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Evaluation of Emerging Biomarkers to Identify Aspirin-Exacerbated Respiratory Disease

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Master of Science

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Evaluation of Emerging Biomarkers to Identify Aspirin-Exacerbated Respiratory Disease

By

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M.D., Tulane University School of Medicine, 2010
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An abstract of
A thesis submitted to the Faculty of the James T. Laney School of Graduate Studies of Emory University in partial fulfillment of the requirements for the degree of Master of Science in Clinical Research
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Abstract

Evaluation of Emerging Biomarkers to Identify Aspirin-Exacerbated Respiratory Disease

By

Joshua M Levy, MD, MPH

Introduction: Aspirin-Exacerbated Respiratory Disease (AERD) is a severe inflammatory syndrome with inadequate diagnostic options for the 1.4 million Americans affected by this disease. Limited access to diagnostic options is a serious problem for patients. In fact, the gold standard for the identification of AERD, diagnostic aspirin challenge, is under-utilized due to limited availability of specialized providers and concerns of precipitating a life-threatening anaphylactic reaction. The dysregulated production of immunoregulatory eicosanoids promotes chronic airway inflammation in AERD and may represent novel biomarkers for unrecognized disease. In the following study, we evaluate the expression of urinary leukotriene E4 (uLTE4) and the type-2 cannabinoid receptor (CB2R), two such eicosanoids with the potential to identify clinically unrecognized AERD among high-risk patients with nasal polyps and asthma. We then propose a trial seeking to evaluate a multi-variable screening panel to identify undiagnosed AERD.

Methods: Multi-site observational trial of consecutive adult patients with either clinically apparent AERD or high-risk disease. Study sites included Emory University and Scripps Health. Clinical and demographic information was collected with the measurement of uLTE4 and CB2R gene expression from collected urine and nasal epithelium, respectively.

Results: A total of n=70 participants completed all study activities from June 2017 to December 2020. Significant differences in participant demographics were found for enrollment site (100 vs. 61.4%, $p<0.001$) and prevalence of respiratory reactions to alcohol (3.9 vs 50%, $p<0.001$). Mean concentration of uLTE4 was similar among cohorts with mean (SD) values of 187.8 (1689.3) vs. 138.3 (889.9) for High Risk vs. AERD cohorts, $p=0.222$. A significant difference in CB2R gene expression was found between cohorts with mean (SD) values of 41 (119) vs. 131 (279) for High Risk vs. AERD cohorts, $p=0.0042$. Further evaluation of high-risk participants with elevated CB2R gene expression resulted in a new diagnosis of AERD in one patient with previously unidentified disease.

Conclusion: uLTE4 and CB2R represent two emerging biomarkers for the identification of unrecognized AERD among high-risk patients with nasal polyps and asthma. Future study incorporating the results of diagnostic aspirin challenge with ten emerging biomarkers has the potential to improve patient care by identifying previously unrecognized disease.

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Table of Contents

Abstract

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Introduction

Aspirin-Exacerbated Respiratory Disease (AERD) is a severe inflammatory syndrome with inadequate diagnostic and treatment options for the 1.4 million Americans and 10-20% of asthmatics affected by this disease.[1] AERD is clinically characterized by moderate to severe asthma, nasal polyps and anaphylactic reactions to aspirin and other nonsteroidal anti-inflammatory drugs (NSAIDs). Symptoms initially present in early adulthood and follow a progressive course marked by increasing airway inflammation, multiple sinus surgeries and irreversible upper and lower airway remodeling.[2]

Limited access to diagnostic tools and disease-modifying therapies is a serious problem for patients. In fact, the gold standard for the diagnosis and treatment of AERD, diagnostic aspirin challenge and high-dose therapy, is poorly utilized due to limited availability of specialized providers and concerns of precipitating a life-threatening anaphylactic reaction. Specifically, despite being beneficial in 80% of cases, diagnostic aspirin challenge and high-dose therapy is not offered by approximately 37.5% of providers.[3] Furthermore, 28.2% of survey respondents, who were members of the American Academy of Allergy, Asthma & Immunology, also stated that they would decline to refer patients for this procedure.[4] Many patients are therefore left without access to approved standard of care diagnostics and subsequent therapies despite evidence that they improve quality of life, decrease reliance on corticosteroids and extend the duration between surgeries. Innovative diagnostic and treatment options are urgently needed to advance patient care for a condition that costs more than $4.5 billion annually.[5]

AERD is associated with the dysregulated production of immunoregulatory eicosanoids, promoting chronic upper and lower airway inflammation. Metabolism of eicosanoids, such as immunomodulatory leukotrienes and endogenous cannabinoids (endocannabinoids), are carefully balanced to control inflammation (Figure 1).[6] In AERD, however, eicosanoids are shunted towards the leukotriene pathway. This shunting increases the production of inflammatory cysteinyl leukotrienes both at rest and following NSAID exposure, which correlates with severity of disease.[7,8] Dysregulated eicosanoids thereby represent emerging disease-specific biomarkers for screening and the early identification of AERD.

Urinary Leukotriene E4 (uLTE4) is the metabolic end-product of the pro-inflammatory leukotriene pathway and is over-expressed in AERD.[9] Several prior studies have evaluated uLTE4 as a potential biomarker for the diagnosis of *clinically apparent* AERD (i.e., patients self-reporting acute intolerance reactions following NSAID exposure) with a large distribution of diagnostic thresholds (Table 1).[10-16] Despite the potential for uLTE4 to identify different phenotypes of upper and lower airway disease, its utility in identifying *clinically unrecognized* AERD among high-risk patients (those with nasal polyps and asthma but unable to identify life-threatening NSAID sensitivity) has not been reported. This is a critical gap in current knowledge as screening tools are desperately needed to decrease reliance on diagnostic aspirin challenges among individuals at high risk of developing clinically apparent AERD.

