8-9, 10-11, 12-13, 14-15, 16-17 years).

The children were recruited from kindergartens and public schools. Written informed consent was provided by parents and the child (if 12 years or older). Exclusion criteria were a history of asthma or related respiratory symptoms defined by the International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire[10]; history of treatment with asthma-specific medication (β-agonists, steroids, leukotriene receptor antagonists); other chronic pulmonary diseases or recent airway infection (cold, flu, sore throat within the last 7 days), active smoking, or inability to comply with the study procedure.

The study was approved by the local ethics committee in each country and local health authorities where needed.

FE_{NO} measurements

FE_{NO} was measured by using the online single breath method with NIOX (Nitric Oxide Monitoring System; Aerocrine, Sweden) according to ATS guidelines.[8] The subject was seated comfortably, with the instrument at a convenient position in front of the subject. No nose clip was used.

The subject inserted the mouthpiece and inhaled NO–free air to total lung capacity over a period of 2 to 3 seconds through the mouthpiece of the NIOX instrument. The subject then started exhalation against a positive mouthpiece counter pressure of 10 to 20 cm H_2O to ensure that the soft palate was closed against the nasal cavity, thus preventing contamination of exhaled NO with NO of nasal origin. Nose clip was not used.[11] Exhalation flow was 50 mL/s. Children younger than 10 years performed a 6-second exhalation, and FE_{NO} was calculated during the last 2 seconds of the exhalation. Children $\geq$10 years performed an exhalation of 10 seconds, and FE_{NO} was calculated during the last 3 seconds of the exhalation. A measurement was accepted if (1) the mean flow was 0.045 to 0.055 L/s, (2) the instant flow was 0.0375 to 0.0625 L/s, and (3) the instantaneous mouth pressure was 5 to 20 cm H_2O. Exhalations were approved if they did not deviate more than 2.5 ppb or 10% and were completed within a 15-minute period. The interval between exhalations was at least 30 seconds. Each subject performed no more than a total of 6 exhalations, and the total number of exhalations performed to obtain 3 acceptable FE_{NO} values was recorded. FE_{NO} was calculated as the mean of 3 correct exhalations.

All measurements were performed between 8:00 AM and 5:00 PM. Information on eating or any strenuous physical activity during the last 60 minutes before testing was obtained. The subject rested in the sitting position for 5 minutes before the measurement procedure.

Nitric oxide–free air was used in all measurements to minimize the influence of ambient NO on the results.

After the measurements, children were asked if they had any adverse event in association with the procedure.

Questionnaire

Each subject completed the ISAAC core questionnaires for asthma, rhinitis, and eczema.[10] Atopy was defined as any positive answer regarding rhinitis/conjunctivitis and/or hay fever. Additional information was obtained on recent infection, passive smoking, parental disposition to asthma or atopy, and ethnicity. The questionnaire was completed by the parents. When data were missing, these were filled in by a telephone interview afterward. The parents were asked to contact the investigator in case a subject developed a respiratory tract infection within 3 days after the FE_{NO} measurement.

Data analyses

Analyses were performed by using data from all subjects who completed the study according to the protocol (n = 405), except feasibility analyses, which were performed on a data set consisting of the total number of subjects having performed or having tried to perform FE_{NO} measurements (n = 522).

Because the distribution of FE_{NO} was skewed, all analyses were performed with log-transformed data (e^{ln}), and results are presented as point estimates and 1-sided CIs with back-transformed values. Reference values of FE_{NO} are presented as geometric means and upper 95% 1-sided confidence limits for individuals. Individual upper 95% confidence limits were calculated as $e^{(LN(mean) + t \times LN(SD))}$, where t is the Student t with the appropriate number of degrees of freedom (n – 1) and n the number of observations in a group.

All analyses were performed with and without outliers, and both results were presented if different. Outliers were defined as FE_{NO} values above arithmetic mean + 2 SD. Factors possibly affecting FE_{NO} were analyzed by linear regression multivariate analyses and Pearson correlation analysis. Group comparisons were analyzed by independent t tests and 4-field tables by the Fisher exact test.

Short-term repeatability (intraindividual SD) was calculated as the SD of 3 measurements within a subject. The CIs for repeatability were calculated by using the χ^2 distribution, because the variance of repeatability was distributed according to this distribution.

Success rates were calculated as the frequencies of children of different ages that were able to perform the measurement procedure according to the protocol. Multivariate regression and partial correlation analyses were performed to investigate possible influence on FE_{NO} from confounding factors such as age, sex, and other factors.

RESULTS

Subject flow chart

Five hundred thirty-four children were recruited for the study. Four hundred five children (191 boys) completed the study with approved measurements. Subject flow diagram and demographic data are presented in Fig 1 and Table I.

Within 1 hour before the FE_{NO} measurement, 16% of the subjects had food or liquid intake (other than water), and 1.5% had performed strenuous physical activity.

Reference FE_{NO} values for age and sex

Table II presents geometric means and upper 95% limits of FE_{NO} reference values according to age for all children (n = 405). Sixteen children had extreme FE_{NO} values (>34.9 ppb) and were considered outliers. Data are also presented without these 16 outliers (n = 389), and