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The prevalence of nasal polyps and the corresponding urinary LTE_4 levels in severe compared to mild and moderate asthma

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Key words

Nasal polyps; severe asthma; asa-intolerance; cysteinil leukotrienes

Summary

Background: *Several comorbid conditions may contribute to worsening asthma symptoms, including nasal polyps (NPs). Cysteinyl leukotrienes (Cys-LTs) play a crucial role in asthma pathophysiology, and specific receptors for CysLTs are reported as up-regulated in nasal polyp tissues. The aim of the present study was to assess the prevalence of nasal polyps in severe vs mild and moderate asthma, and to compare the corresponding levels of urinary Leukotriene E4 (LTE_4).* ***Matherials and methods:*** *A cohort of 386 asthma patients were studied: n=166 with mild, n=146 with moderate and n=74 severe asthma. All patients performed a nasal endoscopy and urine were collected in the morning for the quantitative LTE_4 immunoenzimatic assay (Cayman Chemical, Mi, USA). Intolerance to ASA was also assessed by means of a nasal provocation test with L-ASA.* ***Results:*** *The prevalence of NPs was the following: 8 cases (4.8%) in mild; 14 (9.6%) in moderate, and 33 (44.6%) in severe asthma. Mean urinary LTE_4 levels were increasing according to the disease severity. ASA-intolerance was assessed in 1 patient in mild asthma (0.6%), 14 in moderate asthma (9.6%) and 28 in severe asthma (37.8%).* ***Conclusions:*** *Nasal polyps represent a comorbid which is highly frequent in severe asthma. Both their prevalence and the corresponding mean LTE_4 levels in urine proved in strict, direct relationship with asthma severity. In severe asthma, nasal polyps represent a condition which is associated with the highest excretion of urinary LTE_4 and ASA intolerance.*

Introduction

GINA guidelines define severe asthma as a condition in which any of the following are manifest: continuous symptoms prior to treatment; frequent exacerbations and nocturnal symptoms; impairment of lung function (FEV_1 ≤ 60% predicted or PEF ≤ 60% of personal best, and PEF variability ≥ 30%) (1).

Severe asthma, although present in a relatively small proportion of the whole asthma population, comprises those subjects with the highest morbidity and who are in particular need of careful evaluation (2). Bronchial asthma is a costly disease and the related social impact is ever increasing, particularly in terms of indirect costs in severe asthma (3).

A number of pathological factors may contribute to poor control of asthma, including nasal polyps (NPs), which usually affect asthma severity in real life (4). Generally speaking, NPs consist in the prolapse of the mucosal lining of the nose and nasal sinuses (in particular from the lining of the ethmoid sinuses), which protrude down into the middle meatus and present as a smooth, round, pale

and translucent swelling, and are characterized by infiltration of the mucosa with eosinophils, lymphocytes and mast cells (5). NPs are not only associated with atopy but also with asthma; aspirin intolerance; cystic fibrosis; allergic fungal sinusistis, and Churg-Strauss syndrome (6).
Cysteinyl leukotrienes (cys-LTs), namely leukotriene C4 (LTC4), LTD4 and LTE_4, play an extremely important role in the pathophysiology af asthma. Cys-LTs cause potent bronchoconstriction; mucosal edema; vasodilatation; vascular permeability and increased mucus secretion within the airways of asthmatic patients (7). In particular, LTE_4 has been identified as a major metabolite of LTC4, and urinary LTE_4 (u-LTE_4) is now regarded as the most reliable analytic parameter for monitoring the endogenous synthesis of cys-LTs (8).
Basal urinary levels of LTE_4 excretion are significantly higher in aspirin-intolerant asthma patients (AIA) than in aspirin-tolerant asthma subjects (9). Interaction of CysL-Ts receptors (Cys-LT1 and Cys-LT2) with their ligand LTC4, LTD4 and LTE_4 play a disease-regulating role also in chronic rhinosinusitis (CHRS) and NPs, particularly in the aspirin intolerance syndrome, which is often related to these conditions (9). Cys-LTs receptors have been described as up-regulated in nasal polyp tissue, and their expression is related with eosinophilic inflammation (10).
CHRS/NPs are involved in cys-LTs overproduction of asthmatic patients and are not strictly associated with aspirin intolerance itself but rather with clinical features (11). Urinary LTE_4 exexcretion proves directly proportional to the extent of nasal structural changes occurring in ASA-intolerant asthmatics, being subjects with NPs those with the highest LTE_4 values, immediately followed by those with hypertrophic rhinitis (12).
The prevalence of NPs is considered to be around 4% in the general population, but near 30% in patients with NPs and asthma (13); nevertheless, their true prevalence in severe asthma is still unknown.
The aim of the present study was to assess the incidence of NPs in severe vs mild and moderate asthma, and to compare the corresponding levels of urinary Leukotriene E4 (LTE_4).

