Factors Associated with Positive Adenosine Challenge Test in Young Children with Suspected Asthma

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Background: To investigate the predictive factors associated with positive adenosine monophosphate challenge using the auscultation method (AMP-PCW) test results.

Methods: This is a prospective study of young children with suspected asthma who underwent AMP-PCW test. Patients with a positive AMP-PCW test were compared with those with a negative AMP-PCW. A multivariate logistic regression model was performed to identify the independent determinants of positive AMP-PCW.

Results: A total of 159 patients completed the AMP-PCW test. The median age was 53 months. In total, 54.0% of patients had a positive AMP-PCW. The prevalence of atopic dermatitis and family history of asthma and allergy were significantly higher among the positive AMP-PCW group ($P=0.04$, $P=0.02$, and $P=0.007$, respectively), as were the prevalences of elevated immunoglobulin E (IgE), peripheral blood eosinophils percentage ($P=0.003$, $P<0.001$, respectively), and number of emergency department (ED) visits/hospitalizations before AMP-PCW test ($P=0.006$). A significant inverse correlation exists between peripheral blood eosinophils percentage and serum IgE levels with the AMP end-point concentrations ($r=-0.302$, $P=0.001$, and $r=-0.312$, $P=0.001$, respectively). In multivariate logistic regression model, peripheral blood eosinophils percentage, IgE levels, and the number of ED visits/hospitalizations before the AMP-PCW test were found as independent predictors for positive AMP-PCW test result.

Conclusions: Our results suggest that bronchial responsiveness to AMP-PCW is related to proxy markers of airway inflammation (elevated eosinophils and IgE levels) and clinical exacerbation of asthma before the test. This may support the role of AMP-PCW in detecting inflammatory changes and monitoring their trend among young children with suspected asthma.

Keywords: asthma, adenosine, challenge test, hyper-responsiveness, wheezing

Introduction

BRONCHIAL HYPER-RESPONSIVENESS (BHR) measurement has become an important tool in the diagnosis and management of asthma.^{1–5} Adenosine 5'-monophosphate (AMP) challenge is an indirect method for evaluation of BHR as it activates different tissue cells that release inflammatory mediators that, in turn, act on the smooth bronchial muscle and cause muscle contraction.⁶

AMP challenge is both sensitive and specific for the diagnosis of BHR and asthma among adults and children,^{7–10} and its utilization among young infants after 2 years of age by nebulized adenosine monophosphate challenge using the auscultation method (AMP-PCW) is both safe and effective.^{11–13}

As performing AMP-PCW, a test with high negative predictive value (NPV) and moderate positive predictive

value (PPV) for future asthma¹⁴ requires cooperation from both infants and parents and involves inhalation of provocative AMP in an increasing concentration,¹⁵ it is important to identify in advance which infants will benefit most from its performance, mostly those who will probably have a negative AMP-PCW (in light of the high NPV of the test).

Previous studies have evaluated the correlation between factors such as atopy, immunoglobulin E (IgE) levels and inflammatory markers, and AMP responsiveness with conflicting results reported.^{16–22}

The aim of this study was to further delineate independent characteristics associated with positive AMP-PCW challenge among young children with recurrent respiratory complaints who were tested for suspected asthma to identify those infants less likely to have a positive AMP-PCW who will benefit from its performance.

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Materials and Methods

Patients

This is a prospective cohort study. The study group comprised patients aged 2 to 8 years who were referred by pediatric pulmonary clinics in Jerusalem to the Hadassah Medical Center between January 2006 and October 2012 to perform AMP-PCW. These patients experienced recurrent respiratory symptoms such as shortness of breath, wheeze, or cough and were suspected for asthma.

Patients with positive AMP-PCW test were compared with those with a negative test result. Exclusion criteria included patients diagnosed with any other chronic respiratory diseases such as bronchiectasis, cystic fibrosis, ciliary dyskinesia, and bronchopulmonary dysplasia.

Data collection

For the purpose of this study, we abstracted patients' anamnesis reports, parents' filled questionnaire, AMP-PCW reports, laboratory results, and outpatient clinics data. At time of admission for AMP-PCW, a detailed entry questionnaire was conducted including data regarding the age at onset of respiratory symptoms, duration and severity of respiratory symptoms, known triggers for respiratory complaints, presence of other atopic conditions, relevant family history, and home and environmental exposure. The aforementioned questionnaire was filled out for all patients admitted. In addition, children were also objectively assessed by physical examination.

