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Exhaled markers in the monitoring of airways inflammation and its response to steroid's treatment in mild persistent asthma

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Abstract

The measure of inflammatory cytokines in the exhaled breath condensate has been recently proposed for use in monitoring asthma and the therapeutic response to steroids.

The aim of the present study was to investigate the usefulness of measuring exhaled IL-6, IL-4 and pH in mild persistent asthma. Furthermore the effects on these markers of inhaled steroids were assessed.

The study enrolled 28 asthmatic (15 males, 38±12 years) and 15 healthy subjects (5 males, 35±6 years). IL-6, IL-4 and pH were measured in the exhaled breath condensate of the subjects studied.

Significantly higher concentrations of IL-6 and IL-4 were observed in the breath condensate of asthmatic patients (7.1±1.1 and 64.4±8.3 pg/ml) compared to controls (2.7±0.6 and 31.7±3.5 pg/ml), $p<0.001$. Furthermore, exhaled IL-4 fell significantly after treatment with inhaled steroids for 6 months (47.9±3.2 pg/ml, $p<0.001$) while exhaled IL-6 did not (6.4±1.0 pg/ml, $p=0.8$). The exhaled pH turned out to be lower in asthmatic subjects than in controls (7.39±0.11 vs. 7.85±0.14; $P<0.001$) but trended towards control levels after steroid treatment (7.65±0.16, $P<0.001$).

We conclude that the measurement of exhaled IL-4 and pH in mild asthmatic subjects could be a useful way of monitoring their airway inflammation as well as their response to the treatment.

Keywords: IL-4; IL-6; pH; Asthma; Airway inflammation

1. Introduction

The pathogenesis of asthma is centred on airways inflammation. In fact, bronchial inflammation seems to be the *primum movens* that triggers airway obstruction and bronchial hyper-reactivity (Rees and Price, 1996).

Several indices, including serum eosinophils, eosinophil cationic protein (ECP) levels and bronchial responsiveness, have been used to study the mechanisms underlying inflammation in asthma and to explore the anti-inflammatory effects of steroids (Gruber et al., 1999; Sont et al., 1999).

In the past few years the measurement of various inflammatory cytokines has been proposed in the study of asthma. Airways inflammation is, in fact, orchestrated and perpetuated by several cytokines produced by activation of T helper lymphocytes (Wong et al., 2001a,b; Stankiewitz et al., 2002). One of these Th_2-type cytokines, interleukin (IL)-4 is over-expressed in asthma where it plays an important role determining eosinophilic inflammation and inducing bronchial responsiveness (Lee et al., 2001; Webb et al., 2001; Zimmermamm et al., 2003).

Several proinflammatory cytokines, including IL-6, the IL-9, the IL-12, the IL-13, the IL-15, the IL-17 and the IL-18,

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seem to be involved in inducing and perpetuating the inflammatory cascade in allergic asthma (Wong et al., 2001a,b). IL-6 in particularly, is responsible for neutrophils recruitment and activation and seems to be able to direct Th_2 immune response (Heijink et al., 2002; Woodruff et al., 2001; Stankiewitz et al., 2002).

Several more or less invasive techniques have been used to obtain samples when looking for these cytokines with a view to monitoring airways inflammation in asthma in an easy manner.

Most of these methods present some challenges: i) lung biopsy is extremely invasive; ii) broncho-alveolar lavage is not well accepted by patients and gives problems in terms of dilution; iii) sputum induced through hypertonic saline inhalation may itself cause airways inflammation and decreased lung function (Carpagnano et al., 2003; Shahid et al., 2002). The exhaled breath condensate has been recently proposed as a reliable and non-invasive method. It is easy to collect and has a good acceptance level on the part of the patients. Many inflammatory mediators, as well as the IL-4, IL-6, leukotriene (LT)-B_4 and nitric oxide (NO) products, have been measured in samples obtained by this method (Carpagnano et al., 2003; Hanazawa et al., 2000; Shahid et al., 2002). Recently breath condensate pH measurement has been proposed as a new promising inflammatory marker (Kostikas et al., 2002).

