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# IDENTIFICATION OF CROSS-REACTIVE CARBOHYDRATE DETERMINANTS IN SUBJECTS REPORTING WORK-RELATED RESPIRATORY SYMPTOMS

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**Abstract**
**Objectives:** The role of cross-reactive carbohydrate determinants (CCDs) in diagnostics of occupational allergy remains unclarified and its clinical relevance is still questioned. The aim of the study was to assess the frequency of positive response to CCDs in the subjects with suspected occupational allergy and the relationship between other diagnostic test results and final diagnosis. **Material and Methods:** The study group included 201 patients. They underwent clinical examination, skin prick test (SPT) to common and occupational allergens, specific serum immunoglobulin (sIgE) determinations, spirometry and specific inhalation challenge test. Moreover, sIgE to CCDs from bromelain was assessed in all subjects. **Results:** Occupational respiratory allergy was recognized in 64.3% of CCD-positive and 52.4% of CCD-negative patients. Positive SPT results to common and occupational allergens were found in 64.3% and 35.7% of CCD-positive subjects, respectively. In all subjects with CCDs, the sIgE to grass pollens as well as to occupational allergens were detected. The total IgE level > 100 kU/l was significantly associated with the presence of sIgE to CCDs. **Conclusions:** sIgE to CCDs were found in 7% of subjects suspected to suffer from occupational respiratory allergy. The presence of CCDs is not significantly associated with occupational respiratory allergy. It is also not more frequent in subjects reporting work-related respiratory symptoms in whom occupational allergy was not confirmed. The elevated total IgE level was related with CCD positivity. In patients with suspected occupational allergy, the presence of sIgE to CCDs in serum did not indicate the irrelevance of positive sIgE to occupational allergens.

**Key words:**
**Occupational allergy, Cross-reactive carbohydrate determinants, CCDs, Work-related respiratory symptoms**

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## INTRODUCTION

The interpretation of results obtained by specific immunoglobulin E (sIgE) testing requires a cautious and sensible approach. Positive results most often indicate IgE-mediated sensitization; however, the clinical relevance is likely only in the context of clinical symptoms, since the false-negative and false-positive results can happen [1,2]. It is emphasized that the immunologic response may only reflect exposure and/or the immunologic nature of the tested allergen. It may have also resulted from the presence of IgE directed against carbohydrate determinants.

In the early 1980s, Aalberse et al. described the presence of IgE directed against the cross-reactive carbohydrate determinants (CCDs) in patients sera [3,4]. The cross-reactive carbohydrate determinants may cause production of anti-CCD IgE and many allergic patients develop specific serum immunoglobulin E (sIgE) directed against plant and/or insect protein-linked glycans [5]. It has been postulated that the presence of anti-CCD IgE is not clinically relevant, especially when it is related to negative reactions to skin tests and lack of clinical symptoms [6,7].

We have recently reported the preliminary results related to the presence of CCDs in 81 patients suspected to be affected by occupational allergy. The initial results indicated that sIgE to CCDs could be found in about 10% subjects and detection of CCDs in serum was not helpful in diagnostics of occupational allergy [8]. Since data on the anti-CCD IgE prevalence in a large cohort of subjects with allergy symptoms due to workplace exposure are limited and the role of CCDs in diagnostics of occupational allergy remains unclarified, the authors have decided to expand the study group.

The aim of the present study was to evaluate the frequency of positive response to CCDs in the subjects with suspected occupational allergy and its relation to the results of other diagnostic tests and final diagnosis.

## MATERIAL AND METHODS

The study group included 201 patients (bakers, farmers, healthcare workers (HCWs), carpenters and single other occupations, i.e., veterinarian, poultry farm worker, cleaner, seamstress, ceramics decorator, leather cutter, pharmacist) suspected to be affected by occupational respiratory allergy (asthma and/or rhinitis), diagnosed at our Department of Occupational Diseases between 2008 and 2010. After completion of the diagnostic procedures, the study group was divided taking into account the final diagnosis (subjects with occupational respiratory allergy vs. suspected occupational allergy) and anti-CCD IgE determination results (CCD-positive vs. CCD-negative subjects) to compare statistically the various parameters.

