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ORIGINAL ARTICLE

Comparison of mycology between different types of chronic rhinosinusitis

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Abstract

Background: The aim of this study was to culture fungi from the nasal discharge of patients with chronic rhinosinusitis (CRS) using both a traditional and Ponikau et al's method, and subsequently compare the culture results between CRS with nasal polyps (CRSwNPs) and without nasal polyps (CRSsNPs), and between eosinophilic and noneosinophilic CRS.

Methods: Eighty-one CRS patients with CRS who underwent functional endoscopic sinus surgery were enrolled. Before surgery, the severity of each patient's CRS was evaluated through an endoscopic examination and CT scan. Swab samples were collected from the middle meatus for traditional fungal cultures using cotton-tipped sticks. Afterward, the ipsilateral nasal cavity was irrigated, with the irrigated fluid processed using Ponikau et al's method for fungal culture.

Results: The endoscopic and CT scores were significantly higher in CRSwNPs than CRSsNPs, but were not different between eosinophilic CRS and noneosinophilic CRS. Using Ponikau et al's method, 61/81 (75.3%) of the specimens grew fungi. Among them, 20 of 32 (62.5%) CRSwNPs specimens and 41 of 49 (83.7%) CRSsNPs specimens grew fungi. For eosinophilic CRS specimens, 35 of 46 (76.1%) grew fungi, and 26 of 35 (74.3%) noneosinophilic CRS specimens grew fungi. The fungal culture rate was borderline significantly higher in CRSsNPs than CRSwNPs ($p = 0.058$) but was not significantly different between eosinophilic CRS and noneosinophilic CRS ($p = 1$). However, *Cladosporium* was significantly more common in CRSsNPs than CRSwNPs ($p = 0.048$).

Conclusion: Our results showed that the mycology of CRS was different between CRSwNPs and CRSsNPs.

Keywords: Chronic rhinosinusitis; Eosinophilic; Fungal culture; Mycology; Nasal polyps

1. INTRODUCTION

Chronic rhinosinusitis (CRS) is a chronic inflammatory disorder of the sinonasal mucosa.[1] CRS has been phenotypically divided into CRS with nasal polyps (CRSwNPs) and without nasal polyps (CRSsNPs), and endotypically classified as either eosinophilic or noneosinophilic CRS.[2] Phenotypically, CRS is divided based on the presence or absence of nasal polyps. Eosinophilic CRS is a subtype of recalcitrant CRS and generally has a tissue eosinophil >10 cells per high power field with worse disease severity and poorer treatment outcomes than noneosinophilic CRS.[3] However, high tissue eosinophilia has been observed in nonpolypoid sinonasal mucosa and has been shown in up to 27.5% of CRSsNPs.[3]

It has been considered that bacteria have important roles in the pathogenesis of CRS.[4,5] The biofilm formation, superantigen secretion, and dysbiosis of the nasal microbiota may all result in the occurrence of CRS.[6,7] However, over the last 20 years, fungi have also been proposed to cause CRS by dysregulating immune response, inducing breakdown of epithelial membrane, and exacerbating local inflammation of sinonasal mucosa.[8–10]

When bacteria are easily cultured from nasal discharge through traditional laboratory methods, it is difficult to culture fungi from nasal secretions using standardized laboratory techniques.[8] In 1999, Ponikau et al reported another method to culture the nasal irrigants of 210 CRS patients and found a positive fungal culture rate of 96%.[11] In this study, we attempted to perform fungal cultures from the nasal discharge of CRS patients using both traditional and Ponikau et al's methods, and subsequently compare the culture results between CRSwNPs and CRSsNPs, and between eosinophilic and noneosinophilic CRS.

2. METHODS

2.1. Patients

CRS patients who had failed medical treatment, and subsequently received bilateral primary functional endoscopic sinus surgery, were included in the study between August 2018 and May 2021. CRS was diagnosed according to the EPOS criteria based upon the history, endoscopic examination, and CT

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scan.[12] The duration of CRS symptoms was longer than 12 weeks, and both endoscopic and CT examinations showed evidence of sinonasal inflammation. Those with an age below 20 years, and a history of immunodeficiency or antibiotic treatment within a week before surgery were excluded. We also excluded those diagnosed pathologically as a sinonasal tumor or fungal sinusitis. This study was approved by the Institutional Review Board (I) of Taichung Veterans General Hospital (protocol code CF17328B). Written consent was obtained from each patient.

