



## Utility of low-dose oral aspirin challenges for diagnosis of aspirin-exacerbated respiratory disease

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### ABSTRACT

**Background:** Aspirin-exacerbated respiratory disease (AERD) is diagnosed through graded aspirin challenges that induce hypersensitivity reactions and eicosanoid level changes. It is not known whether diagnostically useful changes also occur after low-dose aspirin challenges that do not induce hypersensitivity reactions.

**Objective:** To investigate the utility of low-dose oral aspirin challenges for diagnosing AERD by measuring different clinical parameters and eicosanoid changes.

**Methods:** Sixteen patients with AERD and 13 patients with aspirin-tolerant asthma underwent oral challenges with low-dose (20 or 40 mg) aspirin and diagnostic oral graded aspirin challenges (up to 325 mg of aspirin). Forced expiratory volume in 1 second, nasal peak flow, the fraction of exhaled nitric oxide (FeNO), and eicosanoid levels in plasma and urine were analyzed.

**Results:** In patients with AERD but not in those with aspirin-tolerant asthma, 40-mg aspirin challenges induced a significant mean (SEM) decrease from baseline in FeNO (19% [5.1%];  $P = .001$ ) without causing any hypersensitivity reaction. The FeNO decrease also occurred after higher-dose aspirin challenges (27.8% [4.9%];  $P < .001$ ). The sensitivity and specificity of 40-mg aspirin-induced FeNO changes for identifying AERD were 90% and 100% with an area under the curve of 0.98 (95% CI, 0.92–1.00). The low-dose challenge also induced a significant leukotriene E<sub>4</sub> urine increase in patients with AERD (from 6.32 [0.08] to 6.91 [0.15] log-pg/mg creatinine;  $P < .001$ ), but the sensitivity and specificity of these changes were less than for the FeNO changes.

**Conclusion:** The low-dose aspirin-induced decrease in FeNO in patients with AERD may be useful for the diagnosis of aspirin allergy without inducing a hypersensitivity reaction.

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### Introduction

Approximately 10% of adults with asthma have aspirin-exacerbated respiratory disease (AERD) characterized by more severe persistent asthma.<sup>1</sup> Distinct differences in several eicosanoids

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are seen in patients with AERD compared with those with aspirin-tolerant asthma (ATA), including differences in baseline levels, after clinical reactions to aspirin, and after aspirin desensitization and treatment.<sup>2–9</sup> AERD is characterized by higher baseline urinary leukotriene E<sub>4</sub> (LTE<sub>4</sub>) levels compared with patients with ATA.<sup>4,10</sup> During a standard oral graded aspirin challenge, urinary LTE<sub>4</sub> levels increase further.<sup>3,8,11</sup> Levels of another eicosanoid, prostaglandin D<sub>2</sub> (PGD<sub>2</sub>) increase in patients who develop bronchospasm after aspirin challenge.<sup>12–14</sup> After an aspirin challenge, urine levels of prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) metabolites do not change in patients with AERD but decrease in those with ATA.<sup>7</sup>

The diagnosis of AERD usually requires the induction of a clinically apparent hypersensitivity reaction to aspirin, which has significant morbidity because of the often severe and prolonged nature of these reactions. In this study, we investigated the effect of low-dose oral aspirin on selected clinical measurements and on

eicosanoid changes of patients with AERD to determine whether it was possible to diagnose aspirin sensitivity without inducing a clinically apparent hypersensitivity reaction.

Hypersensitivity reactions in patients with AERD are dose dependent, and most patients with AERD do not react to a low oral dose (<30 mg) of aspirin.<sup>15,16</sup> We therefore challenged patients with 2 low aspirin doses (20 and 40 mg). Most patients with AERD tolerate 20 mg of aspirin,<sup>15</sup> but this dose still blocks platelet cyclooxygenase (COX) 1 and inhibits thromboxane production in healthy volunteers.<sup>17</sup> We used the 40-mg aspirin dose because this is the initial dose used during standard oral aspirin challenges.<sup>16,18</sup>

In addition to measuring eicosanoid levels, we also analyzed other measures of reactions to aspirin, including forced expiratory volume in 1 second (FEV<sub>1</sub>), nasal peak flow (NPF), and fraction of exhaled nitric oxide (FeNO). Previous reports suggested that FeNO values increase during a 2-day oral aspirin desensitization procedure and during inhaled aspirin-lysine challenges in patients with AERD.<sup>19,20</sup> On the other hand, another study found that nasal nitric oxide exhibited a dual pattern of changes during nasal challenges with aspirin-lysine.<sup>21</sup> For all the clinical and eicosanoid parameters, we measured changes after low-dose aspirin challenges that did not induce any apparent clinical symptoms and after standard diagnostic challenges that induced clinically apparent aspirin-induced hypersensitivity reactions.

## Methods

### Patients

Patients with AERD and ATA with a history of physician-diagnosed asthma were recruited into the study. All patients with AERD had history of nasal polyposis and nonsteroidal anti-inflammatory drug (NSAID)–induced respiratory reactions. All patients suspected of having AERD were confirmed as having AERD with a standard oral graded aspirin challenge. All aspirin-tolerant patients occasionally used NSAIDs without any adverse reactions. They were asked to stop occasional NSAID use 2 weeks before and for the duration of this study. All patients signed informed consent forms at the time of enrollment in the study, approved by the Albert Einstein College of Medicine Institutional Review Board.

