Biomarkers for predicting response to aspirin therapy in aspirin-exacerbated respiratory disease

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Funding information
This work was supported by the National Science Centre, Poland [grant number UMO-2015/19/B/NZ5/00096] and Jagiellonian University Medical College, Cracow, Poland [grant number N41/DBS/000510].

Abstract
Background: Aspirin desensitization followed by daily aspirin use is an effective treatment for aspirin-exacerbated respiratory disease (AERD).
Objective: To assess clinical features as well as genetic, immune, cytological and biochemical biomarkers that might predict a positive response to high-dose aspirin therapy in AERD.
Methods: We enrolled 34 AERD patients with severe asthma who underwent aspirin desensitization followed by 52-week aspirin treatment (650 mg/d). At baseline and at 52 weeks, clinical assessment was performed; phenotypes based on induced sputum cells were identified; eicosanoid, cytokine and chemokine levels in induced sputum supernatant were determined; and induced sputum expression of 94 genes was assessed. Responders to high-dose aspirin were defined as patients with improvement in 5-item Asthma Control Questionnaire score, 22-item Sino-Nasal Outcome Test (SNOT-22) score and forced expiratory volume in 1 second at 52 weeks.
Results: There were 28 responders (82%). Positive baseline predictors of response included female sex (p = .002), higher SNOT-22 score (p = .03), higher blood eosinophil count (p = .01), lower neutrophil percentage in induced sputum (p = .003), higher expression of the hydroxyprostaglandin dehydrogenase gene, HPGD (p = .004) and lower expression of the proteoglycan 2 gene, PRG2 (p = .01). The best prediction model included Asthma Control Test and SNOT-22 scores, blood eosinophils and total serum immunoglobulin E. Responders showed a marked decrease in sputum eosinophils but no changes in eicosanoid levels.
Conclusions and Clinical Relevance: Female sex, high blood eosinophil count, low sputum neutrophil percentage, severe nasal symptoms, high HPGD expression and low PRG2 expression may predict a positive response to long-term high-dose aspirin therapy in patients with AERD.

Keywords
aspirin therapy, aspirin-exacerbated respiratory disease, gene expression analysis of cells, induced sputum, responders
1 | INTRODUCTION

Aspirin-exacerbated respiratory disease (AERD) is characterized by the presence of asthma, chronic rhinosinusitis with nasal polypsis, and acute respiratory reactions induced by aspirin and other cyclooxygenase-1 inhibitors. The disease is usually associated with eosinophilic inflammation of the bronchial mucosa, but sometimes, a non-eosinophilic subphenotype based on induced sputum (IS) cells is also observed. The hallmarks of the disease are complex alterations of the eicosanoid pathways, including reduced levels of anti-inflammatory eicosanoids, particularly prostaglandin E2 (PGE2), along with increased levels of proinflammatory cysteinyl leukotrienes (CysLT) and prostaglandin D2 (PGD2), due to eosinophil, mast cell and platelet-adherent granulocyte activation. Type 2 innate lymphoid cells (ILC2 s) have been implicated in the pathogenesis of AERD. The dysregulation of the pro- and anti-inflammatory eicosanoids was reported in various body fluids, such as IS supernatant (ISS), and tissue samples from ethmoidal and maxillary sinuses in AERD patients. Also direct in vivo measurements of ISS bioactive lipid mediators were shown to provide useful information on AERD subphenotypes.

The mechanisms of AERD are currently being investigated in the context of various treatment methods. One of the well-established therapeutic options is aspirin desensitization followed by daily aspirin therapy. The potential mechanisms underlying the clinical benefit of this approach include the down-regulation of CysLT1 receptor, inhibition of PGD2, and interleukin-4 via the transcription factor signal transducer and activator of transcription 6, as well as, paradoxically, systemic activation of type 2 (T2) inflammation. T2-driven inflammation asthma is associated with eosinophil and mast cell activation as well as type 2 cytokine release, for example IL-5. Although most patients report improvement of symptoms, there are individuals who do not benefit from aspirin therapy.

