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Relationship between Methacholine Challenge Testing and exhaled Nitric Oxide in adult patients with suspected bronchial asthma

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Key words

Asthma; Bronchial Hyperreactivity (BHR); Methacolin Challenge Testing (MCT); Exhaled Nitric Oxide (FeNO)

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Summary

Usually, hyperresponsiveness to inhaled methacholine is considered closely associated with a diagnosis of bronchial asthma. Recently, it has been clearly pointed out that bronchial hyperreactivity (BHR) is not a constant feature of asthma and that this condition is not always related to airways inflammation.

In the present study we evaluated 42 Patients (21 positive and 21 negative for bronchial hyperreactivity, BHR) with the aim to determine the effect of Methacholine Challenge Testing (MCT) on the levels of exhaled nitric oxide ().

Higher FeNO levels were found before methacholine provocation in the group that eventually resulted positive to the challenge, while after the challenge in both groups FeNO decreased in similar way, with no statistical difference.

These data confirm that MCT is a relevant test for asthma diagnosis, but it is not always related to the severity of bronchial inflammation, while FeNO levels in our study have limited clinical significance when evaluated out of asthma exacerbation.

Introduction

Airways hyperresponsiveness is one of the features that may contribute to the diagnosis of asthma. Methacholine Challenge Testing (MCT) is the best established method of assessing airway responsiveness (1,2,3)

When spirometry, performed before and after administration of a bronchodilator, has not confirmed or eliminated the diagnosis but symptoms (wheezing, chronic cough, chest tightness) continue to suggest asthma, MCT is usually performed in patients who are medication free and don't present a recent exacerbation of disease.

The MCT has excellent sensitivity but poor positive predictive value for asthma, while its negative predictive power is high and always useful in differential diagnosis (4,5)

Improvement in the clinical severity of asthma is associated with improvement of airways responsiveness, and clinical studies of asthma therapies often use MCT as an objective outcome measure, but responsiveness to direct bronchoconstrictor stimuli does not indicate presence and severity of airway inflammation (6).

In fact, challenge with indirect stimuli as Adenosine and Mannitol can provide a better correlation with inflammatory markers, with increase of sputum eosinophil count and exhaled nitric oxide (7).

Nitric oxide is synthetized from L-Arginine in both neuronal and not neuronal tissues through the action of NO synthetase. The epithelium of the respiratory tract is an important source of NO, which increases with the presence of inflammatory cells. Production of NO is assessable by measuring the fraction of NO in exhaled air (FeNO) and elevated levels of this diffusible gas have been proposed as a non invasive marker of airways inflammation (8,9,10). Timing of sampling may significantly alter FeNO measurements, and repeated spirometry maneuvres may reduce FeNO levels (11).

Studies in asthmatic children reported that FeNO values are reduced after MCT (12), but it is not clear if the change of FeNO values is a consequence of repeated maneuvres and hyperventilation, or it is due to methacholine induced bronchial constriction.
More studies are necessary to define relationship between results of bronchial provocation tests, i.e. MCT, and values of FeNO.
With the aim of providing a better definition of a different risk of acute asthma exacerbation, in this study we measured bronchial hyperreactivity and inflammation in a group of Patients with clinical suspect of asthma and with negative or positive MCT, and we determined the effect of MCT on FeNO levels in all Patients.

Materials and methods

Subjects

42 Patients (20 M, 22 F, mean age 37.04 years) with symptoms (chronic cough, nocturnal wheezing, or dyspnea for more than 3 weeks) and visited in our Outpatients Office, have been selected for a Methacoline provocation test after a normal spirometry before and bronchodilator test after with 400 mcg of salbutamol.
21 Patients were smokers, 11 ex smokers, 10 no smoker, 21/42 were atopic (10 mites, 6 grasses, 4 molds, 1 cat), see **table 1**.
Before and after MCT, FeNO levels were measured in all patients, mean of two consecutive test (?).
All Patients received written information on the test and gave their informed consent.

Methacholine Provocation Test

The bronchial provocation test was performed using MasterScreen Body connected with an aerosol provocation system APS PRO. Two different Methacholine Chloride (Lofarma) concentrations (0.2 and 1%) were diluted in sodium phosphate buffer solution. The Patient breathed against a device a single dose of 30, 30, 60, 120, 150, 300, 600, 1200 mcg up to a cumulative dose of 2490 mcg of the substance. After a minute from each concentration, Patient performed control spirometry.
The test was interrupted if FEV1 decreased more than 20% from the value found after buffer solution, before the first dose of the drug. In this condition, patient was invited to inhale 400 mcg of salbutamol and spirometry was repeated after 20 minutes.

FeNO Measurement

FeNO was measured using HypAir FeNO (Medisoft S.A., Belgium). Each Patient was asked to inhale deeply against the machine via a filtered breathing mouthpiece; after a whole inspiration with a pressure maintained between 4 and 10 cm/H_2O the subject had to exhale continuously, maintaining an optimal exhalation pressure with a flow at 50 ml/s; at this point the instrument started sampling automatically. Double measurements of NO were averaged and expressed as parts per billion (ppb).

Study design

FeNO levels were measured before MCT as a baseline, then immediately after completion of the provocation test. When test was positive for hyperreactivity (cumulative dose of methacholine reduced FEV1 more than 20% when compared with baseline value after isotonic saline) FeNO was performed before inhalation of the bronchodilator.

Statistical Analysis

All data are expressed as means +/- SE, χ^2 test was performed for Patients with pre-Methacholine FeNO > or < 30 ppb, ANOVA test for FeNO pre/post MCT.
Statistical Analysis was performed by courtesy of Valentina Mirisola, Engineer - Mediservice, Genova.

