Antinuclear Antibodies in Asthma Patients- A Special Asthma Phenotype?

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ABSTRACT

Several studies reported the appearance of asthma and autoimmune conditions in the same patient, but the clinical significance of this association was not yet assessed.

One hundred asthmatic patients were observed for one year evolution with death, severe exacerbations, intake of > 1000 micrograms of beclometasone or equivalent (high ICS) and FEV1 decline >100 ml, in relation with ANA (ELISA), sputum and blood eosinophilia (EO), NSAID intolerance, BMI >25, chronic rhinosinusitis, smoking status and FEV1 <30% predicted (low FEV1).

After 1 year of observation, there were 5 deaths, 28 severe asthma exacerbations requiring hospitalisations, 24 cases requiring high inhaled corticosteroid intake, and 19 patients with fast FEV1 decline (>100 ml/year). Multiple regression analysis pointed out several different independent risk factors for severe asthma evolution: for death presence of ANA (P=0.037), NSAID intolerance (P<0.001) and low FEV1 (P=0.021); for evolution with severe exacerbations ANA (p=0.011), sputum EO (P<0.001), smoking (P=0.044) and NSAID intolerance (P=0.022); for high ICS intake ANA (P=0.036), sputum EO (P=0.026) and low FEV1 (P=0.006); for FEV1 decline >100 ml ANA (P=0.006), sputum EO (P=0.037), BMI>25 (P=0.046) and NSAID intolerance (P=0.017).

The presence of ANA is an independent risk factor in asthma for evolution with death, severe exacerbations, high inhaled corticosteroid intake and FEV1 decline >100 ml.

Key words: Antinuclear antibodies; Asthma phenotype; Risk factors; Severe asthma

INTRODUCTION

Immune dysregulation in asthma and autoimmune diseases shares many common key pathogenic mechanisms: genetic background, mast cells and T cells, co-stimulatory molecules, cytokines and autoantibodies.1

Polymorphism within the IL-4Ra is associated with atopy and asthma, systemic lupus erythematosus (SLE) and Chron disease.2 Mast cells, pivotal for the allergic inflammation in asthma, have been recently involved in the pathogenesis of rheumatoid arthritis and multiple sclerosis.3,4 A defect in T regulatory cells or the recently described IL-17 cells are central for the pathogenesis of both asthma and autoimmune diseases.5,7 iNKT cells regulate the autoimmune response in SLE and colitis and are increased and promote bronchial hyperreactivity.
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(BHR) in asthma. Serum levels of CTLA-4, CD28, CD80 and CD86 are increased both in adults and children with asthma and in SLE which directly reflect disease severity. CTLA-4 Ig, used in the treatment of SLE and psoriasis, inhibited the allergen-specific Th2 cytokine production from T cells isolated from bronchial biopsies from asthmatics. Anti TNF-α treatment, highly efficient for rheumatoid arthritis and psoriasis, decreases symptom score and BHR and increases FEV1 and quality of life in severe asthma patients.

Antibodies against the beta2-adrenergic receptor are increased in the serum of asthma patients compared to controls and depress receptor function with more than 50% and down-regulate the receptor number by 30%. Autoantibodies anti-cytokeratin are significantly increased in non-allergic and in aspirin-sensitive asthma, and also in toluene disocianate (TDI)-induced asthma compared to asymptomatic exposed subjects and to un-exposed healthy subjects.

Objective of the study was to evaluate the prognostic value of antinuclear antibodies (ANA) for severe evolution of asthma.

PATIENTS AND METHODS

The study was approved by the local ethics committee and all subjects signed an informed consent before enrolment. We present an observational, prospective study conducted in 100 asthma patients, diagnosed at inclusion using the history and an increase of ≥ 12% of FEV1 15 minutes after a bronchodilator.

In order to ensure that the study was not conducted in a population with high ANA incidence background, a control group of 30 healthy subjects, matched for age and sex ratio was used. We excluded patients with ANA positive connective tissue diseases and asthma patients with positive ANCA antibodies or other symptoms relevant for Churg-Strauss syndrome.