We aimed to identify AERD patients who are most likely to derive clinical benefits from high-dose aspirin therapy. For this purpose, we assessed numerous genetic, immune, cytological and biochemical biomarkers that might allow for the differentiation between responders and non-responders to aspirin treatment in AERD.

2 | METHODS

2.1 | Study group

Patients were recruited from the AERD cohort (n = 151) treated at the Department of Internal Medicine (Cracow, Poland), with aspirin hypersensitivity confirmed by provocation test. Of the 38 enrolled patients, 34 completed 52-week aspirin treatment and were included in statistical analysis, while 4 patients resigned (see Appendix A). All participants were in clinically stable condition, and their baseline forced expiratory volume in 1 s (FEV1) was at least 70% of the predicted value on challenge/desensitization days. None of the patients received systemic corticosteroids or leukotriene modifiers or experienced any exacerbation in the 6 weeks preceding the study. A history of treatment with biologicals was an exclusionary criterion. In all patients, the same regimen was used both in the 6 weeks before and during aspirin therapy (see Table 1 and Appendix A). All patients had severe asthma, diagnosed according to the 2020 Global Initiative for Asthma (GINA) update, and chronic rhinosinusitis with nasal polypsis. Baseline patient characteristics are presented in Table 1.

The study was approved by the Jagiellonian University Bioethics Committee (approval number: 122.6120.277.2016), and written informed consent was obtained from all participants.

2.2 | Study design

All patients underwent aspirin desensitization followed by 52-week aspirin treatment (650 mg/d). The run-in period was 52 weeks. During the study, all patients underwent two main hospitalizations: at baseline (challenge/desensitization days) and at the end of the study (at 52 weeks). Additionally, all patients underwent obligatory medical checkups at 8 and 16 weeks. After 52 weeks, patients remained under ambulatory care at our clinic and continued aspirin treatment.

The following evaluations were performed during hospitalizations: (1) clinical assessment (asthma control, nasal symptoms, spirometry); (2) cytological assessment (inflammatory phenotype detection based on IS cells); and (3) biochemical tests: blood eosinophil count, neutrophil count and total immunoglobulin E (IgE) levels; ISS levels of cytokines (IL-4, IL-5, IL-13, IL-17E/IL-25, IL-33; thymic stromal lymphopoeitin) and chemokines (CCL17, CCL22, CCL26) of the T-helper-2 (Th2) pathway as well as eicosanoids (PGD2, PGE2, 8-iso-PGE2, tetranor-PGD-M, tetranor-PGE-M, leukotriene LTBA4, CysLT); and, finally, urinary LTE4 levels.

Asthma exacerbations, computed tomography scans of the sinonuses, total IgE levels in ISS and the mRNA expression of 94 genes (see Appendix C) in sputum cells were evaluated at baseline and at 52 weeks (Figure 1).
Clinical evaluation

Exacerbations in the year preceding the study and during the 52-week treatment were defined as asthma symptoms requiring hospitalization or systemic steroid therapy. Asthma control was evaluated using the Asthma Control Test (ACT) and 5-item Asthma Control Questionnaire (ACQ-5). The 22-item Sino-Nasal Outcome Test (SNOT-22) was used to assess nasal symptoms. Computed tomography scans of the sinuses were assessed independently by two experienced radiologists according to the Lund-Mackay score.

Participants were classified as responders to high-dose aspirin treatment if they met at least one of the following criteria based on the validated values of the primary end-points: (1) increase in prebronchodilator FEV$_1$ by at least 100 mL; (2) increase in the ACQ-5 score by at least 0.5 points; and (3) reduction in the SNOT-22 score by at least 9 points.

Aspirin desensitization

Subjects with AERD underwent aspirin desensitization protocol as previously reported.

Induced sputum collection

IS was collected according to the European Respiratory Society recommendations. Additional information can be found in Appendix A.

T2 inflammatory profile for severe asthma

T2 inflammatory profile for severe asthma was defined as blood eosinophil count of 150 cells/µL or higher and IS eosinophil percentage of 2% or higher based on the GINA report.

