Respiratory Medicine (2007) **101**, 1455–1461

ELSEVIER

respiratoryMEDICINE

The characteristics of different diagnostic tests in adult mild asthmatic patients: Comparison with patients with asthma-like symptoms by gastro-oesophageal reflux

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Received 31 August 2006; accepted 21 January 2007
Available online 13 March 2007

KEYWORDS
Asthma;
Gastro-oesophageal reflux;
Gastro-oesophageal reflux disease;
Diagnostic tests;
Sensitivity;
Specificity

Summary
Background: Diagnosing asthma cannot be always easy. It is important to consider the validity of the diagnostic tests, and/or how much more commonly they are positive in patients with asthma compared to healthy subjects and, particularly, to patients with asthma-like symptoms.
Objective: To evaluate the validity of diagnostic tests for asthma, in terms of sensitivity, specificity, positive and negative predictive values, in patients with bronchial asthma compared to patients affected by gastro-oesophageal reflux disease (GERD) with asthma-like symptoms, and healthy control subjects without asthma and gastro-oesophageal reflux (GER).
Design: Single-center, cross-sectional, observational study.
Patients: We studied 60 patients with mild asthma, 30 patients with GERD and asthma-like symptoms and 25 healthy control subjects.
Measurements: We measured provocative concentration of methacholine causing a 20% fall in the forced expiratory volume in 1 s (MCh PC_{20}/FEV_1), the amplitude percent mean of

Abbreviations: GER, gastro-oesophageal reflux; GERD, gastro-oesophageal reflux disease; FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity; MCh, methacholine; MCh PC_{20}/FEV_1, provocative concentration of MCh causing a 20% fall in FEV_1; A%M of PEF, amplitude percent mean of peak expiratory flow; ECP, eosinophil cationic protein

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0954-6111/$ - see front matter
doi:10.1016/j.rmed.2007.01.014

peak expiratory flow (A%M of PEF), derived from twice-daily readings for >2 weeks, the FEV_1/forced vital capacity (FEV_1/FVC) ratio, the eosinophil count in blood and in induced sputum and the serum eosinophil cationic protein (ECP) levels.
Results: FEV_1/FVC ratio, A%M of PEF, blood eosinophils counts and serum ECP levels were less sensitive and specific when the reference population was composed of patients with asthma-like symptoms by GER. While, MCh PC_{20}/FEV_1 and induced sputum eosinophils count were the most sensitive (both 90%) and specific (89% and 92%, respectively) tests.
Conclusion: Our findings demonstrate that MCh PC_{20}/FEV_1 and the induced sputum eosinophil count are the most useful objective tests in patients with mild asthma. All patients with asthma presented both an MCh PC_{20}/FEV_1 <1500 μg and eosinophils count in the induced sputum >1%.

Introduction

Gastro-oesophageal reflux disease (GERD) is a condition with relatively high incidence and it may be associated with chronic/recurrent respiratory symptoms.[1,2] GERD can induce asthma-like symptoms, such as coughing and breathlessness, through two major mechanisms: inhalation of gastric content in the airways and vagal reflexes precipitated by acid in the lower oesophageal portion and/or in the larynx.[3] These reflexes, through the release of tachykinins and other neurotransmitters by nociceptive airways afferent nerves, mediate not only cough, bronchospasm, mucus secretion and vasodilation, but also a ''neurogenic inflammation'' characterized by the recruitment of polymorphonuclear leukocytes.[4,5]

Differential diagnosis between bronchial asthma and asthma-like symptoms induced by GERD is not always easy, and it is based on the use of some investigative tests. The extent to which of these tests alter the probability of a diagnosis of bronchial asthma depends on the validity of the test, and/or how much more commonly the test results are positive in patients with bronchial asthma compared to healthy subjects and patients with conditions that are commonly confused with asthma, such as GERD with asthma-like symptoms.[6] However, many patients with bronchial asthma present gastro-oesophageal reflux (GER).

