

Letter to the Editor

Asthma and biofilm-forming bacteria are independently associated with revision sinus surgeries for chronic rhinosinusitis

To the Editor:

Chronic rhinosinusitis (CRS) is a common but multifactorial disease that is often refractory in nature, requiring prolonged medical and surgical therapy.¹ Although many hypotheses have been suggested,¹ little is known about the epidemiology and pathophysiology of refractory CRS. Asthma has been associated with CRS severity, as measured by computed tomography scores or revision endoscopic sinus surgery (ESS) rates.^{1,2} Recently, bacterial biofilms, 3-dimensional aggregates of bacteria that can form on sinus mucosa, have been associated with worse computed tomography scores and persistent sinus infection.^{3,4} However, no single agent can account for the various clinical courses of CRS.¹ Many previous studies on CRS had small samples and did not adjust for confounding factors, which could inflate the results. Thus, more rigorous determination of whether asthma and biofilm-forming bacteria are independently associated with revision ESS could increase knowledge of refractory CRS pathogenesis and ultimately improve medical management. Our aim was to test the hypothesis that asthma and biofilm-forming bacteria are associated with revision ESS after adjusting for potential risk factors for CRS in a large cross-sectional cohort of patients with CRS undergoing ESS.

We recruited consecutive patients with CRS (≥ 18 years) undergoing ESS in the Department of Otorhinolaryngology–Head and Neck Surgery at the Hospital of the University of Pennsylvania between January 2007 and February 2010. Patients with the following diseases were excluded¹: cystic fibrosis, congenital or acquired immunodeficiency, congenital mucociliary disease, noninvasive fungal balls and invasive fungal disease, systemic vasculitis and granulomatous diseases, cocaine abuse, or neoplasia.

Chronic rhinosinusitis diagnosis was based on the Rhinosinusitis Initiative guidelines.⁵ ESS was classified as either revision or primary on the basis of medical records, endoscopic examinations, and computed tomography scans. We collected the following factors from patients' medical records¹: age, sex, asthma, nasal polyps, allergic rhinitis, aspirin intolerance, gastroesophageal reflux, diabetes, and smoking status. Asthma diagnosis was confirmed by documentation of one of the following⁶: asthma treatments, asthma symptoms, multiple records of asthma diagnosis, or lung function tests for asthma.

Each patient had 2 sinus secretion samples collected under endoscopic guidance to avoid contamination at the time of ESS. One sample underwent standard bacterial culture, whereas the other underwent *in vitro* biofilm-forming bacteria detection based on the modified Calgary Biofilm Detection Assay, as described previously.⁴ Positive bacterial culture was defined as any isolates of *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Moraxella catarrhalis*, *Pseudomonas aeruginosa*, other gram-negative rods, or anaerobes. Antibiotic use in the month before sample collection was recorded to evaluate its effects on biofilm-forming bacteria and culture results. This study was approved by the University of Pennsylvania Institutional Review Board. All participants provided written informed consent.

Unconditional logistic regression was used to calculate unadjusted and adjusted odds ratios (ORs) with 95% CIs. All variables with $P < .20$ in univariate logistic regression analyses were selected to enter the multiple logistic regression model. We tested for interaction between asthma and biofilm-forming bacteria. Selected variables were retained in the model if $P < .05$ on the basis of the likelihood ratio backward elimination procedure. All analyses were performed by using Stata 11.0 (Stata Corp, College Station, Tex). For further details of the **Methods** and **Results**, see this article's Online Repository at www.jacionline.org.

We enrolled 510 consecutive patients with CRS. Sixty-eight percent of patients underwent revision ESS, of whom 40% underwent more than 1 revision ESS. Patients' characteristics and univariate analyses are summarized in **Table I**. Antibiotic use in the month before sample collection was not associated with biofilm-forming bacteria (OR, 1.10; 95% CI, 0.70–1.74; $P = .672$) but decreased the odds of positive culture results by 44% (OR, 0.56; 95% CI, 0.38–0.81; $P = .002$). Aspirin intolerance ($P = .662$), sex ($P = .160$), and the interaction term between asthma and biofilm-forming bacteria ($P = .307$) were removed from the multiple logistic regression model during backward elimination. The adjusted results from the final model are shown in **Table II**.

