



Fungal Speciation Using Quantitative Polymerase Chain Reaction (QPCR) in Patients With and Without Chronic Rhinosinusitis

Andrew H. Murr, MD; Andrew N. Goldberg, MD, MSCE; Stephen Vesper, PhD

Objectives/Hypothesis: The objectives of this study were to determine the mycology of the middle meatus using an endoscopically guided brush sampling technique and polymerase chain reaction laboratory processing of nasal mucous; to compare the mycology of the middle meatus in patients with sinus disease with subjects without sinus disease; to compare the responses on two standardized quality-of-life survey forms between patients with and without sinusitis; and to determine whether the presence of fungi in the middle meatus correlates with responses on these data sets. **Study Design:** The authors conducted a single-blind, prospective, cross-sectional study. **Methods:** Patients with sinus disease and a control group without sinus disease were enrolled in the study. A disease-specific, validated Sinonasal Outcomes Test survey (SNOT-20) was completed by the subjects and a generalized validated Medical Outcomes Short Form 36 Survey (SF-36) was also completed. An endoscopically guided brush sampling of nasal mucous was obtained from the middle meatus. Fungal specific quantitative polymerase chain reaction (QPCR) was performed on the obtained sample to identify one of 82 different species of fungus in the laboratory. Statistical analysis was used to categorize the recovered fungal DNA and to crossreference this information with the outcomes surveys. **Results:** The fungal recovery rate in the study was 45.9% in patients with sinus disease and 45.9% in control subjects. Patients with chronic rhinosinusitis had a mean SNOT-20 score of 1.80 versus the control group mean

score of 0.77 ($P < .0001$). SF-36 data similarly showed a statistically significant difference between diseased and control populations with controls scoring a mean of 80.37 and patients with chronic rhinosinusitis scoring a mean of 69.35 for a P value of .02. However, no statistical significance could be ascribed to the presence or absence of fungi recovered, the type of fungi recovered, or the possible impact of fungi on the quality-of-life survey results. **Conclusion:** The recovery rate of fungi from the middle meatus of patients with chronic rhinosinusitis and a control population without chronic rhinosinusitis is 45.9% using QPCR techniques. No direct causation with regard to fungal species or presence was proven; however, a species grouping for future studies is proposed based on trends in this data and other reports. Disease-specific outcomes surveys revealed a statistically significant difference between the two groups. **Key Words:** Fungus, sinusitis, polymerase chain reaction, middle meatus, group I and group II fungi.

Laryngoscope, 116:1342–1348, 2006

INTRODUCTION

Despite many years of investigatory effort, the pathophysiology of chronic rhinosinusitis (CRS) remains elusive. The purpose of this study is to define the speciation of fungal elements found in patients with accurately diagnosed CRS and to compare the flora distribution with patients with no sinus disease or complaints. Previous testing techniques using fungal cultures sample nonspecific areas in the nose from the nasal vestibule to the nasopharynx. To alleviate this source of uncertainty concerning quantity and location of sampling, a quantitative polymerase chain reaction (QPCR) method was used to process the specimens obtained from the middle meatus under endoscopic guidance for speciation. Our results are presented with analysis and statistical review.

Sinusitis is the most common chronic condition for which a patient seeks the input of a physician. It is com-

From the Department of Otolaryngology/Head and Neck Surgery (A.H.M., A.N.G.), University of California, San Francisco School of Medicine, San Francisco, California, U.S.A., and the United States Environmental Protection Agency (S.V.), Cincinnati, Ohio, U.S.A.

Editor's Note: This Manuscript was accepted for publication April 4, 2006.

Send Correspondence to Andrew H. Murr, MD, General Otolaryngology/Head and Neck Surgery, A-717, 400 Parnassus Ave., San Francisco, CA 94143-0342, U.S.A. E-mail: ahmurr@ohns.ucsf.edu

DOI: 10.1097/01.mlg.0000225896.91392.6a

monly quoted that sinusitis accounts for between 22 and 30 million patient visits per year.¹ A huge pharmaceutical industry exists to produce drugs that have an effect on sinus conditions with sales figures in the billions of dollars. Panels and cooperative groups have met to try to create a standardized terminology to define sinusitis. The disease is often conveniently broken down into “acute sinusitis” and “chronic sinusitis” based on the time course of existing symptoms despite treatment. Chronic rhinosinusitis is often defined as a disorder of the sinuses that has been present for 12 weeks and persists despite treatment. It is presumptive that acute or subacute rhinosinusitis clears within 3 months.²

Radiologic studies have been used to help define and diagnose sinus disease and computed tomography (CT) scan findings often play into the definition of whether rhinosinusitis is present or has cleared. Yet, findings on radiologic studies are not specific as to the causation of disease.³ In fact, a simple upper respiratory infection can produce CT findings indistinguishable from CRS on film review and yet be entirely reversible with either little or no directed treatment.⁴ Perhaps the reason for this confusion is that the causation of rhinosinusitis itself is currently not properly defined. Although the model of a simple bacterial or viral infection is suitable for understanding acute rhinosinusitis, an all-encompassing model of CRS clearly does not exist because this form of the disease presents with much greater variability. Chronic rhinosinusitis may or may not be accompanied by nasal polyposis, atopic allergy, fungal infection, chromosomal abnormality, immune dysfunction, or other metabolic dysfunction.