### Protocol

The study consisted of 3 visits. Visits were scheduled at least 1 week apart to allow an aspirin washout period. Oral aspirin challenge with 20 mg was performed at visit 1 and with 40 mg at visit 2. During visit 3 all patients underwent a standard oral graded aspirin challenge to confirm aspirin sensitivity using a previously described protocol.<sup>16</sup> In brief, for the graded challenge, 40 mg of aspirin (dissolved Original Alka-Seltzer) was initially administered, and then the dose was doubled every 90 minutes (80, 160, and 325 mg). The diagnosis of AERD was confirmed by the presence of a positive aspirin challenge response, defined as a hypersensitivity reaction with at least one of the following symptoms: (1) 20% or greater decrease in FEV<sub>1</sub>, (2) decrease in FEV<sub>1</sub> of less than 20% combined with naso-ocular reactions, and/or (3) isolated naso-ocular reactions. Patients who experienced a hypersensitivity reaction were treated based on their specific symptoms. The aspirin challenge in patients with ATA followed the same dosing schedule with a cumulative dose of aspirin of 605 mg. Because the threshold for reactions to aspirin is variable in patients with AERD,<sup>15</sup> if patients developed a hypersensitivity reaction on low-dose aspirin exposure, their findings were analyzed as part of the aspirin challenge that led to a clinically apparent hypersensitivity reaction. To confirm that including low-dose reactors data did not unduly influence the outcomes, we analyzed the data both ways: including and excluding low-dose reactors to aspirin.

All patients continued to take their prescribed inhaled corticosteroids (ICSs) with or without long-acting  $\beta$ -agonists (LABAs) at the time of the study. On the mornings of the study visits, ICSs and both LABAs and short-acting  $\beta$ -agonists were withheld. To enhance the safety of aspirin challenges in the patients with AERD,<sup>22,23</sup> all study participants (patients with AERD and ATA) took 10 mg of montelukast daily during the week before the standard graded oral aspirin challenge (visit 3) or continued to take it throughout the study if it was part of their usual medical regimen. All 5-lipoxygenase inhibitors were held for the duration of the study, if applicable. Spirometry, NPF values, and respiratory symptoms were recorded at baseline and then after aspirin administration (20 or 40 mg) at 15 to 30 minutes, 30 to 60 minutes, and more than 60 minutes during the first 2 visits. During the confirmatory standard oral graded aspirin challenge (visit 3, 40–325 mg), recordings were made at the same time points after a hypersensitivity reaction or when the 325-mg dose of aspirin was reached. Spirometry was performed at each time point according to standard guidelines with a Puritan Bennett Renaissance II spirometer (Puritan Bennett, Pleasanton, California).<sup>24</sup> NPF was measured at each time point with an In-Check nasal inspiratory flow device (Clement Clarke International Ltd, Essex, England) according to the manufacturer guidelines.<sup>25</sup> The best of 3 efforts was recorded during spirometry and NPF measurements. The FeNO values were recorded by using NIOX (Aerocrine AB, Morrisville, North Carolina) according to the manufacturer guidelines at the same time points before performing spirometry and measuring NPF. FeNO was not measured during the 20-mg aspirin challenge in either group because of budgetary constraints. Blood and urine specimens were collected at the beginning of each visit. After the aspirin dose was administered during the first 2 visits, blood was collected at 15, 30, 60, and 120 minutes; urine was collected at 0 to 1 hour, 1 to 2 hours, and 2 to 3 hours. At the third visit, specimens were collected at the same time points after a hypersensitivity reaction or when the 325-mg dose of aspirin was reached.

### Blood and Urine Eicosanoid Measurements

Plasma and urine specimens were immediately frozen at  $-80^{\circ}\text{C}$ , then shipped overnight for analysis by Cayman Chemical Inc (Ann Arbor, Michigan). Tetranor prostaglandin D<sub>2</sub> metabolite (PGDM) and LTE<sub>4</sub> were measured in urine, and PGE<sub>2</sub>-metabolite (PGEM) was measured in plasma by enzyme-linked immunosorbent assay.<sup>26–28</sup> All measurements were made in triplicate. The results are expressed as picograms per milligram of creatinine for LTE<sub>4</sub> and tetranor PGDM and picograms per milliliter of plasma for PGEM.

### Statistical Analysis

Baseline characteristics were compared between patients with AERD and patients with ATA by using independent sample *t* tests or Wilcoxon rank sum tests when data were nonnormally distributed. Logarithmic transformation was used for LTE<sub>4</sub> data. All summary statistics were expressed as mean (SEM) or medians (interquartile range [IQR]). General linear models were used for multiple comparisons to reflect the fact that the outcome measurements were repeated over time within individuals. Receiver operating characteristic curves were plotted to choose the best cutoff points. The sensitivity, specificity, positive predictive value, and negative predictive value were calculated. All statistical analyses were performed with STATA statistical software, version 14.0 (StataCorp, College Station, Texas). *P* < .05 was considered significant for all analyses.

## Results

Sixteen patients with AERD and 13 patients with ATA were studied. Participant characteristics are presented in Table 1. Both

**Table 1**  
Baseline characteristics of study patients

| Characteristic                                                               | Patients with AERD (n = 16) | Patients with ATA (n = 13) | P value |
|------------------------------------------------------------------------------|-----------------------------|----------------------------|---------|
| Age, mean (SEM), y                                                           | 37.8 (3.2)                  | 42.6 (2.4)                 | .20     |
| Sex (female/male), No.                                                       | 10/6                        | 7/6                        | .50     |
| Race, No. (%)                                                                |                             |                            | .20     |
| African American                                                             | 5 (31.2)                    | 2 (15.4)                   |         |
| Hispanic                                                                     | 6 (37.5)                    | 7 (53.8)                   |         |
| White                                                                        | 5 (31.3)                    | 3 (23.1)                   |         |
| Other                                                                        |                             | 1 (7.7)                    |         |
| Aspirin hypersensitivity duration, mean (SEM), y                             | 4 (1.6)                     | NA                         |         |
| Presence of nasal polyps, %                                                  | 87.5                        | 23.1                       | <.001   |
| ICS or LABA use, %                                                           | 87                          | 76                         | .50     |
| Montelukast use, % <sup>a</sup>                                              | 37.5                        | 50                         | .60     |
| Baseline FEV <sub>1</sub> , mean (SEM), % predicted                          | 73.0 (3.1)                  | 92.5 (9.4)                 | .30     |
| Nasal peak flow, mean (SEM), L/min                                           | 81.7 (12.7)                 | 143 (28.1)                 | .04     |
| Absolute eosinophil count in peripheral blood, mean (SEM), k/mm <sup>3</sup> | 0.6 (0.13)                  | 0.15 (0.2)                 | <.01    |
| Peripheral blood eosinophils, mean (SEM), % leukocyte                        | 10.0 (1.3)                  | 2.0 (1.7)                  | <.001   |
| Serum IgE, median (IQR), IU/L                                                | 198 (102–372)               | 288 (172–716)              | .20     |
| FeNO, median (IQR), ppb                                                      | 29 (21–104)                 | 18 (13–23)                 | .01     |
| Urinary LTE <sub>4</sub> , median (IQR), pg/mg creatinine                    | 660.4 (462.6–833.4)         | 426.2 (356.2–455.2)        | <.01    |
| Urinary tetranor PGDM, mean (SEM), pg/mg creatinine                          | 6440.3 (697.5)              | 6698.2 (828.2)             | .80     |
| Plasma PGEM, mean (SEM), pg/mL                                               | 38.8 (5.6)                  | 54.9 (9.1)                 | .30     |