Patients' medical records and questionnaires were reviewed for demographics (age and gender), relevant personal and family history, and asthma-related risk factors (eg, allergy, familial asthma, household pet exposure, atopy, and smoking exposure).

Smoking exposure was defined as any parent actively smoking (indoor or outdoor smoking). Pet exposure was defined as owning any household furred pet or a bird. Atopy was defined as the presence of any of the following: allergic rhinitis, atopic dermatitis, and eczema. Familial association was defined as either first degree relative. Elevated IgE levels were defined in age-specific reference intervals; 2–3 years >20 IU/mL, 3–5 years >30 IU/mL, and >5 years 70 IU/mL. Elevated peripheral blood eosinophils percentage was defined as $\geq 5.5\%$,^{23,24} which is the reference range for eosinophilia in the central laboratory at our center. All the aforementioned data were prospectively recorded in an electronic database in a real-time manner. We also reviewed pediatric pulmonologist outpatient clinic data, including laboratory test results, diagnosis, and medical treatment.

Challenge test

All AMP-PCW tests were performed by a pediatric pulmonologist using a previously described method.^{15,25} In brief, an AMP fresh solution (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) was administered by nebulization by a DeVilbiss 646 Nebulizer (DeVilbiss Co., Somerset, PA) attached to a compressor (PulmoAide compressor; DeVilbiss) that produced an output of 5 L/min through a face mask for a period of 2 min. AMP solution concentrations were sequentially doubled starting from 0.39 mg/mL up to 200 mg/mL or until the end point of AMP-PCW was

achieved. End point of AMP-PCW was defined as one or more of the following: (1) continuous wheeze detected over the trachea or the chest using a stethoscope, (2) oxygen saturation drop of at least 5% from baseline, as measured by a pulse oximeter, and (3) an increase in the respiratory rate of 50% or more from baseline.

A positive AMP-PCW was defined when the end point was reached at an AMP concentration of 200 mg/mL or less. The severity of BHR was defined according to the AMP concentration reached at the end point: mild, 100–200 mg/mL; moderate, 25–50 mg/mL; and severe, 0.39–12.5 mg/mL.^{14,26,27} It is important to note that parents were instructed and asked in the questionnaire to confirm that none of the patients was taking inhaled steroids, leukotriene receptor antagonists, or antihistamine drugs within 1 month of AMP-PCW.

Statistical analysis

The values or frequencies between the 2 groups were compared with the $-\chi^2$ test and Fisher's exact test for categorical variables and the 2-sample *t*-test for continuous variables with a normal distribution. Mann–Whitney U test was used to compare medians of continuous variables. Pearson's correlation test was used to evaluate the relationship between inflammatory markers and AMP concentration at end point. A 2-sided *P*-value <0.05 indicated statistical significance. The statistically significant variable between study groups was included as independent variables in multivariate logistic regression model to identify the determinants of positive AMP-PCW. The data were analyzed using Software Package for Statistics and Simulation (IBM SPSS version 23; IBM Corp, Armonk, NY). We divided the cohort into 2 groups by age at AMP-PCW (young, aged ≤ 48 months versus Elder, aged >48 months) and compared between the groups in relation to characteristics and AMP=PCW results.

Ethical consideration

Ethical institutional review board of the Hadassah University Medical and the management board of Meuhedet HMO, waiving informed consent, were obtained for this study (0346-10-HMO).

Results

Within the study period, 161 patients with recurrent respiratory symptoms suspected for asthma were referred to our hospital and underwent an AMP-PCW test. Of them, 159 completed the AMP-PCW test and constituted the study cohort, as 2 (1.2%) patients did not complete the AMP-PCW test. The clinical and demographic characteristics of patients are presented in Table 1. Baseline characteristics and AMP-PCW test results did not vary between young (≤ 48 months) and elder (>48 months) groups, except median age of first wheezing episode; young patients presented earlier with first wheezing episode (median 27 months versus 48 months, $P < 0.001$).