Much recent work has been done to help define the role of antifungal treatment in CRS.⁵ Fungi may participate in the pathophysiology of CRS in a variety of ways either as a direct invasive infection as is seen in mucormycosis or as an IGE-mediated inflammatory agent as seen in allergic fungal sinusitis (AFS).^{6–8} Other mechanisms of fungal participation in sinus inflammation have been proposed, including the concept of allergic mucin fungal sinusitis and the concept described in culture-based studies, which implicates a causative mechanism based on eosinophil-mediated chronic inflammation.^{9,10} This latter concept was unique enough to warrant issuance of a U.S. patent for the application of antifungal medication “for treating and preventing inflammation of mucosal tissue”.¹¹

Although the presence of fungus itself in the nasal cavity of a patient would have at one time been thought to be unusual or even alarming, it has now been shown that the presence of fungal elements in the nasal respiratory tract is in fact common.^{10,12,13} A culture-based study has implied that all humans with or without sinus infection retain fungus in the nasal cavity as part of the background flora.^{10,12} A QPCR study has shown that a large percentage of patients with or without sinusitis have fungal elements present in the nasal cavity.¹³ Furthermore, a second QPCR-based study has shown that the quantity of fungus in the nasal cavity is the same in patients with or without sinus disease.¹⁴ A culture-based study has shown that newborn infants do not have fungus in their nasal cavity when born; however, by the age of 6 weeks, infants

universally have nasal fungus that can be cultured using special techniques.¹²

Some previously idiopathic diseases have been associated through QPCR identification techniques with groupings of fungal species. Vesper et al. in a study of water-damaged, moldy homes found that there are 26 molds associated with water-damaged environments and that 10 fungal species are present in essentially all homes.¹⁵ They were able to link the species recovered in water-damaged environments with idiopathic pulmonary hemorrhage.¹⁵ This study categorized the fungi found in a water-damaged environment as group I molds and refers to other fungi found commonly in a household environment as group II molds^{15,16} (Table I). We have used this same grouping to look at the occurrence of molds in this study of patients with CRS and compared the results with findings in a group of control patients.

MATERIALS AND METHODS

Patients were recruited for the study during routine care appointments in an outpatient clinical practice centering on rhinology and general otolaryngology/head and neck surgery. Informed consent was obtained from all patients for obtaining nasal samples as part of clinical care and the study was approved by the Institutional Committee on Human Research. Individuals were recruited for the study in two broad groups: patients with CRS and patients and other participants without CRS. Patients with CRS had a history of chronic sinusitis for greater than 3 months despite medical management. All patients had nasal endoscopic signs of CRS such as visible mucopurulent drainage or nasal polyposis on physical examination. All patients with rhinosinusitis had evidence of CRS on CT examination. Patients with immune compromise, minors, and pregnant patients were excluded from the study. Individuals without rhinosinusitis were undergoing nasal endoscopy as part of a general otolaryngology/head and neck surgery office examination for reasons unrelated to rhinosinusitis or allergy, had no complaints or history pertaining to CRS, and had no history of a recent upper respiratory infection. All patients in the control population had a negative nasal endoscopy examination for signs of sinus disease.

Subjects filled out two validated quality-of-life questionnaires: a Standard Form-36 Health Survey (SF-36) general quality-of-life outcomes measure and a Sino-Nasal Outcomes Test (SNOT-20) rhinosinusitis-specific quality-of-life instrument.^{17,18} Subjects were given 0.5% Neo-Syneprine aerosolized spray and 1% tetracaine aerosolized spray to allow comfort during the initial endoscopic examination. A 30° Hopkins rod attached to a camera and light source were used to guide an Interdental Brush (Aclean; Henry Schein Inc., Melville, NY) into the middle meatus under direct visualization. The brush had been steam-sterilized and packaged in a peel pack in keeping with standard office instrument processing. After swabbing the middle meatus and removing mucous from this area, the brush was placed in a 2-mL conical bottom, screw-cap tube (506–636; PGC Scientifics, Gaithersburg, MD). The specimen was then refrigerated and transported to the laboratory on ice packs.

