

In vitro study was performed including measurements of serum total IgE (178 kU/l) and serum specific IgE by Enzyme Allergo Sorbent (EAST) or CAP system (Pharmacia, Uppsala, Sweden) to extracts from marigold flour: 7.7 kU/l; *A. vulgaris* pollen: 6 kU/l, *H. annuus* pollen: 7.5 kU/l, olive pollen: 2.2 kU/l, prawn: 0.81 kU/l, peach: 5.03 kU/l, strawberry: 3.05 kU/l and pineapple: 0.87 kU/l.

SDS-PAGE Immunoblotting with marigold flour extract revealed an IgE-binding band of 60 kDa (Fig. 1). Cross reactivity between *H. annuus* pollen extract and marigold flour extract was demonstrated by EAST-inhibition assay.

This is the first reported case of IgE-mediated occupational allergy to marigold flour. It was demonstrated by prick test, nasal challenge test and specific IgE determination. A 60 kDa – IgE binding band has been observed by Immunoblotting.

The EAST-inhibition results showed the existence of cross reactivity between extracts from marigold flour and *H. annuus* pollen suggesting the influence of his pollen allergy in the development of the marigold flour allergy symptomatology.

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Occupational asthma caused by octopus particles

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Key words: food allergy; occupational asthma; seafood.

Patients who are allergic to fish can suffer asthma attacks when they breathe air-borne particles of fish (1).

In restaurants or other industries, occupational asthma can occur among people working in cooking, processing or canning fish and shellfish (2–4).

A 22-year-old female smoker was assessed in our unit because she presented symptoms of rhinoconjunctivitis as well as episodes of breathlessness and persistent cough accompanied by wheezing. These symptoms appeared throughout the previous year and she needed to use beta2-inhaler daily.

She had had a cat at home for over a year, which was well-tolerated. For 2 years, she had been working in a canning fish and shellfish factory where she was directly handling and cooking seafood.

The patient noticed that the symptoms worsened during workdays, but over the weekends, she noticed improvement. The symptoms appeared 10–20 min after the time she started handling octopus and even when other colleagues cut it nearby. At the same workplace, she tolerated handling other seafood.

Despite stopping smoking and starting with appropriate treatment, she continued using beta2-agonist, especially on the days she cut octopus.

The allergic study revealed: skin prick tests (SPT) for raw octopus: 20 mm,

cooked octopus: 10 mm, raw squid: 6 mm, raw shrimp: 10 mm, cat dander 8 mm. Skin prick test were negative for other seafood and other common allergens.

Immunoglobulin E (IgE) were determined by using ImmunoCAP method (Phadia Diagnostics, Uppsala, Sweden): total IgE: 176 ku/l, specific IgE for cat epithelium: 9.36 ku/l, squid: 0.51 ku/l, shrimp: 1.45 ku/l, tropomyosin: 0.84 ku/l, octopus: 24.60 ku/l.

Metacholine challenge test was positive with a PC₂₀ of 0.62 mg/ml. Peak-flow serial measurements were conducted over 15 working days and another 'off work' period and great differences were observed between them.

We monitored the patient in our office, while she was cutting cooked octopus as she used to do at her workplace for 3 h with forced expiratory volume in 1 s (FEV₁) measurements every 15 min. The result was negative, with no respiratory symptoms and unchanged FEV₁.

Finally, we performed a bronchial challenge test with an extract of octopus. The extract was performed boiling octopus with saline 0.9%, filtered and centrifuged. The supernatant was dialysed and passed through a 0.45-µm millipore filter.

We ran the administration of increasing concentrations of the octopus extract in De Vilbiss nebulizer for 2 min. Forced expiratory volume in 1 s was measured at 2, 5, 10 and 30 min after each dose. The onset dose was determined by SPT of one of 10 successive dilutions of the extract until negative. Skin prick test with the extract was 12 mm in the patient and negative in another 10 patients, who were used as controls.

With the undiluted concentration (protein concentration 2 mg/ml), FEV₁ fell more than 20% with no late response. A control challenge with an extract of fish (halibut) was also performed with negative results. On an atopic volunteer as a control, we conducted another octopus bronchial challenge that was negative.

Octopus extract (20 µg) was separated by SDS-PAGE and the protein profile revealed bands between 10 and 50 kDa. For immunoblot studies, the membrane strip was incubated with the undiluted patient serum overnight.