Matherials and methods

A cohort of studied 386 asthma patients was studied and divided in: n=166 with mild (80 males; 18 -76 years; FEV_1= 85.6 % pred. ± 8.9 sd); n=146 with moderate (72 males; 19 – 73 years; FEV_1= 69.7% pred. ± 10.1 sd), and n=74 with severe asthma (23 males; 21 – 69 years; FEV_1= 55.1% pred. ± 7.3 sd), according to GINA guidelines.
All patients performed a nasal endoscopy according to the European Position Paper on Rhinosinusitis and Nasal Polyps (14).

Urinary LTE_4

Urinary LTE_4 concentration was measured by enzyme immunoassay (ACETM Competitive Enzyme Immunoassay, Cayman Chemical, Ann Arbor, Mich, USA), as reported by Pradelles et al (15). This assay is based on the competition between LTE_4 and an LTE_4-acetylcholinesterase (AChE) conjugate (LTE_4 tracer) for a limited amount of LTE_4 antiserum. Because the concentration of the LTE_4 tracer is held constant while the concentration of LTE_4 varies, the amount of LTE_4 tracer that is able to blind to the LTE_4 antiserum will be inversely proportional to the concentration of LTE_4 in the well. This antibody-LTE_4 complex blinds to a mouse monoclonal anti-rabbit IgG that has been previously attached to the well. The plate is washed to remove any unbound reagents and then Ellman's reagent (which contains the substrate to AChE) is added to the well. This reagent consists of acetylthiocholine and 5,5'-dithio-bis-(2-nitrobenzoic acid). Hydrolysis of acetylthiocholine by AChE produces thiocholine. The non-enzymatc reaction of thiocholine with 5,5'-dithio-bis-(2-nitrobenzoic acid) produces 5-thio-2-nitrobenzoic acid, which has a strong absorbance at 412 nm. AChE has several advantages over other enzymes commonly used for enzyme immunoassays. Unlike horseradish peroxidase, AChE does not self-inactivate during turnover. In addition, the enzyme is highly stable under the essay conditions, has a wide pH range (pH 5-10), and is not inhibited by common buffer salts and preservatives. The product of this enzymatic reaction has a distinct yellow colour and absorbs strongly at 412 nm. The intensity of this colour, determined spectrophotometrically, is proportional to the amount of LTE_4 tracer bound to the well, which is inversely proportional to the amount of free LTE_4 present in the well. LTE_4 was measured by enzyme immunoassay on all samples according to the manufacturers' instructions and expressed in pg/mg creatinine (pg/mg). Because treatment with β_2-agonists or with anti-inflammatory drugs, including oral or inhaled corticosteroids, sodium cromoglycate, or oral leukotriene receptor antagonists themselves, does not affect u-LTE_4 levels (16), these

medications were not withheld at the time of the urine sample collection in this study.

