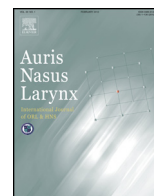




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Delayed type of allergic skin reaction to *Candida albicans* in eosinophilic rhinosinusitis cases

Nozomu Wakayama*, Shoji Matsune, Kimihiro Okubo

Department of Otolaryngology, Nippon Medical School Musashikosugi Hospital, 1-396, Kosugimachi, Nakahara-ku Kawasaki-shi, Kanagawa Prefecture 211-0063, Japan

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ABSTRACT

Objective: Eosinophilic chronic rhinosinusitis (ECRS) is frequently complicated by asthma, and recognized as refractory and persistent rhinosinusitis. However, the detailed pathophysiology of ECRS has not been elucidated yet. In this study, we investigated the association between recurrent ECRS and intradermal testing to multi-antigens including *Candida albicans*.

Methods: The subjects were 49 cases of bilateral chronic rhinosinusitis including 24 ECRS cases. They underwent endoscopic sinus surgery and submitted to pathological examination. Prior to surgery, peripheral blood eosinophil count, total and antigen-specific IgE levels (11 categories), and intradermal tests (5 categories) were carried out in all patients. These patients were followed-up for longer than 3 months. We compared the results of preoperative and postoperative clinical examination data between ECRS and non-ECRS (NECRS) cases.

Results: Positive reaction of the delayed type of intradermal testing to *C. albicans* was significantly more often observed in ECRS than NECRS cases. ($P < 0.01$) Additionally, these positive reaction cases exhibited significantly higher recurrence of nasal polyps and symptoms of ECRS ($P < 0.05$).

Conclusion: These results suggest the involvement of (Coombs) type IV allergic reaction to *C. albicans* in the pathophysiology of ECRS.

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1. Introduction

Chronic rhinosinusitis with nasal polyps (CRS wNP) [1,2] and the marked infiltration of eosinophils in the paranasal mucosa is termed eosinophilic chronic rhinosinusitis (ECRS) [3–8]. It is frequently complicated by asthma, and recognized as refractory rhinosinusitis that may recur. Recently, diagnostic criteria were proposed through the JESREC study [4], but the detailed mechanism remains to be clarified. Currently, only the systemic administration of oral steroids is effective. For treatment, endoscopic sinus surgery (ESS) and postoperative oral steroid therapy are performed.

Nonatopic eosinophilic inflammation of the airway may be involved in the pathogenesis of ECRS [5,7]. Concerning the detailed mechanism, the involvement of arachidonic acid metabolism disorder [8], *Staphylococcus aureus* enterotoxin (SAE) superantigen [9], and fungus [10–13] have been reported. These factors may influence the innate/acquired immune systems, causing eosinophilic inflammation and refractory recurrent conditions.

Asthma, which is a frequent complication, has been reported to be related to *Candida albicans* [14–16]. It has been reported that *C. albicans* stimulates the production of IL-5 by helper T-cells (Th-cells) of asthmatic patients, inducing eosinophilic inflammation [17], and that eosinophilic inflammation and allergic airway inflammation are exacerbated by the proliferation of fungi in the intestinal flora, particularly, *C. albicans*.

* Corresponding author.

E-mail address: waka84@nms.ac.jp (N. Wakayama).

[18]. The proliferation of *C. albicans* in the intestinal flora may exacerbate allergy through an increase in the blood prostaglandin E2 level, and increase the level of macrophage migration-inhibiting factor (MIF), which induces delayed allergic responses, through the activation of macrophages (an increase in the number of M2 macrophages) [18–24].

In this study, we investigated the association between recurrent ECRS diagnosed based on the diagnostic criteria established in the JESREC study and (immediate, delayed) intradermal test responses to *C. albicans*. We performed this study to consider delayed hypersensitivity reaction-enhancing effects of *C. albicans* in patients with ECRS.

2. Subjects and methods

Of patients who underwent ESS in the Department of Otorhinolaryngology, Nippon Medical School Musashi Kosugi Hospital between August 2012 and July 2015, the subjects were 49 with bilateral chronic rhinosinusitis, excluding those with unilateral rhinosinusitis, inflammation of the maxillary sinus alone, such as odontogenic maxillary sinusitis, a single paranasal lesion, cystic diseases, choanal polyps, or fungal sinusitis and those who had received steroids before surgery.

Allergic fungal rhinosinusitis (AFRS) cases were not include in this study [25].

1) Pathological diagnosis, definitive diagnosis of ECRS

A specimen of the ethmoid sinus mucosa was collected during surgery, and submitted for pathological examination. According to the criteria established in the JESREC study, patients with a tissue eosinophil count of ≥ 70 at a 400-fold magnification were definitively diagnosed with ECRS [4]. Based on the results of pathological examination, ECRS was differentiated from non-ECRS (NECRS). The background of ECRS and NECRS patients ($n = 24$ and 25 , respectively) is shown in Table 1. There were no differences in the age or sex between the two groups.