Results

Table 1 describes general characteristics of the 42 Patients, divided for analysis in 21 positive and 21 negative to MCT. No statistical difference was shown within the groups for age, gender, smoke and allergy. Most part of positive MCT were classified as Relevant Hyperreactivity (PD20 FEV1 induced by dose less than 400 mcg of Methacholine in 85.7% of 21 positive patients).
FeNO before MCT shows no statistical difference between patients positive or negative for bronchial hyperreactivity (ANOVA p-value 0234) (**table 2**).
In **table 3** we divided Patients with FeNO above or below 30 ppb, in patients with less than 30 ppb there was not significant difference between positive and negative challenge for bronchial hyperresponsiveness (p = 0.072); all three patients with FeNO > 30 ppb were positive to MCT.
In **table 4** we can see that FeNO significantly decreased in positive and negative group after challenge (p = 0.006) but (**table 5**) the mean decrease of FeNO post MCT was not significantly different between positive and negative patients (ANOVA p value = 0.374).
In two well balanced groups no differences were found in FeNO levels in a statistical comparing of smoker/not smoker and allergic/not allergic subgroup of patients.

Table 1 - *General characteristics of the patients.*

Variable	Negative		Positive		p-value	Total	
	N	%	N	%		N	%
Age, years Mean (± SD)	37.52 (± 13.618)		38.81 (± 17.005)		0.788	38.17 (± 15.230)	
Gender							
Female	9	42.9	13	61.9	0.217	22	52.4
Male	12	57.1	8	38.1		20	47.6
Smoke							
Ex smoker	5	23.8	6	28.6	0.728	11	26.2
No smoker	11	52.4	12	57.1		23	54.8
Smoker	5	23.8	3	14.3		8	19.0
Allergy							
Yes					0.355		
No	12	57.1	9	42.9		21	50.0
	9	42.9	12	57.1		21	50.0
PD20, mcg Mean (± SD)	-		296.88 (±309.073)		-	-	
Bronchial Hyperreactivity							
Slight	-	-	1	4.8	-	-	-
Moderate	-	-	2	9.5		-	-
Relevant	-	-	18	85.7		-	-

Table 2 - *FeNO mean pre-Methacholine Challenge.*

	BHR		Total	ANOVA p-value
	Positive	Negative		
Mean FeNO	18.33	12.47	15.40	0.234
SD	5.336	21.567	15.799	

Table 3 - *Percentage of patients with FeNO pre-MCT < or > 30 ppb.*

		BHR		Total	X^2 p-value
		Negative	Positive		
< 30 ppb	N	21	18	39	
	%	100.0	85.7	92.9	
FeNO pre-MCT ≥ 30 ppb	N	0	3	3	0.072
	%	-	14.3	7.1	
N Total		21	21	42	
	%	50.0	50.0	100.0	

Table 4 - *FeNO pre- and post- Methacholine Challenge.*

		BHR		Paired samples p-value
		Pre	Post	
FeNO	Mean	15.40	12.21	0.006
	SD	15.799	9.907	

Table 5 - *Delta FeNO pre-post- Methacholine Challenge.*

		BHR		ANOVA p-value
		Negative	Positive	
Delta FeNO	Mean	-2.19	-4.18	0.374
	SD	3.243	9.655	

Discussion

MCT is usually performed when spirometry is in normal range but symptoms leave a suspect of bronchial asthma. This test can confirm the presence of bronchial hyperreactivity, but is not able to define whether symptoms are related to bronchial inflammation. By trying to relate these two parameters, we were looking for a better definition of risk for asthmatic acute exacerbations. The data show that FeNO values were within normal range (< 30 ppb) in 39/42 patients admitted to the study, and mean value of positive patients was higher than in negative group after MCT (18.33 vs. 12.47 ppb; ANOVA p-value 0234). These basal values were expected, because of the choice of investigating a population without acute exacerbation and with aspecific symptoms, only 3 patients had basal values of FeNO more than 30 ppb and all three had a rapid onset of bronchoconstriction (with 62.4, 122.4, 143.1 mcg of the product); we can assume that a greater number of patients with more than 30 ppb FeNO before test should give a significant statistical difference between the two groups (chi-square p value 0.072 with not balanced population); in other studies (13) FeNO > 34 has high predictive value for PD20 MHC < 16 mmol. MCT confirms its role for the diagnosis of asthma, with 21/42 Patients positive to the challenge. FeNO decreases significantly in Patients with positive and negative challenge (15.4 vs. 12.21 pp, paired samples p-values 0.006) but FeNO post MCT was not significantly different between the positive and negative patients after MCT (ANOVA p-value 0.374).

The reduction of FeNO may be associated with bronchial constriction during the provocation test, but also the group with MCT negative shows a similar reduction, therefore we can assume that the reduction is a consequence of bronchoconstriction and repeated spirometry maneuvers, but not related to bronchial inflammation.

Contrarily to other authors, we can't state that high levels of FeNO were associated with bronchial hyperresponsiveness (few patients > 30 ppb), while we have similar results on reduction of FeNO after the challenge. FeNO < 30 ppb seems to have limited clinical significance for diagnosis in asthma like symptoms, mostly in smokers.

So, FeNO has been correctly proposed as a non invasive marker of airway inflammation during asthma attacks, particularly in the inflammatory response to allergens, but at the moment we can't propose the use of the test out of exacerbation of the diseases and we don't suggest the contemporary use during bronchial challenge with methacholine. A different result may be possible during provocation tests with allergens, in this case the inflammation of the bronchial epithelium may be significantly greater and FeNO may grow in a direct relation with allergic inflammation. Further investigations are needed to prove this hypothesis.

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