Evaluation of inflammation by level of Interleukin 4 in exhaled breath condensate in patients affected by bronchial asthma treated according to GINA guidelines

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Abstract

GINA guidelines suggest that optimal asthma control can be gained by regular monitoring of symptoms, rescue medication use, airways obstruction and variability upon time of airways flows. Our aim was to check if therapy according to GINA rules is able to lower airways inflammation, measured by the level of IL4 in expired breath condensate (EBC). One hundred patients affected by bronchial asthma in different levels were recruited as they come to the ambulatory ward. They were cured according to GINA guidelines for one year, using inhaled fluthicasone as inhaled steroid. Symptoms were monitored by asthma control test score (ACT), airways obstruction by FEV1, bronchial reactivity by PD20, airways inflammation by IL4 in EBC. ACT showed complete control in the first three GINA levels, improving incompletely in the GINA level 4 (from 20±2 to 21±12; from 17±2 to 22±2; from 14±4 to 18±3; from 10±3 to 14±3 units respectively in the first to fourth levels of asthma). FEV1 improved, but both baseline and after therapy levels were worst in severe persistent asthma (from 96±5 to 96±4; from 92±6 to 93±3; from 74±6 to 83±8; from 45±10 to 60±5% of normal standards respectively from the 1st to 4th level). PD20 and IL4 were fairly normalized by therapy in the s and third levels, improved in the last one (PD20 from 437±329 to 460±269, from 364±308 to >1600, from 436±252 to 890±220; from 45±25 to 60±40 mcg respectively, IL4 in EBC from 60±6 to 40±12, from 65±10 to 41±9, from 72±8 to 45±6, from 78±20 to 52±5 respectively). IL4 and PD20 were significantly related. Experimental data allowed the assessment of the correlation of inflammation with bronchial reactivity and the relevance of addressing therapy upon IL4. Severe persistent asthma behave as a different entity with worst baseline inflammation, partially refractory asthma and persistent inflammation, needing specific immunologic weapons. Bronchial inflammation was fairly reduced but not normalized after one year of therapy.

Introduction

In the therapy of bronchial asthma (BA), current GINA guidelines[1] assign a specific level of gravity according to symptoms, airways obstruction and peak flow (PEF) variability. GINA levels suggest the appropriate intensity of therapy necessary for the treatment to achieve good asthma control. Thereafter changes of therapy depends on routine follow-up and reassessment to determine if a patient is well-controlled, partly controlled or uncontrolled. BA is characterized by persistent inflammation, reversible obstruction, and bronchial hyper-reactivity,[1-3] weakly related with symptoms FEV1 and PEF variability .

Improvement of knowledge about underlying inflammation can be obtained using the bronchial hyper reactivity as a monitor.[4,5] However, the goals of therapy would be better achieved by addressing the therapy using a direct marker of bronchial inflammation.

Within allergic inflammation IL4 is an appropriate marker since it plays a major role in priming naive T0 lymphocytes to Th2 with activation of the cascade IL4, IL5, IL9, IL13,[2,6] while IL5 is mainly responsible of eosinophils recruitment and both IL9 and IL13 for bronchial hyperreactivity. The less invasive, most reproducible and most effective way to measure bronchial inflammation is to perform the dosage of IL4 in Expired Breath Condensate (EBC), as already shown in other studies.[3,7-10]

Our aim is to assess if long term therapy, conducted according GINA guidelines in compliant BA patients of different severity is able to lower and normalize bronchial inflammation assessed by means of IL4 in EBC.

Materials and Methods

One hundred non smoking caucasian patients which never smoked but were affected by allergic bronchial asthma, according to GINA guidelins , were enrolled as they were consecutively refered to the outpatient clinic of the respiratory disease division. They were symptom free without bronchial airways infection in the previous month. Informed consent was obtained. Compliance and motivation were obtained through effective information and periodic calls and it was checked by careful observation of drug consumption (weighting the canisters and counting the prescriptions). Only patients compliant to the assigned therapy were studied. A group of 20 healthy subjects were submitted to the same procedures as control group. For ethical reasons it was not possible to compare the results to a set of patients kept without therapy for one year.

