Tissue levels of Alternaria allergen Alt a 1 reflect recurrence of refractory airway diseases.

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Title:

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To the Editors,

The quantification of Alternaria allergen in the local airway tissues is quite unknown, while *Alternaria alternata* is a widespread fungal species in the airway discharge and known to be one source of aeroallergens which contribute to development of asthma (1,2) and chronic rhinosinusitis (CRS) (3,4). We have recently been established the method for quantifying allergen in the local airway tissues (5). Since Alt a 1 is the main sensitizing allergen component and it is useful in diagnosis and immunotherapy (6), we have measured the levels of Alt a 1 in the local airway tissues and examined whether the quantification of Alt a 1 could reflect the allergic airway inflammation and recurrence of refractory allergic respiratory diseases.

We obtained nasal polyp tissues from 64 patients with refractory CRS with nasal polyp (CRSwNP) and homogenized them. The tissue levels of Alt a 1, Alternaria specific IgE, IL-4, IL-5, IL-13, IL-33, galectin-10 in nasal polyp supernatant were measured. The tissue eosinophil numbers were also counted. We searched for the existence of postoperative nasal polyp for in the medical records in order to examine the usefulness of Alt a 1 as a predictor of nasal polyp recurrence after surgery.

First, we measured the levels of Alt a 1 in airway tissues and ROC curve based on nasal polyp recurrence data was developed, with a cut-off value of 1.84 ng/g of local Alt a 1 (AUC = 0.75, Figure 1A). According to the presence or absence of nasal polyp recurrence, patients were divided into two groups and the levels of Alt a 1 were compared. The tissue levels of Alt a 1 were significantly higher in recurrence group. ($p < 0.01$, Figure 1B). Kaplan–Meier curves at the cut-off point of the local tissue Alt a 1 levels shows that of the recurrence-free rate in the low- Alt a 1 group is lower rate than that in the high- Alt a 1 group ($p < 0.05$, *log-rank test,* Figure 1C).

We further measured Alternaria specific IgE level in the airway tissues in order to determine the sensitization to Alt a 1. The levels of Alt a 1 in nasal polyps had a significant positive correlation with the levels of Alternaria specific IgE (rs=0.56, p<0.0001, Figure 2A). We divided patients into two groups according to the local tissue levels of local Alt a 1, and compared the levels of Alternaria-specific IgE antibody between two groups. As result, the levels of Alternaria-specific IgE antibody were significantly higher in high- Alt a 1 group than those in low- Alt a 1 group. (p<0.0001, Figure 2B)

Fungi induce alarmins which promote the development of type 2 response via increase in the number of eosinophils, accompanied by an increase in innate lymphoid cells (ILCs) and effector cells such as mast cells (7,8). We measured the level of type2 cytokines (IL-4, IL-5, IL-13) in the airway tissues and analyzed between high- and low- Alt a 1group respectively in order to assess the contribution of type2 inflammation

to local allergic reaction to *Alternaria alterna* . Patients were divided into two groups according to the tissue levels of Alt a 1. The levels of IL-4 were significantly higher in high- Alt a 1 group than those in low- group. IL-4 plays an essential role in IgE class switching and production. The levels of IL-5 and IL-13 didn't show significant difference but tended to be higher in the high- Alt a 1 group. (Figure 2C)

Epithelial cells which activated by antigens induce proinflammatory responses due to the production of alarmins. *Alternaria alternata*exposure evokes IL-33 secretion and extracellular DNA from the airway epithelium, which functions as an alarmin to stimulate type 2 immunity in airway diseases (9). The levels of IL-33 in NPs were significantly higher in high- Alt a 1 group than those in the low- group (Figure 2C). Galectin-10 is relatively eosinophil specific protein which released from cytolytic cells is expected to be a biomarker for activated eosinophils in eosinophilic inflammatory diseases (10). The levels of galectin-10 in the airway tissues were significantly higher in high Alt a 1 group than low group. (Figure 2C)

This is the first report to have quantified the levels of Alt a 1 in airway tissues and investigated the association to the type2 molecules of allergic reaction. *Alternaria alterna* is common fungi as an environmental antigen and the increase in the local tissue levels of Alt a 1 were associated with the recurrence of refractory CRSwNP. These results might explain the tissue levels of Alt a 1 can be a predictor of recurrence for refractory respiratory airway disease.