2) Preoperative examination items

Prior to surgery, the following items were investigated in all patients:

1. Peripheral blood eosinophil count, total IgE level, and antigen-specific IgE level

The method of the IgE test were used an Oriton IgE kit[®] (Nippon Chemiphar, JAPAN). Examination items of antigen-specific IgE were House dust, *Dermatophagoides pteronyssinus*, Cedar, white cedar, alder, *Ambrosia*, *Dactylis glomerata*, cat and dog skin, *C. albicans*, and *Aspergillus*.

2. Nasal discharge eosinophil count

Nasal discharge samples were taken from the middle nasal meatus by cotton swab. Eosinophil in nasal discharge were checked by smear stained by Wright's stain solution and Giemsa's stain solution (Muto Pure Chemicals Co., Ltd. Japan).

3. Intradermal test

The method of the intradermal test is as follows. Examination items were House dust, Cedar, *C. albicans*, *Alternaria*, *Aspergillus* and Control which was negative control (Torii Pharmaceutical Co., Ltd. Japan). The control consisted water solution including 0.9%NaCl and 0.5% phenol. The other allergens diluted 10,000 times. The items were subcutaneously injected 0.02 ml on the anterior surface of the forearm. Immediate reactions were evaluated 15 min after intradermal test, and delayed reactions after 48 h. Patients with a flare diameter of ≥ 20 mm or an urticarial lesion diameter of ≥ 9 mm were regarded as showing positive reactions [26].

4. Culture tests

In 19 of the 49 subjects, culture tests of maxillary/ethmoid sinus fluid and mucosa with inflammation were conducted. Culture test was performed more than a week to detect fungi by using these mediums, Pourmedia Sheep Blood Agar M58, Pourmedia Modified DRIGALSKI Aga, Candia GS Agar, Sabouraud Dextrose Agar (Eiken Chemical Co. Ltd. Japan).

For all cases of 49, pathological examination was conducted to detect fungi as well.

3) Postoperative evaluation of recurrence

Regular examinations were conducted at the outpatient clinic of our hospital for more than 3 months after surgery. Refractory rhinosinusitis induces topical mucosal thickening even after ESS, leading to recurrent polyps. In patients with refractory ECRS, polyps may recur after ESS, transiently subsiding through the use of oral/nasal steroids. However, the discontinuation of steroid therapy may result in recurrence, requiring additional steroid therapy or making withdrawal from steroids difficult in some patients. Therefore, patients who received the systemic administration of steroids during postoperative follow-up were regarded as showing an unfavorable course.

4) Comparison and analysis

We compared the results of preoperative examinations and postoperative follow-up between the ECRS and NECRS groups. For statistical analysis, the Mann–Whitney U-test, Fisher's exact probability, and Yates' chi square test were used.

Prior to this study, its protocol was approved by the Ethics Review Board of Nippon Medical School Musashi Kosugi Hospital (Permission number: 252-25-20). Furthermore, UMIN registration was conducted.

Table 1

Classification of ECRS and NECRS based on the results of pathological (N=49).

	Number of patients	Age	Bronchial asthma	Aspirin-induced asthma	Sex (M:F)
ECRS	24	49.3 years (71-28)	9	1	13:11
NECRS	25	49.5 years (78-18)	2	0	18:7

ECRS = eosinophilic rhinosinusitis, NECRS = non-eosinophilic rhinosinusitis.

Table 2

Comparison of peripheral blood eosinophil counts, total IgE level and antigen-specific IgE score between the ECRS and NECRS groups.

	Eosinophil counts (%)	Total IgE level (IU/ml)	Antigen-specific IgE level			
			<i>Dermatophagoides pteronyssinus</i> (IU/ml)	Cedar (IU/ml)	<i>Candida</i> (IU/ml)	<i>Aspergillus</i> (IU/ml)
ECRS	7.7 (±3.51)	179.46 (±240.24)	2.78 (±6.63)	5.92 (±9.30)	0.21 (±0.55)	0.21 (±0.55)
NECRS	4.3 (±3.56)	189.59 (±223.45)	1.95 (±6.51)	5.35 (±12.23)	0.03 (±0.14)	0.35 (±1.60)
P Value	P<0.01	P=0.35	P=0.15	P=0.24	P=0.22	P=0.31

There was a significant difference in the peripheral blood eosinophil count between the ECRS and NECRS groups.

There were no significant differences in serum total/antigen-specific IgE levels between the ECRS and NECRS groups.

Examination items of antigen-specific IgE were House dust, *Dermatophagoides pteronyssinus*, Cedar, white cedar, alder, *Ambrosia*, *Dactylis glomerata*, cat and dog skin, *Candida albicans*, and *Aspergillus*.