The aim of the present study was to investigate the usefulness of measuring the concentrations and levels of some non-invasive markers of airways inflammation, IL-4, IL-6 and pH in the exhaled breath condensate of subjects with asthma.

Furthermore this study was designed to verify the validity of these markers in monitoring the response to steroids of these subjects. To this end some asthmatic patients enrolled (group A) were given a 6-month treatment with inhaled steroids. In addition the study also analysed the possible variations in the exhaled condensate IL-4, IL-6 concentrations and pH levels to compare them with changes in bronchial responsiveness and in other regularly used systemic indices of inflammation, including plasma eosinophils, IgE and eosinophilic cationic protein (ECP).

2. Methods

2.1. Study population

The population studied was made of 28 patients affected by perennial allergic mild persistent asthma (15 males, 38 ± 12 years) and 15 healthy subjects with no history of lung disease (5 males, 35 ± 6 years).

Both the patients and the controls were Caucasian subjects selected among the patients of the Respiratory Disease Division of the Hospital of San Pietro Vernotico (Brindisi, Italy). Written informed consent was obtained from all subjects. The study was approved by the Institutional Ethics Committee. The diagnosis of asthma and the assessment of its severity were done at the time of the enrolment according to Global Initiative for Asthma (GINA) (Von Mutius, 2000). Inclusion criteria for asthmatic subjects: history of asthma >1 year, age between 18 and 65 years, diurnal symptoms of asthma > once per week and nocturnal > twice per month, $FEV_1 \geq 80\%$ and PEF variability between 20% and 30%, PD_{20} <800 μg. Exclusion criteria for asthmatic subjects: smoking habit, history of nasal polyposis, sinus disease, bronchial or respiratory tract infection or a severe exacerbation of asthma resulting in hospitalization in the month preceding the test and/or during the period of the study, history of cancer, immunosuppressive therapy, pregnancy, mental retardation. Exclusion criteria for healthy controls: smoking habit, pulmonary diseases, immunosuppressive therapy, pregnancy, and mental disorders.

At the time of their enrolment in the study population (T_0), the medical history of the subjects was taken and their general physical conditions were assessed. The subjects also underwent skin prick test (and allergy score), a radioallergosorbent test (RAST) assay, circulating eosinophils count, IgE and ECP, pulmonary function test [forced expiratory volume in 1 s (FEV_1); forced vital capacity (FVC)], blood gas analysis, methacholine challenge and breath condensate collection. After the first visit 14 asthmatic subjects started therapy with steroids (inhaled fluticasone 500 μg/day) (group A) while the other 14 remained untreated (group B). Then either all asthmatic (group A and group B) or healthy subjects were seen again and underwent all previous tests, after 2 months (T_1) and 6 months (T_2) of steroid administration.

During the study only further inhaled short-acting B2-agonist was allowed. None of patients included was treated with oral steroids. The patients had taken at least 80% of his medication in order to be considered compliant. All adverse events were recorded and their relationships to tested medication were assessed.

2.2. Study design

This non-controlled study was designed to investigate the worth of exhaled IL-4, IL-6 and pH in monitoring subjects with asthma and their response to inhaled steroids treatment. We considered significative for an improvement a drop of at least 10% of exhaled interleukins' concentrations.

2.3. Assessment of the atopic status

Skin prick tests were performed as previously described (Dreborg and Frew, 1993).

Serum total IgE concentrations were determined in all subjects by latex nephelometry (Behring Institute, L'Aquila, Italy). Eosinophil count on peripheral blood samples was also performed. Allergen-specific IgEs were measured by FEIA-CAP System (Pharmacia, Uppsala, Sweden) (Sterk et al., 1993).

2.4. Methacholine bronchial challenge

A rapid dosimetric methacholine bronchial provocation test was performed in all subjects according to Sterk et al. (1993) 2 days before breath condensate collection. All bronchial challenges were performed at the same hour of the day under the same environmental conditions.