The subjects were administered a questionnaire that included e.g., a history of respiratory symptoms (rhinitis, itching, nasal blockage, cough, wheezing, shortness of breath, chest tightness), skin symptoms, personal and family history of atopy, exposure to pet allergens at home, medication use, and smoking habits.

Skin prick tests (SPT) were performed on the volar part of the forearm with a standard battery of common allergens including tree and grass pollens, *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, moulds, weeds (Allergopharma, Reinbek, Germany) and with occupational allergens depending on patient's profession: bakery series (α-amylase, oatmeal, wheat, corn, barley and rye flour), farmers occupational allergens (mixture of grain, hay, horse epithelium, swine epithelium, sheep, goat, rabbit epithelium, fur, barley, corn, oat, rye, wheat, cereals, straw); HCW allergens (latex, chloramine, formaldehyde, glutaraldehyde, chlorhexidine, benzalkonium chloride solutions), wood dust series (spruce, oak, pine, fir and beech wood) (Allergopharma, Germany; Stallergens, France). Allergen diluents were used as the negative control, while 1 mg/ml histamine dihydrochloride solution was the positive one. The largest wheal diameter

was assessed after 15 min. A weal diameter of 3 mm or more and equal to or greater than half of that formed by histamine was defined as positive, hence indicating sensitization.

Depending on SPT results, patients were grouped as mono-sensitized, i.e., hyperreactive to an individual common allergen (dust mite, pollen, mould or feather allergen) or as multiple-sensitized, i.e., hyperreactive to 2, 3 or 4 different groups of common allergens.

Total serum IgE was evaluated using the ImmunoCap (Phadia, Sweden). Total IgE level > 100 kU/l was considered as elevated. Specific serum IgE against flours and α-amylase were measured among bakers (fx20, k87 Phadia, Sweden), farmers were tested for grain, animal fur and/or bird feather allergens (e4, e83, ex71 Phadia, Sweden), mixed disinfectants and latex were examined in HCWs (pax6: chloramine, formaldehyde, glutaraldehyde, phthalic anhydride; k82 Phadia, Sweden), different kinds of wood were tested among carpenters (spruce-k35, fir-k44, oak-k33, pine-k36; Allergopharma, Germany). Among 15 subjects with other occupations, the sIgE were selected according to occupational exposure. The sIgE levels ≥ 0.35 kU/l were regarded as positive. Moreover, MUXF3 CCDs from bromelain were determined in all subjects (Ro214, Phadia, Sweden). Additionally, among CCD-positive patients (with presence of sIgE to CCDs from bromelain in serum) the horseradish peroxidase (HRP) and sIgE to grass pollens were measured (Ro400, gx1, Phadia, Sweden).

Specific inhalation challenge tests (SICT) with occupational allergens were performed in a work-site simulation setting (room space 6 $m^2$ with temperature 22–25°C) with the patient's own samples. The patient was sifting approximately 500 g of solid materials (e.g., flours and improvers, farmer's allergens, wood dusts) and for liquid substances the test was done by painting the solution onto a 2 $m^2$ piece of cardboard in a challenge chamber for 30 min or until the asthmatic reaction symptoms appeared. Potato flour or lactose were used as placebo, the test was performed one day before SCIT.

The subjects who did not show significant (≥ 20%) fall in forced expiratory volume in 1 s ($FEV_1$) during the challenge test, underwent a repeated challenge test lasting 2 h on the next day. In patients with changes in $FEV_1$ ranging between 10 and 20% after that challenge, the exposure was prolonged up to 3 h.

The study participants did not receive any systemic or local medication. Inhaled short-acting $\beta_2$-agonists were stopped at least 8 h before the study, inhaled long-acting $\beta_2$-agonists – 48 h, inhaled steroids – 5 days, systemic steroids – 14 days. Antihistamine medications were stopped 7–42 days before challenge test, depending on the time for which the drugs were active.