Few studies have compared the sensitivity and specificity of different tests in patients with bronchial asthma and none have done so using a reference population of patients affected by GERD with asthma-like symptoms.

Bronchial asthma can often be diagnosed on the basis of symptoms. However, measurements of lung function, and, particularly, of an abnormal, short-term, variable airflow obstruction, greatly enhanced diagnostic confidence. Spontaneous variable airflow obstruction can be assessed using a twice-daily peak expiratory flow (PEF) monitoring at home. Otherwise, treatment-induced variable airflow obstruction can be assessed in the laboratory by measuring the bronchoconstrictor response to short-acting airway smooth muscle spasmogens, such as methacholine (MCh).[7]

We compared the validity of provocative concentration of methacholine causing a 20% fall in the forced expiratory volume in 1 s (MCh PC_{20}/FEV_1) in terms of sensitivity and specificity, positive and negative predictive values, together with FEV_1/forced vital capacity (FVC) ratio, blood and induced sputum eosinophil counts, and serum eosinophil cationic protein (ECP) levels in 60 patients with mild bronchial asthma, 30 patients affected by GERD with asthma-like symptoms, and 25 healthy subjects, without asthma and GERD or GER.

Material and methods

Patients

Patients and healthy subjects were recruited from the patients attending the Units of Allergology and of Gastroenterology of the Dipartimento di Medicina Clinica e delle Patologie Emergenti of the University of Palermo (Italy) and from the Units staff. The study was carried out during the period between January 2002 and December 2003.

Our institutional policy and the ethical committee in our institution do not require that an ethics committee authorize the study. However, institutional policy requires the patient's written informed consent for us to perform the tests; we obtained consent in every case.

Mild bronchial asthma was diagnosed in 60 asthmatic patients. The subjects had consistent clinical features, were symptomatic at the time of the evaluations, had basal FEV_1 values ⩾80% of predicted, and presented one or more of the following conditions: a 15% increase in FEV_1 10 min after receiving 200 μg of inhaled salbutamol. All patients had a stable asthma, defined as stable asthma symptoms and no change in asthma medication during 2 weeks before study entry. Furthermore, no respiratory tract infection was reported within 3 months before study entry. None of the asthmatic patients reported dysphagia, and/or epigastric pain, heartburn and/or regurgitation, and/or water brash. However, in 48% of these, the 24-h oesophageal pH study demonstrated the presence of GER.

GERD with asthma-like symptoms was diagnosed in 30 patients. All patients had symptoms of GER (heartburn and/or regurgitation at least twice a month, and/or water brash, and/or dysphagia, and/or epigastric pain) and abnormal amounts of acid reflux, documented by 24-h oesophageal pH study, and defined as total percent time pH<4 during the 24-h study period exceeded 1.10% or upright acid exposure exceeded 1.70%, or supine acid exposure exceeded 0.60%.[1] The asthma-like symptoms (i.e. cough and/or breathless) did not deteriorate following the withdrawal of asthma therapy (oral corticosteroid, and/or oral theophylline, and/or short-acting β_2-agonists). Both respiratory and gastric symptoms improved with the treatment with proton pump inhibitors (PPI).

Exclusion criteria of the patients included: smoking, a history of scleroderma, oesophageal, gastric or pulmonary surgery, or the presence of other pulmonary (i.e. chronic bronchitis, or other forms of chronic lung disease), cardiac or systemic disorders, that could interfere with interpretation of results or preclude completion of the study.

Twenty-five healthy control subjects had no symptoms suggesting past or current bronchial asthma or asthma-like symptoms by GERD. In these subjects, GERD and GER were excluded to endoscopy and 24-h oesophageal pH study.

