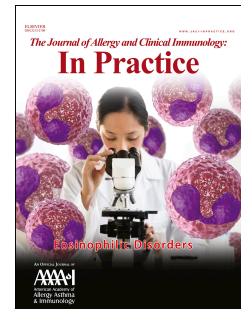


# Journal Pre-proof

Effect of LTRA In L-ASA Challenge For Aspirin Exacerbated Respiratory Disease Diagnosis

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**Effect of LTRA In L-ASA Challenge For Aspirin Exacerbated Respiratory  
Disease Diagnosis**

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**Conflict of Interest**

Authors whose names appear at the beginning of the document, do not have affiliations or participation in any organization or entity. There is no financial, personal or professional interest, in any of the topics of study of this manuscript.

**Highlights box****1. What is already known about this topic?**

Several types of challenges have been used for the diagnosis of hypersensitivity to aspirin and/or NSAIDs, including oral, intravenous, bronchial, and nasal routes of administration. However, there is no internationally unified consensus to perform nasal challenge tests with lysine-acetylsalicylate (L-ASA) either with or without the use of a leukotriene receptor antagonist (LTRA) prior to the nasal challenge.

**2. What does this article add to our knowledge?**

The present study has demonstrated that patients without antileukotriene treatment showed a greater positive response to the L-ASA challenge than those taking this drug, increasing both accuracy and the sensitivity of the test.

**3. How does this study impact current management guidelines?**

Anti-leukotriene therapy reduces the response to nasal L-ASA challenge significantly. Thus, it is recommended to avoid the use of LTRA when performing a nasal L-ASA challenge.

**Key words:** AERD, Aspirin hypersensitivity, NSAIDs, LTRA, Montelukast, Nasal challenge, L-ASA, Rhinomanometry, Spirometry, Clinical Score.

**Abbreviations**

|               |                                            |
|---------------|--------------------------------------------|
| <b>L-ASA</b>  | Lysine-acetylsalicylate, Lysine-aspirin    |
| <b>AERD</b>   | Aspirin-exacerbated respiratory disease    |
| <b>LTRA</b>   | Leukotriene receptor antagonist            |
| <b>NSAIDs</b> | Non-steroidal anti-inflammatory drugs      |
| <b>NPT</b>    | Nasal provocation test                     |
| <b>PGE2</b>   | Prostaglandine E2                          |
| <b>INER</b>   | National Institute of Respiratory Diseases |
| <b>TNF</b>    | Total nasal flow                           |
| <b>FEV1</b>   | Forced Expiratory Volume in one-second     |
| <b>FVC</b>    | Forced vital capacity                      |
| <b>ASA</b>    | Acetyl-salicylic acid                      |

## Abstract

**Background.** Aspirin-exacerbated respiratory disease (AERD), consists of asthma, chronic rhinosinusitis with polyps and hypersensitivity to Aspirin and/or non-steroidal anti-inflammatory drugs (NSAIDs). Nasal Lysine Aspirin Challenge is an effective tool for the diagnosis of hypersensitivity to aspirin and/or NSAIDs in patients with AERD. However, there is not a unified international consensus version to perform nasal provocation tests.

**Objective:** To investigate the effect a leukotriene receptor antagonist (LTRA), montelukast, on lysine-acetylsalicylate (L-ASA) nasal challenge.

**Methods:** We included eighty-six patients divided into three samples: group A (AERD without LTRA), group B (AERD with LTRA) and the control group (NSAID-tolerant asthmatics). Nasal provocation test (NPT) with L-ASA was performed with 25 mg of L-ASA every 30 minutes in four times followed by rhinomanometry and spirometric measurements and evaluation of symptoms using a novel clinical scale.

**Results:** In group A, 94.5% of patients (35/37) developed a positive response to nasal provocation test (drop >40% in total nasal flow) while only 46% of group B subjects (13/28) showed a positive response to the nasal challenge ( $p < 0.001$ ). Control subjects did not show any response to L-ASA challenge. A novel clinical score demonstrated accuracy in classifying the hypersensitivity to aspirin and/or NSAIDs when patients avoid LTRA (33/37).

**Conclusion:** AERD patients without LTRA showed a greater positive response to the L-ASA challenge than those taking this drug, therefore LTRA treatment should be discontinued prior to the challenge for optimal diagnostic accuracy.