Quantitative Polymerase Chain Reaction Assays and Standard Curves

Methods used in this study have been reported for preparing conidia or spore suspensions from fungal cultures, extracting DNA, performing QPCR analyses, and preparing standard calibration curves for target conidia or spore equivalents versus delta cycle threshold values ($\Delta C_T = C_{T,target} - C_{T,reference}$) using coextracted DNA from *Geotrichum candidum* as an exogenous reference.^{19,20}

TABLE I.
Group I and Group II Molds as Classified by Vesper et al.¹⁵

Group I Fungi (26 species)	Group II Fungi (10 species)
Predominate in water-damaged environments	Ubiquitous in homes
<i>Aspergillus flavus</i> group	<i>Aspergillus ustus</i>
<i>A. flavus</i> and <i>A. oryzae</i>	<i>Penicillium chrysogenum savar.2</i>
<i>Aspergillus fumigatus</i> group	<i>Acremonium strictum</i>
<i>A. fumigatus</i> and <i>Neosartorya fischeri</i>	<i>Alternaria alternata</i>
<i>Aspergillus niger</i> group	<i>Cladosporium cladosporioides-svar.1</i>
<i>A. niger</i> , <i>A. foetidus</i> , <i>A. pheonicis</i>	<i>Cladosporium cladosporioides-svar.2</i>
<i>Aspergillus ochraceus</i> group	<i>Cladosporium herbarum</i>
<i>A. ochraceus</i> and <i>A. ostianus</i>	<i>Epicoccium nigrum</i>
<i>Aspergillus penicillioides</i>	<i>Mucor</i> and <i>Rhizopus</i> group
<i>Aspergillus restrictus</i> group	<i>M. amphibiorum</i> , <i>M. circinelloides</i> , <i>M. hiemalis</i> ,
<i>A. restrictus</i> , <i>A. caesillus</i> , <i>A. conicus</i>	<i>M. indicus</i> , <i>M. mucedo</i> , <i>M. racemosuus</i> ,
<i>Aspergillus sclerotiorum</i>	<i>M. ramosissimus</i> , <i>R. azygosporus</i> ,
<i>Aspergillus sydowii</i>	<i>R. homothalicus</i> , <i>R. microsporus</i> ,
<i>Aspergillus unguis</i>	<i>R. oligosporus</i> , <i>R. oryzae Rizopus stolonifer</i>
<i>Aspergillus versicolor</i>	
<i>R Eurotium</i> group	
<i>E. amstelodami</i> , <i>E. chevalieri</i> , <i>E. herbariorum</i> ,	
<i>E. rubrum</i> , <i>E. repens</i> .	
<i>Penicillium brevicompactum</i>	
<i>Penicillium</i> group 2	
<i>P. crustosum</i> , <i>P. camembertii</i> , <i>P. commune</i> ,	
<i>P. echinulatum</i> , <i>P. solitum</i>	
<i>Penicillium corylophilum</i>	
<i>Penicillium purpurogenum</i>	
<i>Penicillium spinulosum</i> group	
<i>P. spinulosum</i> , <i>P. glabrum</i> , <i>P. lividum</i> ,	
<i>P. pupurescens</i> , <i>P. thomii</i>	
<i>Penicillium variabile</i>	
<i>Paecilomyces variotii</i>	
<i>Aureobasicium pullulans</i>	
<i>Chaetomium globosum</i>	
<i>Cladosporium sphaerospermum</i>	
<i>Scopulariopsis brevicaulis</i>	
<i>Scopulariopsis chartarum</i>	
<i>Stachybotrys chartarum</i>	
<i>Trichoderma viride</i> group	
<i>T. viride</i> , <i>T. atroviride</i> , <i>T. koningii</i>	
<i>Wallemia sebi</i>	

Methods for estimating the amplification factors and extrapolating spore or conidia sensitivities of the assays from the standard curves have also been described.²¹ All primer and probe sequences used in the assays as well as known species comprising the assay groups are at the web site: <http://www.epa.gov/nrlcwww/moldtech.htm>. Primers and probes were synthesized commercially (Applied Biosystems, Foster City, CA; Integrated DNA Technologies, Coralville, IA; Sigma Genosys, Woodlands, TX).