Resting spirometry (Vicatest 2A, The Netherlands) was performed in all subjects. Bronchial response was measured by serial monitoring of $FEV_1$ before and 5 min, 30 min, 1 h, 2 h, 4 h, 6 h and 24 h after the provocation. The non-specific bronchial hyperreactivity (histamine challenge) was evaluated on the day before the SICT and 24 h after the test.

Occupational allergic rhinitis was recognized in subjects reporting work-related nasal symptoms with positive nasal response to provocation test, i.e., total score of more than 3 points and significant increase in total count and proportion of eosinophils (2-fold increase and eosinophilia at least 5% after challenge) in nasal lavage fluid. Recognition of occupational asthma was based on SICT with evaluation of bronchial response (at least a 20% decrease in $FEV_1$), or a 3-fold increase in non-specific bronchial hyperreactivity accompanied by increased sputum eosinophilia (at least 3% of eosinophils after the SICT). The group of patients with suspected occupational allergy included subjects reporting work-related respiratory symptoms, for whom the SICT did not induce significant bronchial response as well as subjects reporting work-related nasal symptoms with negative nasal response to SICT.

## ETHICS

The Regional Bioethical Committee approved the study protocol (approval decision number 15/2008). All of the participants gave their informed consent prior to the study.

## STATISTICS

Statistical analyses were performed using Statistica 8. Continuous variables were expressed as mean values ± standard deviations while the nominal variables were specified as numbers and percentages. Chi$^2$ test (or Fisher's exact test) and Wilcoxon rank sum test were used to compare subjects with occupational respiratory allergy and patients with suspected occupational allergy as well as in the study population in relation to the presence of sIgE to CCDs in serum. A p-value $< 0.05$ was considered as significant.

## RESULTS

The group under the study consisted of 117 bakers, 35 farmers, 29 HCWs and 20 subjects representing other occupations. Specific IgE to MUXF3 CCDs were found in 14 subjects (7%), while the sIgE to HRP was found among 13 CCD-positive patients. Occupational asthma and/or rhinitis were recognized in 9 (64.3%) CCD-positive patients and 98 (52.4%) subjects without sIgE to CCDs. The other 5 subjects with positive sIgE to CCDs were diagnosed with non-occupational respiratory disease. No occupational respiratory allergic disease was recognised amid 47.6% of patients without sIgE to CCDs in serum. Table 1 gives the characteristics of the study group.

The sIgE to grass pollens as well as to occupational allergens were detected in all subjects with anti-CCD

**Table 1.** Characteristics of patients and duration of reported symptoms in the study population

| Variable | Total (N = 201) | CCD-positive (N = 14) | CCD-negative (N = 187) |
|---|---|---|---|
| Age (years) [M±SD (min.–max)] | 40.3±10.8 (20–61) | 38.6±8.6 (23–53) | 40.5±10.9 (20–61) |
| Occupation [n (%)] | | | |
| baker | 117 (58.2) | 8 (57.1) | 109 (58.3) |
| farmer | 35 (17.4) | 4 (28.6) | 31 (16.6) |
| health care worker | 29 (14.4) | 1 (7.1) | 28 (15.0) |
| carpenter | 5 (2.5) | 1 (7.1) | 4 (2.1) |
| other | 15 (9.9) | 0 | 15 (8.0) |
| Occupational respiratory allergy disease [n (%)] | 107 (53.2) | 9 (64.3) | 98 (52.4) |
| Occupational asthma [n (%)] | 79 (39.3) | 7 (50.0) | 72 (38.5) |
| Occupational rhinitis [n (%)] | 85 (42.3) | 6 (42.9) | 79 (42.2) |
| Occupational contact dermatitis [n (%)] | 26 (12.9) | 2 (14.3) | 24 (12.8) |
| Work-related respiratory symptoms [n (%)] | 94 (46.8) | 5 (35.7) | 89 (47.6) |
| Asthma [n (%)] | 27 (13.4) | 4 (28.6) | 23 (12.3) |
| Rhinitis [n (%)] | 42 (20.9) | 4 (28.6) | 38 (20.3) |
| Contact dermatitis [n (%)] | 10 (5.0) | 0 | 10 (5.3) |
| Duration of symptoms (years) [M±SD (min.–max)] | 7.0±6.4 (1–36) | 9.9±11.2 (1–36) | 6.7±5.8 (1–35) |
| Latency period (duration of exposure before the occurrence of symptoms) (years) [M±SD (min.–max)] | 13.3±8.9 (1–37) | 10.0±9.1 (1–28) | 13.6±8.8 (1–37) |