We considered positive for asthma a PD_{20} <800 μg.

Table 1
Clinical and functional data in asthmatic subjects and healthy controls

	Asthmatic subjects ($n=28$)	Healthy subjects ($n=15$)
FEV_1 (%predicted)	81.5±1.7	101±18
FVC (%predicted)	95.5±2.1	119±9
PaO_2 (mmHg)	84.8±10.6	96.1±2.7
$PaCO_2$ (mmHg)	36.1±3.2	36.3±2.5
House dust mites' sensibilization (n)	20	0
Grass pollens' sensibilization (n)	5	0
Cat furs' sensibilization (n)	3	0
IgE (kU/L)	164±128	11±6
ECP (ng/mL)	110±115	6.2±2.1
Blood eosinophil count ($\times 10^9$/l)	307±187	170±40
MethacholinePD_{20} (μg)	85.6 (geometric mean)	>1600

2.5. Lung function

Pulmonary function tests were performed within 1 day of the measurement of the breath condensate using a spirometer (Sensormedics, USA).

2.6. Exhaled breath condensate

The exhaled breath condensate was collected using a condenser (EcoScreen, Jaeger, Wurzburg, Germany). The subjects breathed through a mouthpiece and a two-way non-rebreathing valve, which also served as a saliva trap. They were asked to breathe at a normal frequency and tidal volume, wearing a nose clip, for a period of 10 min. If subjects felt saliva in their mouth they were instructed to swallow it. Condensate, at least 1 ml, was collected as ice at −20 °C, transferred to Eppendorf tubes and immediately stored at −70 °C. Samples were analysed within 3 months from collection.

To exclude saliva contamination amylase activity was analysed in exhaled breath condensate.

2.7. Measurement of IL-4 and IL-6

A specific enzyme immunoassay (EIA) kit (Cayman Chemical, Ann Arbor, MI, USA) was used to measure IL-4 and IL-6 concentrations in breath condensates. The IL-6 assays was validated directly by gas chromatography/mass spectrometry (Carpagnano et al., 2003). The intra-assay and inter-assay variability was ≤10%. The detection limit of the assays was 20 pg/ml and 1.5 pg/ml, respectively.

2.8. pH measurement

A stable pH was achieved in all cases after deaeration/decarbonation of breath condensate specimens by bubbling with argon (350 ml/min) for 10 min, as previously reported (Kostikas et al., 2002). pH was then measured within 10 min of condensate collection by means of a pH meter (Corning 240, Science Products Division, New York with a 0.00 to 14.00 pH range and a resolution/accuracy on the order of 0.01±0.02 pH).

2.9. Statistical analysis

Data were expressed as arithmetic means±S.D.; only the value for PD_{20} was expressed as geometric mean. Mann–Whitney tests were used to compare groups and correlations between variables were performed using Spearman's rank correlation test. Significance was defined as $P<0.05$. Cytokines' EIA analysis of two aliquots of the some sample (for all 10 healthy subjects analyzed) was repeated by the some operator with two different EIA kit in the same day. A Bland and Altman analysis was used to assess the level of agreement between the measurement of the same samples (Bland and Altman, 1996).

3. Results

Clinical and functional data of asthmatic and healthy subjects are reported in Table 1.

The FEV_1, the ECP concentrations and the provocative dose of methacholine causing a 20% fall in FEV_1 (MethacholinePD_{20} ($MhcPD_{20}$)) showed an improvement in asthmatic patients (group A) after therapy with steroids (T_0 vs. T_1 and T_2). PaO_2, $PaCO_2$, IgE, blood eosinophils did not change during the course of inhaled steroid therapy in asthmatic (Table 2).