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After the assessment of allergy by prick tests and IgE level, a wash out period lasting one month was allowed, to avoid the effects of previous drugs, treating the airways obstruction by salbutamol inhalation only at needing. Symptoms control, airways obstruction, degree of airways reactivity, level of IL4 in EBC were assessed and the patient was assigned to a specific GINA level. Therapy was administered according to GINA rules and the measurements were repeated after one year of therapy. During the period of therapy, evaluation of adherence to therapy was determined by periodic visits and detailed information. Patients affected by bronchial intermittent asthma were treated by inhalation of salbutamol spray as needed. Patients with persistent asthma were treated by fluthicasone propionatae (FP) and salmeterol (25 mg+250 umg twice/day). In case of moderate asthma, montelukast (10 mg/day) was assigned in addition; theophylline (20 0mgx2/day) was prescribed only as needed. In the last step zarfilukast (20 mgx2/day) plus salmeterol-fluticasone (25 mg+250 mcg twice a day) association, theophylline and prednisolone (5 mg/day) were used (Table 1). Within one year some patients experienced seasonal relapses due to allergenic load and were treated according to GINA guidelines. During measurement they were free of seasonal allergen load.

The Asthma Control Test (ACT) was administered according to literature[11,12] to check for the control of symptoms. Skin prick tests were performed (according the EAACI suggestion) by a panel of allergen extracts (ALK-Abello,

Spain) responsible for allergy in the Mediterranean regions, measuring wheal flare after fifteen min in the forearm. The diameter was compared to saline, as negative control, and histamine, as a positive control. A positive reaction was considered by the presence of one or more wheal flares larger than 5 mm. Circulating IgE was dosed by immunocolorimetric method (Beckman, USA).

Forced expired vital capacity (FVC),forced expired volume in one s (FEV1), Peak expiratory flow rate (PEFR) were measured by turbine spirometer (MIR, Italy) and the data were referred to ERS normal standards. Bronchial Provocation Test (BPT) was conducted according to the stepwise cumulative inhalation of buffered methacholine chloride (Mefar dosimeter, Brescia, Italy) to trace a semi logarithmic plot of FEV1 deflection against the cumulative inhaled dose. PD20 was assumed as the provocative dose causing 20% decrease of FEV1, interpolated from the relation between natural logarithm of the dose and percentage of fall of FEV1. The test was conducted according the ERS standards.

Exhaled breath condensate was collected by a condenser (Ecoscreen, Jaeger, Hoechberg, Germany), while patients breathed for ten min, using a nose clip at normal breathing rate and tidal volume through a two way non re breathing valve. A saliva trap was put in the expiration line and the dosage of amylase allowed the exclusion of samples with mouth secretion contamination. The volume of the condensate (average 2cc) was collected in a plastic tube and kept at a -70°C in Eppendorf tubes. The dosages were examined within three months from collection. A double sandwich enzymatic immunoassay test was used to measure IL4 (EIA), (Cayman Chemical, Ann Arbor, MI, USA). The intra-assay variability was within 10%. The dosages were repeated twice and the measured coefficient of variation was 5%.

Data were expressed as mean± standard deviation. The comparison of means was conducted by means of T-test by statistical package (Epistat, Richardson,Texas, USA). Least square method analysis allowed the analysis of regressions between variables. Differences are reported as significant for a level of probability (P) less than .05 and highly significant if less than .001. The study was approved by the Ethic Committee of Ninetto Melli Hospital .Written informed consent was obtained from all patients. The paper was approved by the Institutional Review Board (IRB).

Results

The majority of the 100 patients referred for asthma care to our clinic were found to be GINA levels 1-3. The average age of patients increased in the consecutive steps from 28 years in the first level to 52 years in severe persistent asthma. Biometry was similar. Forced Vital Capacity (FVC) was significantly lower in severe persistent asthma. Airways obstruction (FEV1) was significantly impaired on mild and severe persistent asthma. Atopy, assessed by IgE and blood eosinophils, showed the highest values in severe persistent asthma.