M – mean; SD – standard deviation; min. – minimum value; max – maximum value.
CCDs – cross-reactive carbohydrate determinants.

IgE in serum. Positive SPT results to common and occupational allergens were found in 9 (64.3%) and 5 (35.7%) of CCD-positive patients, respectively. Table 2 shows the detailed characteristics of the subjects with the anti-CCD IgE in serum.

The comparison of the reported allergy symptoms, the results of SPT to common and occupational allergens as well as the total and sIgE determinations among CCD-positive patients and those without sIgE to CCDs did not show significant differences (Table 3). At least one positive SPT response to common allergens was found in 64.3% of CCD-positive subjects and in 55.1% of those without sIgE to CCDs in serum, while sensitization to occupational allergens was detected in 35.7% and 50.8% of patients, respectively. Total IgE level was elevated in both groups and a significant percentage of subjects with more than 100 kU/l IgE was found, particularly among CCD-positive subjects (85.7%). Only sensitisation to weed pollens and elevated mean total IgE level were more frequent in CCD-positive subjects (42.9% and 85.7%, respectively) than in those without sIgE to CCDs (15.5%, $p < 0.05$, and 47.6%, $p = 0.01$, respectively).

The frequency of CCD-positivity as well as the kU/l-values of anti-CCD IgE results did not vary significantly between patients with evident clinical occupational allergy and those with suspected occupational allergy (Table 4). Only the sensitisation to occupational allergens and the multi-sensitisation to common allergens were more frequently associated with occupational allergy.

**Table 2.** The questionnaire data and results of SPT and sIgE among CCD-positive individuals (based on reference 8)

| Variable | Patient number (N = 14) | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 |
| Occupation | | | | | | | | | | | | | | |
| baker | + | + | + | + | + | + | + | + | | | | | | |
| farmer | | | | | | | | | + | + | + | + | | |
| nurse | | | | | | | | | | | | | + | |
| carpenter | | | | | | | | | | | | | | + |
| Positive SPT to at least one common allergen | + | – | + | + | – | – | – | + | + | + | + | + | + | – |
| Positive SPT to at least one occupational allergen | – | – | + | – | – | – | + | – | + | – | + | + | –* | – |
| Total IgE level (IU/ml) | | 813.8 | 252.6 | 278.0 | 334.7 | | 407.0 | 1 000.0 | 141.6 | 340.5 | 871.9 | 318.2 | 1 000.0 | 814.0 |
| Positive sIgE to grass pollens (class) | 3 | 2 | 3 | 2 | 2 | 1 | 3 | 2 | 4 | 2 | 2 | 2 | 6 | 2 |

## DISCUSSION

Immunologic IgE-dependent mechanisms have been confirmed for many causes of occupational respiratory allergy, particularly for high-molecular-weight (HMW) allergens. Hence, the assessment of sensitization is an important stage in diagnosis of occupational allergy and includes skin tests and/or specific IgE measurement. However, it is pointed out that reliable interpretation of the immunologic tests