## Introduction

Aspirin-exacerbated respiratory disease (AERD), consists of asthma, chronic rhinosinusitis with polyps and hypersensitivity to aspirin and/or others non-steroidal anti-inflammatory drugs (NSAIDs).<sup>(1)(2)(3)</sup> The pathophysiology of AERD is not completely understood, nevertheless, alterations in arachidonic acid metabolism could be responsible for NSAID-induced reactions. NSAIDs block the production of prostaglandins, by inhibiting the cyclooxygenase pathway, including PGE<sub>2</sub>, which hampers leukotriene production in these patients, leading to dysregulation of the 5-lipoxygenase pathway. These events cause the overproduction of cysteinyl leukotrienes, which eventually trigger the sinus and bronchial inflammatory process during exacerbations.<sup>(4)(5)(6)(7)(8)(9)</sup>

Several challenges have been used for the diagnosis of hypersensitivity to aspirin and/or NSAIDs, including oral, intravenous, bronchial and nasal routes of administration.<sup>(10–14)(15–17)</sup> Single-blind, placebo-controlled oral aspirin challenge is the gold standard for the diagnosis of aspirin and/or NSAID hypersensitivity. The European Academy of Allergy and Clinical Immunology, developed in 2007, the first guideline where it recommends nasal challenges for the diagnosis of AERD in patients with severe asthma in whom oral and bronchial provocation is usually contraindicated.<sup>(12)</sup> Advantages of the nasal challenge include greater safety as a consequence of less severe bronchospasm events and it can be done on an outpatient basis.<sup>(14–17)</sup>

Until now there is no internationally unified consensus to perform nasal challenge tests with lysine-acetylsalicylate (L-ASA) also named Lysine-aspirin, a soluble aspirin prodrug. L-ASA is not marketed in several countries, including the United States, which has led to the development of protocols that use ketorolac in its liquid state (formulations for ocular and intramuscular/intravenous use) diluted with saline solution for nasal administration.<sup>(18)(19)(20)(21)</sup> In Europe, the use of L-ASA in nasal challenges has been widely established since the 1990s. However, there is still heterogeneity in the method of performing the procedure, such as 1) administration of different cumulative doses, ranging from 16 to 150 mg of L-ASA, 2) discrepancy in the time intervals of administration (10 to 45 minutes), 3) no specification of the

withdrawal of the drug before the test, such as leukotriene receptor antagonists (LTRA) or intranasal steroids, 4) different measurement methods, without validated scales.<sup>(14)(17,18,19)(21,23)</sup> We hypothesize that the use of LTRA may modify the response to nasal L-ASA challenge. In the present study we performed nasal challenge in AERD patients treated or not treated with LTRA, in order to establish the effect of antileukotriene therapy on the nasal aspirin challenge.

## Methods

### Participant and clinical diagnosis

Eighty-six patients with controlled asthma (average age 43.3 years) were recruited for the study by the Department of Allergy and Immunogenetics of the National Institute of Respiratory Diseases (INER), Mexico City. The clinical and demographic characteristics of the patients are shown in **Table I**. The diagnosis of asthma was established in accordance with the 2018 update of the Global Strategy for Asthma Management and Prevention Guidelines. Patients discontinued intranasal steroids and antihistamines at least 7 days before nasal provocation but not, inhaled treatment for asthma - all patients were given inhaled steroids / long-acting beta-agonists (fluticasone/salmeterol) as a long-term treatment to maintain control of asthma. No patient was receiving oral steroid treatment 4 weeks prior to the study. A complete physical exam and medical history were performed; patients were specifically asked for the duration of symptoms, disease monitoring and medication regimens used in their treatment, as well as lung function tests. At the time of recruitment, all subjects were clinically stable, with a Forced Expiratory Volume in one-second (FEV1) > 70% of predicted. The presence of sinonasal disease with or without polyposis was confirmed by computed tomography and/or nasal endoscopy. None of the subjects showed symptoms of upper respiratory tract infection and they did not undergo sinonasal surgery for at least 6 months previously. All subjects signed an informed consent letter.

### Study design

The patients were divided into three groups.

**Group A**(AERD s/LTRA): patients diagnosed with AERD, polyposis and a history of at least 2 episodes of asthma exacerbation and nasal symptoms with the intake of 2 or more different NSAIDs in the previous 6 months and / or positive oral aspirin challenge. If patients were taking LTRA, they were asked to discontinue it at least 7 days before the provocation test.

**Group B** (AERD w/LTRA): patients diagnosed with AERD who suffered from nasal polyps, history of at least 2 episodes of asthma exacerbation, history of nasal symptoms after the intake of 2 or more different NSAIDs in the previous 6 months and/or positive oral aspirin challenge, who were treated with 10 mg LTRAs (daily) at least one week before the procedure.