DNA Extractions and Quantitative Polymerase Chain Reaction Enumeration of Molds

Eighty-two molds selected because of their common environmental presence were assayed in each of the samples.¹⁵ Positive

control suspensions, containing approximately 10⁴ or 10⁵ spores or conidia/mL of each of the standard cultures, were prepared.²¹ Samples and positive control suspensions were extracted by a rapid bead-milling method.¹⁹ Briefly, 0.3 g of glass beads (G-1277; Sigma, St. Louis, MO) and 100 and 300 μ L of lysis and binding buffer, respectively, from an Elu-Quik DNA Purification Kit (Schleicher and Schuell, Keene, NH) with 10 μ L of a 2 \times 10⁸ conidia/mL reference suspension of *G. candidum* were added to the 2-mL tube containing the sampling brush. The tubes were shaken in a Mini Bead-Beater (Biospec Products, Bartlesville, OH) for 1 minute at a maximum speed and then centrifuged for 1 minute at 8,000 \times g to pellet the glass beads and debris. The supernatants were further purified using a DNeasy kit (Qiagen, Valencia, CA).

TABLE II.
Number of Cell Equivalents Detected in Control Subjects by Patient Identification Code for Each Detected Mold Species But Only for Samples in Which at Least One Mold Was Detected.

Fungal ID	81	82	85	86	87	29	43	44	49	50	51	52	53	55	56	60	65
<i>Aspergillus niger</i>																	
<i>Aspergillus penicillioides</i>			6	7	4												
<i>Eurotium (Asp.) amstelodami</i>	2																
<i>Aureobasidium pullulans</i>						10							3				10
<i>Penicillium crustosum</i> (group II)																	
<i>Stachybotrys chartarum</i>							1	1							1		
<i>Trichoderma viride/koningii</i>											15		2				
<i>Aspergillus ustus</i>																	
<i>Cladosporium cladosporioides-1</i>	1	1			1				1	1		1				1	
<i>Cladosporium cladosporioides-2</i>																	
<i>Cladosporium herbarum</i>				1													
<i>Epicoccum nigrum</i>	3														1		
<i>Mucor</i> group																	
<i>Aspergillus terreus</i>																	
<i>Penicillium expansum</i>																	

QPCR assays for target organism and *G. candidum* reference DNA in the extracts were prepared using a “Universal Master Mix” of PCR reagents (Applied Biosystems, Foster City, CA) and performed in an Applied Biosystems Prism model 7700 sequence detection instrument. Numbers of spores or conidia detected in mucous brush samples (N) were calculated using the equation: $\log_{10}(N) = (\Delta C_T - a)/b$, where ΔC_T was the difference in observed C_T values between the target and reference organisms ($C_{T,target} - C_{T,reference}$) for the respective mucous brush sample and “a” and “b” were the mean y-intercept and slope parameter values from the standard calibration curves for each target assay group. Parallel analyses of method negative control samples, containing AE buffer only, were performed at a frequency of approximately one per each six test samples analyzed. All positive results were confirmed by two additional replications.

RESULTS

Seventy-four subjects were enrolled in the study and had laboratory specimens processed for fungi detection. Sixty-five subjects filled out the SF-36 completely and 71 subjects filled out the SNOT-20 survey completely. Thirty-seven subjects were in the control group and 37 patients had CRS.

The laboratory tested for 36 fungal species initially and then expanded the range to investigate for 82 fungi. Of the initial 36 species, positive identification was achieved for 13 of the species sought. When the investigation was broadened to search for an additional 46 fungal species, only two new organisms were identified: *Aspergil-*

TABLE III.
Number of Cell Equivalents Detected in Patients With Chronic Rhinosinusitis by Patient Identification Code for Each Detected Mold Species But Only for Samples in Which at Least One Mold Was Detected.

Fungal ID	1	3	7	8	9	14	16	19	22	27	34	36	42	45	48	67	68
<i>Aspergillus niger</i>		1															
<i>Aspergillus penicillioides</i>												7					
<i>Eurotium (Asp.) amstelodami</i>												2			2		
<i>Aureobasidium pullulans</i>													4		2		43
<i>Penicillium crustosum</i> (group II)															1538		
<i>Stachybotrys chartarum</i>													1		1		
<i>Trichoderma viride/koningii</i>									8	4							
<i>Aspergillus ustus</i>				2		1											
<i>Cladosporium cladosporioides-1</i>	9	1			2		1				1					2	1
<i>Cladosporium cladosporioides-2</i>													1				
<i>Cladosporium herbarum</i>																254	1
<i>Epicoccum nigrum</i>		3						6							1		
<i>Mucor</i> group																	1
<i>Aspergillus terreus</i>			3														
<i>Penicillium expansum</i>									286								

TABLE IV.
Occurrence of Group I and II Molds in Patients With Chronic Rhinosinusitis (CRS) and Control Subjects.