Design of study

This was a single-center, cross-sectional, observational study. Patients and controls were evaluated at a first ambulatory visit and then after 14 days. On the first day of attendance, all patients and controls completed a detailed demographic questionnaire assessing asthma and GER symptoms and underwent clinical examination. Then, spirometry, allergen skin prick tests, peripheral blood eosinophil counts, and serum ECP levels were performed, and the subjects were asked to record, on a diary, twice-daily PEF values. On the second attendance, 14 days later, a MCh inhalation test was performed, followed by sputum induction after recovery from MCh test. All laboratory measurements were performed by a blinded observer. Patients with bronchial asthma continued to receive their usual therapy throughout the investigation period, although short-acting β_2-agonists were withheld for 6 h before the evaluation of airway responsiveness. Subjects with GERD and asthma-like symptoms were studied at least 1 month after stopping all asthma medication, but continued to receive their therapy for GERD, with PPI.

Spirometry

Lung function measurements and MCh PC_{20}/FEV_1

FEV_1 was measured with a Gould 2400 automated system (Sensormedics BV, Bilthoven, Netherlans), taking into account the highest of 3 successive measurements, provided that the difference between measurements was within 100 mL. FEV_1, FVC, FEV_1/FVC ratio, and PEF were determined.

Patients and control subjects measured their PEF at home, twice daily, in the morning, on waking, and in the evening, at bedtime, for 14 days, as the best of three blows, using a mini-Wright peak flowmeter (Clement Clarke Ltd; London, UK), and were asked to register PEF measurements on a diary, assigned at the first visit, and collected at the second visit.[11]

MCh challenge was performed according to the Chai's method.[8–10] Increasing concentrations of MCh were administered with a MEFAR nebulizer (Markos, Monza, Italy). After taking baseline measurements of FEV_1, subjects inhaled five puffs of saline solution. The diluent value was considered as the control, and, if the FEV_1 variations were within 10% of baseline, the patients were enrolled in the study. Subjects then inhaled increasing concentrations of MCh, ranging from 16 to 5120 μg/mL, at 5-min intervals, until a 20% decrease from the baseline FEV_1 was recorded. The MCh PC_{20}/FEV_1 was determined by computer-assisted logarithmic interpolation of the log dose–response curve. If the patients did not react, a censored value of 5120 μg was given.[9,10]

Skin prick tests

Skin Prick Tests (Alk Abellò, Milan, Italy) for common aeroallergens of West Sicily [trees (*Olea europea* and *Cupressus*), grass pollens (*Lolium perenne*, *Cynodon*, *Phleum pratense*), mugwort (*Compositae*), wall pellitory (*Parietaria judaica*), house dust mite mites (*Dermatophagoides pteronyssinus* and *farinae*), moulds (*Alternaria alternata*, *Aspergillus*, *Cladosporium herbarum*, *Candida*, *Penicillium*), dog and cat dander, and *Blatella germanica*] were performed on the volar aspect of the forearm after anti-histamines had been withheld for at least 5 days. Glicerine and histamine (10 mg/mL) were used as negative and positive controls, respectively. A positive response was defined as any wheal with a diameter 3 mm greater than the negative control, 15 min after the application.[12]

Laboratory analyses

Peripheral blood eosinophil counts

A venous blood sample was collected, and absolute peripheral blood eosinophil counts were determined with Technicon-H1 blood cell counter (Bayer Leverkusen, Germany). The normal range is $0.10–0.40 \times 10^{-3}$ cells/μL.[12–15]

Sputum induction and processing

After baseline FEV_1 and FVC measurements, salbutamol was given by inhalation (200 μg by metered-dose inhaler), and subjects inhaled hypertonic (4.5%) saline nebulized solution for periods of progressively increasing length (1, 2, 4, 8, and 16 min.). FEV_1 was measured 1 min after each inhalation. Increasing concentrations were administered with a MEFAR nebulizer (Markos, Monza, Italy). The collected sputum samples were examined within 2 h. Selected portions of the sputum sample originating from the lower respiratory tract were chosen through examination with an inverted microscope, were weighed, and then, processed using 1% dithiothreitol (Sigma Chemicals, Poole, UK). Total cell count and viability (Trypan blue exclusion method) were determined with a Burkers chamber hemocytometer. The cell suspension was placed in a Shandon cytocentrifuge (Shandon Southern Ltd., Runcorn, UK) and cytospin preparations were made at 450 rpm for 6 min. Cytospin slides were fixed with methanol, were stained with May-Grunwald-Giemsa for an overall differential cell count of 500 nucleate nonsquamous cells, and were examined, under oil immersion by light microscopy, at magnification of $400\times$, by an observer unaware of the clinical characteristics of the subjects. Only samples with a cell viability $>50\%$ and $<20\%$ squamous cell contamination were considered. The normal range for eosinophil count was $<1\%$.[16,17]

