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Exhaled nitric oxide as a diagnostic tool for wheezing in preschool children: A diagnostic accuracy study

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ABSTRACT

Background: Airways inflammation may precede pulmonary dysfunction in wheezing individuals. The fraction of exhaled nitric oxide (FE_{NO}) has been described as a useful method for wheezing diagnosis in children, however, its application requires evidence. This study aimed to determine the accuracy of FE_{NO} in identifying wheezing in preschoolers.

Methods: A cross-sectional study was carried out with children from 3 to 5 years old, from Brazilian day care centers and public schools. They were evaluated by FE_{NO} measurement through the single breath method, and by ATS-DLD-78-C questionnaire that is used as a gold standard to phenotype wheezing patterns.

Results: The sample consisted of 243 non-wheezing children, 118 non-recurrent wheezing and 62 recurrent wheezing. The means of FE_{NO} and confidence intervals of 95%, were 5.4 (CI 95%, 5.2–5.6); 7.5 (CI 95%, 6.9–8.2) and 11.2 (CI 95%, 9.6–12.7), respectively. The sensitivity, specificity, positive and negative predictive FE_{NO} values in the 6 parts per billion (ppb) cut-off point that best diagnosed wheezing of non-wheezing children, were: 65.5%, 84.3%, 75.6% and 76.7%, respectively, with an area under the curve (AUC) = 0.77. At 10 ppb, the best cut-off points for differentiating recurrent wheezing of non-recurrent wheezing were: 56.4%, 81.3%, 61.4%, 78.0%, respectively, with an AUC = 0.69. The post-test probability for each FE_{NO} cut-off points was increased by 33% for wheezing and 20% for recurrent wheezing diagnosis when associated with clinical examination.

Conclusion: FE_{NO} can provide a reliable and accurate method to discriminate the presence and type of wheezing in preschoolers with 92% of acceptable in this study population.

1. Introduction

Wheezing is a common symptom in children in the early years of life. Among the most frequent reasons for its emergence are bronchiolitis and asthma. Whatever the cause, wheezing has an impact on quality of life and creates a high demand to seek medical care, especially when the episodes become recurrent [1,2]. Early identification of wheezing in young children provides the opportunity for both environmental and clinical intervention reducing the progression of inflammatory pulmonary disease [3].

In preschool children the diagnosis of respiratory diseases is limited since some diagnoses are essentially clinical, for example presence of bronchiolitis and diagnostic tests such as spirometry with bronchodilator reversibility and bronchial provocation are prone to problems of cooperation, execution and reproducibility in preschool children [4–6]. Additionally, studies show that children with wheezing or mild to moderate asthma often have normal baseline values of forced expiratory volume in the first second (FEV1) [7–10].

The inflammatory process of the respiratory tract may be present in wheezing children even before the pulmonary function

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Abbreviations list

ATS-DLD-78-C	American Thoracic Society and Division of Lung Diseases for children Questionnaire
AUC	area under curve
CI	confidence interval
ERS	European Respiratory Society
FeNO	fraction of exhaled nitric oxide
LR−	Negative Likelihood ratio
LR+	Positive Likelihood ratio
PPB	parts per billion
ROC curve	receiver operating characteristics curve

impairment is diagnosed, because episodes of recurrent wheezing determine the release of eosinophilic mediators and inflammatory biomarkers, including, nitric oxide (NO) [11–13]. For this reason, the measurement of the fraction of exhaled nitric oxide (FE_{NO}) has been investigated as a useful tool in the diagnosis and monitoring of respiratory tract inflammation, especially in populations in which other pulmonary function tests are not easily applied [11,14,15].

Studies have been shown a direct relationship between high levels of FE_{NO} and recurrent wheezing in preschool children, and therefore, have been used to identify an increased risk of asthma at school-age and adolescents [16]. However, although the FE_{NO} measurements can provide positive perspectives for the pulmonary monitoring of this population, the interpretation of FE_{NO} values should be further clarified in the different types of wheezing [17–19].