ACT scores decreased in the successive levels; moderate persistent and severe persistent asthma scores were significantly lower than under intermittent. After therapy ACT scores showed a fairly constant and significant increase in each level, but the difference between severe persistent and the other levels was still significant (from 20±2 to 21±12; from 17±2 to 22±2; from 14±4 to 18±3; from 10±3 to 14±3 units respectively in the first to fourth levels of asthma). After therapy the control of symptoms (ACT) was almost completely achieved in the first two levels of severity, quite satisfactory in the third one and improved but not completely controlled in the last one.

Airways bronchial obstruction (FEV1) was significantly worse in moderate and severe persistent asthma before therapy. The treatment significantly increased FEV1 in severe persistent asthma. The comparison within the different levels after therapy showed a persistent airways obstruction in severe persistent asthma (from 96±5% to 96±4%; from 92±6% to 93±3%; from 74±6 to 83±8%; from 45±10% to 60±5% of normal standards respectively from the 1st to the 4th level). Airways obstruction (FEV1) improved in persistent asthma, but the difference between severe persistent and the other levels was still significant.

The bronchial hyperreactivity (PD20) showed a significant difference between each level, worsening progressively. Therapy did not improve PD20 in the first level, but significantly improved in the other three consecutive levels, although the PD20 still remained significantly lower in severe persistent asthma (PD20 from 437±329 to 460±269, from 364±308 to >1600, from 436±252 to 890±220;

Table 1. Features of patients and therapy used.

	Healthy controls	Intermittent	Mild persistent	Moderate persistent	Severe persistent	Units
Age	35±15	28±13	35±15	41±15	52±14	Years old
Gender	10:10	11:12	20:20	9:12	7:9	M:F
Height	168±5	170±5	170±7	168±5	165±6	Cm
BMI	26±7	25±6	28±7	24±5	24±4	Cm^2/kg
FVC	110±7	98±2	97±2	85±4	75±10	%pred±sd
FEV1	105±4	96±5	92±6	74±6	45±10	%pred±sd
IgE	70±30	389±510	254±300	291±400	419±475	U/lt
Eos	120±25	372±194	325±208	352±224	415±442	Cell/cc
Salbut. rescue	40±10			20±20	40±20	Mg/day
Salmeterol			50	50	50	Mg/day
Fluticasone			250	500	500	Mcg/day
Montelukast				10		Mg/day
Zarfilukast					60	Mg/day
Theophylline				400±100	400±100	Mg/day
Prednisolone					7±7	Mg/day
N.	20	23	40	21	16	N

BMI, body mass index; FVC, forced vital capacity; FEV1, forced exp. volume in one s; Eos, circulating eosinophils/cc; Salbut. rescue, dose of salbutamol inhaled to resume bronchospasm.

from 45±25 to 60±40 mcg respectively). IL4 in EBC was significantly increased in severe persistent asthma (IL4 in EBC from 60±6 to 40±12, from 65±10 to 41±9, from 72±8 to 45±6, from 78±20 to 52±5 pg/mL respectively). Allergic inflammation, according to IL4 levels, is fairly reduced in the first three levels, but it was still active in the level four. Allergic inflammation and bronchial hyper reactivity showed a reciprocal trend.

Discussion

The current study demonstrated that conventional therapy conducted according to GINA guidelines in compliant patients is able to control bronchial inflammation in the first three asthma levels with a trend toward normalization. The last level requires additional therapeutic weapons.

Several investigative tools have been proposed for the assessment of the degree of inflammation in bronchial asthma such as the levels of Nitric Oxide (NO) and Carbon Monoxide (CO) in the expired gas, the examination of induced sputum (IS) for assay of interleukins and mediators as well as the count of eosinophils, the examination of Broncho- Alveolar Lavage (BAL) and bronchial biopsy. We disagree with the use of IS since the inhalation of hypertonic solutions for induction of secretions determines bronchospasm; expired gas analysis seems almost limited, while EBC looks suitable because it is well tolerated and easily reproducible, despite technological problems, currently under standardization and solution.[13]