Serum ECP

Serum ECP assay was determined by CAP system FEIA (Pharmacia, Uppsala, Sweden) as previously described. The normal range for the assay is <16 μg/mL.[12–15]

Statistical analysis

The basal spirometric values, the peripheral blood eosinophil counts and the serum ECP levels were presented as mean ± SEM.

The MCh PC_{20}/FEV_1, and the induced sputum eosinophil counts were log normally distributed, log-transformed, and presented as geometric mean ± SEM. For log transformation, a value of 0.01% was assigned to a induced sputum eosinophil count of 0%.

The maximum PEF amplitude percent mean (maximum PEF A%M), expression of diurnal PEF variability, was derived from the maximum within-day PEF variability observed during the 14-days period after the first and before the second visit, measured as the difference between the highest and the lowest daily PEF measurements, and was expressed as a percentage of the mean PEF.

The comparison between the three groups were examined using Kruskal–Wallis test. For statistical analyses, a value of $P<0.05$ was considered statistically significant.

Sensitivity and specificity (with 95% confidence intervals), positive and negative predictive values, accuracy and likelihood ratios of positive and negative results were calculated using SYSTAT 10 software package for Windows (SPSS Inc, Chicago, IL). We consider for each analysis that the prevalence of asthma was 52.1%.

Finally, we used the following formulas to determine global sensitivity (Eq. (1)) global specificity (Eq. (2)) and global predictive positive value (Eq. (3)) of two tests used in succession (MCh and eosinophils-induced sputum).[18]

$$\text{global specificity} = \frac{\text{true positive to second test}}{\text{patients with diagnosis of asthma}}, \quad (1)$$

$$\text{global sensitivity} = \frac{\text{true negative to first test} + \text{true negative to second test}}{\text{patients without diagnosis of asthma}}, \quad (2)$$

$$\text{global predictive positive value (PPV)} = \frac{\text{true positive to second test}}{\text{total positive to second test}}. \quad (3)$$

Results

Subjects details are shown in Table 1. All the groups were sex- and age-matched. Atopy was demonstrated in 56% of the patients with bronchial asthma, in 2% of the patients with GERD and asthma-like symptoms, and in 6% of healthy control subjects.

Patients with GERD and asthma-like symptoms had received an asthma treatment, many of them were treated with regular oral theophylline and/or inhaled β_2 short-acting and/or oral corticosteroids (i.e. prednisone) therapy, but without clinically relevant results.

In Table 2 we reported the mean ± SEM of FEV_1/FVC ratio, maximum PEF A%M, peripheral blood eosinophil counts, and serum ECP levels and the geometric mean ± SEM of MCh PC_{20}/FEV_1 and induced sputum eosinophil counts. Significant differences were found between asthmatics patients and healthy control subjects ($P<0.001$) and between asthmatics patients and patients with GERD and asthma-like symptoms ($P<0.001$). No differences were found between healthy control subjects and patients with GERD and asthma-like symptoms.

Normal range and the distribution of individual values in the three groups considered for FEV_1/FVC ratio, maximum PEF A%M, MCh PC_{20}/FEV_1, induced sputum and blood eosinophil counts, and serum ECP levels are shown in Fig. 1(a)–(f).

The sensitivity, specificity, positive and negative predictive values and likelihood ratios of the test results are shown in Table 3.

The MCh PC_{20}/FEV_1 and induced sputum eosinophil counts were the most sensitive (both 90%) and specific (89% and 92%, respectively) tests.