**Group C** (NSAID-tolerant asthmatics or control group): patients with sinonasal disease and NSAID-tolerant asthma. NSAID tolerance was defined as the absence of exacerbation (nasal and/or bronchial) with the intake of 2 or more different NSAIDs in the last 6 months. If patients were taking LTRA, they were asked to discontinue it at least 7 days before the provocation test.

#### Aspirin Challenge Protocol

Nasal provocation test (NPT) with lysine acetylsalicylate (Coraspir ® Armstrong, Mexico) was performed as described in **Figure I**. A positive NPT was defined as one in which the decrease of total nasal flow (TNF) was >40% measured by anterior rhinomanometry, a decrease in FEV1 >20% measured by spirometry and/or an increase in clinical symptoms for which a novel clinical score was used during this study. The challenge was interrupted if TNF dropped > 25% after nasal administration of 100 µL of saline solution 0.9% (this type of reaction is considered a non-specific nasal hyper-reactivity as demonstrated by Miller et al).<sup>(14)</sup>

#### Anterior Rhinomanometry

The nasal flow was measured using a Jaeger Rinoscreen® rhinomanometer based on nasal provocation patterns. A measurement of the nasal flow of each nostril was obtained separately, the sum of the two measurements represents the total nasal flow. A 40% reduction of this, after each dose of L-ASA, determined the challenge as positive. For all groups, the baseline TNF value was the first rhinomanometric

measurement performed before the administration of any substance (saline or L-ASA). In groups A and B, the final TNF corresponded to the measurement obtained by meeting the positive criteria for the challenge; In group C, the rhinomanometric record obtained 90 minutes after the last application of L-ASA (cumulative dose of 100 mg) was assigned as final TNF.

#### Spirometry

Respiratory function was evaluated according to the NIOSH guidelines using a Jaeger® spirometer. The predicted values of Perez-Padilla for the Mexican population were used. The forced vital capacity (FVC) [% of predicted], the forced respiratory volume in one second (FEV1) [% of predicted] and the FVC / FEV1 [%] ratio were recorded. If there was a decrease with respect to the baseline in FEV1 >20% after each administration of L-ASA, it would be considered as a criterion of positivity and the challenge would be concluded. Basal FEV1 value was obtained from the first spirometric record before the administration of any substance (saline or L-ASA) in both groups. For group A and B, final FEV1 corresponded to the one presented at the time of meeting positive criteria for the challenge; in group C it was the record obtained 90 min after the cumulative dose of 100 mg of L-ASA.

#### Clinical score (CS)

In order to measure the intensity of clinical symptoms triggered by L-ASA nasal challenge, a novel clinical scale was developed. Ten clinical items were evaluated by two independent allergists every 30 minutes after each administration of L-ASA, each item was given a score of 0-3. The items included: 1. Rhinorrhea, 2. Sneezing, 3. Pruritus, 4. Nasal obstruction, 5. Facial colour, 6. Size of the right inferior turbinate, 7. Colour of the right inferior turbinate, 8. Size of the left inferior turbinate, 9. Colour of the left inferior turbinate, 10. Conjunctival colour. These features are common clinical manifestations of AERD. Summation of the score of each item was made from baseline and 30 min. after each administration of L-ASA.

## Statistical analysis

All statistical analyses were performed with STATA 11.1 (StataCorp, Texas, USA). Continuous variables were expressed as mean ( $\pm$  SD) or median (range) and categorical variables were expressed as numbers (percentages). Normality test was performed in each variable (Shapiro-Wilk) and according to the result parametric or non-parametric statistics were used. The difference between two independent means was analyzed by t-test or medians by Mann-Whitney test. In the case of non-quantitative variables, proportions were compared using chi square or Fisher's exact. Spearman's rank coefficient was used to investigate the correlations between the variables. Probability values of less than 0.05 were considered statistically significant. Youden's Index was used to identify the optimal cut off point. Sensitivity, specificity and accuracy were performed using standard procedures.

## **Results:**

The protocol was successfully completed in 86 subjects: 3 AERD patients were excluded from the study because they showed nasal hyper-reactivity during nasal provocation test. The 86 subjects were divided into 3 groups: **Group A:** 37 AERDs/LTRA (27 women and 10 men; mean 37,  $\pm$  11 years). **Group B:** 28 AERDw/LTRA (19 women and 9 men; mean 49,  $\pm$  12 years). **Group C:** 21 NSAID-tolerant asthmatics (9 women and 12 men; mean 44,  $\pm$ 15 years). The proportion of female patients was higher in both groups with AERD compared to group C. The average age in groups A, B and C were 37 $\pm$ 11, 49 $\pm$ 12 and 44  $\pm$ 15, respectively. All patients with AERD (n=86) had nasal polyps, while 76% of control subjects (n=16) had nasal polyps **Figure II**. The clinical features of the subjects are shown in **Table I**.