3.1. Interleukin-6

IL-6 was measurable in the breath condensate of all subjects. Concentrations were significantly greater in asthmatic patients (7.1±1.1 pg/ml) than in healthy subjects (2.7±0.6 pg/ml), $P<0.001$ (Fig. 1A). However, they did not decrease after treatment with inhaled steroids (group A) either for 2 months (T_1) (6.8±1.1 pg/ml, $P=0.3$) or for 6 months (T_2) (6.4±1.0 pg/ml, $P=0.8$) (Fig. 2A). No changes in exhaled IL-6 concentrations were observed at T_1 and at T_2 in group B and healthy subjects. No correlation was found between and across exhaled IL-6 concentrations and lung function parameters (FEV_1 and FVC), circulating eosinophils count, IgE and the ECP concentrations, and the MethacholinePD_{20}.

Reproducibility of exhaled IL-6 measurements was assessed in 10 non-smoking normal (6 males, 35±7 years) subjects. In the majority of measurements, the differences between the two IL-6 values remained within ±2 S.D. (mean difference of −0.03±0.24 pg/ml). The coefficient of variation for IL-6 measured was 5.9%.

Table 2
Clinical and functional data in asthmatic subjects treated with steroids

	Asthmatic subjects (T_0)	Asthmatic subjects (T_1)	Asthmatic subjects (T_2)
FEV_1 (%predicted)	82.0±1.8	88.2±5.6[a]	88.5±4.4[a]
FVC (%predicted)	95.2±2.3	97.3±5.8	98±15
PaO_2 (mmHg)	84.6±11.1	85±10	84±10
$PaCO_2$ (mmHg)	35.9±3.8	37±4	36±3
IgE (kU/L)	162±126	160±80	166±70
ECP (ng/mL)	107±113	28.4±21.9[b]	19.7±16.9[b]
Blood eosinophil count ($\times 10^9$/l)	309±185	280±150	290±90

Definition of abbreviations: FEV_1=forced expiratory volume in 1 s; FVC=forced vital capacity; ECP=eosinophil cationic protein. All data are expressed as means±standard deviation.

[a] T_0 vs. T_1–T_2=$p<0.05$.

[b] T_0 vs. T_1–T_2=$p<0.01$.

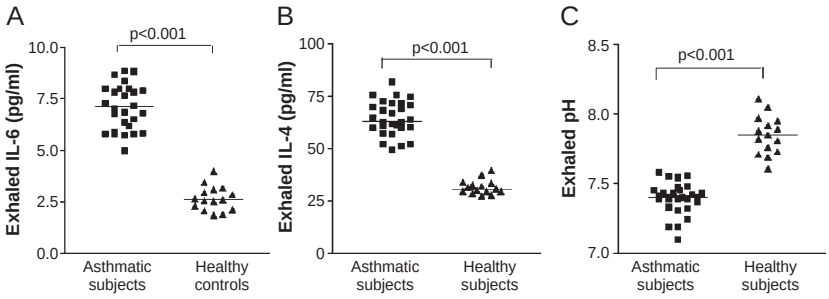

Fig. 1. Concentrations of exhaled IL-6 (A), IL-4 (B) and pH (C) in asthmatic and healthy subjects.

3.2. Interleukin-4

IL-4 was measurable in the breath condensate of all subjects. Concentrations were significantly greater in asthmatic patients (64.4±8.3 pg/ml) than in healthy subjects (31.7±3.5 pg/ml), $P<0.001$ (Fig. 1B). However, they started to decrease significantly after treatment with inhaled steroids (group A) for 2 months (T_1) (51.8±5.5 pg/ml, $P<0.005$) to further fall after 6 months (T_2) (47.9±3.2 pg/ml, $P<0.001$) (Fig. 2B). Twelve asthmatic subjects showed a reduction of IL-4 concentrations in exhaled breath condensate >10%. No changes in exhaled IL-4 concentrations were observed at T_1 and at T_2 in group B and healthy subjects. Positive correlations were observed between exhaled IL-4 levels and plasmatic eosinophils ($r=0.7$; $P<0.005$), ECP ($r=0.6$, $P<0.05$), IgE ($r=0.7$, $P<0.005$) and exhaled IL-6 ($r=0.7$, $P<0.001$). The correlation between IL-4 and IL-6 was present also at T_1 but was less significant ($r=0.5$, $p<0.05$).