The maximum PEF A%M was less valid than the identification of an obstructive spirogram, and the sensitivity of this parameter was low (20%) whereas the specificity was higher (83%). Blood eosinophils counts and serum ECP levels presented a high specificity (90% and 92%, respectively) but a low sensitivity (66% and 70%, respectively).

Finally, we calculated the global sensitivity, the global specificity, and the global PPV evaluating the presence of eosinophils in induced sputum only in subjects with MCh $PC_{20}/FEV_1 > 1500\,\mu g/mL$. The global sensitivity was 80%, the global specificity was 100% and the global PPV was 100%.

Table 1 Characteristics of the subjects.

	Healthy control subjects ($N = 25$)	Asthma patients ($N = 60$)	GERD patients ($N = 30$)
Age yr (range)	34.5 (19–55)	37.1 (23–57)	38.6 (26–57)
Men no.	12	48	21
Atopy (%)	6	56	2
Current smokers (%)	0	0	0
Inhaled steroid use (%)	0	38	0
Theophylline use (%)	0	20	26
Oral steroid use (%)	0	21	28
β_2-short acting on demand (%)	0	100	90
Proton pump inhibitors (%)	0	0	100
Prokinetic agent (%)	0	0	60

Table 2 Mean or geometric mean ± SEM of the evaluated parameters.

	Healthy control subjects	Asthma patients	GER patients
FEV_1/FVC ratio*	81.3 ± 1.3	76.6 ± 0.4	79.9 ± 0.5
Maximum PEF A%M*	9.1 ± 1.2	14.6 ± 0.6	10.5 ± 1.1
MCh PC_{20}/FEV_1†	1897.6 ± 96.1	304.7 ± 68.1	1957.8 ± 123.2
Blood eosinophils*	0.20 ± 0.02	0.46 ± 0.005	0.20 ± 0.016
Serum ECP levels*	4.6 ± 0.8	17.4 ± 0.8	5.6 ± 0.8
Induced sputum eosinophils†	0.13 ± 0.09	5.37 ± 0.38	0.14 ± 0.08

*Mean ± SEM.
†Geometric mean ± SEM.