Nasal Provocation test with L-ASA

Each patient performed the NPT according to the method described by Casadevall and co-workers by means of the acoustic rhinomanometry (12, 17). This method consists in the measurement of acoustic reflections from the nasal cavity of a sound pulse created by a spark in a sound tube connected to the nasal cavity via a nosepiece. Unlike conventional rhinomanometry, acoustic rhinomanometry does not require generation of nasal flow, and therefore its use is less limited by the presence of nasal polyps and nasal obstruction. The response was evaluated by the Eccovision Acoustic Rhinomanometry System (TM Hood Laboratories, USA) with the measurement of (18):

1) calculated resistance, based on a tube with the same area and laminar flow (REQ, $mmH_2O/l/min$);
2) the total volume of the nostrils (VOL, cm^3) represents the nasal cavity volume in the analysis segment;
3) the minimal cross sectional area (cm^2);
4) its distance from the nosepiece (cm).

Rhinomanometric measurements were performed while the subject was in apnoea after a non-forced expiration. The rhinomanometer was calibrated daily with a calibration tube provided by the manufacturer. The analysis of data was performed using the Kwikstat program (TM Texasoft). Baseline nasal function was measured with Acoustic Rhinomanometry, while the subjects were in a sitting position, then 80 μl of L-ASA solution (180 mg/ml L-ASA) was applied locally through nose droplets on the inferior nasal concha in both nostrils. The total deposited dose of L-ASA was equivalent to 25 mg of acetylsalicylic acid. Acoustic Rhinomanometry was then performed bilaterally every 10 minutes for the next 2 hours. L-ASA was prepared freshly each day by dissolving crystalline L-ASA in 0.9% sodium chloride to produce a solution containing 180 mg/ml.

NPT was considered positive when: nasal resistance increased more than 40% in at least one nostril as compared with the corresponding baseline value; when the volume of one nostril decreased more than 10% from baseline; both parameters sustained for at least two consecutive measurements, and were accompanied by clinical symptoms persisting at least 30 minutes. The dose of aspirin, the duration of the observation period, and criteria for positivity of the test were established on the basis of previous experiments (12, 17).

Pulmonary function (FEV_1; forced expiratory volume in 1 sec) was measured simultaneously by means of a computerized pneumotachograph (Masterlab, Jaeger). The maximal fall in REQ, VOL and FEV_1 observed in the 2 hours following the NPT were calculated.

Statistical analysis

Mean values ± sd obtained before and after NPT for each variable were compared by t test, and $p<0.05$ was assumed as the lowest limit for the statistical significance.

Results

The prevalence of nasal polyps was: 8 cases (4.8%) in mild asthma; 14 (9.6%) in moderate asthma, and 33 (44.6%) in severe asthma (Tab. 1).

According to a semi-quantitative score for classification (14), the endoscopic appearance score was: 7,6 ± 2,3sd in mild asthma; 10,1 ± 1,9sd in moderate asthma, and 12,6 ± 2,2sd in severe asthma, respectively (severe vs moderate $p<0.001$; moderate vs mild $p< 0.05$).

Furthermore, in severe asthmatics, nasal polyps were much more frequent and also more clinically relevant, such as characterized by a more frequent bilateral occurrence, by the presence of severe oedema and consistent discharge.

Table 1 - Prevalence of nasal polyps

	Polyps n.	Polyps %	Prevalence AIA	u-LTE_4 (pg/ml)
Mild asthma	8/166	4.8	0.6%	129.1±74.8
Moderate asthma	14/146	9.6	1.4%	330.7±72.3 °
Severe asthma	33/74	44.6	37.8%	432.3±88.1 * ^

* $p<0.001$ Severe vs Mild; ^ $p<0.001$ Severe vs Moderate; ° $p< 0.001$ Moderate vs Mild asthma

Urinary LTE_4 levels proved to increase according to asthma severity: 129.1 pg/ml ± 74.8sd in mild asthma; 330.7 pg/ml ± 72.3 sd in moderate (severe vs mild $p<0.001$), and 432.3 pg/ml ± 88.1 sd in severe asthma (severe vs moderate $p<0.001$; moderate vs mild $p< 0.001$).
Moreover, the higher proportion of ASA intolerance (37.8%) was found in severe asthma, vs 1,4% in moderate and 0,6% in mild asthma.