Endogenous cannabinoids (endocannabinoids) may impact airway inflammation in AERD by directly producing prostaglandins (PGs) and regulating mediators of airway inflammation (Figure 1).[17,18] The endocannabinoid 2-arachidonoylglycerol (2-AG) is metabolized by cyclooxygenase-2 (COX-2), the primary target of NSAID activity and key

regulator of physiologic eicosanoids. Additionally, activation of type-2 cannabinoid receptors (CB2R) on mast cells and other inflammatory cells decrease Th2 cytokine profiles, leukotriene synthesis and leukocyte migration, all features associated with AERD and other forms of persistent asthma.[19] Our team has previously identified the increased transcription of the CB2R gene, *CNR2*, in AERD nasal polyp epithelium, a novel finding that is independent of tissue inflammation.[20] In the following study we therefore seek to evaluate both uLTE4 and CB2R as potential independent biomarkers to identify clinically silent AERD among high-risk patients. We will then use the findings of the above to propose a future trial seeking to evaluate a multi-variable screening panel to identify undiagnosed AERD among patients at high risk of developing this progressive disease.

Methods

This multi-site, observational study was approved by the Emory University Institutional Review Board (IRB00092578 and IRB00102406). Study sites included Emory University School of Medicine, Atlanta, GA and Scripps Health, San Diego, CA. Scripps Health was selected as a supporting site due to its active AERD practice and established research protocols.

uLTE4 Evaluation

Consecutive patients presenting to either the Emory Nasal, Sinus & Allergy Center or the AERD Center at Scripps Health were prospectively evaluated for study enrollment from June 2017 to December 2020. Study cohorts include adult patients (>18 years old) with high-risk or clinically apparent AERD. For the purposes of this study, high-risk AERD

was defined as chronic rhinosinusitis with nasal polyposis (CRSwNP) and asthma without a reported history of NSAID sensitivity. Clinically apparent AERD was defined by the combination of CRSwNP, asthma and self-reported sensitivity reactions to over-the-counter NSAIDS. Sensitivity reactions included any acute respiratory, dermatologic or gastrointestinal reactions within hours of known NSAID exposure.[1] Non-English speaking patients, patients currently taking daily NSAID therapy and those taking leukotriene modifying drugs within 90-days prior to trial screening were excluded from participation. Participants were assessed for study enrollment by trained personnel at the time of clinic presentation. Study participation was limited to enrollment activities without follow-up.

Study variables included baseline demographic and self-reported quality-of-life measures, including the 22-item Sinonasal Outcomes Test[21] and Asthma Control Test.[2] Objective measures of sinonasal disease included the endoscopic Lund-Kennedy score.[22] A 30mL urine sample was collected from all participants at the time of study enrollment and stored at -80°C pending batch laboratory analysis. Samples collected at Scripps Health were transferred on dry ice to maintain sample integrity.

The LTE4 ELISA Kit (Item 514010, Cayman Chemical Co.; Ann Arbor, MI) was used to determine the uLTE4 concentration, with processing of samples in triplicate and at 1:10 and 1:30 dilution in phosphate buffered saline per manufacturer recommendations. Assay range is defined as 7.8 – 1,000 pg/mL. The Creatinine Colorimetric Assay Kit (Item 500701, Cayman Chemical Co.; Ann Arbor, MI) was used to normalize uLTE4 levels based on urinary creatinine concentration for a reported uLTE4 concentration of pg/mg-creatinine.

To significantly detect a true difference of means of 150 pg/mg-creatinine corresponding to the reported difference in uLTE4 concentration between a population of patients with aspirin tolerant asthma and AERD,[23] a 2-tailed test for dependent means requires a minimum of 20 subjects assuming 90% power (1–β error probability), a 5% alpha level and an equal variance assumption with sigma value 141. The calculated sample size was increased by 20% to allow for specimen processing error and study drop-out, with a resulting target sample size of 48 participants.

CB2R Evaluation

This exploratory aim seeks to evaluate the association of CB2R expression (as measured by the *CN2R* gene) with undiagnosed AERD. Study activities in support of this investigation were completed at Emory University following the same inclusion and exclusion criteria as defined above. Once enrolled, the same clinical, demographic and patient-reported outcome measures were collected. Participants then underwent the collection of nasal epithelial cells via established protocol.[24] Collected samples were stored at -80°C in RNALater (Item R0901, Sigma-Aldrich; Rockville, MD) pending further processing and analysis, as previously described.[20]

Due to the absence of specific antibodies to identify CB2R protein expression,[25] we utilized quantitative RT-PCR to measure mRNA of the CB2R gene *CNR2* following established protocols.[20] Expression of reference genes 40S ribosomal protein S18 (*RPS18*) and Cyclooxygenase-1 (*PTGS1*) were measured and used to normalize *CNR2* expression. A priori power analysis was not completed due to the exploratory nature of this experiment.

Statistical Analysis

All study data is maintained in a password-protected relational database (Project REDCap, Nashville, TN). Urinary LTE4 is reported as mean group concentrations and normalized based on urinary creatinine (pg/mg-creatinine). All statistical analyses were completed using commercially available software (SPSS v.22; IBM Corp., Armonk, NY). Normal distribution was evaluated by the Shapiro-Wilk test. Between-group comparisons were performed using ANOVA or the non-parametric Mann–Whitney U-test. Correlation analysis was performed using Spearman's rank correlation coefficient. Comparison of categorical variables was performed using the Chi-Square or Fisher's exact probability test. All study data was evaluated descriptively with verification of data normality or skewness for all ordinal and continuous measures. Differences were considered significant when the p-value was less than 0.05.