Furthermore a negative correlation was found between exhaled IL-4 and $MhcPD_{20}$ ($r=-0.5$, $P<0.05$) while no correlation was observed with lung function parameters (FEV_1 and FVC).

Reproducibility of exhaled IL-4 measurements was assessed in 10 non-smoking normal (6 males, 35±7 years) subjects. In the majority of measurements, the differences between the two IL-4 values remained within ±2 S.D. (mean difference of −0.03±1.05 pg/ml). The coefficient of variation for IL-4 measured was 4.2%.

3.3. Exhaled pH

pH was measurable in the breath condensate of all subjects. pH levels were significantly lower in asthmatic patients (7.39±0.11) than in healthy subjects (7.85±0.14), $P<0.001$ (Fig. 1C). However, they increased slightly after treatment with inhaled steroids (group A) for 2 months (T_1) (7.41±1.15) and significantly after 6 months (T_2) (7.65±0.16, $P<0.001$) (Fig. 2C). No changes in exhaled pH levels were observed at T_1 and at T_2 in group B and healthy subjects. A negative correlation was found between exhaled pH, circulating eosinophils ($r=-0.9$, $P<0.0001$) and exhaled IL-4 ($r=-0-5$, $P<0.05$) but not with exhaled IL-6, lung function parameters (FEV_1 and FVC) and the $MhcPD_{20}$.

The reproducibility of orally exhaled pH measurements was assessed in 10 healthy subjects (6 males, 35±7 years). In the majority of measurements, the mean difference between the two measurements was −0.01±0.4. The coefficient of variation for exhaled breath condensate pH was 0.4%.

4. Discussion

This study showed significantly higher concentrations of IL-6, IL-4 and lower levels of pH in the breath condensate of asthmatic subjects than in healthy controls. Exhaled IL-4 exhibited a reduction after 2 months of daily administration of inhaled corticosteroids that further decreased after 6 months of treatment. By contrast, exhaled IL-6 did not follow the downward trend.

Exhaled pH increased slightly after treatment with inhaled steroids for 2 months and significantly after 6 months. However no changes in exhaled IL-6, IL-4 and pH were observed at T_1 and at T_2 in untreated asthmatic and healthy subjects. Exhaled IL-4 positively correlated with exhaled IL-6, $MhcPD_{20}$, plasmatic ECP, IgE and eosinophils concentrations. Instead, the exhaled pH showed a negative correlation with exhaled IL-4 and circulating eosinophils.

Airways inflammation plays a key role in the pathogenesis of asthma (Rees and Price, 1996). The diagnosis and the monitoring of this disease are however commonly based on clinical observation on lung function tests and bronchial responsiveness that, however, do not give a direct measure of the airways inflammation (Brand et al., 1999). The evaluation of the inflammatory markers present in the

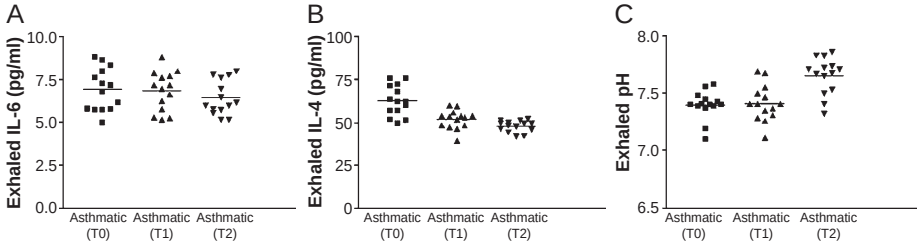

Fig. 2. Concentrations of exhaled IL-6 (A), IL-4 (B) and pH (C) in asthmatic patients (group A) before (T_0) and after 2 (T_1) and 6 months (T_2) of inhaled corticosteroids treatment.

exhaled breath condensate of subjects with asthma could obviate this limit as these markers reflect directly the airways inflammation. Furthermore, unlike plasma measurement, dosing these inflammatory markers directly in the airways avoids systemic metabolism.