Discussion

Severe asthma is not a frequent condition in the whole population of asthma patients, even though its burden in terms of health care utilization; hospitalization or access to emergency department, and frequent exacerbations is quite elevated (3).
A disproportionately large amount of the total cost of illness is generated by a relatively small proportion of patients with severe symptoms. The underlying structural changes still are not well understood (in particular their response to inhaled corticosteroids), but probably comorbidities play a crucial role in deteriorating lung function and quality of life. Some patients may continue to require high-intensity asthma treatment because of persistent symptoms owing to particular conditions, such as rhinosinusitis, gastro-oesophageal reflux or psychosocial problems, despite the best available therapeutic strategies for managing these conditions (19).
Results of present study confirmed the high incidence of ASA-intolerance in severe asthma. AIA develops according to a characteristic sequence of symptoms characterized by the presence of: persistent rhinitis (which usually starts at an average age of 30 years and which is followed by asthma); ASA sensitivity, and nasal polyps (9). Rhinorrhea and nasal congestion are usually the earliest symptoms of AIA, which generally persist and become perennial; they are difficult to treat, and frequently combine with recurrent or chronic sinusitis, anosmia, and nasal polyps. Asthma and ASA sensitivity tend to become manifest later (an average of 1 to 5 years after the onset of rhinitis), being AIA-patients with nasal polyps a much more difficult clinical condition to treat when compared to that of subjects with AIA-induced rhinitis only.
Clinical signs of AIA are bronchoconstriction and, in many cases, naso-ocular, gastrointestinal, and/or skin reactions, which occur shortly after ingestion of ASA or of non-steroidal anti-inflammatory compounds. As patients with AIA are intolerant to all drugs that inhibit cyclooxygenase, it has been postulated that this sensitivity may stem from the up-regulation of the 5-lipoxygenase pathway of arachidonic acid metabolism with the resultant production of mediators such as the cys-LTs. In particular, genetic predisposition to cys-LTs pathway up-regulation can be related to the overactive expression of the LTC4S-444C allele (20). AIA is therefore associated with an elevated urinary excretion of LTE_4, both in basal conditions and after ingestion, inhalation (9), or nasal instillation (21) of ASA.
Most of the proinflammatory actions of CysLTs are mediated by their binding to the Cys-LT1 receptors (22-23). CysLT1-receptor antagonists attenuate asthma which is elicited by aspirin challenge in aspirin-sensitive subjects (24-25), even though the elevated number of nasal inflammatory leukocytes expressing Cys-LTs receptors in ASA-sensitive patients with chronic rhino-sinusitis is probably also critical in the pathogenesis of aspirin sensitivity (26).
The underlying role of Cys-LTs is confirmed by the positive effect of Montelukast, a selective Cys-LT1 receptor antagonist, on nasal function, nasal reactivity to L-ASA, and on blood markers of eosinophilic inflammation in mild- to-moderate AIA in the presence of nasal symptoms (27).
Even though the number of eosinophils, neutrophils and plasma cells in NPs is significantly higher than in nasal mucosa, NPs represent a comorbid characterised by an extremely high level of systemic inflammation and for this reason they should be always taken into account in the management of severe asthma. Then, the role of NPs in triggering severe asthma should not be mainly attributed to the trivial occurrence of a mechanical post-nasal drip, but mainly to the occurrence of a sustained local and systemic inflammation. It is easily mirrored by the high levels of urinary LTs and of other plasma eosinophilic mediators measurable in these conditions.
As NPs confirm to represent a true risk factor for increasing asthma severity, this evidence supports the need of a much more careful assessment of asthma patients, with particular attention to their nasal conditions. Actually, in order to define the most convenient therapeutic strategy and to obtain the best control of the disease, the united assessment of airways (such as proximal and distal) should be mandatory in all asthma patients.
In conclusion, nasal polyps represent a comorbid condition which is more highly frequent in severe asthma. For a better disease management it is crucial to investigate the presence of concomitant disorders, such as ASA-intolerance and NPs which can exacerbate asthma. Their preva-

lence, together with their urinary LTE_4 concentrations, increases in strict relationship with the level of asthma severity, even though nasal polyps represent a peculiar condition which proves to be associated with the highest excretion of urinary LTE_4 and with the highest prevalence of ASA intolerance.

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