Results

uLTE4 Evaluation

During the study period, a total of n=70 participants were enrolled and completed all study activities. Demographics of enrolled subjects by study cohort (high risk vs. AERD) are presented in Table 2. Significant differences were found for enrollment site (100 vs. 61.4% at Emory, $p<0.001$) and prevalence of reported respiratory reactions to alcohol (3.9 vs 50%, $p<0.001$). No significant clinical differences were seen (Table 3).

Mean concentration of uLTE4 was similar among study cohorts, with mean (SD) values of 187.8 (1689.3) vs. 138.3 (889.9) for high risk vs. AERD cohorts, $p=0.222$ (Figure 2). Abnormal distribution of uLTE4 was found (W statistic=0.4754; p-

value<0.0001). Of note, the majority of these values were outside the defined range for this assay.

Correlation of patient-reported SNOT-22 scores with clinical and demographic measures was evaluated to identify a potential predictive model for cohort assignment and is presented in Table 4. For this analysis a p-value < 0.2 was used to conservatively identify covariates for model evaluation. Potential covariates included ACT score (r=-0.48, p=0.014) and absolute eosinophil count (r=0.43, p=0.129). However, due to a lack of power and statistical significance, we were unable to fit a predictive model.

CB2R Evaluation

A total of 26 participants were included in this exploratory analysis. A significant difference in *CN2R* expression was found between study cohorts with mean (SD) values of 41 (119) vs. 131 (279) for High Risk vs. AERD cohorts with p=0.0042 (Figure 3). Despite this difference, n=2 participants in the high-risk cohort displayed elevated *CN2R* expression above the mean value for those with diagnosed AERD, suggesting the possibility of clinically undiagnosed disease. Further clinical evaluation of these participants was subsequently completed, resulting in a new diagnosis of AERD in one patient with previously unidentified disease. The other patient was lost to follow-up and not reached for further evaluation.

Definitive study

Given the above findings, it was decided to proceed with the design of a definitive study to incorporate multiple emerging biomarkers into a novel screening platform for undiagnosed AERD among patients with asthma and nasal polyps. To ensure accurate

cohort assignment, participants will be followed through completion of a postoperative diagnostic aspirin challenge, the gold standard for AERD diagnosis. Both endoscopic sinus surgery and diagnostic aspirin challenge will be completed per standard of care. The proposed observational study design is presented in Figure 4.

Ten genes, inflammatory lipids and clinical features previously associated with AERD will be explored as predictive variables to generate a novel biomarker panel to screen for undiagnosed AERD among subjects with asthma and nasal polyps (Table 5).[16,20,26-32] Relative to subjects without NSAID sensitivity, EP2 and PTGS2 are downregulated in AERD, while *ALOX5*, *CNR2* and the ratio of plasma prostaglandin D2/E2 are increased.[1,26,28] These findings are associated with altered arachidonic acid metabolism characteristic of AERD (Figure 1). Alcohol-induced respiratory symptoms are reported by 83% of subjects with AERD, with associated nasal congestion, rhinorrhea or shortness of breath.[31] Subjects with AERD are more likely to undergo multiple sinus surgeries.[33] Finally, patient sex may influence presentation as COX-2 expression is increased in men.[34] Subject age and symptom duration are not included due to a lack of power to detect differences among similar group means.[33]

The above study was funded by a special emphasis panel of the National Center for Advancing Translational Sciences under award 1R03TR004022. Patient enrollment is currently ongoing.

Discussion

The reliance on patient history and resource intensive procedures to diagnose patients with suspected AERD is a significant limitation of current clinical practice. Aspirin provocation challenge, the gold standard to diagnose AERD, is under-utilized, and up to

42% of subjects with asthma and nasal polyps fail to report that they have comorbid NSAID sensitivity.[32,35] This may occur due to NSAID avoidance, delayed onset of symptoms, or other unknown causes. The lack of sensitive biomarkers to identify unrecognized NSAID sensitivity is, therefore, a critical barrier to early diagnosis and improved patient care.

In the above study we provide preliminary data in support of a larger observational trial, which seeks to utilize the results of aspirin provocation challenge and ten emerging biomarkers to create a novel screening platform to identify unrecognized AERD among high-risk patients with nasal polyps and asthma. If successful, this screening tool would address an unmet need for patient identification, as evidenced by diagnosis of a young female with previously unrecognized NSAID sensitivity and elevated *CN2R* expression.

While the above findings have successfully supported R03-level funding for future study, there are several critical limitations that must be understood. First and foremost, the utilization of uLTE4 concentrations outside the assay range of the commercial ELISA is a significant limitation of rigor and prevents the quantitative evaluation of these values. While future study is currently being completed to address this limitation, the qualitative assessment of these values without a notable difference in mean uLTE4 concentrations between study cohorts does support the presence of patients with undiagnosed AERD in those with nasal polyps and asthma. Additionally, the omission of high-risk participants recruited from Scripps Health is a limitation of study generalizability. The large AERD practice at Scripps was leveraged to increase study enrollment, but unfortunately was limited to participants with clinically apparent

AERD. Future study will address this issue by including additional sites with a stratified enrollment scheme to ensure equal distribution of cohort enrollments from each site.

Conclusions

Aspirin-exacerbated respiratory disease is a chronic and debilitating condition with unmet needs for readily available options for patient diagnosis. Emerging biomarkers, such as uLTE4 and CB2R, have the potential to screen high risk patients with asthma and nasal polyps, thus decreasing the current reliance on inaccessible procedures such as diagnostic aspirin challenge, the gold-standard for AERD diagnosis. By incorporating a panel of ten emerging biomarkers with the results of diagnostic aspirin challenge in a prospective, observational study, we seek to generate a novel screening platform to address this ongoing limitation in patient care.

