Clinical and biochemical factors for response to aspirin desensitization in aspirin-induced asthma patients – pilot study

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Additional key words:
asthma
aspirin
induced sputum
nasal lavage

Introduction
Aspirin-induced asthma was traditionally termed as Samter’s triad or Aspirin-Exacerbated Respiratory Disease (AERD). Recently members of the Task Force of the European Academy of Allergy and Clinical Immunology/World Allergy Organization (EAACI/WAO) proposed to use the name Non-steroidal anti-inflammatory drugs (NSAIDs)-Exacerbated Respiratory Disease (NERD), to uniform the definitions [1]. NERD is a clinically distinct syndrome, which consists of a classic tetrad: moderate to severe asthma, chronic rhinosinusitis, sinonasal polyps, and intolerance to aspirin and/or other nonsteroidal anti-inflammatory drugs [2]. The prevalence of this condition in the adult asthmatic population is 7% and twice that number in individuals suffering from severe asthma [3]. The evolution of the disease is characteristic, which may suggest a common underlying pathomechanism. The typical patient with NERD is an adult in the third or fourth decade of life, who develops chronic rhinosinusitis, often after a viral respiratory infection, resulting in hyposmia or anosmia in the majority of cases [4]. Persistent asthma develops during the evolution of sinus disease [5]. The ingestion of aspirin or other nonselective cyclooxygenase (COX) inhibitors by a sensitive individual results in the exacerbation of the already existing inflammation in the upper and lower respiratory airways, which may appear as a wide spectrum of symptoms ranging from conjunctivitis and rhinitis to laryngospasm and bronchospasm [2]. Despite subsequent avoidance of aspirin and other NSAIDs, asthma and nasal and bronchial inflammatory disease persist, often requiring systemic corticosteroid therapy [5].

A number of theories have been proposed to explain the pathogenesis of NERD, though most evidence points towards an abnormal metabolism of arachidonic acid (AA) by COX and lipoygenase (LO) pathways [6-8]. It is suggested that a pharmacological mechanism of hypersensitivity is associated with COX-1 inhibition by aspirin and other NSAIDs. This leads to decreased biosynthesis of protective prostaglandins (especially PGE2) as well as overexpression of 5-LO and leukotriene C4 synthase, resulting in an overproduction of cysteinyl leukotrienes (CysLTs) by inflammatory cells, most likely mast cells and eosinophils [9-11]. The dysregulation of these pro- and anti-inflammatory pathways is observed in the bronchi, sinuses and nasal polyps of aspirin intolerant patients [9]. Moreover, the airways of NERD subjects show chronic inflammation with marked eosinophilia.

Aspirin desensitization is considered to be an effective and well-tolerated therapy for patients with Non-steroidal anti-inflammatory (NSAIDs)-Exacerbated Respiratory Disease (NERD). The aim of the present study was to investigate the influence of aspirin desensitization on inflammatory cell count in induced sputum and nasal lavage in fifteen NERD individuals subjected to one-year aspirin therapy. The decrease in induced sputum count of eosinophils and macrophages was observed. Clinical efficacy of aspirin therapy in improving nasal symptoms and quality of life in NERD patients was also confirmed.

Metoda desensytyzacji kwasem acetylosalicylowym uważana jest za efektywną i dobrze tolerowaną opcję terapeutyczną u pacjentów z chorobą dróg oddechowych zastrzałną przez niesteroidowe leki przeciwna表明 (NERD). Celem badania była ocena wpływu desensytyzacji kwasem acetylosalicylowym na liczbę komórek zapalnych w indukowanej płocinie oraz popłuczynach nosowych u piętnastu pacjentów z NERD poddanych rocznej terapii kwasem acetylosalicylowym. Zaoberwowano spadek liczby eozynofilów oraz makrofagów w płocinie indukowanej. Potwierdzono została także efektywność terapii kwasem acetylosalicylowym w poprawie objawów nosowych oraz jakości życia pacjentów z NERD.