Fungal ID	CRS Patients Occurrence	Control Subjects Occurrence
Group I		
<i>Aspergillus niger</i>	1	0
<i>Aspergillus penicillioides</i>	1	3
<i>Eurotium (Asp.) amstelodami</i>	2	1
<i>Aureobasidium pullulans</i>	3	3
<i>Penicillium crustosum</i> (group II)	1	0
<i>Stachybotrys chartarum</i>	2	3
<i>Trichoderma viride/koningii</i>	2	2
Group II		
<i>Aspergillus ustus</i>	2	0
<i>Cladosporium cladosporioides-1</i>	7	7
<i>Cladosporium cladosporioides-2</i>	1	0
<i>Cladosporium herbarum</i>	2	1
<i>Epicoccum nigrum</i>	3	2
<i>Mucor amphibiorum/. . group</i>	1	0
Unclassified		
<i>Penicillium expansum</i>	1	0
<i>Aspergillus terreus</i>	1	0

lus terreus and *Penicillium expansum*. The most common organism identified was *Cladosporium cladosporioides-1* with 14 individuals having evidence of this organism in their samples. There was no statistical difference between the presence of *Cladosporium cladosporioides-1* in the diseased and control groups with a Fisher's exact ratio of 1.000. The second most common organisms isolated were *Aureobasidium pullulans*, which was identified in six individuals, and *Epicoccum nigrum* and *Stachybotrys chartarum*, which were identified in five individuals each. Statistical significance was not achieved between diseased and control populations in this small group using Fisher's exact method to examine the distribution of these organisms. Tables II and III summarize the identified organisms found in this study in the control and CRS groups, respectively.

Fisher's exact method was used to crossreference the species of fungus isolated and determine whether the presence of any particular fungus could predict whether a subject was in the CRS or control group. This did not reveal statistical significance. Crossreferencing SNOT-20 and SF-36 scores with the presence or absence of fungal identification did not show a statistical trend between diseased and control populations using the Mann-Whitney method.

The yield of the QPCR method for detecting fungi in this population was 45.9% in both the CRS and the control groups. Seventeen of 37 patients in the diseased group had fungus identified in the laboratory for a recovery rate of 45.9% and 17 of the 37 subjects in the control group had fungi recovered for a yield of 45.9%.

There were 15 mold species found in these samples. Half of the control and half of the CRS patient samples had no detectable molds. Most species occurred at less

than 10 cells per sample in control subjects (Table II). In a few CRS cases, however, relatively large numbers of cells were detected (Table III). No individual species was significantly correlated with CRS. Therefore, molds were grouped as previously described (Table I).^{15,16}

Mold was detected 30 times in patients with CRS and 22 times in control subjects (Table IV). Seven were members of the group I molds associated with water damage and were found in both the patients with CRS and the control subjects. There were two species, *Aspergillus terreus* and *Penicillium expansum*, detected but these were not part of the group classification system. Positive results for any group I mold were seen in 12 of 37 samples from patients with CRS and 12 of 37 for controls. For group II species, the incidence was 16 of 37 in patients with CRS versus 10 of 37 for the control individuals. This gives a Fisher's exact test (one-tailed) *P* value of .24 when comparing group II molds in the patients with CRS with control subjects. No individual mold assay was able to discriminate between CRS and control patients any better than the presence/absence of any group 2 species. This was not statistically significant.

Statistically significant responses were found on the SNOT-20 survey when comparing the sinusitis and control groups using the Mann-Whitney calculation. Diseased patients reported worse symptoms with a mean score of 1.807 compared with controls responding with a mean score of 0.768 (*P* value of <.0001). The SF-36 results also showed statistically significant differences in responses with the overall score of the patients with CRS at a mean of 69.350 and the control group reporting a mean score of 80.373 (*P* = .0201). Certain dimensions of the SF-36 revealed a difference in scoring between the two groups with the sinusitis group reporting statistically significant impaired function in the categories of role limitation resulting from physical health or PH (*P* = .0236), social functioning or SF (*P* = .0360), and general health or GH (*P* = .0005).

DISCUSSION

This study was designed to test the yield of an endoscopically directed brush sampling technique of the middle meatus for the presence of fungus as identified with a highly accurate QPCR laboratory protocol. In addition, the study was designed to compare populations of patients with sinus disease with a cohort of subjects without sinusitis-related symptoms or signs of sinus disease. Comparison between these groups was accomplished using standardized outcomes surveys (SNOT-20 and SF-36) and also laboratory analysis for fungal DNA of the obtained mucous specimens.