## Result of nasal provocation test (NPT)

There were no statistically significant differences in baseline total nasal flow (TNF) between any of the three groups. **Table I**. However, intranasal challenge with L-ASA caused a significant decrease in TNF in both subjects with AERD but not in

the NSAID-tolerant group. Nonetheless, there was a greater drop in TNF in group A (-57%, range -43 to -66)% compared to group B (-39.8%, range -3.6 to -66), probably due to the LTRA protective effects in this last group of patients ( $p < 0.05$ ), **Figure III**. In fact, 94.5% of group A patients ( $n=35$ ) developed a positive response to nasal provocation test while only 46% of the subjects in group B ( $n=13$ ) showed a positive response to the nasal challenge, **Figure II**. The subjects who did not respond underwent an oral aspirin challenge with 50 mg and 100 mg, at 90 and 150 minutes respectively, when completing the nasal challenge (54% of Group B and 5.5% of Group A). Two patients in group A had a significant decline in TNF with the oral challenge of two doses of L-ASA, while 6 subjects of Group B had a significant decrease in TNF (3 with the first dose of 50 mg and the other 3 with the second dose of 100 mg), consequently, 9 subjects of group B did not have a significant reduction in TNF. **Table I**.

The L-ASA nasal concentrations to induce a significant decline in TNF in group A and B, was 50 mg (50 – 75) and 75 mg (50 – 150), respectively. The subjects of the control group did not show a response to the L-ASA challenge. **Figure IV** shows the cumulative dose of L-ASA inducing a positive response, including nasal and oral challenge; there was a significative Log-rank between two groups ( $p < 0.001$ ).

The accuracy of the test was evaluated; by obtaining the values of sensitivity and specificity, using a TNF > 40% as a positive parameter. Interestingly, patients without LTRA therapy, distinguished themselves from control subjects with 0.91 accuracy, 91.8% sensitivity and 100% specificity, while AERD patient with LTRA showed an accuracy of 0.73, with 53.5 % sensitivity and 100% specificity, **Figure V**.

#### Clinical score.

The LTRA effect on the response to L-ASA nasal challenge was also measured using a clinical scale we have developed to detect the severity of symptoms. Patients not treated with LTRA showed a clinical score greater than those treated with LTRA (median, 10 versus 5),  $p, < 0.0001$ ), Table 1. In contrast, there was no

significant difference between this last group of patients and the NSAID tolerant asthmatics (median 5 versus 3,  $p > 0.05$ ). The optimal clinical score cut-off point increase was  $> 7$  points, figure 3B.

The agreement between the clinical score and a fall of  $> 40\%$  TNF as measured by rhinometry in group A was 94%: out of the 35 patients, 33 showed a TNF decrease  $> 40\%$  and the clinical scale increased  $> 7$  points. In contrast, the agreement between these two variables in group B was only 62%: 8 of the 13 showed a TNF decrease  $> 40\%$  and the clinical scale was lower than 7 points. Next, the diagnostic accuracy criteria were assessed by ROC analysis. The group not treated with LTRA (group A) obtained a sensitivity of 89.1% with a specificity of 93.1% and a percentage of 93.1% correctly classified, while those treated with LTRA (group B) the sensitivity decreased to 42.8% with a specificity of 100% and a percentage of 67.3% correctly classified. **Figure V.** These results indicate that patients with LTRA have a lower clinical response to L-ASA nasal challenge.

#### Security of nasal challenge

Nasal provocation with L-ASA was safe and well-tolerated in all AERD patients including those with and without LTRA treatment. There were no changes in oxygen saturation or asthma symptoms in any of the patients. No significant modification in FEV1 was observed with L-ASA challenge in NSAIDs sensitive patients (FEV1 falls  $< 1.6\%$  in group A and  $< 0.72$  in group B). **Table I.** The same analysis was made, excluding patients without severe asthma from group A and we found no difference.

#### **Discussion**

This study demonstrated that AERD patients not treated with LTRA showed greater sensitivity to L-ASA challenge compared to those treated with this drug. Indeed, most patients not treated with LTRA, developed a positive response with L-ASA concentrations higher than 50 mg, which is a higher dose than that recommended by the published guidelines (16 mg) and other works published

previously. <sup>(12,13,16,18)</sup> It should be mentioned that all AERD patients received bronchodilator treatment that kept asthma symptoms fully controlled.