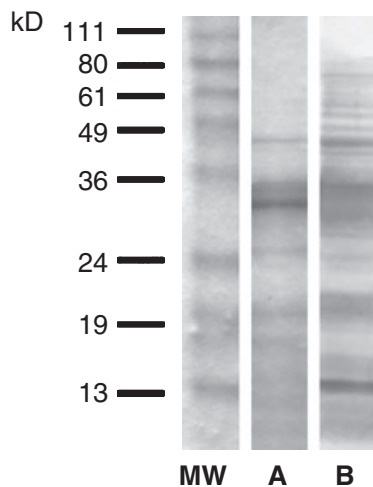


Figure 1. (A) SDS-Page. (B) Immunoblot of octopus extract showing IgE-binding bands with the patient's serum. MW, molecular weight.

Immunoblot showed IgE-binding bands of 43 and 32 kDa (Fig. 1) that could correspond to tropomyosin (38–40 kDa) as the responsible allergen for the sensitization.

In other published cases, a band of 38 kDa in a case of squid allergy, bands of 38 and 49 kDa in abalone allergy (bivalve mollusc) (4) and bands of 35 and 39 kDa in scallops allergy (5) have been detected. To our knowledge, we did not find any case of occupational asthma by inhalation of air-borne particles of octopus.

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Comparison of biomarkers between allergic rhinitis only and allergic rhinitis with concomitant asthma

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Key words: asthma; biomarkers; phenotypes; rhinitis.

Despite many similarities between allergic rhinitis (AR) and allergic asthma (AA), defined in the ARIA guidelines for an integrated diagnostic and therapeutic approach (1), there are still several differences suggestive of distinct phenotypes within the allergic airways disease (2). This *post hoc* analysis was undertaken to further characterize the differences in biomarkers between patients with AR only and patients with AR and concomitant AA (AR & AA). In addition, the relationship between nasal nitric oxide (nNO) and nasal eosinophils in nasal brushings (NAB) was explored, on the analogy of the lower airways in asthmatic patients (3).

This post hoc analysis suggests distinct immunologic profiles in the allergic rhinitis (AR)-only and the AR & AA phenotypes.

Ten patients with AR-only (four M/six F; age 19–43 years) and 10 with AR & AA (six M/four F; age 20–50 years; using short-acting β_2 -agonists $<2\times$ pw only) participated in a biomarker validation study. All patients were clinically stable, nonsmokers and not using controller therapy for >6 weeks. The following baseline data were collected (at the same time of the day, within 2 h) and compared between the two study groups: serum specific IgE (BsIgE, IU/ml; Pharmacia, Uppsala, Sweden), skin prick tests with six different allergens [wheat response (mm); ALK Abelló, Nieuwegein, the Netherlands; nNo (ppb); Ecomedics CLD 88 SP, Duernten, Switzerland], biomarkers [ECP (μ g/ml), $\alpha 2$ -macroglobulin (ng/ml), IgE (IU/ml), MUC5AC (AU/ml)] from nasal lavage (NAL) fluid and percentage eosinophils in NAB (% nonsquamous cells). A Wilcoxon sum rank test was performed for nonparametrical comparison of the allergy-related biomarkers between AR-only and AR & AA. All results were expressed as medians [interquartile range]. The correlation between nNO and NAB-eosinophils in all subjects was tested by the Spearman's rank correlation.

There were no statistical differences between the AR-only group (four M/six F; mean age $\pm$ SD = 25.8 ± 8.1 years) and the AR & AA-group (six M/four F; mean age $\pm$ SD = 25.8 ± 8.8 years) in terms of age ($P = 1.00$) and gender ($P = 0.371$). In the AR-only group, there was a significantly larger wheal response ($P = 0.006$) and a higher BsIgE-level to grass pollen ($P = 0.038$) as compared with AR & AA. In contrast, the AR & AA group had higher HDM-specific BsIgE-levels ($P = 0.049$) and overall more allergies ($P = 0.017$) as compared with the AR-only group. All results are shown in Table 1. Because of a large variability, no statistically significant differences could be found in the local biomarkers in this relatively small sample size. Additionally, no correlation could be demonstrated between nNO and NAB eosinophils ($r = -0.222$, $P = 0.426$).

Despite a small sample size, our data underscore and extend previous evidence from pediatric studies (4, 5), suggesting distinct phenotypes of the allergic air-