Dodatkowe słowa kluczowe:
astma
kwasy acetylosalicylowe
plwocina indukowana
popłuczyny nosowe

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Aspirin desensitization is considered to be an effective and well-tolerated therapy for patients with NERD. It is recommended for individuals with poorly controlled asthma and sinusitis, those who require multiple nasal surgeries or need COX-1 inhibitors for anti-platelet therapy [14]. It has been shown that such treatment results in a reduction of rhinosinusitis, a decreased rate of polyp formation, lower doses of oral corticosteroids as well as improvement in patient quality of life [15-17]. Nonetheless, it should be borne in mind that aspirin therapy is associated with an increased risk of gastrointestinal (epigastric pain, nausea, bloating, ulcers), renal (elevation in serum creatinine, interstitial nephritis) or dermatological (urticaria) events, to name just a few. Despite many studies regarding the pathophysiology of NERD, the exact mechanism driving the beneficial effects of long-term aspirin therapy has not yet been clearly elucidated. The aim of the present study was to investigate the influence of aspirin desensitization on inflammatory cell count in induced sputum (IS) and nasal lavage (NL) in NERD subjects.

Materials and Methods
The study group consisted of 15 patients with Non-steroidal anti-inflammatory-Exacerbated Respiratory Disease who presented to the Pulmonology Department at the University Hospital in Cracow, Poland. The characteristics of the study population are shown in Table I.

The diagnosis of aspirin intolerance was confirmed by oral aspirin provocation tests. All patients were instructed to withhold medications altering bronchial responsiveness prior to aspirin challenge. Short-acting β2 agonists were not used for 8h, long-acting β2 agonists and theophylline for 24h, and antihistamines as well as cromones for 5 days before the test. Inhaled steroids were allowed at the maximum dose of 2000μg budesonide equivalent per day. None was treated with systemic corticosteroids or leukotriene-modifying drugs.

All the participants gave informed consent and the study was approved by the Jagiellonian University Ethics Committee.

Study design
All participants were hospitalized four times over the period of one year. On the first visit, patients underwent placebo-controlled oral aspirin challenge in order to establish the minimum dose of aspirin that provoked typical symptoms of aspirin hypersensitivity. Each study participant was given increasing doses of aspirin according to applied aspirin challenge protocol. Clinical symptoms such as dyspnoea, nasal blockage, rhinorrhea, sneezing, ocular secretion and skin flushing were assessed. Aspirin desensitization started on the first visit when the provoking dose was established. The target dose was 650mg of aspirin daily that is 325 mg of aspirin twice a day, which the patient ingested every day throughout the study. At baseline and during each follow-up visit (2nd, 6th and 12th month) IS and NL were collected from all subjects. Study participants were also asked to complete the questionnaire including nasal (sneezing, rhinorrhea, nasal blockade, nasal itching and postnasal drip) and non-nasal symptoms (any complaints concerning eyes, ears, throat, chronic cough, headache or impairment of mental function). The asthma diagnosis and control was assessed according to GINA 2014 guidelines.

Induced sputum collection
IS collection was performed according to European Respiratory Society (ERS) recommendations [18]. Sputum induction was conducted by inhalation of hypertonic saline solution which concentration was consecutively increased from 3% to 5% with a use of ultrasonic nebulizer (Ultraneb 2000; De Vilbiss, Somerset, PA, USA). As saline inhalation may cause the constriction of bronchi, pre-treatment with inhaled salbutamol and monitoring of lung function during the procedure was applied. Sputum was expectorated onto ice-cooled Petri dish and transferred to the laboratory. Mucus plugs were manually separated from saliva and processed to obtain cytospin slides for differential cell count. The cell pellet was resuspended in balanced saline solution and May-Grünwald stain was used to ascertain cell count. The sample was included in the further analysis if squamous cell contamination was <20%. The number and percentage of bronchial epithelial cells, macrophages, neutrophils, eosinophils, lymphocytes and monocytes in the total of nonsquamous cell count were reported.

Nasal lavage collection
In order to obtain NL samples, patients were asked to flex their neck 30° from the vertical position and do not breathe though their nose. Five millimetres of sterile isotonic saline was instilled into each nasal cavity by using a syringe. The solution was retained for approximately 10 seconds in nostrils without swallowing. The nasal lavage fluid (NLF) was subsequently collected from both nasal cavities by forward flexing the head, exhaling and rinsing NLF into a sterile cup. It was then stored on ice, weighed and processed within an hour. NFL was filtered using 40μm cell strainer and the NFL filtrate was pelleted by centrifugation. Cytocentrifuge slides were stained for differential cell count. The number and percentage of bronchial epithelial cells, neutrophils, eosinophils, lymphocytes and monocytes were reported.

Nasal and non-nasal symptoms and quality of life assessment
At each visit all study participants were asked to fill in questionnaire assessing their nasal and non-nasal symptoms on a seven-point scale: 1- lack of symptoms, 7- symptoms are intolerable.

Similarly, the quality of patient life with reference to nasal symptoms was assessed on a seven-point scale, but the classification was reverse: 1- very bad quality of life, 7- very good quality of life.