Patients' characteristics in relation to the AMP-PCW result are presented in Table 2. The median age of the study group was 53 (42–63 interquartile range) months, with 97 (61%) males. Eighty-six patients (54.0%) had positive AMP-PCW results. Of those with positive AMP-PCW

TABLE 1. DEMOGRAPHIC AND LABORATORY DATA OF PATIENT REFERRED FOR AMP-PCW

Characteristics	Total cohort (n = 159)	Aged ≤48 months (n = 64)	Aged >48 months (n = 95)	P
Median age, months (IQR)	53.0 (42–63)	39 (36–45)	60 (54–71)	<0.001
Median age at first wheezing episode, months (IQR)	41 (25–57)	27 (24–33)	48 (42–59)	<0.001
Median duration of symptom, months (IQR)	29 (25–33)	27 (23–31)	29 (26–33)	0.51
Gender, male (%)	97 (61%)	36 (56.2%)	61 (64.2%)	0.31
Smoking exposure (%)	43 (27.0%)	19 (26.7%)	24 (25.3%)	0.53
Pets exposure (%)	24 (15.1%)	9 (14.1%)	15 (15.8%)	0.76
Atopy (%)	41 (25.8%)	17 (26.6%)	24 (25.3%)	0.85
Allergic rhinitis, n (%)	16 (10.0%)	7 (10.9%)	9 (9.5%)	0.77
Atopic dermatitis, n (%)	25 (15.7%)	10 (15.6%)	15 (15.8%)	0.97
Asthma in family (%)	61 (38.4%)	25 (39.1%)	35 (36.8%)	0.77
Allergy in family (%)	57 (35.8%)	26 (40.6%)	30 (31.6%)	0.24
Elevated IgE (%)	42 (26.4%)	14 (21.9%)	28 (29.5%)	0.28
Elevated eosinophils %	63 (39.6%)	24 (37.5%)	39 (41.0%)	0.65
Persistent medical treatment before AMP-PCW (%)	101 (63.5%)	44 (68.7%)	57 (60.0%)	0.26
ED visits/hospitalization before AMP-PCW (%)	50 (31.4%)	23 (35.9%)	27 (28.4%)	0.32
Number of risk factors, median (IQR)	2 (1–3)	2 (1–3)	2 (1–3)	0.88
Positive AMP-PCW (%)	86 (54.1%)	38 (59.4%)	48 (50.5%)	0.27

The data is presented in median and interquartile range as it best represents the population.

AMP-PCW, adenosine monophosphate challenge using the auscultation method; ED, emergency department; IgE, immunoglobulin E; IQR, interquartile range.

results, 31.4% were defined as mild BHR, 17.4% moderate BHR, and 51.2% severe BHR. Demographic characteristics (eg, age, age at first wheezing episode, duration of symptoms, and gender) and the prevalences of several asthma-related risk factors (eg, exposure to smoking, pet exposure, composite of atopy, and allergic rhinitis) were comparable between those with positive and negative AMP-PCW test results. Furthermore, the study groups were comparable in terms of the prevalence of chronic antiasthmatic treatment before test performance. The prevalences of atopic dermatitis, family history of asthma, and allergy were significantly higher among those with a positive AMP-PCW result ($P=0.04$, $P=0.02$, and $P=0.007$, respectively), as were the prevalence of elevated IgE, peripheral blood eosinophils

percentage ($P=0.003$, $P<0.001$, respectively), and the number of emergency department (ED) visits/hospitalizations before AMP-PCW test ($P=0.006$). Overall, 13% had no asthma-related risk factors. The proportions of risk factors prevalence are presented in Table 2. The median number of risk factors was higher among the positive AMP-PCW group (3 versus 1, $P<0.001$).

The relationships between AMP end-point concentrations and peripheral blood eosinophil percentage and serum IgE levels were examined in the positive AMP-PCW group. A significant inverse correlations for both peripheral blood eosinophils percentage and serum IgE levels were found with the AMP end-point concentrations, ($r=-0.302$, $P=0.001$, and $r=-0.312$, $P=0.001$, respectively). Peripheral blood