T2 asthma based on IS cells

T2 asthma was defined as eosinophil percentage of 3% or higher and neutrophil percentage lower than 64%, while non-T2 asthma,
as eosinophil percentage of 3% or lower irrespective of neutrophil percentage (≥64% or <64%). Mixed phenotype (eosinophils ≥ 3% and neutrophils ≥ 64%) was considered as transient between eosinophilic and neutrophilic phenotypes and was not included in this classification. Non-T2 asthma was traditionally defined as asthma without features of T2 asthma.\(^{35}\)

2.8 | Biochemical and genetic evaluation

The analysis of total IgE, cytokines, chemokines and eicosanoids\(^{17}\) as well as targeted gene expression analysis of IS cells is described in Appendix A.

2.9 | Prediction model development

All the above clinical, genetic, immune, cytological and biochemical parameters were included in the analysis. The model was created by subsequent addition of each parameter, starting from demographic data and routine measurements (questionnaires, spirometry, blood eosinophil count) to more specialized evaluations (inflammatory, biochemical and genetic biomarkers). The optimal model was developed using stepwise model selection based on the Akaike information criterion (AIC), which provides a means for model selection by estimating the quality of each candidate model among the models created from the available data. The preferred model is the one with the minimum AIC value. The AIC rewards goodness of fit (assessed by the likelihood function) but also includes a penalty, namely, an increasing function of the number of estimated parameters. The penalty eliminates overfitting (increasing the number of parameters almost always improves the goodness of fit).\(^{36}\) Discriminative ability was calculated with the area under the receiver operating characteristic curve.

2.10 | Statistical analysis

Summary statistics were presented as mean with standard deviation, median with 25th and 75th percentiles, OR number in each category with the percentage of total. We transformed mediator concentrations to their logarithm or used Box-Cox transformation to approximate the distributions to normality before analysis. Normality was checked using the Shapiro-Wilk test. A general linear model and the post hoc Tukey test were applied to assess differences in mediator levels in time and between groups. Logistic regression was used to identify the best predictors. Categorical data were analysed using the chi-square, Fisher exact and McNemar tests. A p-value lower than .05 was considered significant. In the case of group comparisons, the size of the effects was expressed by differences between the means (assuming a normal distribution) or medians. For pairwise comparisons, the means or medians of the differences were estimated. A 95% confidence interval was also calculated for each effect. In the gene expression analysis, a twofold change in the relative expression of a given target (ie log2fold <−1 or >1) with a p-value lower than .05 (estimated either with an unpaired or paired t-test, depending on the experimental setting) was considered significant. p-values were adjusted to account for multiple hypotheses using the Benjamini-Hochberg false discovery rate. Statistical analysis was performed using Dell Statistica (v.13) and GraphPad Prism (v.8.4.2).

3 | RESULTS

3.1 | Response to aspirin treatment

Of the 34 patients, 28 (82%) were classified as responders to aspirin. The optimal model for the prediction of response to treatment included the ACT score (A), SNOT-22 score (B), blood eosinophil

FIGURE 1 An overview of the trial design and procedures. CT, computed tomography
count (C) and serum total IgE (D). Based on the logit model, the discriminative linear function was constructed: \[ \text{LOGIT}[P(\text{RESP})] = 1.5250 - 0.4526 \times A + 0.1631 \times B + 0.0204 \times C + 0.0072 \times D \geq 0. \] The sensitivity for the prediction of response to aspirin was 96.4% (81.7%-99.9%); specificity, 100.0% (54.1%-100.0%); and accuracy, 97.1% (84.7%-99.9%).