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Positive sIgE to occupational allergens | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| sIgE to α-amylase (k87) concentration (class) | – | – | – | 1.6 (2) | – | – | 0.56 (1) | – | | | | | | |
| sIgE to flours (wheat, rye, rice, barley) (Fx20) | + | + | + | + | + | + | + | + | | + | + | | | |
| sIgE to latex (k82) concentration (class) | | | | | | | | | | | | | 22 (4) | |
| sIgE to cow epidermis (e4) | | | | | | | | | + | + | + | | | |
| sIgE to swine epidermis (e83) | | | | | | | | | | + | + | + | | |
| sIgE to feathers (ex71) | | | | | | | | | – | – | – | | | |
| sIgE to pine wood (k36) | | | | | | | | | | | | | | + |
| HRP result – concentration ($kU_A/l$) (class) | 2.38 (2) | 1.42 (2) | 1.00 (2) | 1.26 (2) | 1.36 (2) | 0.67 (1) | 1.78 (2) | 1.74 (2) | 0.69 (1) | 1.29 (2) | 2.68 (2) | 1.21 (2) | 0.16 (0) | 0.72 (1) |
| CCD result – concentration (class) | 2.77 (2) | 0.58 (1) | 0.70 (1) | 0.65 (1) | 0.64 (1) | 0.56 (1) | 0.54 (1) | 0.96 (2) | 0.40 (1) | 0.58 (1) | 1.89 (2) | 0.68 (1) | 0.38 (1) | 0.58 (1) |
| Occupational respiratory allergy disease | + | + | + | – | – | + | – | + | + | + | + | – | + | – |
| Non-occupational respiratory allergy disease | | | | + | + | | + | | | | | + | | + |

"+" – positive result; "–" – negative result.

SPT – skin prick tests; sIgE – specific serum immunoglobulin E; CCDs – cross-reactive carbohydrate determinants; HRP – horseradish peroxidase.

* SPT with latex not tested in this patient.

**Table 3.** Prevalence of reported symptoms, SPT results for common and occupational allergens and evaluation of total and specific IgE levels in study population depending on anti-CCD IgE presence

| Variable | CCD-positive (N = 14) | CCD-negative (N = 187) | p |
|---|---|---|---|
| Questionnaire data [n (%)] | | | |
| family history of atopy | 1 (7.1) | 46 (24.6) | n.s. |
| pets at home | 4 (28.6) | 56 (30.0) | n.s. |
| smoking status | | | |
| current-smokers | 1 (7.1) | 32 (17.1) | n.s. |
| ex-smokers | 7 (50.0) | 59 (31.0) | n.s. |
| sex: male | 10 (71.4) | 116 (64.0) | n.s. |
| Reported allergy symptoms [n (%)] | | | |
| cough | 9 (64.3) | 119 (63.6) | n.s. |
| dyspnea | 13 (92.9) | 148 (79.1) | n.s. |
| rhinitis | 14 (100.0) | 146 (78.1) | n.s. |
| conjunctivitis | 9 (64.3) | 81 (43.3) | n.s. |
| skin symptoms | 4 (28.6) | 72 (38.5) | n.s. |
| Positive SPT results [n (%)] | | | |
| to at least 1 common allergen | 9 (64.3) | 100 (53.5) | n.s. |
| mono-sensitization to common allergens | 5 (35.7) | 61 (32.6) | n.s. |
| multiple-sensitization to common allergens | 4 (28.6) | 39 (20.9) | n.s. |
| sensitization to 2 common allergens | 4 (28.6) | 31 (16.6) | n.s. |
| sensitization to 3 common allergens | 0 | 6 (3.2) | n.s. |
| sensitization to 4 common allergens | 0 | 2 (1.1) | n.s. |
| feathers | 1 (7.1) | 4 (2.1) | n.s. |
| grass pollens | 4 (28.6) | 33 (17.6) | n.s. |
| tree pollens I[1] | 3 (21.4) | 26 (13.9) | n.s. |
| tree pollens II[2] | 4 (28.6) | 32 (17.1) | n.s. |
| moulds I* | 0 | 7 (3.7) | n.s. |
| moulds II** | 0 | 4 (2.1) | n.s. |
| *Dermatophagoides pteronyssinus* | 6 (42.9) | 52 (27.8) | n.s. |
| *Dermatophagoides farinae* | 6 (42.9) | 55 (29.4) | n.s. |
| weeds | 6 (42.9) | 29 (15.5) | < 0.05 |
| *Acarus siro* | 5 (35.7) | 36 (19.3) | n.s. |
| *Lepidoglyphus destructor* | 5 (35.7) | 39 (20.9) | n.s. |
| *Thyrophagus putrescentiae* | 5 (35.7) | 37 (19.8) | n.s. |
| at least 1 occupational allergen | 5 (35.7) | 95 (50.8) | n.s. |
| only common allergens | 5 (35.7) | 43 (23.0) | n.s. |
| only occupational allergens | 1 (7.1) | 35 (18.7) | n.s. |
| common and occupational allergens | 4 (28.6) | 60 (32.1) | n.s. |