Numerous studies now have been accomplished to describe the mycology of the human nose.^{10,12-14} The investigations can essentially be broken down into two types: those that use culture identification methods and those that use QPCR identification methods.^{10,12-14,22-35} Although the techniques used by two investigators processing specimens by a culture method reveal a yield of close to 100% in either diseased or control populations, others that have attempted to duplicate this yield have not been as successful.^{12,13,22,25-31,33,35}

Perhaps the most interesting comparison of patients using the culture method studied a cohort of newborn infants

and followed them over time to see if they would develop laboratory evidence of nasal fungal colonization.¹² In this study, most newborns did not have fungus in their nose on birth but developed fungal colonization over time without showing signs of clinical disease. It could be argued that the fungal laboratory culture technique leads to contamination and that this is why the yields approach 100% in some studies. Yet, the fact that the early newborn cultures did not become positive over time argues strongly against laboratory contamination as an explanation for near 100% yields. Rather, it may be the collection technique itself that leads to specimens being contaminated at the outset. The high-yield culture studies use a nasal irrigation technique to collect mucous.^{10,12} A small amount of saline is irrigated into the nose for several seconds and then collected for laboratory processing. This irrigation technique likely samples the nasal vestibule, lower nasal mucosal surfaces of the inferior turbinate, and perhaps the nasopharynx and olfactory cleft. It is rather indiscriminate as to the specific origin of the washing irrigant. This present study reveals a fungal yield of approximately 45%. Our yield is similar to other QPCR techniques reported in the past.^{13,14,26,32,33} However, the present study was specific to the mycology of the middle meatus because the samplings were obtained under direct endoscopic guidance. Two QPCR-based studies have shown higher fungal yields than revealed in this report, but one was obtained from surgical material and the other used an irrigation technique that was not specific to the middle meatus.^{31,35} Yet, when diseased and nondiseased patients are compared, no statistical difference is revealed between the presence or absence of fungus, the species of fungus, or the number of different fungi present.

Other studies have sought to reveal the mycology of the nose and have shown a similarly variable distribution of fungi as revealed in this report.^{12,28} The investigation by Lackner et al. revealed that approximately 20% of specimens taken from newborn infants grew fungi when sampled immediately after birth, but this rate dropped to approximately 15% on the next several days of life only to rise to the 90% range for a myriad of fungi at 2 to 5 months of life.¹² Jiang et al. noted a higher yield on lavage specimens when compared with a swab-sampling method using a culture technique, but was still only able to approach a 50% yield when using "Ponikau's method" compared with an 11.8% method using the swab.²⁸ A directed swab sampling method of infants reported by Hannula et al. found a 43% positive yield for fungi in the nasopharynx and oropharynx of 2- to 4-month-old infants.³⁶

Obviously, the technique chosen as a method to sample the nose is important with respect to the expected yield. There are, however, certain disadvantages to the culture method. The culture method requires a week's worth of specialized laboratory processing and therefore results are not immediately available. In addition, to reveal the specific identification of the fungi found, QPCR methods may be necessary to finalize the specific genus and species of the growing fungal colonies. QPCR techniques are extraordinarily accurate because the identification is based on specific DNA coding. Therefore, accurate identification is essentially instantaneous. QPCR also avoids prolonged laboratory culturing and has the

potential to reveal a quick result. Also, it does not appear that directed culture techniques can duplicate the yields found in irrigation methods of sampling as evidenced by our results. In fact, the directed culture techniques seem to have a yield lower than the QPCR-based methods that show a yield of approximately 40% to 60%.^{13,14,22,28} It is possible that the irrigation techniques are too indiscriminate to provide accurate information on the mycology of the middle meatus or of a specific nasal region.

This study was successful in revealing the mycology of a specific region of the nose in patients afflicted with sinusitis and in subjects without sinus complaints or evidence of sinus disease. The results are generally similar to other investigations in that a vast variety of organisms was identified. This vast and variable flora precluded identifying a statistically significant culprit or group of culprits underlying rhinosinusitis in the diseased population. Other studies have revealed a predominance of *Aspergillus* species^{25,30} or a general trend toward identifying *Penicillium* species.¹² Others have found *Alternaria*, *Penicillium*, *Aspergillus*, and *Cladosporium* species that seem to predominate.¹⁰ This study showed a predominance of *Cladosporium* species from specific middle meatus sampling. Other fungi, including *Aspergillus*, *Aureobasidium*, *Epicoccum*, and *Stachybotrys*, were also identified frequently. It is interesting that *Alternaria* species were not identified in this study because it is a prominent finding in other studies.^{10,30} No conclusions can be drawn from this flora distribution, but this study does reveal the mycology of a specific region of the nose in that the specimens were directly sampled from the middle meatus.