ECRS = eosinophilic rhinosinusitis NECRS = non-eosinophilic rhinosinusitis.

Mann–Whitney U-test.

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3. Results

1. There was a significant difference in the peripheral blood eosinophil count between the ECRS and NECRS groups (one-tailed, $P < 0.01$; $P = 0.0013$) (Table 2).

2. There were no significant differences in the nasal discharge eosinophil count (Table 3) and serum total/antigen-specific IgE levels (Table 2).

3. An allergic antigen intradermal test demonstrated positive delayed reactions to *C. albicans* in 67% of the patients in the ECRS group. There was a significant difference between the ECRS and NECRS groups (one-tailed, $P < 0.01$; $P = 0.00011$). There was no significant difference in any other antigen intradermal test (Table 4).

4. Evaluation was performed 3 months after surgery. In the ECRS group definitively diagnosed based on the results of pathological examination, recurrence were noted in 15 of the 24 patients; the results were significantly poorer than in the NECRS group (one-tailed, $P < 0.01$; $P = 0.00007$) (Table 5).

In the ECRS group, delayed intradermal reactions to *C. albicans* were detected in 13 of the 15 patients with an unfavorable course, showing a significant difference (one-tailed, $P < 0.05$; $P = 0.0215$) (Table 6).

5. In 19 of the 49 subjects, culture tests of maxillary/ethmoid sinus fluid and mucosa with inflammation were conducted. However, these patients were negative for *C. albicans*. It was impossible to identify *C. albicans* in topical areas on histopathological examination in any of the patients.

Table 3

Number of nasal discharge eosinophil-positive patients.

Eosinophils in nasal discharge	Positive	Negative
ECRS	8	9
NECRS	7	9

ECRS = eosinophilic rhinosinusitis, NECRS = non-eosinophilic rhinosinusitis.

There were no significant differences in the nasal discharge eosinophil count.

Yates 2×2 Chi square test.

4. Discussion

ECRS is a type of CRSwNP characterized by multiple bilateral nasal polyps, and marked infiltration of eosinophils in the paranasal mucosa. This type of rhinosinusitis is more refractory than NECRS with less marked eosinophil infiltration, and may more frequently recur [5]. The incidence of asthma is high, and oral steroids are effective, but the pathogenesis remains to be clarified. In Japan, the JESREC study [4] was published in 2014, and diagnostic criteria for ECRS were established. In this study, the rate of peripheral blood eosinophils in patients with ECRS was significantly higher than in those with NECRS. This was similar to the results of previous studies, including the JESREC study [3–7]. In addition, the postoperative course was unfavorable in the ECRS group, and the number of patients requiring oral steroids was significantly greater. This was also consistent with the results of previous studies [3–7]. In this study, various allergic antigen tests were performed. In the ECRS group, the proportion of patients with delayed intradermal reactions to *C. albicans* was significantly larger. In particular, compare with non-responders belonging to this group, those responders exhibited unfavorable course. These results suggest the involvement of delayed allergic reactions to *C. albicans* in refractory ECRS.

According to previous studies, fungal infection/sensitization in the topical nasal and paranasal mucosa can be ruled out as a mechanism involved in ECRS [8,27]. In this study, there was no topical involvement of *C. albicans* in any patient based on histopathological findings. In 19 subjects, culture tests of maxillary/ethmoid sinus fluid and mucosa with inflammation were conducted, but *C. albicans* was not identified in any patient. Briefly, the pathogenesis of ECRS differs from that of fungal allergy in the topical areas of the paranasal mucosa, that is, allergic fungal rhinosinusitis (AFRS), or fungal infection [25].

In ECRS, positive delayed type reactions to *C. albicans* is caused by type IV allergic reaction to *C. albicans*, this reaction differs from that of AFRS which is caused by type I/III allergic reaction to fungi. This shows that ECRS is be entirely distinct from AFRS.

But, *C. albicans* exists in the skin, digestive tract, and nasal cavity as normal bacterial flora. According to Hirotsu's study,

Table 4
Antigen intracutaneous reaction test.

Preoperative assessment	Intracutaneous result	HD		Cedar		Candida		Alternaria		Aspergillus	
		15 min	48 h	15 min	48 h	15 min	48 h	15 min	48 h	15 min	48 h
ECRS		5	0	8	0	5	16	2	1	2	0
	–	19	24	16	24	19	8	22	23	22	24
	Positive rate	21%	0%	33%	0%	20%	67%	8%	4%	8%	0%
NECRS		9	1	5	1	4	3	0	0	2	0
	–	16	24	20	24	21	22	25	25	23	25
	Positive rate	36%	4%	20%	4%	16%	12%	0%	0%	8%	0%
P Value		P = 0.345	P = 1.000	P = 0.345	P = 1.000	P = 0.946	P < 0.01*	P = 0.235	P = 0.490	P = 1.000	–

ECRS = eosinophilic rhinosinusitis, NECRS = non-eosinophilic rhinosinusitis, HD = house dust. Yates 2 × 2 Chi square test or Fisher exact test.