Asthma patients were observed one year for evolution with death, severe exacerbations requiring hospitalisation, high inhaled corticosteroid intake for asthma control (> 1000 micrograms of beclometasone or equivalent) and fast FEV1 decline (> 100 ml/year) in relation with the presence at inclusion of the following risk factors: ANA, blood and sputum eosinophilia; non-steroidal antinflammatory drugs (NSAID) intolerance, including aspirin intolerance; body mass index (BMI) > 25; chronic rhinosinusitis; smoking status; FEV1 at baseline < 30% predicted (low FEV1).

ANA were measured in both asthma and control groups using a third generation ELISA (Quanta Lite ANA ELISA, INOVA Diagnostics Inc., San Diego, CA; cut-off value 20 UI/ml). ANA pattern was determined using the indirect immunofluorescence technique (NOVA Lite ANA KSL, INOVA Diagnostics Inc., San Diego, CA). Spirometry was conducted at inclusion and at the end of the observation period, outside an exacerbation period, in accordance with the ATS guidelines, using a Microlab MK 6 spirometer. Blood eosinophils were counted at inclusion using a FACS Calibur cytometer (Becton-Dickinson GmbH, Heidelberg, Germany, Cell Quest software; cut-off value was set at 500 eosinophils/mm³). Sputum eosinophils were measured at inclusion on May-Grunwald–Giemsa stained sputum smears, cut-off value <1%.

Chronic rhinosinusitis was diagnosed according to the ENT examination combined with a CT scan.

Data were analysed using Chi test, multiple regression analysis and Kaplan-Meyer analysis.

RESULTS

Baseline Characteristics

Ninety five enrolled asthmatic patients completed the observation period of one year (12.14±1.28 months) and 5 patients died. The mean age of asthma patients was 53.25±13.8 years, there were 56% females, and the mean asthma duration was 13.06±12.20 years. 38 subjects had mild persistent asthma, 36 had moderate persistent asthma and 26 had severe persistent asthma. The incidence of atopic asthma was 34%. The control group included 30 healthy subjects, with mean age 49.14±14.6 years, 70% females. In the asthma group the incidence of positive ANA was 22%, of blood eosinophilia 36%, of sputum eosinophilia 41.9%, of NSAID intolerance 7%, of BMI>25 29%, of chronic rhinosinusitis 28%, of smokers 14% and of low FEV1 8%.

ANA medium titre was 48.62±26.5 IU/ml. ANA pattern under indirect immunofluorescence was speckled in 15(68.18%) cases, homogenous in 4(18.18%) cases and nucleolar in 2(9.09%) cases. ANA incidence in the asthma patients group (22%) was significantly higher compared to the control group (3.3%) (Chi test: p<0.05). ANA incidence was not different between atopic (20.59%) and non-atopic asthma patients (22.73%).

Outcome Measures for Severe Evolution of Asthma

After 1 year of observation of the asthma patients there were 5 deaths, 28 severe asthma exacerbations re-
Risk Factors for Severe Evolution of Asthma

Multiple regression analysis pointed out several different independent risk factors for severe asthma evolution: for death ANA (p=0.037), NSAID intolerance (<0.001) and low FEV1 (p=0.021); for exacerbations ANA (p=0.011), sputum EO (p<0.001), smoking (p=0.044) and NSAID intolerance (p=0.022); for high ICS intake ANA (p=0.036), sputum EO (p=0.026) and low FEV1 (p=0.006); for FEV1 decline > 100 ml ANA (p=0.006), sputum EO (p=0.037), BMI>25 (p=0.046) and NSAID intolerance (p=0.017) (Table 1).

For death Kaplan-Meyer analysis identified as risk factor the presence of ANA (p=0.0008), NSAID intolerance (p<0.001) and low FEV1 (p=0.00026). For exacerbations Kaplan-Meyer analysis identified as risk factor the presence of ANA (p=0.01), sputum EO (p=0.0004) and low FEV1 (p=0.002). For high inhaled corticosteroid intake Kaplan-Meyer test showed significance for the presence of ANA (p=0.002) and low FEV1 (p=0.00076) For FEV1 decline > 100 ml/year Kaplan-Meyer test identified as risk factor the presence of ANA (p=0.02), sputum EO (p=0.028) and low FEV1 (p=0.033) (Table 2).