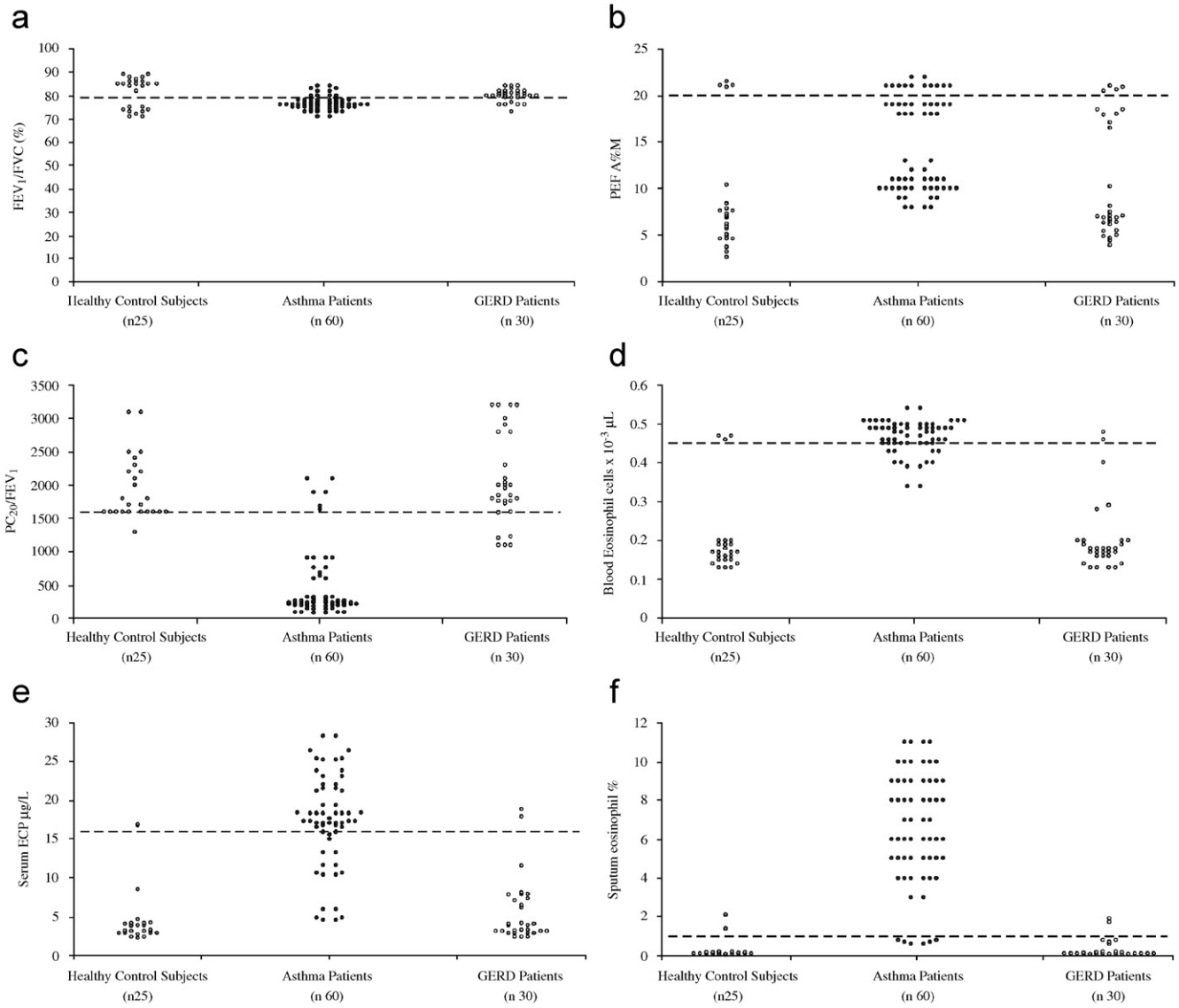

Figure 1 (a–f) Individual values for FEV_1/FVC ratio, PEF A%M, MCh PC_{20}/FEV_1, blood eosinophil counts, serum ECP levels, and induced sputum eosinophil counts, in each patient category.

None of the patients with bronchial asthma presented the combination of MCh $PC_{20}/FEV_1 > 1500\,\mu g/mL$ and presence of eosinophils in induced sputum, and none of the patients with GERD and asthma-like symptoms presented abnormal results for both and simultaneously of these parameters.

Discussion

This is the first study comparing the validity of different tests for the diagnosis of bronchial asthma with reference to a population that has a condition that is often confused with

Table 3 Measurement categories of various tests.

Test	Normal range	Sensitivity (CI95%)	Specificity (CI95%)	Positive predictive value %	Negative predictive value %	Likelihood ratio of positive result	Likelihood ratio of negative result
FEV_1/FVC	$>79\%$[7]	83 (71.4–91.7)	67 (53.2–79.3)	73	78	2.5	0.2
PEF A%M	$<20\%$[7]	20 (10.7–32.3)	83 (71.2–92.2)	57	48	1.2	0.9
Mch PC_{20}/FEV_1	$>1500\,\mu g/ml$[7,9]	90 (79.4–96.2)	89 (77.7–95.8)	90	89	8.2	0.1
Blood eosinophils	$<0.45\times10^{-3}/\mu L$[10]	66 (53.3–78.3)	90 (80.0–96.9)	88	71	7.3	0.3
Serum ECP	$<16\,\mu g/L$[15]	73 (61.9–83.7)	92 (82.4–97.9)	91	73	9.6	0.3
Induced sputum eosinophils	<1[16]	90 (79.4–96.2)	92 (82.4–97.9)	93	89	12.3	0.1

bronchial asthma (i.e. GERD with asthma-like symptoms). The results of this study indicate that, in adults with diagnosis of mild bronchial asthma, and normal or near normal basal spirometric values, MCh broncho-constrictor response (i.e. MCh PC_{20}/FEV_1) and the count of eosinophils in induced sputum are the most valid tests, in terms of both sensitivity and specificity, and, by implication, the most clinically useful tests for discriminating these patients from subjects with asthma-like symptoms by GERD. Making the distinction between bronchial asthma and asthma-like symptoms by GERD is an important problem in medical practice.[7] Patients reporting asthma-like symptoms by GERD are likely to be representative of a wider population with a condition that is commonly confused with bronchial asthma.[1–5]