Abbreviations: AERD, aspirin-exacerbated respiratory disease; ATA, aspirin-tolerant asthma; FENO, fraction of exhaled nitric oxide; FEV<sub>1</sub>, forced expiratory volume in 1 second; ICS, inhaled corticosteroid; IQR, interquartile range; LABA, long-acting  $\beta$ -agonist; LTE<sub>4</sub>, leukotriene E<sub>4</sub>; NA, not applicable; PGDM, prostaglandin D<sub>2</sub> metabolite; PGEM, prostaglandin E<sub>2</sub> metabolite.

<sup>a</sup>This value represents the percentage of patients in each group taking montelukast throughout the study as part of their medical regimen. All patients (AERD and ATA) were asked to take 10 mg of montelukast daily during the week before the standard graded oral aspirin challenge.

groups had a similar distribution of age, sex, race, and baseline FEV<sub>1</sub>, total serum IgE level, and PGEM and urinary tetranor PGDM levels. There were significant differences between the 2 groups in absolute counts and percentages of peripheral blood eosinophils, the number of patients with a history of nasal polyps, FeNO levels, and NPF values. Baseline urine LTE<sub>4</sub> levels were higher in patients with AERD.

Among patients with AERD, there were no clinical hypersensitivity reactions to the 20-mg aspirin challenges. There were 2 patients who clinically had responded to 40 mg of aspirin: one had nasal congestion, rhinorrhea, and nausea/vomiting, and the other reacted with a bronchospasm and nausea/vomiting. Because these 2 patients had a clinical hypersensitivity reaction, their data were analyzed as part of the standard graded aspirin challenge as described in the Methods. Excluding them from the analysis did not alter the results (data not shown). As expected, all patients with AERD experienced a hypersensitivity reaction during the standard graded aspirin challenge, whereas none of the patients with ATA reacted to the graded aspirin challenges.

#### FeNO Responses to Low and Graded Aspirin Challenge

FeNO changes in response to aspirin were measured in all 16 patients with AERD and in 10 patients with ATA. Sixty minutes after the 40-mg aspirin challenge, the FeNO levels decreased in 13 of the 14 patients with AERD who did not exhibit a clinical reaction to this dose (Fig 1A). In contrast, FeNO levels increased or remained the same in 9 of the 10 patients with ATA (Fig 1B). There was a significant decrease from baseline in mean FeNO in patients with AERD by 9.0% (4.0%) at 15 to 30 minutes, 8.1% (3.0%) at 30 to 60 minutes, and 19.0% (5.1%) at greater than 60 minutes ( $P = .001$ ) (Fig 1A). In contrast, there was an apparent increase in FeNO in the patients with ATA, but this was not statistically significant ( $P = .08$ ) (Fig 1B).

A similar pattern of FeNO changes was observed after the standard, hypersensitivity-provoking graded aspirin challenge. During the first hour after the onset of the hypersensitivity reaction, FeNO levels decreased in all 16 patients with AERD (Fig 2A), whereas levels either increased or remained the same in all 10 patients with ATA (Fig 2B). The mean decrease in FeNO in patients

with AERD was –13.9% (4.0%) at 15 to 30 minutes, –27.8% (4.9%) at 30 to 60 minutes, and –21.8% (6.6%) at greater than 60 minutes after the onset of hypersensitivity reaction ( $P < .001$ ) (Fig 2A). For the ATA group, the mean FeNO level changed by 3.9 (5.3) at 15 to 30 minutes, 15.9 (6.4) at 30 to 60 minutes, and 9.0 (10.1) at greater than 60 minutes ( $P = .10$ ) (Fig 2B).

#### Changes in FEV<sub>1</sub> and NPF After Low and Graded Aspirin Challenges

Changes in FEV<sub>1</sub> and NPF were measured in all patients after the low-dose and standard aspirin challenges. There was no significant change in FEV<sub>1</sub> or NPF after 40 mg of aspirin in the AERD group, confirming that this dose did not induce a clinically apparent hypersensitivity reaction (Fig 3A and C). During the standard graded aspirin challenge, FEV<sub>1</sub> decreased in the patients with AERD by 6.2% (1.8%) at 15 to 30 minutes, 18.8% (2.6%) at 30 to 60 minutes, and 5.0 (7.6) at greater than 60 minutes ( $P = .03$ ) (Fig 3A). NPF decreased in patients with AERD after the standard graded aspirin challenge by 27.0% (5.1%) at 15 to 30 minutes, 13.7% (5.8%) at 30 to 60 minutes, and 17.7% (7.9%) at greater than 60 minutes ( $P = .001$ ) (Fig 3C). There was no significant change in FEV<sub>1</sub> or NPF in the patients with ATA during the standard graded aspirin challenge (Fig 3B and D).