DISCUSSION

This study demonstrates that antinuclear antibodies are an independent risk factor for evolution of asthma with death, severe exacerbations requiring hospitalisation, high inhaled corticosteroid intake and fast FEV1 decline. The prognostic value of ANA, demonstrated both by multiple regression analysis and by Kaplan-Meyer test, is comparable with demonstrated risk factors for severe asthma evolution (sputum eosinophils, obesity, chronic rhinosinusitis, NSAID intolerance and decreased pulmonary function).

Few studies evaluated the prognostic significance of the association between autoimmunity and asthma. Autoantibodies to alpha-enolase were significantly increased in severe asthma compared to mild-to-moderate asthma and healthy controls and were the most significant indicator for severe asthma, even after adjusting for the effects of age, sex, atopy and FEV1.20 In asthma patients skin tested with autologous serum skin test the positivity of the skin test was related to significant increased airway hyperresponsiveness.21

In a previous communicated cross-sectional study in severe asthma patients we identified as risk factors for asthma severity ANA (p<0.001), sputum eosinophils (p<0.001), blood eosinophils (p=0.046), chronic rhinosinusitis (p=0.002) and BMI>25 (p=0.038).22 ANA in asthma patients were correlated with high inhaled corticosteroid intake necessary to control asthma, depicting a decreased responsiveness to inhaled corticosteroids.

The association between ANA and fast FEV1 decline highlights an aggressive remodelling process and implies screening for these antibodies in patients with rapid loss

### Table 1. Independent risk factors for severe evolution of asthma (multiple regression analysis).

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<tr>
<th>End-point</th>
<th>Risk Factor</th>
<th>P value</th>
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<tbody>
<tr>
<td>Death</td>
<td>ANA</td>
<td>0.037</td>
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<tr>
<td></td>
<td>NSAID intolerance</td>
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<td></td>
<td>low FEV1 at inclusion</td>
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<td>Severe exacerbations</td>
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<td>smoke</td>
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<td></td>
<td>NSAID intolerance</td>
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<tr>
<td>High inhaled corticosteroid intake</td>
<td>sputum eosinophils</td>
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<tr>
<td></td>
<td>FEV1 at inclusion &lt; 30%</td>
<td>0.006</td>
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<tr>
<td>FEV1 decline &gt; 100 ml/year</td>
<td>sputum eosinophils</td>
<td>0.037</td>
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<td>BMI&gt;25</td>
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<td>NSAID intolerance</td>
<td>0.017</td>
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### Table 2. Kaplan-Meyer estimates for severe evolution of asthma.

<table>
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<td>sputum eosinophils</td>
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of lung function. Both steroid decreased responsiveness and aggressive remodelling sustain the functional relevance of the ANA in asthma. The mechanisms of action remain elusive and further bronchial biopsy and BAL studies are warranted in this subset of asthma patients.

The incidence of antinuclear antibodies in asthma (22%) was significantly higher compared to controls (3%). This finding was also reported by Szczeklik: ANA were present in 55% patients with aspirin-induced asthma, 41% with intrinsic asthma, 39% with atopic asthma, and 11% of the healthy subjects, with the difference between each patient group and the healthy subjects being statistically significant (P< 0.05). In the same study ANA were associated with signs of complement activation, the presence of rheumatoid factor, and circulating immune complexes. In contrast, in our study there were no clinical or serological manifestations of ANA.

The “silent” presence of ANA in asthma related to severe evolution points out to the potential value of ANA screening in asthma, in order to diagnose a more severe phenotype, prone to severe exacerbations and fast decline in lung function. It can also be speculated that antinuclear antibodies could define an asthma phenotype more responsive to immune modulation.

REFERENCES