2.2. Classification of CRS and evaluation of its severity

CRS was classified into either CRSwNPs or CRSsNPs based on intraoperative endoscopic findings, and was divided into eosinophilic CRS if surgical specimens revealed eosinophils at a ≥10/high power field and noneosinophilic CRS if tissue eosinophils had a <10/high power field.

The preoperative severity of CRS was evaluated through an endoscopic examination and sinus CT scan. Endoscopic appearances were scored on a 0-2-point scale according to the Lund-Kennedy staging system.[13] The endoscopic appearances were categorized into polyps (0: no polyps; 1: polyps present within the middle meatus; 2: polyps beyond the middle meatus); nasal secretion (0: no secretion; 1: clear, thin secretion; 2: thick, purulent secretion); and mucosal edema (0: no edema; 1: edematous mucosa; 2: polypoid mucosa). The total endoscopic score of the studied side of the nasal cavity was the sum of all the scores (range 0–6). The preoperative sinus CT scan was quantified using the Lund-Mackay staging system.[14] Five ipsilateral sinuses, the maxillary, anterior ethmoid, posterior ethmoid, sphenoid and frontal sinuses, were individually scored on a 0-2-point scale (0: clear sinus; 1: partial opacification; 2: total opacification). The ipsilateral ostiomeatal complex was graded as either 0 (not obstructed) or 2 (obstructed). The total CT score was the sum of all the scores (range 0–12).

2.3. Traditional fungal culture

At the outpatient clinic on the day before surgery, the traditional fungal culture was performed using a cotton-tipped stick. The stick was placed into the middle meatus, with more severe disease being determined by the preoperative sinus CT scan to collect a swab specimen. The stick was then placed in a glass tube and transferred to the clinical microbiology laboratory. In the laboratory, the stick was removed and brushed a Sabouraud dextrose agar plate and a Sabouraud dextrose agar plate which contained chloramphenicol and cycloheximide. The plates were incubated at 30°C for 30 days. They were examined every day, and all isolates were identified.

2.4. Fungal culture using Ponikau et al's method

After taking a swab specimen for a traditional fungal culture, the patients were asked to breathe deeply inward and hold. The ipsilateral nasal cavity was then irrigated with 20 mL of sterile water using a syringe. The irrigated fluid forcefully exhaled into a sterile pan. The fluid in the pan was poured into a centrifuge tube and transferred to the mycology laboratory. Under a laminar flow hood, the fluid in the centrifuge tube was mixed with an equal volume of diluted dithiothreitol (1.055 mg/mL) and vortexed for 30 seconds. The tube was placed at room temperature for 15 minutes to wait the dithiothreitol to break apart the disulfide bonds to liquefy the mucus, and was centrifuged again at 3000*g* for 10 minutes. The supernatant in the tube was thrown away and the sediment was vortexed for 30 seconds. The sediment was then inoculated onto a Sabouraud dextrose agar plate and a Sabouraud dextrose agar plate which contained chloramphenicol and cycloheximide. The plates were incubated at 30°C for 30 days. They were examined every day, and all isolates were identified using micromorphological characteristics under a microscopy.

2.5. Statistical analyses

All data are presented as mean ± standard deviation. The gender of the patients, and fungal culture rates were compared between CRSwNPs and CRSsNPs, and also between eosinophilic CRS and noneosinophilic CRS using the Pearson Chi-square test. The age of the patients, total endoscopic scores, MCA_2, saccharin transit time, and total computed tomography scores were compared between CRSwNPs and CRSsNPs, and also between eosinophilic CRS and noneosinophilic CRS using the Mann-Whitney U test. SPSS version 17.0 (SPSS Inc., Chicago, IL, USA) was used to analyze all data.

3. RESULTS

Eighty-one CRS patients were included in this study. There were 32 women and 49 men. The age ranged from 21 to 81 years with a mean of 47.9 years. Amongst the studied sides of nasal cavities, nasal polyps were present in 32 sides, while another 49 presented none. The gender and age were not different between patients with CRSwNPs and those with CRSsNPs (Table 1). Surgical specimens from 46 sides of nasal cavities revealed eosinophilic CRS, while noneosinophilic CRS was classified in the remaining 35 specimens. The gender was not different between the eosinophilic CRS and noneosinophilic CRS patients, however the noneosinophilic CRS patients were older than those diagnosed with eosinophilic CRS (Table 1).