#### Changes in LTE<sub>4</sub> After Low and Graded Aspirin Challenges

Urine levels of LTE<sub>4</sub> were measured after low and graded aspirin challenge. There was no significant change in LTE<sub>4</sub> level in either the AERD or ATA group after 20 mg of aspirin (Fig 4A). In patients with AERD, after 40 mg of aspirin, there was a significant mean increase in LTE<sub>4</sub> from 6.32 (0.08) to 6.91 (0.15) log-pg/mg of creatinine at 3 hours ( $P < .001$ ) (Fig 4A). There was no significant increase in LTE<sub>4</sub> in the patients with ATA (Fig 4A).

After the standard oral aspirin challenge, there was a significant mean increase in LTE<sub>4</sub> in patients with AERD from 6.5 (0.1) to 7.81 (0.3) log-pg/mg of creatinine ( $P < .001$ ) (Fig 4B). There was also a significant but smaller mean increase in LTE<sub>4</sub> in patients with ATA after the graded aspirin challenge (cumulative aspirin dose of 605 mg), from 6.0 (0.1) to 6.4 (0.17) log-pg/mg creatinine ( $P = .02$ ) (Fig 4B).

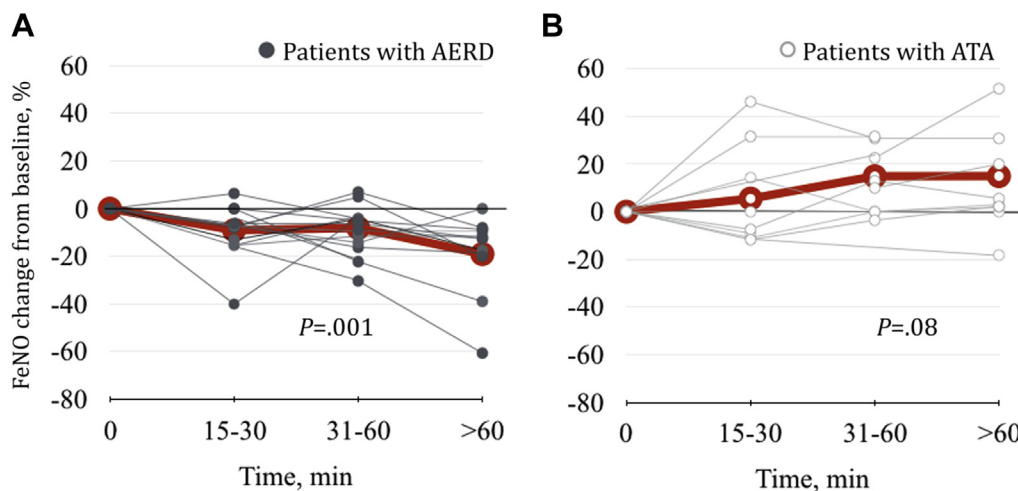


**Figure 1.** Fraction of exhaled nitric oxide (FeNO) change after 40-mg aspirin challenge. A, In the 14 patients with aspirin-exacerbated respiratory disease (AERD), there was a significant decrease in FeNO ( $P = .001$ ). B, In the 10 patients with aspirin-tolerant asthma (ATA) patients, there was no significant change in FeNO ( $P = .08$ ). Gray thin lines indicate individual data points; red thicker lines, mean values.

#### Changes in Tetranor PGDM after Low and Graded Aspirin Challenges

Tetranor PGDM is the major metabolite of PGD<sub>2</sub>. There was no significant change in urine tetranor PGDM concentration in either the AERD or ATA group after the low dose 20-mg or 40-mg aspirin challenges (Fig 5A).

After the standard oral aspirin challenge, 10 patients with AERD exhibited a significant increase in urinary tetranor PGDM 1 hour after the onset of the hypersensitivity reaction (Fig 5B). In these patients, urine tetranor PGDM levels increased from a mean of 5,607.6 (715.6) to 9,065.8 (1,746.9) pg/mg of creatinine ( $P = .001$ ). All of them developed bronchospasm, with an FEV<sub>1</sub> decrease of 20% or more. In 6 other patients with AERD, urine tetranor PGDM levels decreased from a mean of 7,018.4 (1,538.7) to 4,900.6 (1,413.3) pg/mg of creatinine ( $P = .02$ ) (Fig 5B). All 6 of them developed predominantly upper respiratory reactions and an FEV<sub>1</sub> decrease of less than 20%. The change in urine tetranor PGDM levels in patients with ATA after the graded aspirin challenge was similar to the latter AERD group. After the graded aspirin challenge, patients with ATA had a significant mean decrease in urine tetranor PGDM levels, from 6,906.9 (810.9) to 5,144.8 (345.5) pg/mg of creatinine ( $P < .01$ ) (Fig 5B).

The only significant differences between patients with AERD who reacted with PGDM level increase vs decrease were the magnitude of the FEV<sub>1</sub> decrease during the challenge and the history of duration of aspirin hypersensitivity; the duration was shorter in patients who reacted with an increase in urinary tetranor PGDM than in those who had a decrease in urinary tetranor PGDM levels (3 [0.5] vs 15 [3.9] years, respectively;  $P = .02$ ).

#### Changes in Plasma PGEM After Low and Graded Aspirin Challenges

PGEM is the major metabolite of PGE<sub>2</sub>. There was no significant change in PGEM plasma concentrations in either group after low-dose or standard oral graded aspirin challenge (eFig 1). PGEM levels appeared to be lower in the patients with AERD at baseline and after most of the aspirin challenge doses; however, this difference was not statistically significant.