The nasal challenge test with L-ASA has been used for the diagnosis of AERD, based on the evaluation of rhinomanometric changes and nasal clinical symptoms induced by ASA and other NSAIDs, including nasal obstruction, rhinorrhea and sneezing. Nonetheless, the impact of anti-leukotriene treatment on L-ASA challenged had not been compared.

The present study has shown that 94.5% of patients not treated with LTRA developed a positive response to the L-ASA nasal provocation at maximum concentrations, while only 46% of treated patients showed a positive response to nasal challenge. In fact, patients treated with LTRA did not only develop reduce sensitivity to nasal challenge but also required higher concentrations of L-ASA. We have carried out a nasal challenge protocol similar to the one used by Miller et al. in their model, with a maximum cumulative nasal dose of 100 mg. However, they continued oral administration in 12% in their sensitive patients <sup>(14)</sup>. This situation occurred in only two of our patients (5.4%) included in the group without LTRA. The variable that can explain that our population has a greater proportion of positive challenges with the same dose of intranasal L-ASA, is the discontinuation of LTRA treatment one week before provocation; this hypothesis was not considered in the Miller model, where LTRA is given to patients with AERD. It is possible to affirm that the better precision values and clinical correlations in TNF are appreciated when patients taking LTRA are excluded from the analysis. <sup>(14)</sup>

White and collaborators reported three patients with a consistent story for AERD and negative provocations, they received LTRA as part of a challenge/desensitization protocol, the authors called it "silent desensitization". <sup>(18)</sup>

Given the findings of this study and the background reported in the literature, a diagnostic test is recommended in patients whose diagnosis of hypersensitivity to NSAIDs is doubtful. In this test, discontinuing both LTRA and intranasal steroids while maintaining inhaled therapy with inhaled steroids / long-acting beta-agonists to control asthma symptoms, <sup>(22,24)</sup> increased the sensitivity of nasal provocation

but decreasing the risk of developing bronchospasm or any other systemic reaction - it allows a more precise response to nasal challenge with L-ASA.

A clinical score was implemented to further assess the response to L-ASA: this one is different from other clinical scales and includes ten variables, that can be assessed by physical examination, in addition to granting a gradual numerical scale for each item was analyzed. The clinical assessments used in previous studies with L-ASA, used 3 or 4 variables only (rhinorrhea, nasal obstruction, sneezing and pruritus) <sup>(12)(20)</sup> with exception of one report, where intranasal ketorolac was applied for provocation and a numerical scale was used for the protocol.<sup>(25)</sup> In the present study, the ROC analysis allowed us to obtain accuracy in the cut-off point, resulting in an increase of 7 points with respect to the baseline and the agreement between this clinical score and the challenges in the group without LTRA reached 94%. In contrast, AERD patients with LTRA showed a lower concordance and no correlation between the clinical score and TNF, indicating that LTRA lowers the response to the nasal L-ASA challenge. The agreement between TNF drop and increase of clinical score, was 62%, in patients without LTRA that is similar to the 69% reported by Cook et al. <sup>(25)</sup> In this last study, LTRA therapy was not withdrawn before the test. These observations further demonstrate that nasal challenge without the use of LTRA is the most accurate way to obtain quantifiable clinical changes.

It is possible to confirm the safety of the model we have proposed for the diagnosis of aspirin / NSAID hypersensitivity with respiratory symptoms. During the protocol, anaphylaxis, bronchospasm or a significant decrease in FEV1 did not occur in any subject under study. We believe that long-term treatment with inhaled long-acting beta-agonists / steroids may have prevented the development of asthma symptoms. In a previous study, 275 AERD patients were desensitized with either nasal ketocorolac or oral aspirin - 98.8% of patients were pre-treated with LTRA.<sup>(26)</sup> However, 4 patients in the ketocorolac group, required intramuscular epinephrine because of laryngospasm, gastrointestinal symptoms, or both and 40.8% of patients were given bronchodilators. In contrast, none of the aspirin desensitized patients needed epinephrine, 56.9% of them were treated with a

bronchodilator. These findings suggests that ketorolac may cause more severe reactions compared with oral aspirin challenge as patients in this last group did not develop any severe reactions. In our study, local nasal L-ASA was found to be a safe method which did not only prevent the development of any severe reaction but it also allowed to study the effect of a LTRA on the nasal challenge. However, this approach may not apply to ketorolac nasal challenges. The use of L-ASA instead of ketorolac could account for the safety parameters we observed in our patients. Limitations of our study include it was not conducted in a double-blind manner and FEV1, and asthma severity differed between groups. However, it is well established that asthma severity in patients suffering from AERD ranges from mild to severe asthma; there is a difference between the gender proportions of the groups, but the higher prevalence of AERD in women is also known. AERD develops in adulthood, mostly between the ages of 30 and 50. In our study, the average age of the three groups fell within this age range.