The disease severity of CRS is shown in Table 1. The endoscopic and CT scores were significantly higher in CRSwNPs

Table 1

Comparison of clinical features and microbiologies between CRSwNPs and CRSsNPs, and between eosinophilic and noneosinophilic CRS

	CRSwNPs (32)[a]	CRSsNPs (49)	*p*	Eosinophilic (46)	Noneosinophilic (35)	*p*
Age, y	50.3 ± 12.68[b]	46.4 ± 11.74	0.139	45.5 ± 10.52	51.1 ± 13.6	0.006
Endoscopic score	3.6 ± 1.0	2.4 ± 0.81	<0.001	2.8 ± 0.94	3.0 ± 1.22	0.36
CT score	7.5 ± 1.76	5.98 ± 1.70	<0.001	6.99 ± 1.68	6.0 ± 2.07	0.073
Culture rate						
Traditional[c]	6.3%	2%	0.705	4.3%	2%	1
Ponikau[d]	62.5%	83.7%	0.058	76.1%	74.3%	1

CRS = chronic rhinosinusitis; CRSwNPs = chronic rhinosinusitis with nasal polyps; CRSsNPs = chronic rhinosinusitis without nasal polyps

[a]Number of patients.

[b]Mean ± standard deviation.

[c]Traditional fungal culture method.

[d]Ponikau et al's method.

than CRSsNPs, but were not different between eosinophilic CRS and noneosinophilic CRS. Overall, the disease was more severe in patients with CRSwNPs than those with CRSsNPs, but was not significantly different between eosinophilic CRS and noneosinophilic CRS.

The results of fungal cultures are shown in Tables 2 and 3. Using the traditional culture method, only 3 of the 81 specimens grew fungi. In contrast, using Ponikau et al's method, 61 of 81 (75.3%) of the specimens grew fungi. *Cladosporium* and *Penicillium* species were the most common types of fungi. Among them, 20 of 32 (62.5%) CRSwNPs specimens and 41 of 49 (83.7%) CRSsNPs specimens grew fungi. The fungal culture rate was borderline significantly higher in CRSsNPs than CRSwNPs ($p = 0.058$) (Table 1).

The comparison of the mycology between CRSwNPs and CRSsNPs is shown on Table 2. *Penicillium* species was the most common fungus in CRSwNPs, but *Cladosporium* species was the most common fungus in CRSsNPs. *Cladosporium* species was significantly more common in CRSsNPs than in CRSwNPs ($p = 0.048$).

For eosinophilic CRS specimens, 35 of 46 (76.1%) grew fungi, and 26 of 35 (74.3%) noneosinophilic CRS specimens grew fungi. The fungal culture rates were not significantly different between eosinophilic CRS and noneosinophilic CRS ($p = 1$) (Table 1). *Cladosporium* species were the most common fungus in both eosinophilic CRS and noneosinophilic CRS, but *Penicillium* species were more common in eosinophilic CRS than noneosinophilic CRS ($p = 0.309$) (Table 3).

Table 2

Mycology of CRSwNPs and CRSsNPs

	CRSwNPs (32)[a]		CRSsNPs (49)	
Species	**Traditional[b]**	**Ponikau[c]**	**Traditional**	**Ponikau**
	No. of Isolates			
Cladosporium species		5	1	19
Penicillium species		7		10
Aspergillus flavus		1		2
Aspergillus fumigatus				1
Aspergillus niger		2		3
Aspergillus sydowii				2
Aspergillus terreus				1
Aspergillus versicolor		1		
Candida albicans	1	2		3
Candida guilliermodii				2
Candida parapsilosis				4
Alternaria species				2
Chaetomium species		1		1
Conidiobolus species				1
Cryptococcus species				1
Cunninghamella species	1			
Curvularia species				2
Dematiaceous mold				1
Fusarium species				2
Geotrichum species		2		2
Mucor species		1		1
Rhodotorula species				1
Unidentified mold		4		4
Total fungal isolates	2	26	1	65

CRSwNPs = chronic rhinosinusitis with nasal polyps; CRSsNPs = chronic rhinosinusitis without nasal polyps.
[a]Number of specimens.
[b]Traditional fungal culture method.
[c]Ponikau et al's method.