#### FeNO and LTE<sub>4</sub>

Because FeNO and LTE<sub>4</sub> were the only parameters in this study that significantly changed in patients with AERD after the 40-mg aspirin challenge dose, their diagnostic value for identification of

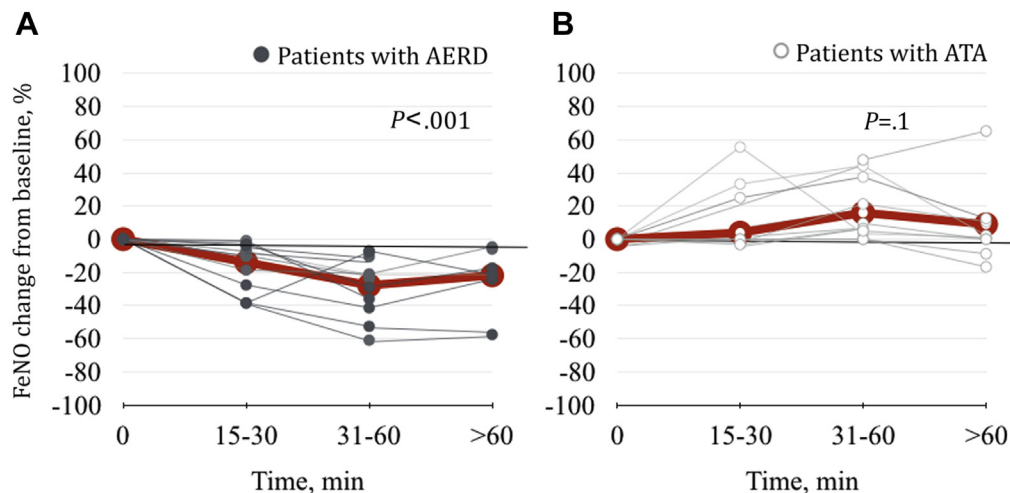


**Figure 2.** Fraction of exhaled nitric oxide (FeNO) change after standard oral graded aspirin challenge. A, In the 16 patients with aspirin-exacerbated respiratory disease (AERD), there was a significant decrease in FeNO ( $P < .001$ ). B, In the 10 patients with aspirin-tolerant asthma (ATA), there was no significant change in FeNO ( $P = .10$ ). Gray thin lines indicate individual data points; red thicker lines, mean values.

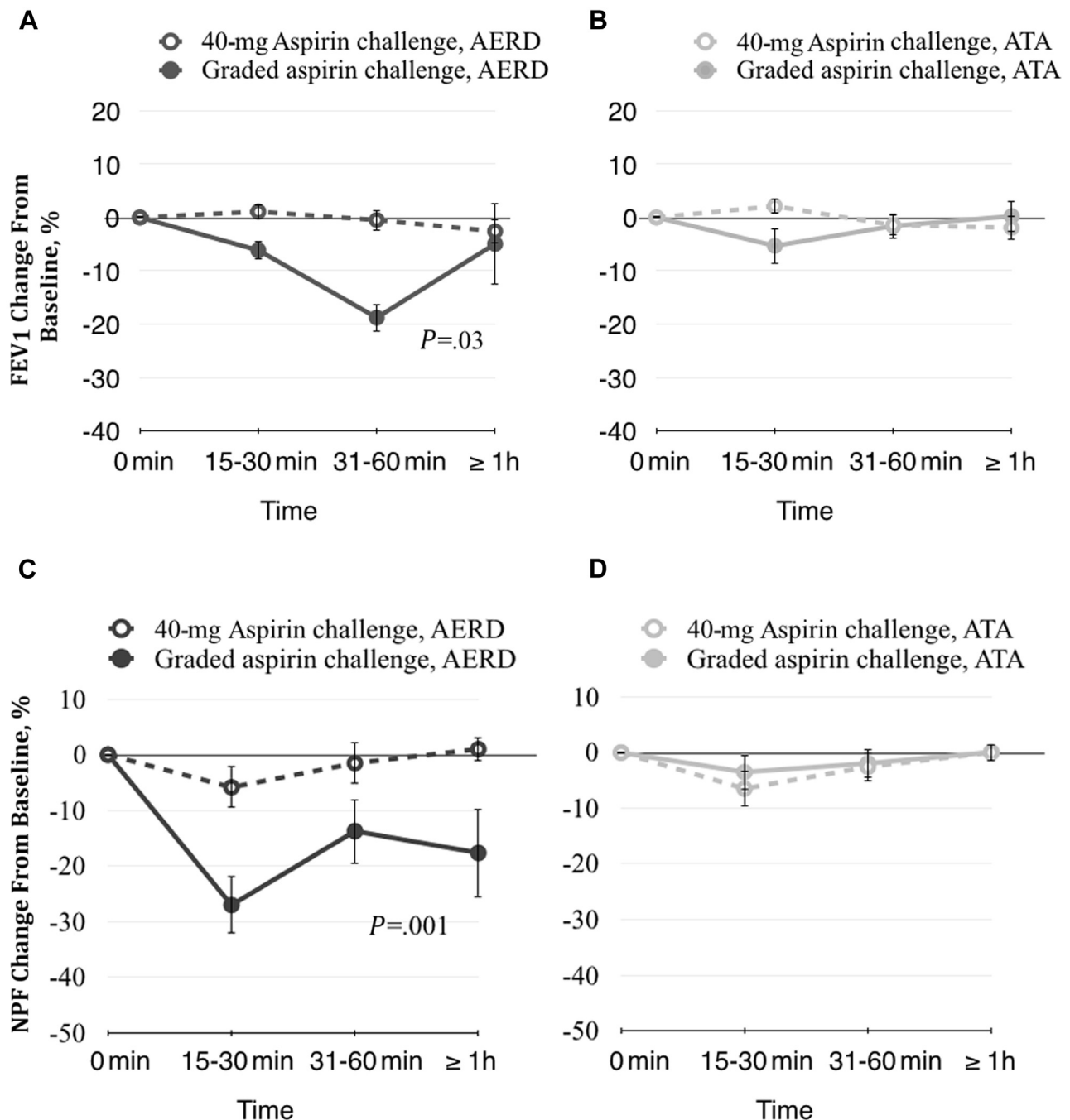


**Figure 3.** Forced expiratory volume in 1 second (FEV<sub>1</sub>) and nasal peak flow (NPF) change after 40 mg of aspirin and after standard oral graded aspirin challenge. A and C, In patients with aspirin-exacerbated respiratory disease (AERD) after the 40-mg aspirin challenge, there was no significant decrease in FEV<sub>1</sub> or in NPF. There was a significant decrease in FEV<sub>1</sub> ( $P = .03$ ) and NPF ( $P = .001$ ) after graded aspirin challenge. B and D, In patients with aspirin-tolerant asthma (ATA), there was no significant change in FEV<sub>1</sub> or NPF after the 40-mg aspirin or graded aspirin challenge.