Studies have adopted heterogeneous criteria for the classification of wheezing, and consequently, different FE_{NO} values have been found, resulting in conflicting information as to the determination of an optimal FE_{NO} cut-off which adequately differentiates the wheezing phenotypes [15–19]. We hypothesized that the FE_{NO}, by eosinophilic airway inflammation assessment, is accurate to identify wheezing and non-wheezing preschool and differentiate recurrent and non-recurrent wheezing. The aim of this study, therefore, was to evaluate and establish a diagnostic FE_{NO} value for wheezing in preschool children compared with information from a validated questionnaire.

2. Method

2.1. Study sample

Based on the best sensitivity and specificity values of a pilot study with 50 volunteers, we calculated from a confidence level of 95% and a maximum permissible error of 5%, a total sample size of 423 preschool children [20]. Volunteers were recruited from 18 day care centers and/or public schools in the city of Recife, Pernambuco, BR, chosen at random, during the period from March to December 2014. All children were present during the data collection period, aged between 3 and 5 years old, wheezing or not. Those with specific diseases such as bronchopulmonary dysplasia, cystic fibrosis, tuberculosis, current respiratory tract infection, acute wheezing, neurological or genetic disease with clinical consequences, and the ones who failed to execute the FE_{NO} maneuver were excluded. All parents or caregivers of the participants signed a letter of informed consent and the study was approved by the Ethics Committee of Universidade Federal de Pernambuco, Brazil, under the protocol number CAAE − 21377513.5.0000.5208.

2.2. Study design

A cross-sectional population-based study was conducted in the school environment. Parents or caregivers of the children answered a questionnaire based in American Thoracic Society and Division of Lung Diseases for children questionnaire (ATS-DLD-78–C) [21]. Participants were classified into three groups: (1) non-wheezing (children who did not have episodes of wheezing in the last 6 months); (2) non-recurrent wheezing (children who presented 1 to 3 episodes of wheezing in the last 6 months); and (3) recurrent wheezing (children who had more than 3 episodes of wheezing in the last 6 months) [22]. Through the questionnaire, gestational age, birth weight, exposure to passive smoking, atopy, and breastfeeding were also evaluated. All interviews were conducted by 2 researchers who were trained about how to approach the parents of study participants.

In the school, each subject's weight was measured with a digital scale accurate to 100 g (Magna, São Paulo, Brazil) and height was measured with a stadiometer (Cardiomed, São Paulo, Brazil) accurate to 1 mm.

2.3. Measurement of the fraction of exhaled nitric oxide (FE_{NO})

FE_{NO} was measured by chemiluminescence through the online single breath method, using a portable analyzer (NIOX-MINO, Aerocrine, Sweden), according to the guidelines of the American Thoracic Society/European Respiratory Society [23] and expressed in parts per billion (ppb). The children were placed sitting comfortably without nose clip, instructed to inhale the ambient air with nitric oxide removed through a mouthpiece, to approximately total lung capacity, and then, performing exhalation with constant flow rate of 50 ml/s for at least 6 s. The FE_{NO} value was represented by the mean of 2 repeated exhalations that agree within 5%. Children who were not successful in performing the maneuver after up to 10 test attempts were excluded.

All evaluations were performed by the same operator, at the subject's school, in a quiet isolated environment, and at the same time of day (morning) thus preventing exposure of the volunteer to extrinsic factors for at least 1 h before the test, such as: smoke, strenuous physical exercise, nutrition and medication use. The operator was not blind to the wheezing rating from the scale.

2.4. Likelihood ratio (LR) and Youden's index

Likelihood ratios are typically used in to assessing the clinical value of performing a diagnostic test. They incorporate sensitivity and specificity of the test to determine whether a test result changes the probability that a condition exists facing a pre-test probability known for gold standard information.

Youden's index is suggested as a way of summarizing the performance of a diagnostic test (Youden's index = Sensitivity + Specificity − 1). Its value ranges from 0 to 1, when zero value, the diagnostic test gives the same proportion of positive results for groups with and without the disease, the test is useless. When 1, indicates that there are no false positives or false negatives, therefore, the test is perfect.

2.5. Statistical analysis

The analysis was performed using SPSS® 20.0 software (Chicago, USA). The mean values for FE_{NO} of the three groups were compared by one-way analysis of variance (ANOVA) and Bonferroni test. For categorical variables, we used the Pearson's chi-square test. Data were expressed as mean ± standard deviation and frequency. Significance was determined by $p < 0.05$.