TABLE 2. PATIENTS CHARACTERISTICS BY AMP-PCW RESULT

Characteristic	Positive AMP-PCW (n = 86)	Negative AMP-PCW (n = 73)	P
Median age, months (IQR)	53.5 (31.25–75.75)	52 (35.5–68.5)	0.49
Median age at first wheezing episode, months (IQR)	40 (24–56)	42 (26–58)	0.41
Median duration of symptom, months (IQR)	28 (24–32)	30 (26–34)	0.4
Gender, male (%)	56 (57.7%)	41 (42.3%)	0.22
Smoking exposure (%)	23 (26.7%)	20 (27.4%)	0.92
Pets exposure (%)	16 (18.6%)	8 (10.9%)	0.18
Atopy (%)	26 (30.2%)	15 (20.5%)	0.16
Allergic rhinitis, n (%)	8 (9.3%)	8 (10.9%)	0.73
Atopic dermatitis, n (%)	18 (20.9%)	7 (9.5%)	0.04
Asthma in family (%)	40 (46.5%)	21 (28.7%)	0.02
Allergy in family (%)	39 (45.3%)	18 (24.6%)	0.007
Elevated IgE (%)	31 (36.0%)	11 (15.0%)	0.003
Elevated eosinophils %	46 (53.4%)	17 (23.2%)	<0.001
Persistent medical treatment before AMP-PCW (%)	60 (69.7%)	41 (56.1%)	0.07
ED visits/hospitalization before AMP-PCW (%)	35 (40.6%)	15 (20.5%)	0.006
Number of risk factors, median (IQR)	3 (2–4)	1 (0–2)	<0.001

The data is presented in median and interquartile range as it best represents the population.

TABLE 3. MULTIVARIATE LINEAR REGRESSION:
POTENTIAL PREDICTOR FACTORS FOR POSITIVE
AMP-PCW IN YOUNG CHILDREN

Independent variable	OR	95% CI	P
Peripheral blood eosinophilia %	2.02	1.22–7.47	0.02
IgE levels	1.27	1.05–3.34	0.04
ED/hospitalization before AMP-PCW	5.1	1.32–21.43	0.009

Only factors found to be statistically significant are shown in the table.

CI, confidence interval; OR, odds ratio.

eosinophils percentage, IgE levels, and number of ED visits/hospitalizations before AMP-PCW testing were found as independent predictors for positive test results after adjustment for age and gender (odds ratio, OR 2.02, $P=0.02$, OR 1.27, $P=0.04$, OR 5.1, $P=0.009$, respectively) (Table 3). Children who harbor all independent determinants combined (elevated eosinophils percentage, IgE levels, and high number of ED visits/hospitalizations before AMP-PCW) were highly likely to have a positive AMP-PCW result [OR 10.6; 95% confidence interval (CI) 6.2–51.9; $P<0.001$] with a PPV of 71.4% (95% CI 65.3%–76.8%) and an NPV of 70.6% (95% CI 48.1%–86.1%).

Discussion

This study shows that elevated eosinophils percentage, IgE levels, and high number of ED visits/hospitalizations are independently associated with a positive AMP-PCW result. We demonstrate high PPV and NPV (71.4%, 70.6%, respectively) for all 3 determinants combined for a positive AMP-PCW test. This finding is helpful in assuming which subset of infant will most probably benefit from the test. As AMP-PCW has a high NPV for asthma diagnosis in the future, those infants who will most benefit are those who will have a negative AMP-PCW and we will rule out future asthma diagnosis (those without our 3 independent determinants). We also show that certain atopic characteristics (atopic dermatitis and familial history of asthma and allergy) did not relate to AMP-PCW results.

BHR to AMP has been shown to have high specificity for the diagnosis of asthma in preschool-age children^{11,14} and has been widely used in clinics as the BHR challenge of choice in young children who were referred for a possible diagnosis of asthma.^{28,29} Our cohort of patients was evaluated prospectively using the AMP-PCW method as part of a study protocol to examine the role in the diagnosis of asthma among young children. AMP-PCW may be easier to perform in terms of children cooperation, particularly in those who are unable to follow spirometry test. AMP-PCW was also performed among children in ages where they can perform spirometry, this in line with our study protocol. A strong correlation was previously shown between spirometry (the gold standard for BHR detection) results and PCW in children who were old enough to perform spirometry.^{13,30} Observations about factors associated with AMP responsiveness are derived from a few small studies among adults with asthma and several studies among children.^{16–19,27,31} Although we did not evaluate determinants associated with AMP responsiveness in relation to the age of the patient at