AERD patients was evaluated. The sensitivity and specificity of FeNO changes for identifying patients with AERD after 40 mg of aspirin for the best-calculated cutoff point (0.8) were 90% and 100%, respectively (Fig 6A), correctly classifying 93% of participants with an area under the curve of 0.98 (95% CI, 0.92–1.00), suggesting that FeNO change at more than 1 hour after the 40-mg aspirin challenge can help discriminate between patients with AERD and those with ATA. The positive predictive value for FeNO change at more than 1 hour was 100%, and the negative predictive value was 83.3%.

The sensitivity and specificity of LTE<sub>4</sub> changes for identifying patients with AERD after 40 mg of aspirin for the best-calculated cutoff point (0.48) were 64.3% and 69.2%, respectively (Fig 6B),

correctly classifying 66.7% with an area under the curve of 0.81 (95% CI, 0.63–0.98). This finding suggests a much more modest and perhaps inadequate ability of LTE<sub>4</sub> changes at more than 1 hour after the 40-mg aspirin challenge to discriminate between patients with AERD and those with ATA. The positive predictive value for LTE<sub>4</sub> change at more than 1 hour was 69.2%, and the negative predictive value was 64.3%.

## Discussion

It is important to correctly identify patients as being aspirin allergic or tolerant. There is the common requirement of NSAIDs for

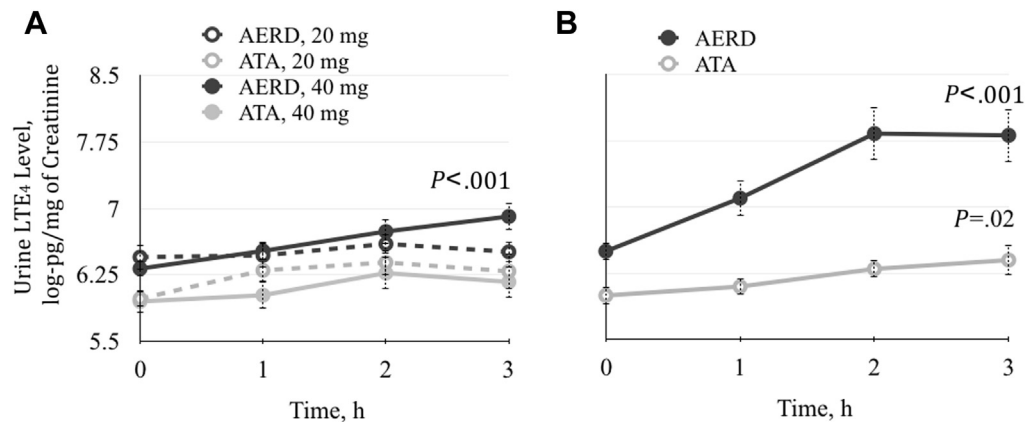


**Figure 4.** Change in leukotriene E<sub>4</sub> (LTE<sub>4</sub>) urine levels after low and standard oral graded aspirin challenge. A, After the 20-mg aspirin challenge, there was no significant change in LTE<sub>4</sub> levels in either group. In patients with aspirin-exacerbated respiratory disease (AERD) after the 40-mg aspirin challenge, there was a significant increase in LTE<sub>4</sub> levels ( $P = .001$ ). B, There was a significant increase in urinary LTE<sub>4</sub> levels after standard graded aspirin challenge in patients with AERD and in patients with aspirin-tolerant asthma (ATA) ( $P < .001$  and  $P = .02$ , respectively).

anti-inflammatory treatment, and many patients also require daily aspirin for cardioprotection. In addition, identifying patients with AERD offers them the opportunity to undergo aspirin desensitization—an important treatment option for AERD that leads to the improvement in upper and lower airway symptoms.<sup>9,29</sup> The current standard method for diagnosing AERD is to observe clinical response (upper and/or lower respiratory symptoms) in patients with asthma after a graded aspirin challenge.<sup>16</sup> This diagnostic approach is problematic because clinical reactions to aspirin can be severe and uncomfortable to the patients. We therefore performed this study to explore the possibility of low-dose aspirin challenges to diagnose AERD without inducing a clinically apparent hypersensitivity reaction.

Although there were no detectable clinical signs of a hypersensitivity reaction after 40 mg of aspirin in most patients, FeNO changes were distinctly different between those with AERD and ATA. FeNO levels significantly decreased from baseline in patients with AERD during 40-mg aspirin challenges and during the standard graded oral aspirin challenge but did not significantly change in patients with ATA. The decrease in FeNO after administration of 40 mg of aspirin was predictive of an eventually positive standard oral aspirin challenge in patients with AERD. It occurred in the absence of detectable changes in FEV<sub>1</sub> or NPF. The decrease in FeNO

during the standard graded aspirin challenge was greater than that after 40-mg challenge, suggesting a dose-dependent response of FeNO levels to aspirin in patients with AERD. FeNO has been traditionally used for monitoring asthma control over time.<sup>30</sup> However, it is also an important biomarker for evaluating acute bronchospasm.<sup>31</sup>

In contrast to our findings, others have reported that FeNO values increase during aspirin desensitization and during inhaled aspirin-lysine challenge in patients with AERD.<sup>19,20</sup> This discrepancy is likely due to a different timing of FeNO measurements between the studies. In the present study, FeNO values were measured shortly after aspirin administration, whereas peak FeNO values in one report were observed at 4 hours after inhaled aspirin-lysine challenge,<sup>20</sup> and another report documented higher FeNO values 24 hours after an allergen challenge.<sup>32</sup> In addition, the differences in observations between the studies could be related to the different route of aspirin administration: inhaled<sup>20</sup> vs oral in this study. FeNO values may also vary because of the differences in devices used in the studies.<sup>33</sup>

However, consistent with our observations, another report also found a decrease in nasal nitric oxide after nasal aspirin-lysine challenges at more than 1 hour in patients with AERD who had positive challenge results.<sup>21</sup> These authors hypothesized that the