From a statistical perspective, it is a daunting task to attempt a study that will have the statistical power to definitively prove a relationship between a specific fungus and sinus disease, especially given the multifactorial causation of sinusitis and the ubiquitous nature of molds in the environment. On the other hand, neither has the converse been proven: neither is it certain that a single fungus or group of fungi is not particularly related to the presence of CRS.

Some investigators have attempted to narrow the statistical beta error by grouping fungi together when looking at causation. Vesper et al. have noted group I fungi related to water damage in homes to be associated with idiopathic pulmonary hemorrhage.¹⁵ By grouping fungi into these types of environmentally based classifications, the task of determining potential causation can be narrowed from a statistical perspective. In this study, 82 molds were screened using QPCR, but only 15 molds were identified and of these 15 molds, only 13 are part of group I or group II. Although not statistically proven, a trend observed in this study was a relation of the presence of group II molds in patients with sinus disease. It will be important to consider this type of classification in an attempt to determine the relationship of molds to sinusitis in a large study with enough statistical power to be confident of the conclusion.

Finally, the descriptive outcomes measures used in this study show that patients with sinus disease have more symptoms that are noted in the SNOT-20 survey and decreased general health as measured by the SF-36 survey

with certain domains being more affected than others. In general, the presence of fungus itself does not seem to correlate with the results of general or specific health outcomes surveys. In this study, the surveys validated the selection of patients for the CRS and control group populations.

CONCLUSION

The yield of endoscopically directed middle meatus brush sampling for QPCR identification of fungi is approximately 45% in patients with CRS and a nondiseased control population. No statistical difference can be ascribed to the presence or type of fungi to help differentiate between these two groups. Consideration of grouping fungi into categories may help to definitively determine whether or not specific molds are in fact related to sinus disease.