the time of AMP-PCW, it was previously shown that the older the child at the time of test performance, the greater the ability of the test to predict asthma.¹⁴ Thus we can estimate that age has a similar effect on the results of this study; increased BHR to AMP may be a normal phenomenon that disappears later in life as was previously reported,^{32,33} and most of the wheezy infants stop wheezing in the subsequent years, as most preschool wheezers show symptoms during respiratory viral infections and do not develop asthma later in life.^{34,35} It was previously demonstrated that atopic sensitization is the single most important determinant of hyper-responsiveness to AMP in children with documented asthma^{17–19}; this in opposed to our finding that only atopic dermatitis is associated with positive AMP-PCW, a finding that was not found to be significant in multivariate analysis. As the only study in the current literature that demonstrated association between atopy and BHR to AMP did not account for confounder bias,¹⁷ we conclude that it might represent a biased finding. In the same aforementioned study, other factors such as gender, age, presence of allergic rhinitis, and familial history of asthma were not associated with hyper-responsiveness to AMP. In addition, the same group of researchers¹⁸ found that peripheral blood eosinophilia was the only predictor of the response to AMP among asthmatic children. This concurs with this study results; as AMP is an indirect challenge that acts on inflammatory cells to release muscle activating substances, it is plausible that markers of inflammation would be correlated with AMP-PCW results. The most recent study examining BHR to AMP demonstrated that total eosinophil count and the percentage of eosinophils, as well as IgE levels, are associated with BHR to AMP, whereas no association was found in other studies,^{16,27} this again reinforces our study findings. Altogether, it can be concluded that AMP challenge provides a good estimation of airway inflammation, coupled with elevation of inflammatory markers rather than atopy. We showed that the number of asthma-related risk factors and respiratory exacerbations requiring ED visits or hospitalizations is correlated with AMP-PCW results. Moreover, we found that ED visits or hospitalizations are an independent risk factor for positive AMP-PCW (and has a higher OR than the other 2 independent risk factors: peripheral blood eosinophils percentage and IgE levels). These findings are novel and never been examined before to the best of our knowledge, yet they come not as a surprise, as patients with more asthma risk factors and clinically unstable disease with recurrent symptoms and exacerbations could represent a subset of patient with BHR rather than just childhood wheezing episodes.

Limitations

This study has some limitations: this study was based on an absolutely small population, yet, it is one of the largest cohort study performed on this topic. Moreover, we did not measure directly airway inflammation biomarkers (eg, sputum eosinophils or exhaled nitric oxide); although the final common pathway is asthma diagnosis by diagnostic tests (spirometry, AMP-PCW) rather than academic laboratory evaluation that could be expensive in terms of medical resources and troublesome in terms of children cooperation. We did not evaluate the study cohort in relation to different age groups, although we believe that as the next step in

management of these children after anamnesis and laboratory evaluation is performing AMP-PCW, the impact of age on clinical evaluation is somewhat limited by this notion. Finally, we did not evaluate skin prick test results as this test is infrequently carried out in our country. Moreover, studies have conflicting conclusions regarding the role of skin prick test atopy and BHR in preschool children.¹⁷

Less than 50% of children in our cohort underwent this evaluation (it is not routinely done as part of young children wheezing evaluation due to resource priority), leaving this variable underreported in our group. A positive response in skin prick test is commonly used to define atopy.¹⁸ This missing information could have valuable contribution to our study, as it was previously shown that positive skin test is associated to AMP responsiveness.¹⁸ Nevertheless, another study found no association between positive skin test and AMP responsiveness value, presumably due to bias as a result from a recent corticosteroids use.³⁶ Skin prick test was found to be associated with direct BHR to methacholine (direct stimuli of smooth muscle by methacholine).³⁶ As AMP-PCW is an indirect challenge test (acts by a way of releasing mediators affecting smooth muscles) on the bronchial tree, and was not found to be associated with skin prick test in the aforementioned study, further clarification of this relationship is warranted.

In conclusion, in this study, we observed a significant and independent correlation between eosinophil percentages, IgE levels, and ED/hospitalization before AMP-PCW to a positive AMP-PCW, which is a surrogate for BHR. These findings can assist in identifying those patients who are most likely to benefit from the performance of the AMP-PCW test (those who are most likely to have a negative AMP-PCW, which, in turn, have a high NPV for future asthma diagnosis). It is important to underline that AMP-PCW is a diagnostic test for asthma with a predictive value that varies by age at performance with a false positive and negative rates as any other diagnostic test. A negative AMP-PCW test in those with the characteristics we have identified might represent a false negative result or might represent a child without asthma suffering from BHR that might be transient or due to other pulmonary pathology. By taking into consideration the number of risk factors present and the aforementioned independent determinants, the pediatric pulmonologist can choose the patients most likely to respond positively to the AMP-PCW test. Moreover, our study adds to the current body of evidence that suggests a significant correlation between inflammatory markers (eg, IgE and eosinophils) and BHR to AMP and put into question the role of atopy in this setting.

Author Disclosure Statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the article.

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