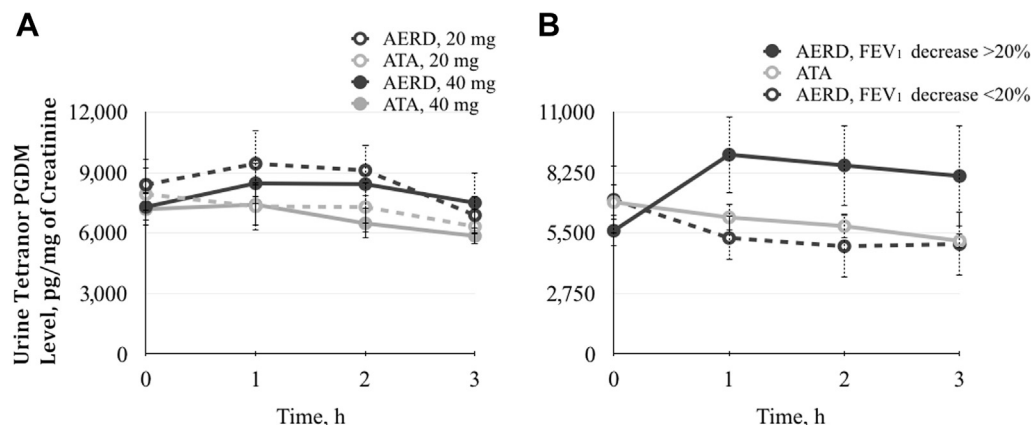


**Figure 5.** Change in tetranor prostaglandin D<sub>2</sub> metabolite (PGDM) urine levels after low and standard oral graded aspirin challenge. A, After low-dose aspirin challenge, there was no significant change in tetranor PGDM levels in either group. B, After standard graded aspirin challenge, there was a significant increase in tetranor PGDM levels in patients with aspirin-exacerbated respiratory disease (AERD) with a forced expiratory volume in 1 second (FEV<sub>1</sub>) decrease of 20% or more ( $n = 10$ ,  $P = .001$ ). There was a decrease in tetranor PGDM levels in 6 patients with AERD with an FEV<sub>1</sub> decrease of less than 20% and in 13 patients with aspirin-tolerant asthma (ATA) ( $P = .02$  and  $P < .01$ , respectively).

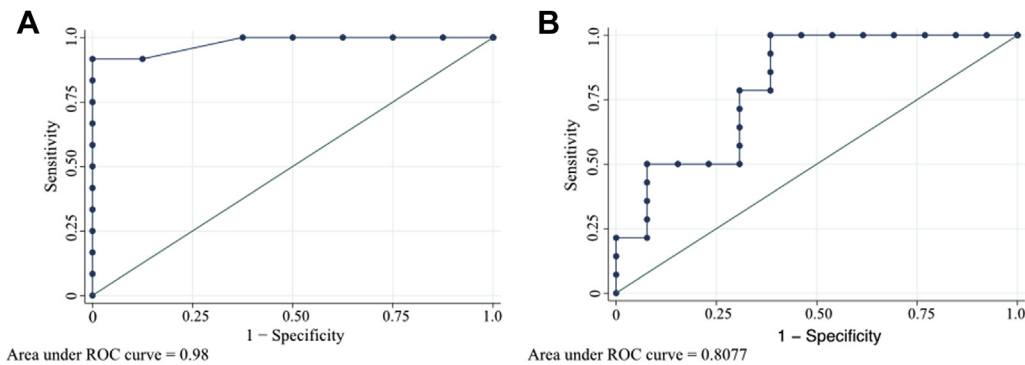


**Figure 6.** Receiver operating characteristic (ROC) curves for fraction of exhaled nitric oxide change (A) and urinary leukotriene  $E_4$  change (B) after the 40-mg aspirin challenge.

decrease was due to nasal mucosa edema that blocked the release of nitric oxide from the sinuses because it was accompanied by a significant decrease in NPF.<sup>21</sup> The concomitant decrease in FEV<sub>1</sub> in the present study also suggests that the FeNO decrease may be due to a FeNO trapping in the airways.

We found that during the 40-mg aspirin challenge FeNO values decreased in patients with AERD, but there was no significant decrease in FEV<sub>1</sub>. Nitric oxide detected in exhaled air is synthesized from L-arginine by nitric oxide synthase enzymes. These enzymes are expressed in endothelial, smooth muscle cells, and inflammatory cells (ie, neutrophils and macrophages) and are activated by inflammatory cytokines.<sup>34</sup> In addition to inflammatory status, FeNO values can be influenced by airway caliber.<sup>31</sup> It is possible that low-dose aspirin triggers mild bronchoconstriction that is too small to be measured by a decrease in FEV<sub>1</sub> but that is sufficient to cause reduced airflow and a subsequent impaired nitric oxide washout from bronchi. Others observed that FeNO values decrease during methacholine and allergen challenges.<sup>31,32,35</sup> In addition, it was recently reported that during lipopolysaccharide challenge in mice, endothelial nitric oxide synthase produces superoxide ion instead of nitric oxide, reducing nitric oxide bioavailability and perhaps leading to a lower FeNO concentration in the exhaled breath.<sup>36</sup>