Our study, the largest cross-sectional study to date, showed that both asthma and biofilm-forming bacteria were associated with a more than 2-fold increased odds of revision ESS after adjustment for CRS risk factors. The interaction between asthma and biofilm-forming bacteria was not significant, indicating that neither asthma nor biofilms modified each other's association with revision ESS. Our study provides further evidence that asthma, bacterial biofilms, infection, nasal polyps, and age independently contribute to refractory CRS.^{1–4,7} However, other potential CRS risk factors were not significant in our study. These factors are also controversial in the literature,¹ perhaps because of differences in study design and outcome measurements.

Interestingly, antibiotic use in the month before sample collection reduced positive culture rates but did not affect biofilm-forming bacteria. Our finding could suggest that the ineffectiveness of antibiotic treatment in CRS is a result of biofilms because bacterial biofilms' inherent resistance to antibiotics is considered to be the root of many chronic bacterial infections.⁸

Our study's strengths include large sample size and the use of multiple logistic regression to analyze independent risk factors for CRS. However, there are several limitations in our study. First, cross-sectional studies cannot discern temporal relationships to assess causality. Second, patients undergoing primary ESS in our study may have revision ESS in future, potentially underestimating the true associations with revision ESS by overestimating the prevalence of CRS risk factors in primary ESS patients. Third, we cannot rule out the possibility of misclassification of asthma and biofilm-forming bacteria. Fourth, potential anatomic and histologic features that may have predisposed to revision ESS^{1,2,9} were not measured in our study. Finally, because sinus secretion samples cannot be obtained from patients without ESS, we could not compare biofilm and culture results between CRS patients with and without ESS as well as normal controls.

TABLE I. Patients' demographics and characteristics

Clinical factors (%)	Overall	Revision ESS		Unadjusted OR (95% CI)	P value
		No	Yes		
Age (y $\pm$ SD)	50 $\pm$ 14	47 $\pm$ 15	52 $\pm$ 14	1.03 (1.01-1.04)	<.001
Sex					
Female	242 (47)	69 (43)	173 (50)	Reference	Reference
Male	268 (53)	93 (57)	175 (50)	0.75 (0.51-1.09)	.134
Smoking					
Nonsmoker	345 (68)	106 (65)	239 (68)	Reference	Reference
Former smoker	110 (22)	38 (24)	72 (21)	0.83 (0.53-1.31)	.430
Smoker	55 (10)	18 (11)	37 (11)	0.90 (0.49-1.66)	.740
Polyps					
No	279 (55)	107 (66)	172 (49)	Reference	Reference
Yes	231 (45)	55 (34)	176 (51)	1.99 (1.35-2.93)	<.001
Allergic rhinitis					
No	275 (54)	81 (50)	194 (56)	Reference	Reference
Yes	235 (46)	81 (50)	154 (44)	0.79 (0.55-1.15)	.226
Asthma					
No	284 (56)	118 (73)	166 (48)	Reference	Reference
Yes	226 (44)	44 (27)	182 (52)	2.94 (1.96-4.41)	<.001
Biofilm					
No	404 (79)	146 (90)	258 (74)	Reference	Reference
Yes	106 (21)	16 (10)	90 (26)	3.18 (1.80-5.62)	<.001
Diabetes					
No	477 (94)	151 (93)	326 (94)	Reference	Reference
Yes	33 (6)	11 (7)	22 (6)	0.93 (0.44-1.96)	.841
Aspirin intolerance					
No	476 (93)	156 (96)	320 (92)	Reference	Reference
Yes	34 (7)	6 (4)	28 (8)	2.28 (0.92-5.61)	.074
GERD					
No	417 (82)	132 (81)	285 (82)	Reference	Reference
Yes	93 (18)	30 (19)	63 (18)	0.97 (0.60-1.57)	.910
Positive culture*					
No	250 (49)	107 (66)	143 (41)	Reference	Reference
Yes	260 (51)	55 (34)	205 (59)	2.79 (1.89-4.12)	<.001
Antibiotics					
No	350 (69)	108 (67)	242 (70)	Reference	Reference
Yes	160 (31)	54 (33)	106 (30)	0.90 (0.60-1.34)	.598

GERD, Gastroesophageal reflux.