The baseline predictors of positive response included female sex (OR = 30, 95%CI: 2.75–328.6, \( p = .002 \)), higher SNOT-22 score (mean difference = 18.6, 95%CI: 1.9–35.4, \( p = .03 \)), higher blood eosinophil count (difference between medians = 210, 95%CI: 50–390, \( p = .01 \)) and lower IS neutrophil percentage (difference between medians = 34.7, 95%CI: 14.7–54.2, \( p = .003 \)). Responders also showed higher expression of the hydroxyprostaglandin dehydrogenase gene, HPGD (mean difference = 0.31, 95%CI: 0.19–0.43, \( p < .001 \), adjusted \( p = .004 \)) and lower expression of the proteoglycan 2 gene, PRG2 (mean difference = 1.32, 95%CI: 0.70–1.94, \( p < .001 \), adjusted \( p = .01 \)) in sputum cells (see Figure 2 and Appendix D).

### 3.2 Evaluation of responders and non-responders at 52 weeks

#### 3.2.1 Clinical outcomes

Responders showed improvement in ACT (mean difference = 2.4, 95%CI: 1.1–3.7, \( p = .001 \)), SNOT-22 (mean difference = 17, 95%CI: 10.6–23.4, \( p < .001 \)) (Figure 3A) and ACQ-5 (mean difference = 0.65, 95%CI: 0.19–1.12, \( p = .007 \)) scores. However, no changes were observed in non-responders. A decline in FEV1 was noted in non-responders (mean difference = 348.6 ml, 95% CI: 43.0–654.2, \( p = .002 \)).

**Figure 2**: Changes in HPGD and PRG2 gene expression in sputum cells at 52 wk of aspirin therapy in comparison with baseline in responders (\( n = 28 \)) and non-responders (\( n = 6 \)). HPGD, hydroxyprostaglandin dehydrogenase gene; PRG2, proteoglycan 2 gene. log rel to GAPDH, relative quantities (ie log2fold change) of individual transcripts were calculated after data normalization to a housekeeping gene (GAPDH).
p = .03) (Figure 3B), while no changes were noted in responders. The total Lund-Mackay score did not change either in responders or non-responders.

3.2.2 Eosinophils and neutrophils in blood and IS

The absolute count of blood eosinophils did not change in responders (p = .316) (Figure 4A), while an increase was noted in non-responders (median of differences=526 cells/μL, 95%CI: 40–1420, p = .04). Responders showed a decrease in IS eosinophil percentage (median of differences = 1.7%, 95%CI: 0.6%–3.6%, p = .001) (Figures 3A and 4A), while no changes were noted in non-responders. Significant correlations between blood and IS eosinophil count were only shown in responders at 52 weeks (r = 0.46, p = .02). No significant changes were observed in blood neutrophil count or IS neutrophil percentage either in responders or non-responders.
3.2.3 | T2 inflammatory profile for severe asthma

There were no changes in the prevalence of blood eosinophilia in responders \((p = .48)\), while an increase was noted in non-responders \((p = .01)\). No changes in the prevalence of sputum eosinophilia were observed either in responders or non-responders \((p = .12\) and \(p = .62\), respectively).

3.2.4 | T2 asthma based on IS

There was a decrease in prevalence of T2 asthma phenotype on high-dose aspirin in responders \((p = .03)\), while no changes were noted in non-responders \((p = .62)\).

3.2.5 | Eicosanoids

No significant changes were observed in the ISS level of any eicosanoids either in responders (Figure 4A) or non-responders (Figure 4B). Urinary \(\text{LTE}_4\) levels increased both in responders (median of differences = 583.5 pg/mg of creatinine, 95%CI: 39–1398, \(p = .03\)) (Figures 3A and 4A) and non-responders (median of differences = 751 pg/mg of creatinine, 95%CI: 10.2–3423, \(p = .03\)) (Figures 3B and 4B).