References

1. White AA, Stevenson DD. Aspirin-Exacerbated Respiratory Disease. New Engl J Medicine 2018;379:1060-1070.

2. Nathan RA, Sorkness CA, Kosinski M, et al. Development of the asthma control test: a survey for assessing asthma control. Journal of Allergy and clinical immunology 2004;113:59-65.

3. Stevens WW, Jerschow E, Baptist AP, et al. The role of aspirin desensitization followed by oral aspirin therapy in managing patients with aspirin-exacerbated respiratory disease: A Work Group Report from the Rhinitis, Rhinosinusitis and Ocular Allergy Committee of the American Academy of Allergy, Asthma & Immunology. J Allergy Clin Immunol 2021;147(3):827-844.

4. Jeremy DW, Andrew AW. A survey of aspirin desensitization practices among allergists and fellows in training in the United States. J Allergy Clin Immunol Pract 2016;4:1253-1255.

5. Chang JE, White AA, Simon RA, Stevenson DD. Aspirin-exacerbated respiratory disease: burden of disease. Allergy Asthma Proc 2012;33:117-121.

6. Levy JM. Endogenous cannabinoids may regulate chronic inflammation in aspirin-exacerbated respiratory disease. World J Otorhinolaryngol Head Neck Surg 2020;6(4):255-257.

7. Szefler SJ, Wenzel S, Brown R, et al. Asthma outcomes: biomarkers. Journal of Allergy and clinical immunology 2012;129:S9-23.

8. Sousa AR, Parikh AA, Scadding GK, Corrigan CJ, Lee TH. Leukotriene-receptor expression on nasal mucosal inflammatory cells in aspirin-sensitive rhinosinusitis. New Engl J Medicine 2002;347:1493-1499.

9. Choby G, Low CM, Levy JM, et al. Urine Leukotriene E4: Implications as a Biomarker in Chronic Rhinosinusitis. Otolaryngol Head Neck Surg 2022;166(2):224-232.

10. Israel E, Fischer AR, Rosenberg MA, et al. The Pivotal Role of 5-Lipoxygenase Products in the Reaction of Aspirin-Sensitive Asthmatics to Aspirin. Am Rev Respir Dis 1993;148(6):1447-1451.

11. Asano K, Lilly CM, O'Donnell WJ, et al. Diurnal variation of urinary leukotriene E4 and histamine excretion rates in normal subjects and patients with mild-to-moderate asthma. J Allergy Clin Immunol 1995;96(5 Pt 1):643-651.

12. Micheletto C, Visconti M, Tognella S, Facchini FM, Dal Negro RW. Aspirin induced asthma (AIA) with nasal polyps has the highest basal LTE4 excretion: a study vs AIA without polyps, mild topic asthma, and normal controls. European annals of allergy and clinical immunology 2006;38:20-23.

13. Sanak M, Bochenek G, Faber J, Plutecka H. Elevated urinary leukotriene E4 excretion in asthma: a comparison of HPLC-mass spectrometry and ELISA. Allergy 2010.

14. Yamaguchi H, Higashi N, Mita H, et al. Urinary concentrations of 15-epimer of lipoxin A(4) are lower in patients with aspirin-intolerant compared with aspirin-tolerant asthma. Clin Exp Allergy 2011;41:1711 1718.

15. Celejewska-Wójcik N, Mastalerz L, Wójcik K, et al. Incidence of aspirin hypersensitivity in patients with chronic rhinosinusitis and diagnostic value of urinary leukotriene E4. Polskie Archiwum Medycyny Wewnętrznej 2012;122:422-427.

16. Divekar R, Hagan J, Rank M, et al. Diagnostic Utility of Urinary LTE4 in Asthma, Allergic Rhinitis, Chronic Rhinosinusitis, Nasal Polyps, and Aspirin Sensitivity. J Allergy Clin Immunol Pract 2016;4(4):665-670.

17. Turcotte C, Blanchet M-R, Laviolette M, Flamand N. The CB2 receptor and its role as a regulator of inflammation. Cell Mol Life Sci 2018;73:4449-4470.

18. Turcotte C, Chouinard F, Lefebvre JS, Flamand N. Regulation of inflammation by cannabinoids, the endocannabinoids 2-arachidonoyl-glycerol and arachidonoyl-ethanolamide, and their metabolites. J Leukocyte Biol 2015;97:1049-1070.

19. Cabral GA, Griffin-Thomas L. Emerging role of the cannabinoid receptor CB2 in immune regulation: therapeutic prospects for neuroinflammation. Expert Rev Mol Med 2009;11:e3.

20. Corrado A, Battle M, Wise SK, et al. Endocannabinoid receptor CB2R is significantly expressed in aspirin-exacerbated respiratory disease: a pilot study. Int Forum Allergy Rhinol 2018;8(10):1184-1189.

21. Hopkins C, Gillett S, Lund VJ. Psychometric validity of the 22-item Sinonasal Outcome Test. Clinical Otolaryngology 2009;34:447-454.

22. Lund VJ, Kennedy DW. Staging for rhinosinusitis. Otolaryngology - Head Neck Surg 1997;117:S35-S40.

23. C Micheletto MVFTSTSBRWDN. The prevalence of nasal polyps and the corresponding urinary LTE4 levels in severe compared to mild and moderate asthma. European annals of allergy and clinical immunology 2010;42:120 124.

24. Massey CJ, Diaz Del Valle F, Abuzeid WM, et al. Sample collection for laboratory-based study of the nasal airway and sinuses: a research compendium. International forum of allergy & rhinology 2020;10(3):303-313.

25. Marchalant Y, Brownjohn PW, Bonnet A, Kleffmann T, Ashton JC. Validating Antibodies to the Cannabinoid CB2 Receptor: Antibody Sensitivity Is Not Evidence of Antibody Specificity. J Histochem Cytochem 2014;62:395-404.