**Table 3.** Prevalence of reported symptoms, SPT results for common and occupational allergens and evaluation of total and specific IgE levels in study population depending on anti-CCD IgE presence – cont.

| Variable | CCD-positive (N = 14) | CCD-negative (N = 187) | p |
|---|---|---|---|
| Total IgE and sIgE measurements | | | |
| total IgE level (kU/l) [M±SD (min.–max)] | 547.6±322.2 (141–1 000) | 190.8±214.6 (6–1 000) | n.s. |
| total IgE > 100 kU/l [n (%)] | 12 (85.7) | 89 (47.6) | 0.01 |
| sIgE to occupational allergens [n (%)] | 14 (100) | 162 (86.6) | n.s. |

[1] Alder, hazel, poplar, elm, willow.
[2] Birch, beech, oak, plane.
* *Alternaria tenuis*, *Botrytis cinerea*, *Cladosporium herbarum*, *Culvularia lunata*, *Helminthosporium*, *Fusarium moniliforme.*
** *Aspergillus fumigatus*, *Mucor mucedo*, *Penicillium notatum*, *Pullularia pullulans*, *Rhizopus nigricans*, *Serpula lacrimans.*
n.s. – not statistically significant. Other abbreviations as in Tables 1 and 2.

**Table 4.** SPT results for common and occupational allergens and evaluation of total and specific IgE levels in subjects with occupational respiratory allergy and suspected occupational allergy

| Variable | Occupational respiratory allergy (N = 107) | Suspected occupational allergy (N = 94) | p |
|---|---|---|---|
| Total IgE and sIgE measurements | | | |
| CCD IgE (bromelain) [n (%)] | 9 (8.4) | 5 (5.3) | n.s. |
| CCD IgE (bromelain) (kU/l) [M±SD (min.–max)] | 0.08±0.36 (0–2.77) | 0.03±0.132 (0–0.68) | n.s. |
| sIgE to occupational allergens [n (%)] | 104 (97.2) | 72 (76.6) | < 0.0001 |
| total IgE level (kU/l) [M±SD (min.–max)] | 229.1±240.1 (10–1 000) | 181.6±204.9 (6–1 000) | n.s. |
| total IgE > 100 kU/l [n (%)] | 54 (50.5) | 47 (50) | n.s. |
| Positive SPT results [n (%)] | | | |
| to at least 1 common allergen | 64 (59.8) | 45 (47.9) | n.s. |
| mono-sensitization to common allergens | 34 (31.8) | 32 (34.0) | n.s. |
| multiple-sensitization to common allergens | 30 (28.0) | 13 (13.8) | < 0.05 |
| sensitization to 2 common allergens | 25 (23.3) | 10 (10.6) | < 0.05 |
| sensitization to 3 common allergens | 4 (3.7) | 2 (2.1) | n.s. |
| sensitization to 4 common allergens | 1 (0.9) | 1 (1.1) | n.s. |
| to at least 1 occupational allergen | 72 (67.3) | 28 (29.8) | < 0.0001 |

Abbreviations as in Tables 1–3.

used in the diagnosis of occupational allergy requires validation, mainly based on the result of SICT [9].