In summary, the local nasal L-ASA challenge, without LTRA therapy, proved to be a reliable test, with high sensitivity and specificity for the diagnosis of NSAIDs hypersensitivity. In contrast, the LTRA reduces the response to nasal L-ASA challenge significantly and leads to false negatives. Thus, it is recommended to avoid the use of LTRA during the local L-ASA nasal challenge.

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### **Ethics**

This investigation adheres to articles 96° and 98°, in the fifth title of the General Mexican Health Law. Human research is based on ethical principles and participants have signed an informed consent letter. The protocols will be performed by health professionals. (Article 100 of the General Health Law). This project has the approval of the INER ethics committee, in protocol B13-15

## Potentials Risk

This study is considered in accordance with the regulations of the General Law of Health in Mexico, in the section of research for health in the second title of the first chapter in article 17, of section II and according to the ethical principles of the Helsinki's Declaration in 1964, as a minimum risk research project.

## Confidentiality

Respecting the principles of bioethics, the principle of autonomy was maintained, always seeking the benefit and not the harm of the patient. This research project is in accordance with the Helsinki declaration and good clinical practices.

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## References

1. Samter M, Beers RF. Concerning the nature of intolerance to aspirin. J Allergy. 1967 Nov 1;40(5):281–93.
2. Stevenson DD, Szczeklik A. Clinical and pathologic perspectives on aspirin sensitivity and asthma. J Allergy Clin Immunol. 2006 Oct;118(4):773–86; quiz 787–8.
3. Szczeklik A. Adverse reactions to aspirin and Aspirin. Ann Allergy. 1987 Nov;59(5 Pt 2):113–8.
4. Stevenson DD, Sanchez-Borges M, Szczeklik A. Classification of allergic and pseudoallergic reactions to drugs that inhibit cyclooxygenase enzymes. Ann Allergy Asthma Immunol Off Publ Am Coll Allergy Asthma Immunol. 2001 Sep;87(3):177–80.
5. Kanaoka Y, Boyce JA. Cysteinyl leukotrienes and their receptors; emerging

concepts. *Allergy Asthma Immunol Res.* 2014 Jul;6(4):288–95.

6. Yoshimura T, Yoshikawa M, Otori N, Haruna S, Moriyama H. Correlation between the Prostaglandin D2/E2 Ratio in Nasal Polyps and the Recalcitrant Pathophysiology of Chronic Rhinosinusitis Associated with Bronchial Asthma. *Allergol Int Off J Jpn Soc Allergol.* 2009 Jan 1;57:429–36.

7. Steinke JW, Bradley D, Arango P, Crouse CD, Frierson H, Kountakis SE, et al. Cysteinyl leukotriene expression in chronic hyperplastic sinusitis–nasal polyposis: Importance to eosinophilia and asthma. *J Allergy Clin Immunol.* 2003 Feb 1;111(2):342–9.

8. Cowburn AS, Sladek K, Soja J, Adamek L, Nizankowska E, Szczeklik A, et al. Overexpression of leukotriene C4 synthase in bronchial biopsies from patients with aspirin-intolerant asthma. *J Clin Invest.* 1998 Feb 15;101(4):834–46.

9. Pérez-Novó CA, Watelet JB, Claeys C, Van Cauwenberge P, Bachert C. Prostaglandin, leukotriene, and lipoxin balance in chronic rhinosinusitis with and without nasal polyposis. *J Allergy Clin Immunol.* 2005 Jun;115(6):1189–96.

10. McDonald JR, Mathison DA, Stevenson DD. Aspirin intolerance in asthma. Detection by oral challenge. *J Allergy Clin Immunol.* 1972 Oct;50(4):198–207.

11. Gajsak K. [The problem of stumps in flexion contracture (author's transl)]. *Lijec Vjesn.* 1977 Feb;99(2):143–5.

12. Nizankowska-Mogilnicka E, Bochenek G, Mastalerz L, Swierczyńska M, Picado C, Scadding G, et al. EAACI/GA2LEN guideline: aspirin provocation tests for diagnosis of aspirin hypersensitivity. *Allergy.* 2007 Oct;62(10):1111–8.