3.2.6 | Gene expression analysis of IS cells

There were no changes in relative gene expression either in responders or non-responders (adjusted \(p > .05\)) (see Appendix E).
We aimed to establish clinical features as well as genetic, immune, cytological and biochemical biomarkers that differentiate responders from non-responders to aspirin treatment among AERD patients. Most participants experienced clinical improvement on aspirin, which is in line with other studies.25,26

At baseline, responders to aspirin in our study were characterized by severe nasal symptoms. The period from sinus surgery to aspirin desensitization was longer in responders than in non-responders. It was suggested that aspirin challenge/desensitization should be proposed to patients shortly after sinus surgery when their aspirin-induced hypersensitivity reactions become less severe.14,37-40 In our study, 28 patients (82%) underwent sinus surgery, among which 13 patients within the year before aspirin treatment initiation. An additional positive prognostic factor in our study was the eosinophil percentage. Notably, the local reduction of T2 asthma in our study was observed only in non-responders, while it remained stable in responders to treatment, the increase of blood eosinophil count and lower sputum neutrophil percentage independent of systemic global and local atopy were potentially associated with good response to aspirin treatment in AERD patients. Indeed, T2 asthma profile, associated with ILC2 and Th2 cells, predicts positive response to high-dose aspirin. The release of IL-25, IL-33 and thymic stromal lymphopoietin from respiratory epithelium (all of which activate ILC2 via its soluble receptor ST2) leads to T2 cytokine release (IL-4, IL-5, and IL-13) from ILC2 cells, followed by mast cell degranulation and, finally, eosinophil attraction.44–46 As IL-5 (responsible for eosinophil maturation and release) is highly released by ILC2 s and Th2 cells, blood and tissue eosinophilia can serve as evidence of inflammation driven by both cell types.47,48 We observed an increase in blood eosinophil count and urinary LTE_4 levels on aspirin, which is in line with other studies.22,49 However, after participants were stratified according to response to treatment, the increase of blood eosinophil count was observed only in non-responders, while it remained stable in responders. This was associated with a decrease in sputum eosinophil percentage. Notably, the local reduction of T2 asthma in our patients did not lead to a switch to other pathologic phenotypes (ie neutrophilic, which has worse clinical outcomes).50 Therefore, the threshold value for blood eosinophil count that best identifies AERD responders should be determined with regard to asthma severity and the confounding local effects of corticosteroids as well as other immune modulators.12,51 Patients with an inflammatory neutrophilic phenotype based on IS are unlikely to respond to aspirin treatment. The threshold for IS neutrophils that best identified responders was 48.5% or lower. Therefore, the identification of a non-T2 asthma profile is essential as it rather excludes aspirin treatment.

Response to aspirin treatment in AERD patients was also influenced by sputum HPGD expression. Higher HPGD expression may predict a greater benefit from aspirin therapy. The HPGD gene encodes prostaglandin-degrading enzyme 15-hydroxyprostaglandin dehydrogenase, which is a functional antagonist of cyclooxygenase COX-2 and might stabilize or even up-regulate COX-2 during long-term aspirin therapy.52 Notably, COX-2 down-regulation has been reported in the nasal polyps of patients with asthma and aspirin hypersensitivity at baseline.53 Arachidonic acid is preferentially metabolized by COX-2 to anti-inflammatory prostanooids such as prostacyclin and PGE_2.54 We speculate that COX-2/HPGD system functions as a complex network that potentially regulates response to high-dose aspirin therapy in AERD. Stable PGE_2 production in the airways during aspirin therapy in our patients might have positive effects including stabilization and/or balancing of mast cell and ILC2 activation.22,55,56 It has already been suggested that the expression of the PTGS2 gene encoding COX-2 increases in human intestinal myofibroblasts subjected to high-dose aspirin.57

On the other hand, a greater benefit from aspirin therapy in AERD patients may be predicted by lower sputum expression of the PRG2 gene. Proteoglycan 2, a protein encoded by PRG2, is the predominant constituent of the crystalline core of the eosinophil granule.58 This protein might be involved in immune hypersensitivity reactions by being directly implicated in epithelial cell damage and bronchospasm in patients with asthma.59 Indeed, a higher PRG2 expression at baseline in AERD patients, indicating potentially greater activity of eosinophils, predicted the lack of response to long-term aspirin treatment. Thus, we hypothesize that eosinophil activity is more important than quantity in bronchial mucosa as far as response to high-dose aspirin is concerned.