Within the different monitors of inflammation we chose IL4 because it plays a pivotal role in the allergic response. It determines the prevalence of allergy through the genic polimorphism[14] and through the ratio between the IL4 receptor (active upon the cells surface) and the soluble IL4 receptor (inefficient). IL4 regulates an immunologic network influencing mastocytes function (increasing IgE affinity), B lymphocytes (decreasing (?) IgE affinity), T lymphocytes (priming T0 into Th2), fibroblasts (it regulates the secretion of pro-collagen I) and endothelial cells (up-regulation of vascular cell adhesion molecule-1 (VCAM-1).[2,3,6,14-20] It is over expressed in the bronchial biopsy in patients affected by asthma and allergic rhinitis and in the blood during the acute phase and after bronchial provocation, while it is reduced during therapy.[3,20] The current experience aims to assess whether the effect of therapy, conducted according to GINA guidelines, using FP as ICS, upon a long time span and in all levels of asthma, is able to lower airways inflammation. According to our results, the first three levels of asthma are under satisfactory control, adopting GINA guidelines: both symptoms and bronchial hyper reactivity and allergic inflammation are significantly reduced by therapy in GINA levels 2 and 3, with a fair concordance with literature data,[21] confirming as well the significant relationship between inflammation (IL4) and hyper reactivity (PD20).[3]

Results are in full agreement with previous experience of levels of IL4 in EBC infant asthma[22] and in moderate persistent asthma in adults,[3] showing raised baseline values of IL4 and pH in EBC with a diminution after a six months lasting period of FP therapy despite normalization of the symptoms and airways hyper reactivity. The current data were obtained after a longer time span and indicate that therapy did not reduce inflammation and bronchial hyper reactivity in GINA level 1, because ICS were not prescribed, raising the question concerning the opportunity to prescribe inhaled ICS in first level as well. In persistent asthma both six months lasting[3] and twelve months lasting (Table 2) therapy were not sufficient to stop bronchial inflammation, indicating the need of a longer time of therapy. The therapy of severe persistent asthma is not complete, despite the addition of oral steroids. Severe persistent asthma behaves as a peculiar disease partially refractory to therapy because of persistent bronchial inflammation leading to structural damages and remodelling.[23,24]

The practical implication is that IL4 levels indicate the need for therapies longer than those suggested by the trends of symptoms or hyperreactivity BHR, since bronchial inflammation is reduced after long term therapy, but not back to normal levels. Severe persistent asthma behaves as a refractory disease, possibly needing a more aggressive therapy.

Conclusions

The therapy conducted according to GINA guidelines, over one year lasting time span, allows reducing bronchial inflammation in mild and moderate levels. The diminution of inflammation is related to the improvement of bronchial hyperreactivity. After one year of therapy the inflammation is not completely overcome and it is necessary to avoid loss of adherence to the therapy, due to disappearance of symptoms, possibly leading to early withdrawal and relapse of the disease. Severe persistent asthma behave as a different entity with the higher baseline inflammation, raised IL4 levels, marked bronchial hyperreactivity, limited improvement after therapy according to GINA guidelines, requesting a precise identification of the phenotype of asthma and the use of more complex and specific weapons to reduce airways inflammation such as anti IgE antibodies.

Table 2. Effect of therapy lasting twelve months in asthmatic patients.

	Healthy controls	Intermittent	Mild persistent	Moderate persistent	Severe persistent	Units
ACT	23±2	20±2	17±2	14±4	10±3	U
ACT th	22±2	21±2	22±2	18±3	14±3	U
P	n.s.	n.s.	*	°	*	
FEV1	113±12	96±5	92±6	74±6	45±10	%pred±sd
FEV1 th	110±15	96±4	93±3	83±8	60±5	%pred±sd
P	n.s.	n.s.	n.s.	n.s.	*	
PD20	>1600	437±329	364±308	436±252	45±25	Mcg
PD20 th	>1600	460±269	>1600	890±220	60±40	Mcg
P	n.s.	n.s.	°	°	n.s.	
IL4EBC	30±15	60±6	65±10	72±8	78±20	pg/mL
IL4EBCth	33±13	60±12	41±9	45±6	52±5	pg/mL
P	n.s.	n.s.	°+	°	°	

ACT, asthma control test score; th, results after twelve month of therapy; P, probability of significance; *P<.05; °P<.001; IL4EBC, levels of Il4 in expired breath condensate; PD20, provocative dose causing a 20% fall of FEV1; n.s., not significant.

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