In this study we measured two inflammatory cytokines, IL-6 and IL-4, in the exhaled breath condensate of subjects with mild persistent asthma.

Our group had already measured exhaled IL-6 in subjects with different respiratory diseases suggesting a crucial roe for this marker in neutrophilic airways inflammation (Carpagnano et al., 2002a,b, 2003). However, this cytokine has never been measured in the exhaled breath condensate of asthmatic subjects although high concentrations of it have already been described in the serum, broncho-alveolar lavage fluid and in the culture supernatants of subjects with severe asthma (Krishnaswamy et al., 1993; Wong et al., 2001a,b). In this study we observed an increase in the exhaled IL-6 levels of the asthmatic subjects enrolled which was completely unexpected considering the specific pattern of eosinophilic inflammation that usually characterizes mild asthma (Jakatonov et al., 1998). The possibility that some patients with mild to moderate asthma may have a neutrophilic airway inflammation has already been advanced by Green et al. who identified a subgroup of patients who also seemed to respond less well to treatment with inhaled corticosteroids (Green et al., 2002). Our patients, unlike those of the study of Green et al., were observed to improve after inhaled corticosteroids. However, the exhaled IL-6 levels in their airways remained unchanged after inhaled corticosteroids treatment thus supporting the notion under which prolong neutrophils survival by inhibition of apoptosis (Stankiewitz et al., 2002; Wong et al., 2001a,b). Further investigations on IL-6 and other neutrophilic markers present in the airways of mild asthmatic subjects are required to verify our assumption of the occurrence of a low-level neutrophilic inflammation in mild asthma as well as to improve our rating of this subgroup of patients.

IL-4 has already been used to study eosinophilic inflammation in asthma. Elevated concentrations of this cytokine have been observed in the cultures of blood mononuclear cells and in the supernatants, in the serum and in the broncho-alveolar lavage of subjects with bronchial asthma (Akpinarli et al., 2002; Stankiewitz et al., 2002; Wong et al., 2001a,b). Recently, high concentrations of IL-4 have been observed also in the exhaled breath condensate of children with mild to moderate asthma (Shahid et al., 2002). When confirming these data, we found, for the first time, elevated concentrations of IL-4 in the exhaled breath condensate of asthmatic adults. However, the values of exhaled IL-4 were slightly higher in asthmatic adults than those reported by Shahid et al., but this is likely to be influenced by age. In fact, long-term exposure to air pollution and/or cigarette smoke due to the age is likely to result in a higher grade airway inflammation reflected by more elevated concentrations of exhaled IL-4 (Hiramatsu et al., 2003; Xu et al., 2004). Consistently with the results of Shahid et al., we found that steroids treatment significantly reduces exhaled IL-4 levels in asthmatics (Shahid et al., 2002). A drop seems to be already present after 2 months and gradually rise over a 6-month period, although not reaching normal values.

We considered significative a drop of IL-4 concentrations in exhaled breath condensate of 10%. This is one limit of our study, to have defined arbitrary the level of reduction to consider significative. However, data in literature are not available and considering the variability of EIA test used we retained opportune to fix this level of reduction.

The validity of IL-4 measure in the monitoring of the response to steroid treatment had been already demonstrated in a previous trial where the serum concentrations of this cytokine had been used to allow withdrawal of steroids (Visser et al., 2002).

In this study, consistently with the results of Shirai, although significative differences in term of IgE and plasmatic eosinophils weren't find after steroids, we observed a positive correlation between IL-4, IgE and plasmatic eosinophils which substantiates the already known role of this cytokine in the immunoglobulin isotype switching to IgE and adhesion of eosinophils to endothelium (Koh et al., 2002; Shirai et al., 2003). This last correlation might furthermore mean that equilibrium does exist between eosinophilic flogosis in the airways and plasma in asthmatic subjects. However, this correlation needed confirmation on larger number of subjects.