Subsequently, we attempted to create a prediction model that would precisely predict the response to aspirin therapy. We used the baseline values of all the analysed clinical, genetic, immune, cytological and biochemical parameters. The proposed model, based on ACT and SNOT-22 scores, blood eosinophil count and total IgE levels, helps identify patients who are most likely to respond to high-dose aspirin. The inclusion of inflammatory phenotypes based on IS, local and systemic eicosanoid levels, or even gene expression does not improve the prediction of response. After further validation, our model could be easily implemented in clinical practice with the use of a simple spreadsheet (eg Excel), allowing a quick recognition of potential responders to high-dose aspirin.

The dysregulation of pro- and anti-inflammatory lipid mediators is the key feature of AERD. Eicosanoid levels in ISS did not change in our patients during aspirin therapy irrespective of treatment response. The sputum cell expression of the genes associated with arachidonic acid pathways also remained stable. Interestingly, during
aspirin challenge, a relatively lower dose of cyclooxygenase-1 inhibitor blocks PGE\(_2\) production\(^{10}\) and thus unbreaks CysLT synthesis, leading to eosinophil and mast cell activation, which mediates symptom exacerbation in AERD.\(^1\) In contrast, long-term high-dose aspirin intake maintains the homeostasis of arachidonic acid metabolism in the airways by stabilizing/balancing the expression of the genes associated with arachidonic acid pathways, resulting in stable levels of sputum pro- and anti-inflammatory eicosanoids.

The local balance of the pro- and anti-inflammatory eicosanoids on aspirin was associated with increased urinary LTE\(_4\) levels. Cahill et al. also reported an increase in urinary LTE\(_4\) levels on aspirin, which was probably associated with systemic mast cell activation.\(^{22}\) On the other hand, long-term aspirin therapy might cause local homeostasis of mast cells,\(^{59}\) which is reflected by stable PGD\(_2\) levels in our patients. Moreover, stable local PGE\(_2\) levels elicits its anti-inflammatory signalling through EP2 receptor by stabilizing mast cells, thus blocking PGD\(_2\) and CysLT as well as controlling T2 cytokine production.\(^{56,60,61}\) Indeed, Th2 and ILC2 cytokines in ISS did not increase on aspirin, which suggests that long-term aspirin administration stabilizes local mediators specific for T2 asthma profile.

Our findings should be interpreted in the context of study limitations. First, our main objective was to define patient-related factors that determine good response to aspirin treatment. As a relatively small number of patients did not respond to treatment, our results should be validated in a larger cohort. Second, it is unclear whether the observed effects of treatment result from nonspecific anti-inflammatory properties of aspirin or are directly associated with desensitization followed by aspirin therapy. Finally, all patients were treated using the same regimen, which does not reflect the clinical heterogeneity of AERD. Future studies should include patients with various levels of asthma severity.

In conclusion, a positive response to high-dose aspirin in AERD patients with severe asthma is associated with T2 asthma profile. Regardless of the response, aspirin treatment balances local eicosanoid production and increases urinary LTE\(_4\) levels. The selection of AERD patients for aspirin treatment could be easily performed using simple clinical and laboratory measurements, with baseline T2 asthma as the main discriminative feature.

ACKNOWLEDGEMENTS

We thank Anita Borkowska-Mosur, Anna Gielicz, Małgorzata Głuszek, Ewa Młicka-Kowalczyk and Hanna Plutecka for technical assistance. We also thank all patients for participating in the study.

CONFLICT OF INTEREST

None of the authors have a conflict of interest in relation to this work.

AUTHOR CONTRIBUTIONS

KET and LM involved in project concept, study design and study implementation; contributed to writing of the first draft of the manuscript. KET, AC and LM involved in data and statistical analysis. All authors contributed to data collection and manuscript editing; reviewed and approved the final version of the manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION
Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Tyrak KE, Pajdzik K, Jakiela B, et al. Biomarkers for predicting response to aspirin therapy in aspirin-exacerbated respiratory disease. Clin Exp Allergy. 2021;51:1046–1056. https://doi.org/10.1111/cea.13886