BIBLIOGRAPHY

1. Benson V, Marano M. *Current Estimates From the National Health Interview Survey, 1995*. Hyattsville, MD: National Center for Health Statistics, 1998, Series 10, No. 199.
2. Benninger MS, Ferguson BJ, Hadley JA, et al. Adult chronic rhinosinusitis: definitions, diagnosis, epidemiology, and pathophysiology. *Otolaryngol Head Neck Surg* 2003;129(suppl):S1–32.
3. Casiano RR. Correlation of clinical examination with computer tomography in paranasal sinus disease. *Am J Rhinol* 1997;11:193–196.
4. Gwaltney JM Jr, Phillips CD, Miller RD, Riker DK. Computed tomographic study of the common cold. *N Engl J Med* 1994;330:25–30.
5. Ponikau JU, Sherris DA, Weaver A, Kita H. Treatment of chronic rhinosinusitis with intranasal amphotericin B: a randomized, placebo-controlled, double-blind pilot trial. *J Allergy Clin Immunol* 2005;115:125–131.
6. Peterson KL, Wang M, Canalis RF, Abemayor E. Rhinocerebral mucormycosis: evolution of the disease and treatment options. *Laryngoscope* 1997;107:855–862.
7. Katzenstein AL, Sale SR, Greenberger PA. Pathologic findings in allergic aspergillus sinusitis. A newly recognized form of sinusitis. *Am J Surg Pathol* 1983;7:439–443.
8. Safirstein BH. Allergic bronchopulmonary aspergillosis with obstruction of the upper respiratory tract. *Chest* 1976;70:788–790.
9. Ferguson BJ. Eosinophilic mucin rhinosinusitis: a distinct clinicopathological entity. *Laryngoscope* 2000;110:799–813.
10. Ponikau JU, Sherris DA, Kern EB, et al. The diagnosis and incidence of allergic fungal sinusitis. *Mayo Clin Proc* 1999;74:877–884.
11. Ponikau JU. Methods and materials for treating and preventing inflammation of mucosal tissue. U.S. Patent Number 6,555,556. Issued April 29, 2003.
12. Lackner A, Stammberger H, Buzina W, et al. Fungi: a normal content of human nasal mucus. *Am J Rhinol* 2005;19:125–129.
13. Catten MD, Murr AH, Goldstein JA, Mhatre AN, Lalwani AK. Detection of fungi in the nasal mucosa using polymerase chain reaction. *Laryngoscope* 2001;111:399–403.
14. Scheuller MC, Murr AH, Goldberg AN, Mhatre AN, Lalwani AK. Quantitative analysis of fungal DNA in chronic rhinosinusitis. *Laryngoscope* 2004;114:467–471.
15. Vesper SJ, Varma M, Wymer LJ, Dearborn DG, Sobolewski J, Haugland RA. Quantitative PCR analysis of fungi in dust from homes of infants who developed idiopathic pulmonary hemorrhaging. *J Occup Environ Med* 2004;46:596–601.
16. Hodgson M, Scott R. Prevalence of fungi in carpet dust samples. In: Johannning E, ed. *Bioaerosols, Fungi and Mycotoxins: Health Effects Assessment, Prevention and Control*. New York: Occupational and Environment Health Center, 1999: 581–592.
17. Ware JE Jr, Snow KK, Kosinski M, Gandek B. *SF-36 Health Survey. Manual and Interpretation Guide*. Boston: The Health Institute, 1993.
18. Piccirillo JF, Merritt MG Jr, Richards ML. Psychometric and clinimetric validity of the 20-item Sino-nasal Outcome test (SNOT-20). *Otolaryngol Head Neck Surg* 2002;126:41–47.
19. Haugland RA, Brinkman NE, Vesper SJ. Evaluation of rapid DNA extraction methods for the quantitative detection of fungal cells using real time PCR analysis. *J Microbiol Methods* 2002;50:319–323.
20. Brinkman NE, Haugland RA, Wymer J, Byappanaahalli M, Whitman NRL, Vesper SJ. Evaluation of a rapid, quantitative real-time PCR method for cellular enumeration of pathogenic *Candida* species in water. *Appl Environ Microbiol* 2003;69:1775–1782.
21. Haugland RA, Varma M, Wymer LJ, Vesper SJ. Quantitative PCR of selected *Aspergillus*, *Penicillium* and *Paecilomyces* species. *Syst Appl Microbiol* 2004;27:198–210.
22. Araujo E, Palombini BC, Cantarelli V, Pereira A, Mariante A. Microbiology of middle meatus in chronic rhinosinusitis. *Am J Rhinol* 2003;17:9–15.
23. Taylor MJ, Ponikau JU, Sherris DA, et al. Detection of fungal organisms in eosinophilic mucin using a fluorescein-labeled chitin-specific binding protein. *Otolaryngol Head Neck Surg* 2002;127:377–383.
24. Braun H, Buzina W, Freudenschuss K, Beham A, Stammberger H 'Eosinophilic fungal rhinosinusitis': a common disorder in Europe? *Laryngoscope* 2003;113:264–269.
25. Vennewald I, Henker M, Klemm E, Seebacher C. Fungal colonization of the paranasal sinuses. *Mycoses* 1999;42(suppl 2):33–36.
26. Polzehl D, Weschta M, Podbielski A, Riechelmann H, Rimek D. Fungus culture and PCR in nasal lavage samples of patients with chronic rhinosinusitis. *J Med Microbiol* 2005;54:31–37.
27. Lebowitz RA, Waltzman MN, Jacobs JB, Pearlman A, Tierno PM. Isolation of fungi by standard laboratory methods in patients with chronic rhinosinusitis. *Laryngoscope* 2002;112:2189–2191.
28. Jiang RS, Su MC, Lin JF. Nasal mycology of chronic rhinosinusitis. *Am J Rhinol* 2005;19:131–133.
29. Wise SK, Kingdom TT, McKean L, DelGaudio JM, Venkatraman G. Presence of fungus in sinus cultures of cystic fibrosis patients. *Am J Rhinol* 2005;19:47–51.
30. Collins MM, Nair SB, Der-Haroutian V, et al. Effect of using multiple culture media for the diagnosis of noninvasive fungal sinusitis. *Am J Rhinol* 2005;19:41–45.
31. Gosepath J, Brieger J, Vlachtsis K, Mann WJ. Fungal DNA is present in tissue specimens of patients with chronic rhinosinusitis. *Am J Rhinol* 2004;18:9–13.
32. Willinger B, Obradovic A, Selitsch B, et al. Detection and identification of fungi from fungus balls of the maxillary sinus by molecular techniques. *J Clin Microbiol* 2003;41:581–585.
33. Hendolin PH, Paulin L, Koukila-Kahkola P, et al. Panfungal PCR and multiplex liquid hybridization for detection of fungi in tissue specimens. *J Clin Microbiol* 2000;38:4186–4192.
34. Perez-Jaffe LA, Lanza DC, Loevner LA, Kennedy DW, Montone KT. In situ hybridization for *Aspergillus* and *Penicillium* in allergic fungal sinusitis: a rapid means of speciating fungal pathogens in tissues. *Laryngoscope* 1997;107:233–240.
35. Kim ST, Choi JH, Jeon HG, et al. Comparison between polymerase chain reaction and fungal culture for the detection of fungi in patients with chronic sinusitis and normal controls. *Acta Otolaryngol* 2005;125:72–75.
36. Hannula J, Saarela M, Jousimies-Somer H, et al. Age-related acquisition of oral and nasopharyngeal yeast species and stability of colonization in young children. *Oral Microbiol Immunol* 1999:176–182.