In the present study exhaled IL-4 was also positively correlated with exhaled IL-6 thus confirming previous evidence on an upregulation of IL-6-mediated IL-4 expression (Heijink et al., 2002).

In order to explore better airways inflammation in asthma we also measured the pH of the exhaled breath condensate of the subjects enrolled in view of the great interest recently generated by pH as a non-invasive and reproducible inflammatory marker (Kostikas et al., 2002). Consistently with previous studies we found more acid pH values in the group of asthmatic subjects than in controls although these values tended to return to norm after steroids treatment (Hunt et al., 2000). A negative correlation was also observed between exhaled pH, exhaled IL-4 and plasma eosinophils. These correlations support our previous suggestion (unpublished) that eosinophils are not extraneous to the lowering of pH. These cells in fact, contain vacuolar (V) H-ATPase activity in their vesicles which is responsible for an increased H^+ release (Chavalittamrong et al., 1979; Kurashima et al., 1996) and for the eosinophil peroxidase in their granule matrix that, in the presence of H_2O_2, is able to oxidize halides and form highly reactive hypohalous acids (Adolphson and Gleich, 1995).

The eosinophilic pathway, as assessed by differential count and ECP dosage, is reported as predictive of the

response to steroids (Meijer et al., 2002). Inhaled corticosteroids have, in fact, been shown to decrease ECP and eosinophils in induced sputum and blood (Pizzichini et al., 1997). Furthermore, inhaled corticosteroids seem to reduce bronchial responsiveness and improve lung function (Brand et al., 1999; Freezer et al., 1995; Jenkins and Woolvock, 1998; Oga et al., 2001).

In agreement with the data reported in the literature, we observed an improvement of lung function values and symptoms, as well as a progressive reduction in ECP and progressive increase of $MhcPD_{20}$ after 2 and 6 months of inhaled corticosteroids. The reduction of bronchial responsiveness observed in this study, is early and particularly sensitive to inhaled corticosteroids opposite to that of the exhaled IL-4 and IL-6 that resulted more gradual and incomplete. However, further studies on longer inhaled corticosteroids administration in mild persistent asthmatic subjects are needed to assess an eventual normalization of exhaled IL-4 and IL-6 after steroidal treatment. It is possible that 6 months of therapy could be sufficient to correct IL-4 levels.

Further studies will have to investigate the length of the therapy and the dosage of corticosteroids. further studies (controlled and randomized) to confirm the role of corticosteroids.

Dependence of bronchial responsiveness on inflammation was already highlighted some years ago when its relationship with immunologic imbalances (increased T4/T8 ratio) and other inflammation features (increased NO output) was shown (Laprise et al., 1999; Nogami et al., 2003). The positive correlation that we observed between $MhcPD_{20}$ and exhaled IL-4 further confirms this dependence in mild asthma. The lack of correlation of $MhcPD_{20}$ with exhaled neutrophilic IL-6, which is a major feature of severe asthma, once again provides evidence of the fact that bronchial responsiveness is made up of two main components: a reversible one, which depends upon inflammation, and an irreversible one, which depends upon remodelling (Ichinose et al., 2000).

Finally, no correlation between pH, IL-6 and IL-4 values and the values of FEV_1 and FVC was observed thus confirming the generally poor value of lung function as a predictor of active inflammation. Exhaled breath condensate pH, IL-6 and IL-4 are likely to reflect the intensity of the ongoing inflammation, while lung function tests are, at least in part, related to the long-standing lung damage.

In conclusion, this study shows that IL-4 and pH measures in the exhaled breath condensate of mild asthmatic subjects truly reflect airways inflammation and reveal the response to inhaled steroids. Considering the complete non-invasiveness of these measures, the assessment of these markers might be useful in the monitoring of asthma and of its anti-inflammatory treatment which, for the time being, still remains linked to lung function tests, systemic inflammatory markers, and bronchial responsiveness.

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