26. Perez-Novo 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;115(6):1189-96.

27. Picado C, Juan M, Roca-Ferrer J, Fuentes M, Xaubet A, Mullol J. Cyclooxygenase-2 mRNA is downexpressed in nasal polyps from aspirin-sensitive asthmatics. Am J Resp Crit Care 1999;160(1):291-296.

28. Cahill KN, Raby BA, Zhou X, et al. Impaired E Prostanoid2 Expression and Resistance to Prostaglandin E2 in Nasal Polyp Fibroblasts from Subjects with Aspirin-Exacerbated Respiratory Disease. Am J Resp Cell Mol 2016;54(1):34-40.

29. Tsuyoshi Yoshimura MYNOS-iHHM. Correlation between the prostaglandin D(2)/E(2) ratio in nasal polyps and the recalcitrant pathophysiology of chronic rhinosinusitis associated with bronchial asthma. Allergol Int 2008;57:429 436.

30. Berges-Gimeno MP, Simon RA. The natural history and clinical characteristics of aspirin-exacerbated respiratory disease. Annals of Allergy 2002;89(5):474-8.

31. Cardet JC, White AA, Barrett NA, et al. Alcohol-induced respiratory symptoms are common in patients with aspirin exacerbated respiratory disease. J Allergy Clin Immunol Pract 2014;2:208-13. (In English).

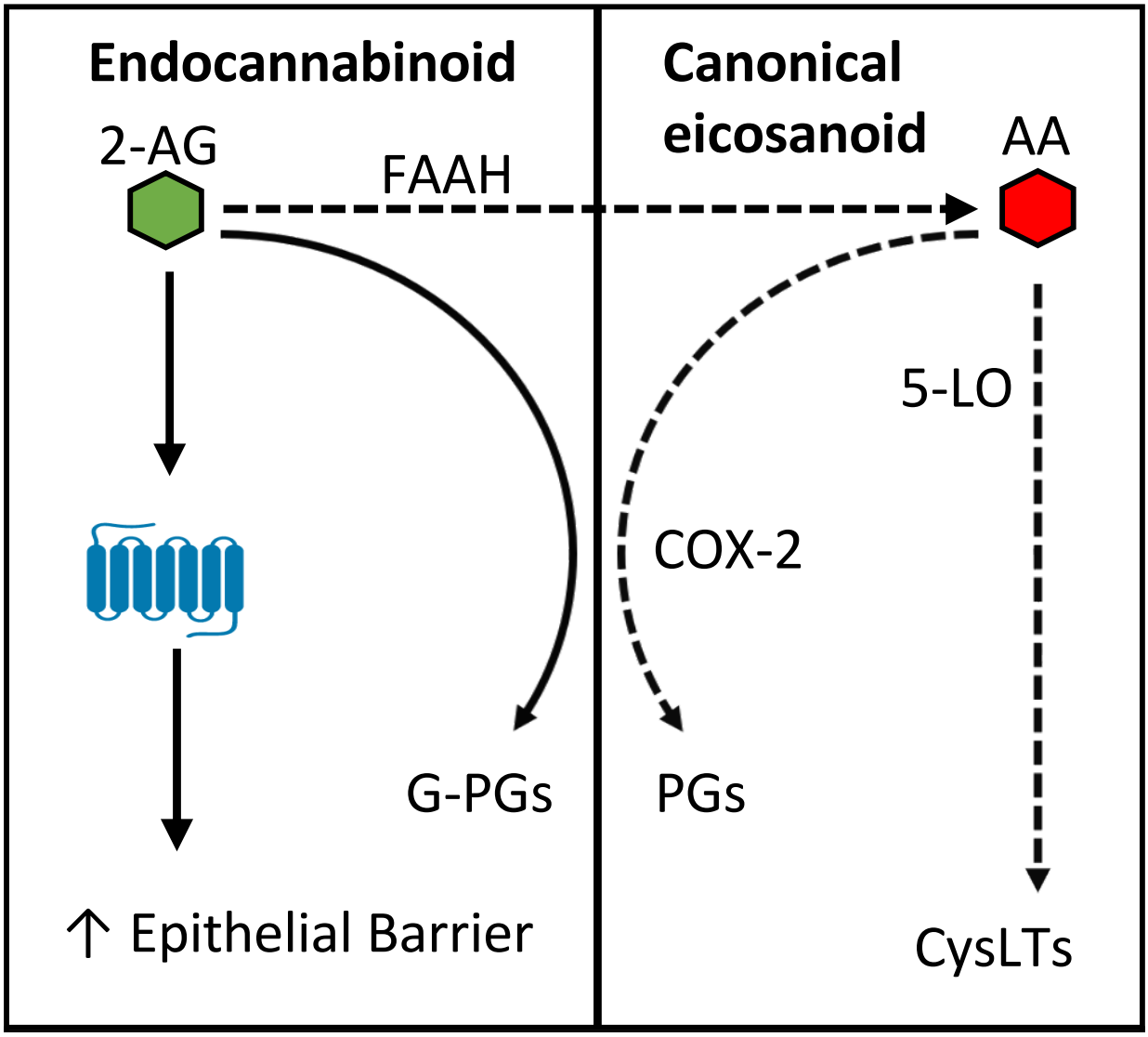
32. Dursun AB, Woessner KA, Simon RA, Karasoy D, Stevenson DD. Predicting outcomes of oral aspirin challenges in patients with asthma, nasal polyps, and chronic sinusitis. Ann Allergy Asthma Immunol 2008;100:420-425.