CONFLICT OF INTEREST

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AUTHOR CONTRIBUTION

YM and TY designed and performed experiments and wrote the manuscript. TA, TY, TE, YK, and SS provided clinical samples. MY and TY edited the manuscript. MA and SU contributed scientific advice.

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INFORMED CONSENT

Informed consent was obtained from all participants in accordance with the principles laid out in the Declaration of Helsinki.

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FIGURE LEGENDS

FIGURE 1. The local tissue level of Alt a 1 and the nasal polyp recurrence after surgery. (A) Based on data from Alt a 1 levels diagnostic test by a ROC curve against the recurrence group after ESS, the cut-off level of local Alt a 1 was 1.84 ng/g. (B) The Alt a 1 group was defined as those with 1.84 or higher ng/g in NP. All others were in the low- Alt a 1 group. P values for the comparison of local Alt a 1 level between two groups (**: $p < 0.01$) (Mann-Whitney test). (C) Kaplan–Meier curves of the recurrence-free rate at the cut-off point of local Alt a 1 levels (*: $p < 0.05$) (log-rank test).**FIGURE 2. Allergic inflammation of CRSwNP and the local tissue Alt a 1 level.** (A) Correlation between local Alt a 1 and Alternaria-specific IgE levels. Spearman's rank correlation coefficient showed the relationship between Alt a 1 and Alternaria-specific IgE levels in airway tissues (A: $rs = 0.56$, $P < 0.0001$). (B) Patients were divided into two groups according to the local tissue level of local Alt a 1. (**** $P < 0.0001$) (Mann–Whitney U test). (C) Characteristics by the levels of local tissue Alt a 1 for chronic rhinosinusitis groups. P values for comparison between low- and high- Alt a 1 antigen groups. (*$p < 0.05$, **** $p < 0.0001$, NP; nasal polyp, HPF; per high-powered field).

FIGURE 1.

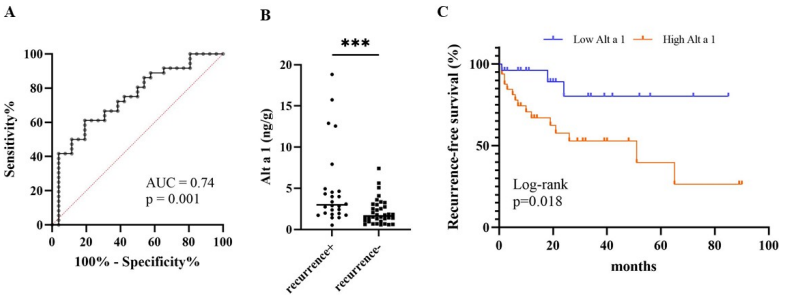
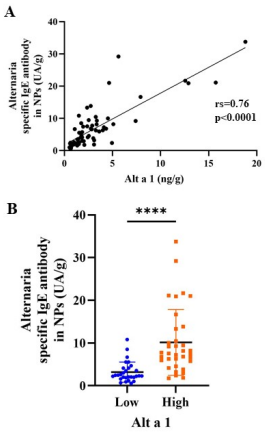


FIGURE 2.



C

		Low- Alt a 1 group (n=29) (Mean ± SE)	High- Alt a 1 group (n=35) (Mean ± SE)
Age	(y)	57.3 ± 2.5	49.5 ± 2.9
Male	No./total (%)	23/29 (79.3)	23/35 (65.7)
Blood Eosinophil	(%)	5.8 ± 0.8	9.1 ± 0.9 *
Tissue eosinophil count	(/HPF)	129.7 ± 24.3	187.1 ± 29.6
IL-4 levels in NP tissues	(pg/g)	41.8 ± 11.6	113.7 ± 33.8 *
IL-5 levels in NP tissues	(pg/g)	311.2 ± 90.6	516.8 ± 106.4
IL-13 levels in NP tissues	(pg/g)	83.9 ± 18.3	258.2 ± 66.3
IL-33 levels in NP tissues	(ng/g)	2.9 ± 0.3	9.8 ± 2.7 *
Galectin-10 levels in NP tissues	(ng/g)	70.2 ± 18.1	484.6 ± 102.6 ****