Generally, the results of the immunologic tests can indicate exposure and sensitization, but by themselves are unable to confirm a diagnosis of occupational asthma. Beach et al. estimated the sensitivity and specificity for sIgE to HMW agents to be 73.3% and 79%, respectively [10]. There are several mechanisms resulting in clinically irrelevant sIgE,

e.g., nonspecific absorption frequently associated with high total serum IgE levels, and cross-reactivity due to panallergens and CCDs [1,11,12].

In addition, serological tests may not be as sensitive as SPT [13] and it is likely that the discrepancies between negative skin test responses and positive sIgE detection can be associated with the presence of sIgE to CCDs [14]. To examine if the presence of sIgE to CCDs in serum might affect results of the determinations of sIgE to occupational allergens, we assessed the frequency of positive response to CCDs in the subjects with suspected occupational allergy and its relation to the results of other diagnostic tests and final diagnosis.

In our paper, we used the radioallergosorbent test for bromelain in all subjects and finally HRP to obtain information on the presence of IgE against the glycan groups. The bromelain is the glycoprotein most widely used to identify IgE reactivity to CCDs and the positive result for a bromelain-specific IgE test indicates either the presence of anti-CCD IgE in the patient's serum or, less likely, that the subjects could be sensitized to bromelain [15]. In contrast, HRP is a polyvalent plant glycoprotein. However, both HRP and MUXF3 CCD ImmunoCAP were evaluated as a valuable screening allergen for the detection of CCD-sIgE [16].

In Altmann review, it has been estimated that over 20% of allergic patients have IgE that binds to carbohydrate compounds [5]. The anti-CCD IgE was found among 28% of patients allergic to bee venom [17], whereas in Kochuyt et al. study the 'CCD positivity' was observed in 47% of subjects with honeybee and yellow jacket venom allergy [18]. The highest prevalence of the anti-CCD IgE to bromelain (45–55%) was observed in studies of carrot and celery allergens [19,20].

Only few data are accessible on the prevalence of anti-CCD IgE among patients suspected to suffer from occupational allergy. In Sander et al. study [21] sIgE to any CCDs (bromelain and HRP) was measured in 30% of the bakers with work-related asthma/rhinitis, compared to 80% of the controls with pollinosis. In Kespohl et al. study, woodworkers sensitized to beech and pine allergens showed a high prevalence of CCD sensitization (73%); however, workers with a single sensitization to wood had no sIgE to CCDs [22]. In our study, sIgE to CCDs was found in 7% of subjects with suspected occupational respiratory allergy disease, mainly among farmers (11.4%). Lower prevalence of IgE sensitization to CCDs has been observed among beekeepers (2.6%) [23].

It is pointed out that the recognition of anti-CCD IgE might be related to the 'atopic' status [18]. Similarly to previous studies, in our study sIgE to grass pollen was also detected in all CCD-positive subjects, although only 4 of them had positive response to grass pollen allergens in SPT [21]. Mari suggested a role for CCD IgE as a marker of the risk of developing multiple allergies [15]. In the present study, a significant correlation was found between the presence of CCDs in serum and sensitisation to weed pollens, although we did not confirm the relationship between the mono- or multiple sensitisation to common allergens and presence of CCD sIgE in serum. Also Kespohl et al. did not find correlation between positive results of sIgE to the most common inhalative allergens and CCDs [22]. Other results demonstrated that in CCD-positive patients with suspected respiratory allergy, male sex and atopy were associated with CCD sensitization and this sensitization was more frequent in pollen-sensitized patients than in those sensitized to mites [24]. In Vidal et al. study, CCD sensitization was not significantly associated with age, rural residence, alcohol consumption or smoking [24]. On the other hand, among beekeepers, the presence of anti-CCD IgE was positively associated with atopy and higher levels of serum total IgE [23].