13. Dursun AB, Woessner KA, Simon RA, Karasoy D, Stevenson DD. Predicting outcomes of oral aspirin challenges in patients with asthma, nasal polyps, and chronic sinusitis. *Ann Allergy Asthma Immunol Off Publ Am Coll Allergy Asthma Immunol.* 2008 May;100(5):420–5.

14. Miller B, Mirakian R, Gane S, Larco J, Sannah AA, Darby Y, et al. Nasal lysine aspirin challenge in the diagnosis of aspirin - exacerbated respiratory disease: asthma and rhinitis. *Clin Exp Allergy J Br Soc Allergy Clin Immunol.* 2013 Aug;43(8):874–80.

15. Patriarca G, Nucera E, DiRienzo V, Schiavino D, Pellegrino S, Fais G. Nasal

provocation test with lysine acetylsalicylate in aspirin-sensitive patients. *Ann Allergy*. 1991 Jul;67(1):60–2.

16. Bochenek G, Niżankowska-Mogilnicka E. Aspirin-exacerbated respiratory disease: clinical disease and diagnosis. *Immunol Allergy Clin North Am*. 2013 May;33(2):147–61.

17. Alonso-Llamazares A, Martinez-Cócer C, Domínguez-Ortega J, Robledo-Echarren T, Cimarra-Alvarez M, Mesa del Castillo M. Nasal provocation test (NPT) with aspirin: a sensitive and safe method to diagnose aspirin-induced asthma (AIA). *Allergy*. 2002 Jul;57(7):632–5.

18. White A, Bigby T, Stevenson D. Intranasal ketorolac challenge for the diagnosis of aspirin-exacerbated respiratory disease. *Ann Allergy Asthma Immunol Off Publ Am Coll Allergy Asthma Immunol*. 2006 Aug;97(2):190–5.

19. Lee RU, White AA, Ding D, Dursun AB, Woessner KM, Simon RA, et al. Use of intranasal ketorolac and modified oral aspirin challenge for desensitization of aspirin-exacerbated respiratory disease. *Ann Allergy Asthma Immunol Off Publ Am Coll Allergy Asthma Immunol*. 2010 Aug;105(2):130–5.

20. Quiralte-Castillo J, Ávila-Castellano MR, Cimbollek S, Benaixa P, Leguisamo S, Baynova K, et al. Nasal Ketorolac Challenge Using Acoustic Rhinometry in Patients With Aspirin-Exacerbated Respiratory Disease. *J Investig Allergol Clin Immunol*. 2017;27(3):169–74.

21. Celikel S, Stevenson D, Erkorkmaz U, White AA. Use of nasal inspiratory flow rates in the measurement of aspirin-induced respiratory reactions. *Ann Allergy Asthma Immunol Off Publ Am Coll Allergy Asthma Immunol*. 2013 Oct;111(4):252–5.

22. White A, Bigby T, Stevenson D. Intranasal ketorolac challenge for the diagnosis of aspirin-exacerbated respiratory disease. *Ann Allergy Asthma Immunol Off Publ Am Coll Allergy Asthma Immunol*. 2006 Aug;97(2):190–5.

23. Milewski M, Mastalerz L, Nizankowska E, Szczeklik A. Nasal provocation test with lysine-aspirin for diagnosis of aspirin-sensitive asthma. *J Allergy Clin Immunol*. 1998 May;101(5):581–6.

24. White A, Ludington E, Mehra P, Stevenson DD, Simon RA. Effect of

leukotriene modifier drugs on the safety of oral aspirin challenges. *Ann Allergy Asthma Immunol.* 2006 Nov;97(5):688–93.

25. Cook KA, Modena BD, Wineinger NE, Woessner KM, Simon RA, White AA. Use of a composite symptom score during challenge in patients with suspected aspirin-exacerbated respiratory disease. *Ann Allergy Asthma Immunol Off Publ Am Coll Allergy Asthma Immunol.* 2017;118(5):597–602.

26. Waldram J, Walters K, Simon R, Woessner K, Waalen J, White A. Safety and outcomes of aspirin desensitization for aspirin-exacerbated respiratory disease: A single-center study. *J Allergy Clin Immunol.* 2018;141(1):250-256.