Statistical analysis
Summary statistics were expressed as mean (M), 95% confidence intervals, median (Me) and minimum and maximum value. Friedman ANOVA test, which takes into account the fact that outcome measurements are repeated over time within subjects, was used for multiple comparisons. P value of <0.05 was considered to be statistically significant.

Results
All participants have accomplished 12-months period of aspirin desensitization and have continued aspirin therapy afterwards. There was a significant decrease in the number (ANOVA, p=0.02) and percentage (ANOVA, p=0.03) of eosinophils in IS between baseline and after aspirin desensitization (Fig. 1). The difference was statistically significant between baseline and 12th month of aspirin desensitization (p<0.001 for absolute count and p=0.01 for the percentage of eosinophils, respectively). The pronounced decrease in the number (ANOVA, p=0.04), but not the percentage (ANOVA, p=0.27) of IS macrophages was also observed (Fig. 2). The difference in absolute count of macrophages was significant between baseline and 6th month (p=0.02) as well as baseline and 12th month (p=0.03) of aspirin therapy.

There were no statistically significant changes in nasal lavage cell count.

Table I
Characteristics of patients at baseline.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Patients (n=15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>47 (29-67)</td>
</tr>
<tr>
<td>Sex (female/male ratio)</td>
<td>10/5</td>
</tr>
<tr>
<td>Asthma duration (y)</td>
<td>7 (4-32)</td>
</tr>
<tr>
<td>ACT score*</td>
<td>22 (14-25)</td>
</tr>
<tr>
<td>Baseline FEV1 (% predicted)</td>
<td>91.2 (66.6-126.6)</td>
</tr>
<tr>
<td>Inhaled steroids (yes/no)</td>
<td>15/0</td>
</tr>
<tr>
<td>Dose of inhaled steroids (mg/day)Δ</td>
<td>1000 (500-1500)</td>
</tr>
<tr>
<td>Nasal polyposis (yes/no)</td>
<td>12/3</td>
</tr>
</tbody>
</table>

Values are expressed as median (min-max value)

*Asma Control Test
**Figure 1**
Significant decrease in the absolute count and percentage of eosinophils in IS from NERD patients between baseline and 12th month of aspirin desensitization.
Values are expressed as mean ±95% confidence intervals.

**Figure 2**
Significant decrease in the absolute count of macrophages in IS from NERD patients between baseline and 6th month as well as baseline and 12th month of aspirin desensitization.
Values are expressed as mean ±95% confidence intervals.

**Figure 3**
Significant improvement of overall nasal symptoms between baseline and 6th month as well as baseline and 12th month of aspirin desensitization.
Out-of-seven rating score.

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Table II
Mean value of variables which changes are statistically significant in the course of aspirin desensitization. Values are expressed as mean±SD

<table>
<thead>
<tr>
<th>Variable</th>
<th>baseline</th>
<th>2nd month</th>
<th>6th month</th>
<th>12th month</th>
</tr>
</thead>
<tbody>
<tr>
<td>IS eosinophils (absolute count)</td>
<td>67.4±81.1</td>
<td>22.6±32.7</td>
<td>32.4±67.2</td>
<td>17.7±35.4†</td>
</tr>
<tr>
<td>IS eosinophils (%)</td>
<td>8.4±10.1</td>
<td>2.9±4.1</td>
<td>5.2±11.2</td>
<td>2.5±4.4</td>
</tr>
<tr>
<td>IS macrophages (absolute count)</td>
<td>263.9±201.8</td>
<td>216.2±138.5</td>
<td>131.7±109.5</td>
<td>126.4±151.3*</td>
</tr>
<tr>
<td>Overall nasal symptoms</td>
<td>3.9±1.5</td>
<td>3.4±1.7</td>
<td>2.9±1.4</td>
<td>2.8±1.6</td>
</tr>
<tr>
<td>Quality of life</td>
<td>3.91</td>
<td>4±1</td>
<td>4.4±0.7</td>
<td>4.7±1.05*</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SD
†comparison of 2nd, 6th or 12th to baseline, p<0.01
*comparison of 2nd, 6th or 12th to baseline, p<0.05

Chronic aspirin desensitization resulted in significant improvements in overall nasal symptoms (ANOVA, p=0.03) and patient quality of life (ANOVA, p=0.01) (Fig. 3). The significant improvement of overall nasal symptoms was observed between baseline and 6th month (p=0.04), baseline and 12th month (p=0.045) as well as 2nd and 6th month (p=0.04) of aspirin desensitization. The significant improvement in patient quality of life was noted between baseline and 12th month (p=0.049) and between 2nd and 12th month (0.024) of aspirin desensitization.