33. Morrissey DK, Bassiouni A, Psaltis AJ, Naidoo Y, Wormald P-J. Outcomes of modified endoscopic Lothrop in aspirin-exacerbated respiratory disease with nasal polyposis. International forum of allergy & rhinology 2016;6(8):820-825.

34. Pace S, Rossi A, Krauth V, et al. Sex differences in prostaglandin biosynthesis in neutrophils during acute inflammation. Scientific reports 2017;7:3759.

35. Waldram JD, White AA. A survey of aspirin desensitization practices among allergists and fellows in training in the United States. J Allergy Clin Immunol Pract 2016;4(6):1253-1255.

Figure 1
Endogenous eicosanoids regulate physiologic inflammation via the production of Leukotriene, Prostaglandin and Endocannabinoid mediators.



2-AG: 2-Arachidonoylglycerol; 5-LO: 5-lipoxygenase; AA: Arachidonic acid; CB2R: type-2 cannabinoid receptor; FAAH: fatty acid amid hydrolase; G-PGs: Prostaglandin glycerol-esters; CysLTs: Cysteinyl leukotrienes; PGs: Prostaglandins.

Adapted from Levy JM. Endogenous cannabinoids may regulate chronic inflammation in aspirin-exacerbated respiratory disease. *World J Otorhinolaryngol Head Neck Surg*. 2020 Sep 8;6(4):255-257.[6]

Table 1

Evaluation of uLTE4 as a biomarker for clinically apparent AERD reveals a heterogeneous distribution of mean concentrations and diagnostic thresholds

Author	Year	n	Controls mean (+SD)	ATA mean (+SD)	AERD mean (+SD)	Dx Threshold (+SD)	Sensitivity / Specificity
Israel et al[10]	1993	8	-	-	469 (141)	-	-
Asano et al[11]	1995	16	83.8 (38.2)	100.0 (59.2)	-	-	-
Micheletto et al[12]	2006	83	66.5 (20.6)	129.1 (74.8)	432.3 (88.1)	-	-
Sanak et al[13]	2010	174	280.3 (43)	674.9	1364.3	274	90.2 / 50.6
Yamaguchi et al[14]	2011	41	53.4	144.3	345.1	-	-
Celejewska et al[15]	2012	24	-	316.5	2371	859	87.5 / 93.75
Divekar et al[16]	2016	194	104	53	588	166	89

AERD: Aspirin-exacerbated respiratory disease; ATA: Aspirin tolerant asthma; Dx: Diagnosis

Table 2
Participant Demographics by Cohort Assignment

Variable	Level	Total cohort (n=70)	High Risk (cohort n=26)	AERD (cohort n=44)	p-value*
Enrollment Site, n(%)	Emory	53 (75.7)	26 (100)	27 (61.4)	<0.001
	Scripps	17 (24.3)	0 (0)	17 (38.6)	
Age, mean(SD)		68 (14.6)	48 (15.3)	51.0 (14.3)	0.423
Gender, n(%)	Female	38 (54.3)	12 (46.2)	26 (59.1)	0.294
	Male	32 (45.7)	14 (53.9)	18 (40.9)	
Race	African American	22 (31.4)	6 (23.1)	16 (36.4)	0.085
	American Indian	1 (1.4)	1 (3.9)	0 (0)	
	Asian	4 (5.7)	1 (3.9)	3 (6.8)	
	Caucasian	35 (50.0)	12 (46.2)	23 (52.3)	
	Unknown	8 (11.4)	6 (23.1)	2 (4.6)	
Ethnicity	Hispanic	1 (1.4)	0 (0)	1 (2.3)	0.081
	Non-Hispanic	56 (80.0)	18 (69.2)	38 (86.4)	
	Unknown	13 (18.6)	8 (30.8)	5 (11.4)	
Smoking History, n(%)	Current	6 (8.6)	1 (3.9)	5 (11.4)	0.212
	Former	14 (20.0)	8 (30.8)	6 (13.6)	
	Never	47 (67.1)	17 (65.4)	30 (68.2)	
	Missing	3 (4.3)	0 (0)	3 (6.8)	
EtOH Resp Rxns, n(%)	Yes	23 (32.9)	1 (3.9)	22 (50)	<0.001
	No	30 (42.9)	24 (92.3)	6 (13.6)	
	Missing	17 (24.3)	1 (3.9)	16 (36.4)	
Age Symptom Onset, mean(SD)		40.6 (14.9)	41.46 (14.5)	39.92 (15.3)	0.69