S aureus* (28%), *P aeruginosa* (13%), and other gram-negative rods (15%) were the most common isolates.TABLE II.** Risk factors associated with repeat ESS in the final multiple logistic regression model

Clinical factors (%)	Revision ESS		Adjusted OR (95% CI)	P value
	No	Yes		
Age (y $\pm$ SD)	47 $\pm$ 15	52 $\pm$ 14	1.02 (1.01-1.04)	.004
Polyps				
No	107 (66)	172 (49)	Reference	Reference
Yes	55 (34)	176 (51)	1.56 (1.03-2.38)	.037
Asthma				
No	118 (73)	166 (48)	Reference	Reference
Yes	44 (27)	182 (52)	2.57 (1.67-3.95)	<.001
Biofilm				
No	146 (90)	258 (74)	Reference	Reference
Yes	16 (10)	90 (26)	2.26 (1.24-4.12)	.008
Positive culture				
No	107 (66)	143 (41)	Reference	Reference
Yes	55 (34)	205 (59)	2.24 (1.48-3.38)	<.001

In conclusion, the associations among asthma, biofilm-forming bacteria, and revision ESS are strong and robust after adjusting for other factors in patients with CRS from a tertiary medical center. Despite its limitations, this study may improve

our understanding of refractory CRS pathogenesis, possibly leading to more effective treatment strategies, such as incorporating the treatments of asthma and biofilm infection into conventional CRS therapies. Prospective cohort studies in

diverse populations are needed to assess the causality of these associations.

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REFERENCES

1. Fokkens W, Lund V, Mullol J. EP3OS 2007: European position paper on rhinosinusitis and nasal polyps 2007: a summary for otorhinolaryngologists. *Rhinology* 2007; 45:97-101.
2. Newman LJ, Platts-Mills TA, Phillips CD, Hazen KC, Gross CW. Chronic sinusitis: relationship of computed tomographic findings to allergy, asthma, and eosinophilia. *JAMA* 1994;271:363-7.
3. Psaltis AJ, Weitzel EK, Ha KR, Wormald PJ. The effect of bacterial biofilms on post-sinus surgical outcomes. *Am J Rhinol* 2008;22:1-6.
4. Prince AA, Steiger JD, Khalid AN, Doghramji L, Reger C, Eau Claire S, et al. Prevalence of biofilm-forming bacteria in chronic rhinosinusitis. *Am J Rhinol* 2008;22: 239-45.
5. Meltzer EO, Hamilos DL, Hadley JA, Lanza DC, Marple BF, Nicklas RA, et al. Rhinosinusitis: establishing definitions for clinical research and patient care. *J Allergy Clin Immunol* 2004;114:155-212.
6. National Asthma Education and Prevention Program. Expert Panel Report 3 (EPR-3): guidelines for the diagnosis and management of asthma-summary report 2007. *J Allergy Clin Immunol* 2007;120:S94-S138.
7. Banerji A, Piccirillo JF, Thawley SE, Levitt RG, Schechtman KB, Kramper MA, et al. Chronic rhinosinusitis patients with polyps or polypoid mucosa have a greater burden of illness. *Am J Rhinol* 2007;21:19-26.
8. Costerton JW, Stewart PS, Greenberg EP. Bacterial biofilms: a common cause of persistent infections. *Science* 1999;284:1318-22.
9. Richtsmeier WJ. Top 10 reasons for endoscopic maxillary sinus surgery failure. *Laryngoscope* 2001;111:1952-6.

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METHODS

This is a cross-sectional study. Patients with CRS were defined as having experienced at least 2 of the typical symptoms—such as headache, nasal obstruction, and nasal drainage—that persisted for 12 weeks or longer and showed edema or erythema of the middle meatus, as identified by means of nasal endoscopy.^{E1} Furthermore, a computed tomography scan showed diffuse mucosal thickening, opacification, or swelling of the ethmoidal and maxillary mucosa with bilateral obstruction of the ostiomeatal complex. Revision ESS was defined as patients with more than 1 ESS.