Summary statistics of variables, which changes are statistically significant in the course of aspirin desensitization are summarised in table II.

Discussion
Advances in methods to obtain cytokines from IS and NL offer better understanding of NERD pathophysiology and allow clinicians to improve care of aspirin-sensitive patients. We conducted a prospective study of fifteen NERD individuals followed for a one-year period to investigate whether inflammatory cell count in IS and NL change during aspirin desensitization.

IS is regarded as a good representation of the inflammation within respiratory tract as it samples both large and small airways [19]. Our study revealed that in the course of aspirin desensitization the absolute number as well as the percentage of IS eosinophils significantly decreased. Since eosinophils play a major role in asthmatic inflammatory process, our findings confirmed the beneficial effect of aspirin therapy. This is consistent with the study of Katiel et al. [16], who investigated the effect of aspirin desensitization on novel biomarkers. Authors showed the decrease in sputum level of metalloproteinase 9 (MMP-9), which is involved in pathologic processes of bronchial asthma and is produced by eosinophils [20]. Moreover, the therapeutic algorithms guided by sputum eosinophilia might be worth considering for NERD patients, as some previous studies have already showed the superiority of tailoring asthma therapy on the basis of sputum eosinophilia in comparison to clinical assessment [21-23]. The benefits comprise reduced frequency of severe exacerbations as well as prolongation of severe exacerbation and no need for more treatment. Additionally, IS eosinophil count is a more sensitive predictor of asthma exacerbation than blood eosinophilia and the lack of correlation as well as poor concurrence between sputum and blood eosinophil count was reported [24].

We have also observed a slight increase of the IS eosinophilia in the 6 month of aspirin desensitization, but the eosinophil count did not reach the baseline value, was statistically insignificant and did not lead to the exacerbation of patient symptoms. As its the first study to investigate the IS cell count changes in the course of long-term aspirin therapy, we cannot compare our results with previously published data. Our finding may suggest that aspirin desensitization do not lead to the permanent decrease in the inflammatory cell count in the airways and there might be transitional periods characterized by the slight increases of these cells, which, however, does not influence clinical outcomes. We have also noted the decrease in the number of macrophages in IS samples taken from NERD patients subjected to aspirin desensitization. Macrophages, which link innate and adaptive immune responses, play a pivotal role in the initiation, maintenance and resolution of inflammation [25]. Alveolar macrophages are the dominant immune cells in the airways and are responsible for lung homeostasis [26]. Pathogen exposure causes their polarization into classically activated macrophages (M1 cells), which express mainly proinflammatory cytokines (TNF-α, IL-1β) and alternatively activated macrophages (M2 cells), which produce both allergic (IL-4, IL-13) and anti-inflammatory molecules (IL-10) [27]. As macrophages produce a spectrum of mediators, having both beneficial and detrimental effect, the single evaluation of the macrophage number in NERD patients cannot be directly transfer to answer specific clinical questions. However, the fact that aspirin desensitization results in the significant change in the IS macrophage count, makes the thorough analysis of these cells, including the expression of their cellular markers mandatory, as it may shed new light on their potential usability in disease monitoring and therapeutic interventions.

Although our study did not show any statistically significant changes in NL cell count in NERD individuals, we have observed a significant improvement of overall nasal symptoms and patient quality of life. It has already been stated before that aspirin desensitization should be considered as the first-line treatment for aspirin-sensitive patients with recalcitrant nasal polyposis and need for repeated sinus surgery [28], since it increases the mean length of surgical reintervention [29,30]. Other authors have also observed the lower number of sinus infections as well as improvements in ability to smell and upper/lower respiratory symptoms after one year of aspirin therapy [31]. The lack of correlation between the NL inflammatory cell count and the improvement of symptoms may be associated with the limited number of cases in our study, thus we are currently examining the problem on a larger group of participants.

Conclusions
In conclusion, a significant decrease in IS count of eosinophils and macrophages in NSAIDs-sensitive individuals subjected to one-year aspirin desensitization suggests that these cells might not play as significant a role in the NSAIDs-exacerbated respiratory disease and the process of aspirin desensitization. We also confirmed clinical efficacy of aspirin therapy in improving nasal symptoms and quality of life in NERD patients.

Acknowledgements
This work was supported by the grant 2015/19/B/NZ5/00096 from the National Science Centre (NCN).

References