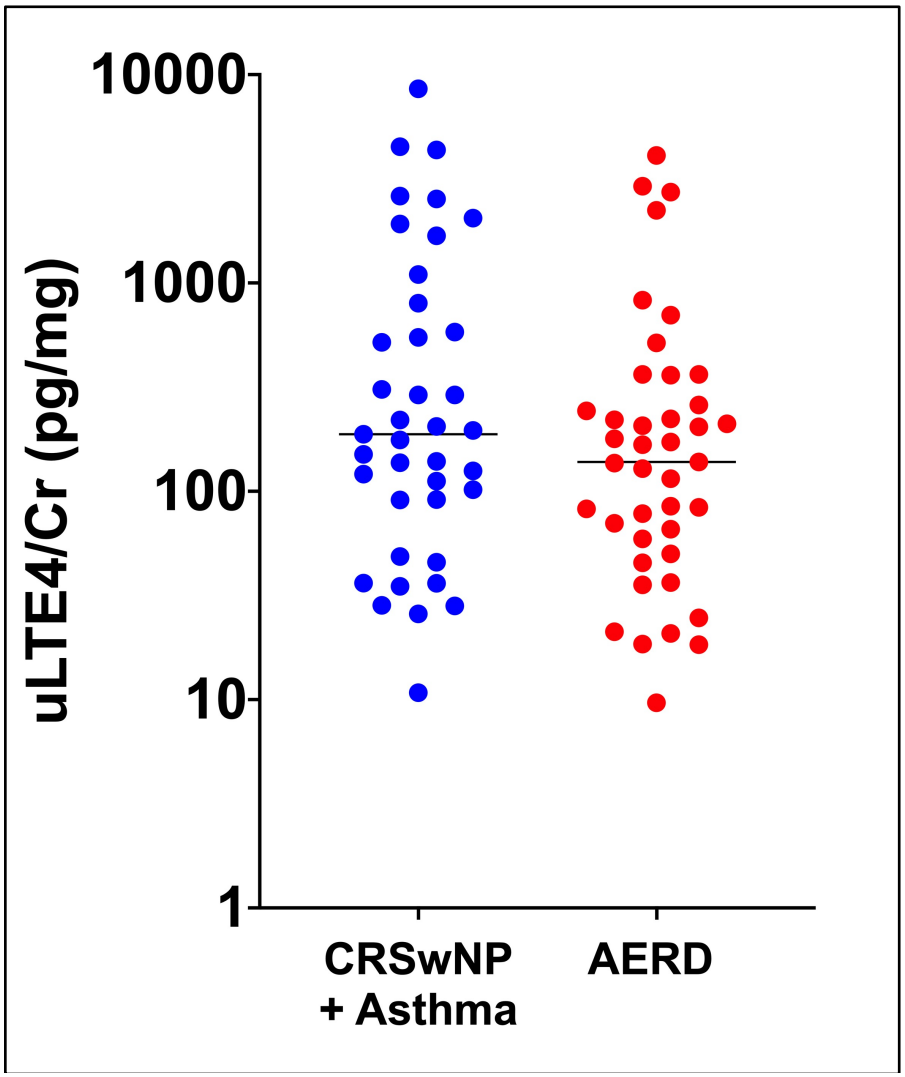
* p-value is calculated by ANOVA for numerical covariates; and chi-square test or Fisher's exact for categorical covariates, where appropriate.

Table 3
Clinical Features of Study Participants by Cohort Assignment

Variable, mean (SD)	n	High Risk (cohort n=26)	AERD (cohort n=44)	p-value*
# Sinus Surgeries	66	2.0 (1.4)	1.9 (1.5)	0.875
Patient Reported				
SNOT-22 Total Score	61	47.7 (19.8)	46.1 (27.3)	0.802
Rhinologic	63	17.4 (6.2)	16.2 (8.1)	0.537
Extra-Nasal Rhinologic	62	8.6 (2.3)	7.3 (4.1)	0.172
Ear/Facial	63	8.6 (5.9)	8.4 (7.0)	0.922
Psychological	62	10.2 (9.4)	13.6 (10.7)	0.215
Sleep	63	11.4 (6.7)	11.7 (8.5)	0.873
ACT Score	26	11.1 (10.6)	17.6 (8.1)	0.107
Objective Measures				
Nasal Endoscopy Score	24	8.4 (4.6)	4.3 (6.3)	0.131
FEV1	35	77.4 (18.4)	83.9 (21.2)	0.387
uLTE4 (pg/mg Cr)*	70	187.8 (1689.3)	138.3 (889.9)	0.222

ACT: Asthma control test; Cr: Creatinine; FEV1: Forced expiratory volume in the first second; SNOT-22: 22-item Sinonasal Control Test; uLTE4: Urinary Leukotriene E4

Figure 2
Mean concentration of uLTE4 was similar among study cohorts with clinically apparent AERD and high-risk disease with nasal polyps and asthma



AERD: Aspirin-exacerbated respiratory disease; Cr: Creatine; CRSwNP: Chronic rhinosinusitis with nasal polyposis; uLTE4: Urinary Leukotriene E4

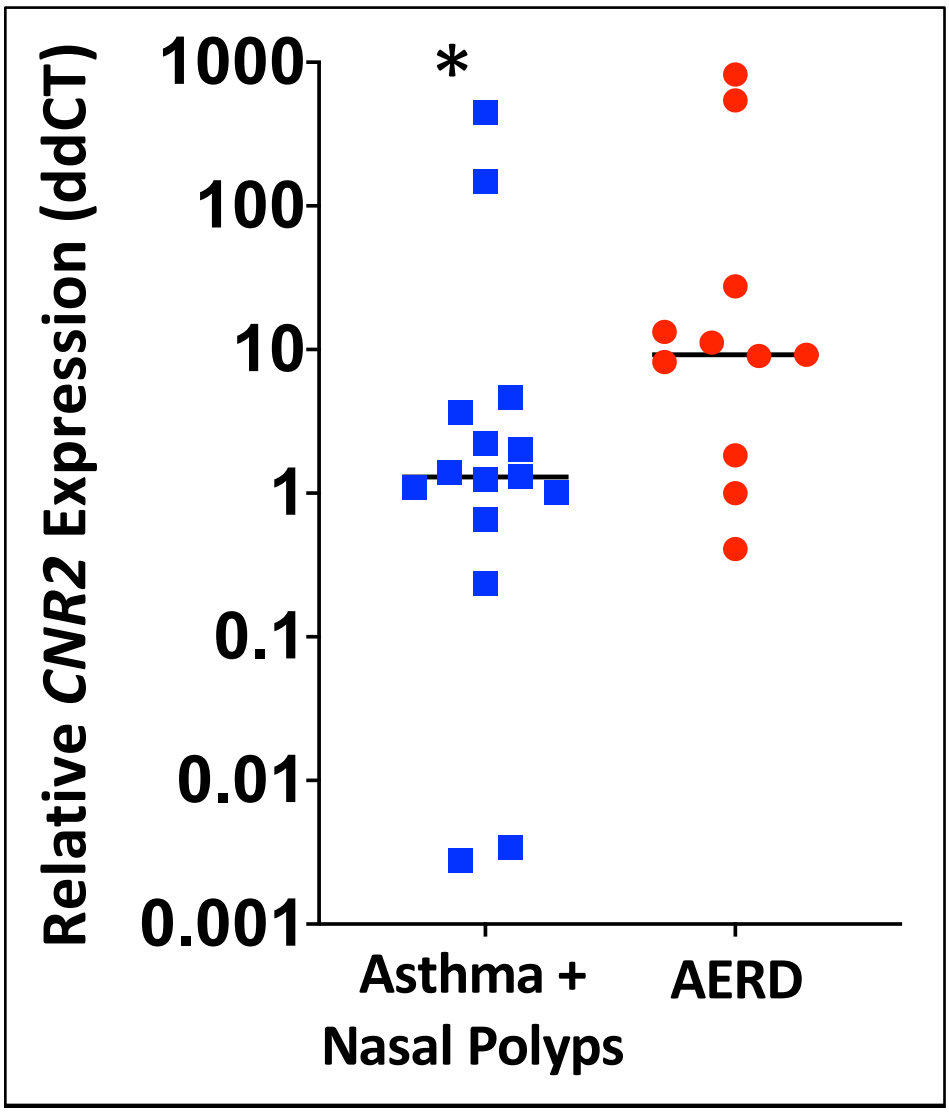
Note: uLTE4 concentrations largely outside the ELISA assay range