Because patients with AERD had no apparent clinical reaction to 40-mg aspirin challenges, the explanations of FeNO decrease due to bronchospasm or changes resulting from an acute lung injury seem to be less plausible. Although investigating the mechanism by which FeNO levels decreased in patients with AERD is beyond the scope of this study, we attempted to find an explanation for the decrease in FeNO. One study investigated the association between inducible nitric oxide synthase (iNOS) and eicosanoids in animals with glomerular immune injury.<sup>37</sup> In that study, authors observed an induction of iNOS expression after a glomerular immune injury. iNOS expression was further enhanced by inhibition of COX by indomethacin or by inhibition of 5-lipoxygenase by a lipoxygenase inhibitor. Because we observed an increase in LTE<sub>4</sub> in patients with AERD after both a 40-mg and standard aspirin challenge, it is possible that high LTE<sub>4</sub> levels may have an opposite effect on iNOS and subsequently on nitric oxide production: high LTE<sub>4</sub> levels may suppress iNOS expression and lead to a decreased nitric oxide production. During the standard challenge, some of the patients with AERD had an increase in tetranor PGDM. This observation indicates that COX inhibition was not complete or that COX could have been activated during the hypersensitivity reaction. Thus, activation of 5-lipoxygenase and COX in patients with AERD could lead to a suppression of iNOS expression and a subsequent decrease in nitric oxide production during both the 40-mg and standard aspirin challenge. Interestingly, several patients with ATA had an apparent increase in FeNO, supporting the hypothesis of enhanced iNOS expression due to the COX inhibition by aspirin and a

subsequent increase in nitric oxide production in individuals who are not allergic to aspirin.

During the 40-mg aspirin challenge, some of the patients with ATA also had a decrease in FeNO. Interestingly, FeNO reduction in patients with ATA was not aspirin dose dependent and became less apparent during a standard graded aspirin challenge.

LTE<sub>4</sub> was the other parameter that revealed significant differences between patients with AERD and ATA. We found that the baseline LTE<sub>4</sub> level was greater in the patients with AERD than in the patients with ATA, although, as also previously reported, there was an overlap in LTE<sub>4</sub> levels between both groups of patients with asthma.<sup>9,38</sup> LTE<sub>4</sub> was the only metabolite measured in this study that significantly increased after 40 mg of aspirin in patients with AERD and not in those with ATA. This increase did not lead to a hypersensitivity reaction in most patients, confirming that such reactions are aspirin dose and LTE<sub>4</sub> level dependent.<sup>3,15</sup> This finding provides further confirmation that leukotriene overproduction is a characteristic feature of AERD. It has been linked to the deficiency of PGE<sub>2</sub> in patients with AERD.<sup>39</sup> PGE<sub>2</sub> prevents leukotriene pathway activation, and its inhibition by aspirin results in a surge of leukotrienes from eosinophils or mast cells in aspirin-sensitive and aspirin-tolerant individuals.<sup>40</sup> The LTE<sub>4</sub> increase after 40 mg of aspirin could be considered as a predictor of clinical reactivity in patients with AERD. However, the ability of LTE<sub>4</sub> change after 40 mg of aspirin to discriminate between patients with AERD and ATA is probably inadequate.

We found that both COX pathway metabolites that we measured in this study (urinary tetranor PGDM and plasma PGEM) behaved in a similar way in the AERD and ATA groups during low-dose aspirin challenges. After aspirin-induced hypersensitivity reactions, patients with a milder bronchoconstriction had urinary tetranor PGDM decrease similar to that of patients with ATA, consistent with a previous report.<sup>12</sup> In addition, we observed that a more severe bronchospasm during the challenge was associated with an increase in tetranor PGDM and a shorter duration of AERD symptoms. Notably, a duration of AERD symptoms of less than 10 years was previously identified as a risk factor for a more severe bronchospasm during aspirin challenges.<sup>15</sup>

Plasma PGE<sub>2</sub> metabolite levels in this study were not significantly different between patients with AERD and ATA at baseline, and they also did not significantly change after aspirin challenges. Although it is believed that AERD is due to a lack of PGE<sub>2</sub>, the findings of studies on PGEM levels and their changes in response to aspirin have not been consistent.<sup>7,41–45</sup>

This study has several strengths: a multiracial and multiethnic population of patients with moderate to severe persistent asthma. To our knowledge, it is the first study to analyze changes in FeNO and eicosanoids during low-dose oral aspirin challenges. The study also has some limitations: a relatively small sample size and a lack

of healthy controls. In addition, it is limited by a lack of placebo challenges preceding aspirin challenges and by underrepresentation of patients with nasal polyposis among aspirin-tolerant patients with asthma.

In conclusion, early aspirin-induced decrease in FeNO may prove to be a useful adjunct for diagnosing AERD if confirmed in larger studies. It may also allow better monitoring and the early initiation of treatment during aspirin challenges to prevent more severe procedure-induced bronchoconstriction.

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## Supplementary Data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.anai.2015.12.026>.

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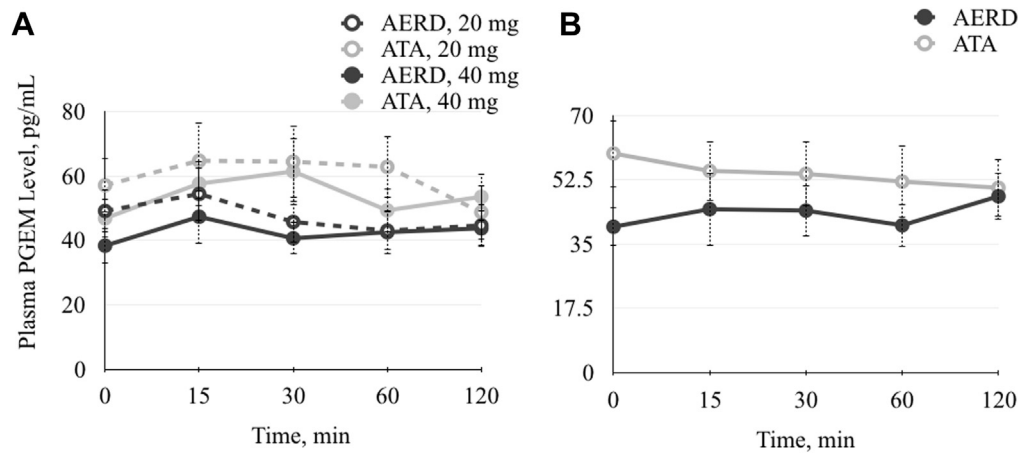


**Figure 1.** There was no significant change in prostaglandin E<sub>2</sub> metabolite (PGEM) plasma levels in either group after low-dose aspirin challenge (A) or after standard graded aspirin challenge (B).