Each patient had 2 sinus secretion samples collected. One sample underwent standard bacterial culture at the hospital microbiology laboratory, whereas the other sample underwent *in vitro* evaluation of biofilm-forming capacity based on the modified Calgary Biofilm Detection Assay, as described previously.^{E2} Bacterial samples were individually placed in a culture tube with Luria-Bertani broth (Fisher, Fairlawn, NJ) for growth and standardization and were then transferred to a well of a flat-bottom 96-well microtiter plate (catalog no. 269787; Nalgene Nunc Intl, Rochester, NY) in quadruplicate. A modified polystyrene microtiter lid with 96 pegs (catalog no. 445497, Nunc TSP system, Nunc, Roskilde, Denmark) was placed into the bacterial isolate growth plate for incubation. Then the peg lid was rinsed 3 times in sterile water, and the lid was placed in 2% crystal violet solution (Remel, Inc, Lenexa, Kan) for 30 minutes to stain the biofilms adherent to the pegs. The peg lid was then discarded, and the eluted crystal violet was read on a microtiter plate reader (Microplate Reader 680; Bio-Rad, Hercules, Calif) of an absorbance of optical density (OD) at 595 nm. Biofilm-forming wild-type *P aeruginosa* (PAO1; positive control) and non-biofilm-forming mutants *sad-31* (*type IV pili*; negative control) and *sad-36* (*flagella K*; negative control) were run simultaneously with the clinical sample as positive and negative controls.

For the statistical analyses, first, univariate logistic regressions were used to assess the associations between revision ESS and the potential risk factors for CRS. Age was analyzed as a continuous variable whereas all the other factors were analyzed as categorical variables. Then, we performed multivariate analysis using multiple logistic regression adjusting for all the potential risk factors for CRS. We also tested the interaction between culture results and biofilm-forming bacteria. The overall significance level for the study was $P < .05$ using a 2-sided test.

RESULTS

Forty-four percent of the patients with CRS had asthma. The modified Calgary Biofilm Assay showed that 21% of the patients with CRS harbored biofilm-forming bacteria *in vitro*. The average OD at 595 nm was 0.87 (SD, 0.71) for the biofilm-forming bacteria ($n = 106$) and 0.08 (SD, 0.06) for the non-biofilm-forming bacteria ($n = 404$). In the univariate analyses (Table I), patients with CRS with revision ESS were significantly more likely to be older (52 vs 47 years) and have polyps (51% vs 34%), asthma (52% vs 27%), biofilm-forming bacteria (26% vs 10%), and positive culture results (59% vs 34%) compared with patients with CRS without revision ESS.

In the multivariate analysis, age, sex, polyps, asthma, biofilm-forming bacteria, positive culture results, and aspirin intolerance were selected for entry in the multiple logistic regression model on the basis of the results of the univariate analyses ($P < .20$). The interaction between culture results and biofilm-forming bacteria was not significant ($P = .157$), and thus the interaction was removed from the multiple logistic regression model during backward elimination. In the final multiple logistic regression model, 5 variables remained significantly associated with revision ESS: age ($P = .004$), polyps ($P = .037$), asthma ($P < .001$), biofilm-forming bacteria ($P = .008$), and positive culture results ($P < .001$; Table II). Thus, revision ESS was independently associated with asthma (adjusted OR, 2.57; 95% CI, 1.67-3.95), biofilm-forming bacteria (adjusted OR, 2.26; 95% CI, 1.24-4.12), positive culture results (adjusted OR, 2.24; 95% CI, 1.48-3.38), polyps (adjusted OR, 1.56; 95% CI, 1.03-2.38), and age (adjusted OR, 1.02; 95% CI, 1.01-1.04).

REFERENCES

- E1. Meltzer EO, Hamilos DL, Hadley JA, Lanza DC, Marple BF, Nicklas RA, et al. Rhinosinusitis: establishing definitions for clinical research and patient care. *J Allergy Clin Immunol* 2004;114:155-212.
- E2. Prince AA, Steiger JD, Khalid AN, Doghramji L, Reger C, Eau Claire S, et al. Prevalence of biofilm-forming bacteria in chronic rhinosinusitis. *Am J Rhinol* 2008;22:239-45.