* P < 0.01.

Table 5
Postoperative course.

Postoperative course	Favorable course	Unfavorable course
ECRS	9	15
NECRS	23	2

ECRS = eosinophilic rhinosinusitis, NECRS = non-eosinophilic rhinosinusitis. The ECRS group significantly poorer than the NECRS group (P < 0.01). Fisher exact test.

fungal were detected in nasal polyp cells of ECRS by PCR (polymerase chain reaction) [12]. Former studies including Hirotsu's one [12,13] reported that immunological steps with fungal antigen is important and involved in pathology of ECRS. As ECRS is considered to be eosinophilic inflammation related to immunological regulatory failure [8], *C. albicans* and component of *Candida* may be involved in immunoregulation.

Previous studies indicated the association between *C. albicans* and nonatopic adult-onset asthma [14–16]. Mori et al. reported that the stimulation of monocytes from nonatopic asthma patients with *C. albicans* activated CD4-positive Th cells, leading to IL-5 production. This suggests that IL-5 causes eosinophilic inflammation of the tracheal mucosa [17]. In particular, refractory ECRS is complicated by nonatopic asthma in many cases. Both ECRS and asthma may be associated with eosinophilic inflammation of the paranasal/tracheal mucosa, respectively; their pathogenesis may be similar. Actually, Okano et al. [13] and Hirotsu et al. [12] reported that the stimulation of fungal extracts which also including *C. albicans* leads to IL-5 production from nasal polyp cells. Therefore, *C. albicans* may induce IL-5 secretion by activating Th cells even in patients with ECRS, causing eosinophilic inflammation,

as reported by Mori et al. However, among Th cells, which secrete IL-5, Th2 cells are dominant [28], and it is unclear whether Th1 cells, which play a central role in the pathogenesis of type IV allergy, are similarly activated. In the future, whether stimulation with *C. albicans* activates Th1 cells should be examined. Also, further studies are needed in order to clarify the factors that only *C. albicans* involved in delayed allergic reaction unlike other fungal (*Alternaria* and *Aspergillus*) in case of ECRS.

Recently, a study reported that the proliferation of fungus, as a type of enteric bacteria, especially *C. albicans*, exacerbated eosinophilic inflammation or allergic airway inflammation [18]. Other studies indicated that the proliferation of *C. albicans* in the intestinal flora promoted the exacerbation of allergy and activation of M2 macrophages through an increase in the blood prostaglandin E2 level [21–23], suggesting that activated macrophages secrete macrophage migration-inhibiting factor (MIF). MIF is a humoral factor involved in delayed allergic reactions [29]. Therefore, the intestinal proliferation of *C. albicans* may induce asthma development/exacerbation, the deterioration of eosinophilic inflammation in the presence of rhinosinusitis, and activation of M2 macrophages through an increase in the prostaglandin E2 level in patients with refractory conditions, enhancing MIF and causing delayed allergic reactions. We suggest that this is also one of factors of the type IV allergic reaction to *C. albicans* in the ECRS. It is very interesting that proportion of *C. albicans* in the intestinal flora induce the local eosinophilic hyper infiltration as reported in internal medicine journal, however, detailed mechanism of these phenomena has been still unclear. In the future, these issues should be resolved through further study to clarify the pathogenesis of ECRS.

Table 6
Delayed intracutaneous reactions to *Candida* and the course in the ECRS group.

	Delayed intracutaneous reactions to <i>Candida</i>	Favorable course (total 9 cases)	Unfavorable course (total 15 cases)	Tissue eosinophil count (400-fold magnification)
ECRS	Positive	33.3% (3/9)	86.7% (13/15)	177.5 (±79.2)
	Negative	66.6% (6/9)	13.3% (2/15)	207.5 (±92.8)

ECRS = eosinophilic rhinosinusitis, NECRS = non-eosinophilic rhinosinusitis.

Positive reaction cases exhibited significantly higher recurrence of nasal polyps and symptoms. (P < 0.05) Fisher exact test.

5. Conclusion

In ECRS, positive delayed type reactions to *C. albicans* were observed significantly more often than in NECRS, which simultaneously correlates significantly with the higher incidence of recurrent ECRS. There were no positive pathological or micro organic examination data indicating local infection or allergic pathology in naso-paranasal sinus mucosa. Recently, increasing *C. albicans* in the intestinal flora is assumed to play an important role in the pathophysiology of eosinophilic inflammation in asthmatic cases. More studies are needed on ECRS from this view point.

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