Table 4
Association of SNOT-22 Score with Study Covariates

Variable	N	Pearson Correlation with SNOT-22	P-Value
Age	62	-0.10	0.445
Number Sinus Surgeries	60	0.00	0.983
Nasal Endoscopy Score	20	0.21	0.368
ACT Score	**26**	**-0.48**	**0.014**
Age of symptom onset	60	-0.08	0.575
FEV1	34	0.12	0.513
AEC	**15**	**0.43**	**0.129**
Serum IgE	14	0.13	0.670

SNOT-22: 22-item Sinonasal Outcome Test; ACT: Asthma Control Test; AEC: Absolute Eosinophil Count; FEV1: Forced Expiratory Volume in 1 Second; IgE: Immunoglobin E

Figure 3
The CB2R gene *CNR2* is upregulated in a subpopulation of patients with asthma and nasal polyps (i.e. high-risk for AERD)



AERD: Aspirin-exacerbated respiratory disease; DDCT: Delta-Delta-Cycle Threshold

Figure 4
Study diagram for definitive observational trial which seeks to utilize the results of diagnostic aspirin challenge to determine accurate cohort assignment

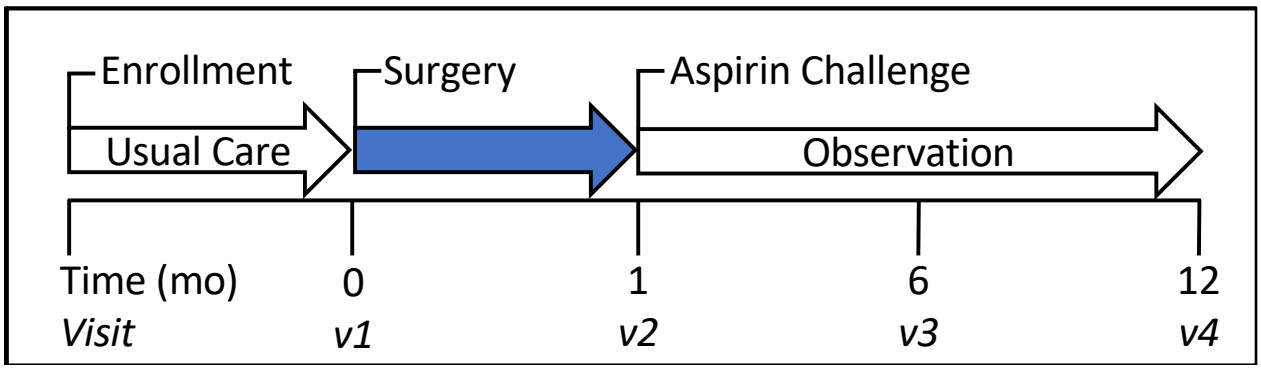


Table 5
Predictive Variables for Biomarker Panel

Predictive variable	**Difference in reported means (AERD vs. ATAwNP) Mean (SD)[#]**	**Calculated N (per cohort)**
Urinary leukotriene E4 ($uLTE_4$), pg/mg creatinine[16]*	347 (1079.4)	116
Predictive variable	**Difference in reported means (AERD vs. ATAwNP) Mean (SD)[#]**	**Detectable Difference (n=232)**
5-lipoxygenase (*ALOX5*), ddCT[26]	1.6 (2.0)	0.9
Cyclooxygenase 2 (*PTGS2*), ddCT[27]	-2.55 (0.52)	0.2
Prostaglandin E2 Receptor (*EP2*), ddCT[28]	-25.6 (13.5)	5.8
Type-2 Cannabinoid Receptor (*CNR2*), ddCT[20]	5.29 (1.19)	0.5
Prostaglandin D2/E2 Ratio[29]	3.5 (3.0)	0.1
Multiple Sinus Surgeries, #[30]	1.4 (1.9)	0.8
Female Sex[31]	16%	10
Self-reported alcohol-induced symptoms[31]	42%	10
Self-reported NSAID intolerance[32]	72%	10

[#] Largest reported SD used to generate conservative estimate; * Primary variable used to calculate power requirements. Subsequent calculations were completed for all other study variables to determine the detectable difference given the enrollment goal of n=232 with 90% power.

AERD: Aspirin-exacerbated respiratory disease; ATAwNP: Aspirin tolerant asthma with nasal polyps; SD: standard deviation