GERD is particularly common in infants, but the problem may persist in many older children and in adults.[19] Mechanism of gastro-oesophageal acid-induced bronchoconstriction have been examined on a pathophysiologic basis, and include microaspiration, vagally mediated reflex, and enhanced bronchial reactivity. However, other mechanisms also may play a role in producing respiratory symptoms in response to gastro-oesophageal acid. For example, neurogenic inflammation, with tachykinin/substance P release, airway oedema and recruitment of polymorphonuclear leucocyte, resulting from acid stimulation, have been demonstrated.

An ambulatory 24-h pH study needs to be performed to confirm the diagnosis of GERD, both in children and in adults, evaluating total percent time $pH<4$ during the 24-h study period, upright acid exposure and supine acid exposure.[1,20,21]

Interestingly, the prevalence of GER, in people with bronchial asthma, is higher than in control populations. Patients with bronchial asthma may be prone to develop GER because of an increased pressure gradient between the abdominal cavity and the thorax, over-riding the lower oesophageal sphincter pressure barrier, alterations in crural diaphragm function, autonomic dysregulation, anti-asthmatic drugs use, and a high prevalence of hiatal hernia.[22] In our study 48% (29/60) of asthmatic patients presented GER evaluated with 24-h oesophageal pH study. However, we have not found any significant difference between asthmatic patients with GER and without GER, as regards to the symptoms and the parameters studied (data not shown).

In our study, the combination of the absence of bronchial hyper-responsiveness to MCh and of the absence of eosinophils in induced sputum exclude the diagnosis of bronchial asthma.[7] In fact, our data demonstrated the presence of eosinophils, both in peripheral blood and in induced sputum of the patients with bronchial asthma comparing with patients with GERD and asthma-like symptoms, can be important for the choice of the therapy. The lung inflammation in patients affected by GERD is predominantly a neutrophil-driven process, while that in bronchial asthma depends mostly on eosinophils and lymphocytes, although more severe forms of asthma tend to be associated with neutrophils as well.[5,23] In particular, in patients with GERD and asthma-like symptoms, the presence of macrophages and neutrophils, activated, in the lung, by the phagocitosis of inhaled gastric organic fat or proteins, led to an increased production of IL-8, having an active pathogenetic role in the recruitment and activation of neutrophils in the airways. Whereas, in severe asthma, epithelial damage has the potential to contribute to demonstrated neutrophilic inflammation through enhanced production of IL-8 via epidermal growth factor receptor-dependent mechanisms.[5,23]

So, we demonstrated that the presence of eosinophils in induced sputum can be a valid marker of bronchial asthma and it is more sensitive and specific than blood eosinophil counts. Similar findings have been reported by other authors.[5,24,25] Interestingly, the combination of the presence of MCh airway hyperresponsiveness and of eosinophilia in induced sputum presents a high specificity in patients with bronchial asthma.

In conclusion, we have demonstrated that the MCh PC_{20}/FEV_1 and the induced sputum eosinophil counts are the most sensitive and specific markers of mild bronchial asthma, able to discriminate asthma from asthma-like symptoms by GERD. Our findings suggest that, in patients with GERD and asthma-like symptoms, both MCh PC_{20}/FEV_1 and induced sputum eosinophil counts are necessary to support or exclude the diagnosis of bronchial asthma.

Acknowledgments

This study was supported by grants from Ministero Italiano Università e Ricerca (MIUR) (fondi ex 60%) to Gabriele Di Lorenzo and Pasquale Mansueto. No support has been received from the pharmaceutical industry.

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