Table 3

Mycology of eosinophilic and noneosinophilic CRS

	Eosinophilic (46)[a]		Noneosinophilic (35)	
Species	**Traditional[b]**	**Ponikau[c]**	**Traditional**	**Ponikau**
			No. of Isolates	
Cladosporium species		13	1	11
Penicillium species		12		5
Aspergillus flavus		1		2
Aspergillus fumigatus				1
Aspergillus niger		3		2
Aspergillus sydowii				2
Aspergillus terreus		1		
Aspergillus versicolor				1
Candida albicans	1	2		3
Candida guilliermodii		2		
Candida parapsilosis		3		1
Alternaria species		1		1
Mucor species				2
Geotrichum species		1		3
Chaetomium species		1		1
Conidiobolus species				1
Cryptococcus species		1		
Cunninghamella species	1			
Curvularia species		1		1
Dematiaceous mold				1
Fusarium species				2
Rhodotorula species		1		
Unidentified mold		7		1
Total fungal isolates	2	50	1	41

CRS = chronic rhinosinusitis.
[a]Number of specimens.
[b]Traditional fungal culture method.
[c]Ponikau et al's method.

4. DISCUSSION

Although the etiology of CRS has been considered to be multifactorial,[15] the role of bacterial infection or colonization has been assumed to be important in the pathogenesis of CRS.[4,5] Fungus has also been suggested as an etiological factor of CRS.[16] Environmental fungi have been linked to the TH2 cell–related airway inflammation in CRS.[17] It has been found that peripheral blood mononuclear cells and T-lymphocytes from CRS patients produce cytokines interleukin (IL)-5 and IL-13 which recruit and activate eosinophils when presented with certain fungal antigen.[18] One the other hand, in a pilot study challenging ex vivo nasal polyp tissue with fungi, *Aspergillus niger* stimulation increased proinflammatory cytokines while suppressing levels of the main remodeling cytokine TGF-β1, but stimulation with *Cladosporium sphaerospermum*, *Alternaria alternata*, and *Penicillium notatum* reduced proinflammatory cytokines while inducing an increase in remodeling cytokines.[17]

In this study, only 3 of 81 specimens grew fungi using the traditional culture method, but 61 of 81 (75.3%) of the specimens grew fungi using Ponikau et al's method. These results are similar to both our previous study and Ponikau et al's first one.[11,19] However, the fungal culture result has not ever been compared between CRSwNPs and CRSsNPs, or between eosinophilic CRS and noneosinophilic CRS. Our results show that the fungal culture rate was borderline significantly higher in CRSsNPs than CRSwNPs ($p = 0.058$) but was not significantly different between eosinophilic CRS and noneosinophilic CRS ($p = 1$).

Regarding the mycology, Ponikau et al's first study found that *Alternaria*, *Penicillium*, *Cladosporium*, *Aspergillus*, and *Candida* were the most common cultured fungi found in the

nasal discharge of CRS patients.[11] Within Europe, the most common cultured fungi were *Aspergillus*, *Penicillium*, *Cladosporium*, and *Candida*.[20] In our previous study, *Candida*, *Aspergillus*, *Penicillium*, and *Cladosporium* were the most common cultured fungi.[19] In this present work, the most common cultured fungi were *Cladosporium*, *Penicillium*, *Aspergillus*, *Cladosporium*, and *Candida*. *Alternaria* has been proposed to play a role in the pathogenesis of CRS,[8] but was not common in the nasal discharge of CRS patients in Europe and Asia.

When the mycology has not been compared between CRSwNPs and CRSsNPs, or between eosinophilic CRS and noneosinophilic CRS in the literature, it was found in this study that *Cladosporium* was significantly more common in CRSsNPs than in CRSwNPs. In contrast, the culture rate of *Cladosporium* was not different between eosinophilic CRS and noneosinophilic CRS. Whether fungi play different roles in the pathogenesis of different types of CRS requires further investigation.

It has been controversial about whether fungi identified in sinonasal cultures are pathogenic. Topical antifungal therapy such as amphotericin B irrigation has been used to treat CRS. Although some studies show benefit from this irrigation, others refute the efficacy.[21] However, the efficacy of antifungal therapy in different types of CRS has never been assessed in a clinical trial.

In conclusion, our results of fungal culture using Ponikau et al's method were compared between CRSwNPs and CRSsNPs and between eosinophilic CRS and noneosinophilic CRS. The fungal culture rate was borderline significantly higher in CRSsNPs than in CRSwNPs ($p = 0.058$) but was not significantly different between eosinophilic CRS and noneosinophilic CRS ($p = 1$). However, *Cladosporium* was significantly more common in CRSsNPs than in CRSwNPs ($p = 0.048$). In contrast, the culture rate of *Cladosporium* was not different between eosinophilic CRS and noneosinophilic CRS. Whether fungi play different roles in the pathogenesis of different types of CRS requires further investigation.

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