In our study, in the majority of CCD-positive subjects, the average total IgE level was elevated, additionally in 2 subjects it was above 1000 kU/l. Moreover, a significant correlation was found to occur between the total IgE

level over 100 kU/l and presence of the sIgE to CCDs. This could be explained by the fact that nonspecific absorption, frequently associated with high total serum IgE, might be responsible for the presence of clinically irrelevant sIgE. On the other hand, the possible effect of high total IgE levels on the detection of CCD IgE has been ruled out in the Mari study [15].

It has been proved that the presence of CCD IgE directly correlates with positive assay for many different allergenic extracts that are unable to trigger an allergic reaction in the skin test [15]. In our study, the sIgE to occupational allergens were detected in all CCD-positive patients, while positive SPT to occupational allergens were found in 5 of those subjects. Additionally, in one nurse, the SPT to latex could probably have been positive (as she reported in history an urticarial reaction associated with wearing latex gloves at work) but it was not confirmed due to the contraindication for prick testing with latex allergens. Finally, in 9 of CCD-positive patients, the occupational asthma and/or rhinitis were recognized from the result of SICT.

In Sander et al. study [21], IgE results to HRP and bromelain were significantly correlated. Unexpectedly, in our study, negative HRP result was found in 1 CCD-positive subject, although high level of sIgE and positive SPT to grass pollens were found in that patient. The reason why the sIgE to HRP was negative is uncertain at this moment. The clinical significance of sIgE to CCDs in occupational allergy remains unclear. Unlike results of other authors, in our study all the CCD-positive patients reported work-related respiratory symptoms; moreover, occupational allergy disease was recognised in 64.3%, and work-exacerbated asthma or rhinitis in (35.7%) of those patients. In Ebo et al. study, CCDs of natural rubber latex allergens were confirmed to mimic latex sensitization [11]. In our study, presence of anti-CCD IgE should be excluded as the reason for cross-reaction to both common and occupational allergens, because non-occupational respiratory allergy was recognized only in 5 patients with positive sIgE to CCDs. In patients with occupational asthma, concomitant sensitizations to occupational allergens seemed to be an independent phenomenon (co-sensitization) proved by positive SICT results.

The results of our study revealed that sIgE to occupational allergens found in sera of CCD-positive patients could not be considered as 'irrelevant' results caused by cross-reactive mechanism. Recognition of occupational respiratory allergy was based on SICT results, therefore the presence of sIgE to occupational allergens confirmed occupational origin of the disease even if the patients did not react to workplace allergens in the skin prick testing. In the unclear cases, the relevance of sIgE could be proved by using properly folded recombinant allergens.

A limitation of our study is a lack of determination of HRP in all subjects. Detection of anti-HRP or anti-bromelain IgE by UniCAP is a practical tool for the detection of anti-CCD IgE [25]. However, it has been pointed out that the HRP contains 6 N-linked glycans, while bromelain carries only 1 carbohydrate chain. Therefore, some authors emphasize that bromelain is an imperfect CCD model [26]. Furthermore, the RAST inhibition test using HRP as an inhibitor was not performed in the present study, while the pre-incubation of the sera with HRP could abrogate IgE binding to occupational allergens.

It was suggested that anti-CCD IgE detection should be implemented in allergy diagnostic process to prevent misdiagnosis, but considering our study results, it is not useful method in occupational allergy. Similarly to our results, also Kochuyt et al. have concluded that positive bromelain CAP test does not exclude clinical reactivity to venoms in allergic patients [18].

## CONCLUSIONS

In conclusion, specific IgE to CCDs can be found in 7% of subjects suspected to suffer from occupational respiratory allergy disease. The presence of anti-CCD IgE in serum is not significantly associated with occupational respiratory

allergy. Additionally it is also not more frequent among subjects reporting work-related respiratory symptoms in whom occupational allergy was not confirmed. The elevated total IgE level is related with CCD-positivity in subjects with suspected occupational allergy.

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