## Figure Legends

**Figure I.** The flowchart shows the sequence of steps for nasal challenge.

**Figure II.** Study design: 89 patients with AERD participated in the study except for 3 subjects who were excluded from the study due to nasal hyper-reactivity. The 86 patients were divided into: AERD s / LTRA, AERD w / LTRA and NSAID-tolerant asthmatics. Number of AERD patients who developed a positive response to nasal L-ASA challenge in group A (n=35) and B (13).

**Figure III. A.** Total nasal flow during nasal provocation test In AERD s / LTRA, AERD w / LTRA and NSAID-tolerant asthmatics. **B,** Clinical score during nasal L-ASA challenge.

**Figure IV. A.** Concentrations of L-ASA which induce a positive nasal response in AERD subjects. **B.** The cumulative dose of L-ASA required to induce a positive nasal challenge in AERD s/LTRA and AERD w/LTRA. Kaplan-Meier survival estimates.

**Figure V.** The accuracy of the nasal provocation test increased using the total nasal flow drop and the clinical score. ROC analysis of two models: AERD s / LTRA and NSAID-tolerant asthmatics vs AERD with LTRA and NSAID-tolerant asthmatics.

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**Table 1.**

Clinical and demographic characteristics

|                                                                                                                                                                                         | Group A<br>(n= 37)<br>AERD s/LTRA | Group B<br>(n= 28)<br>AERD w/LTRA | Group C<br>(n=21)<br>NSAID-tolerant<br>asthmatics | <i>p</i>                                                       |        |        |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------|-----------------------------------|---------------------------------------------------|----------------------------------------------------------------|--------|--------|
|                                                                                                                                                                                         |                                   |                                   |                                                   | A vs B                                                         | A vs C | B vs C |
| Age □ (±SD) years                                                                                                                                                                       | 37 (±11)                          | 49 (±12)                          | 44 (±15)                                          | §                                                              | ns     | ns     |
| Female n (%)                                                                                                                                                                            | 27 (73%)                          | 19 (68%)                          | 9 (43%)                                           | ns                                                             | §      | ns     |
| Nasal polyps n (%)                                                                                                                                                                      | 37 (100%)                         | 28 (100%)                         | 18 (78%)                                          | ns                                                             | §      | ns     |
| Basal TNF median<br>(range) ml/min                                                                                                                                                      | 978 (578-<br>1244)                | 1172 (836-1836)                   | 835 (698-1372)                                    | ns                                                             | ns     | ns     |
| Basal FEV1□(±SD) %<br>predicted                                                                                                                                                         | 99 (±11.96)                       | 80 (±5)                           | 92 (±13)                                          | ¥                                                              | ns     | ¥      |
| Asthma Severity (%)                                                                                                                                                                     | 28 (80%)                          | 28 (100%)                         | 10 (43%)                                          | §                                                              | §      | §      |
| <b>Results of L-ASA Nasal Challenge</b>                                                                                                                                                 |                                   |                                   |                                                   |                                                                |        |        |
| Modification of TNF<br>median (range), %                                                                                                                                                | -57 (-43 - -66)                   | -39.8 (-3.6 --47.5)               | -7.3 (-19.2 - 23.7)                               | ¥                                                              | ¥      | §      |
| Modification of CS<br>median (range), pts                                                                                                                                               | 10 (8 - 13)                       | 5 (1.5 - 8.5)                     | 3 (2 - 4)                                         | ¥                                                              | ¥      | ns     |
| FEV <sub>1</sub> median (range),<br>% after challenge                                                                                                                                   | -1.6 (-9.6 – 1.8)                 | 0.72 (-1.16 – 3)                  | 1.1 (-1.4 – 2.3)                                  | ns                                                             | ns     | ns     |
| Positive nasal<br>challenge                                                                                                                                                             | 35/37 (94.5%)                     | 13/28 (46.4%)                     | 0/21 (0%)                                         |                                                                |        |        |
| Positive oral<br>challenge (post-<br>nasal)                                                                                                                                             | 2/2 (100%)                        | 6/15 (40%)                        | 0/21 (0%)                                         |                                                                |        |        |
| Concentrations of<br>nasal L-ASA to<br>induce a significant<br>decline TNF                                                                                                              | 50 (50 – 75)                      | 75 (50 - 150)                     |                                                   | ns                                                             |        |        |
| TNF: Total Nasal Flow<br>FEV <sub>1</sub> : Forced Expiratory Volume at first second<br>Severe asthma: Asthma that requires high medication to achieve control<br>CS: Clinical symptoms |                                   |                                   |                                                   | §: <i>p</i> <0.05<br>¥: <i>p</i> <0.001<br>ns: not significant |        |        |

