

# SHOULD POSITIVE SKIN PRICK TESTS TO AEROALLERGENS EXCLUDE COMMON VARIABLE IMMUNODEFICIENCY DIAGNOSIS?

R. Rodrigues-Alves (1), S. Lopes-Silva (1, 2), J. Caiado (1), E. Pedro (1),  
C. Pereira-Santos (2), M. Branco-Ferreira (1), A. E. Sousa (2), M. Pereira-Barbosa (1)

**Introduction:** CVID diagnosis is based on a marked decrease of IgG and IgM and / or IgA, along with poor specific antibody production. CVID diagnosis has been questioned in patients presenting specific IgE (sIgE), either evaluated by skin prick-tests (SPT) or peripheral blood quantification. We aimed to evaluate the incidence of IgE-mediated allergy in patients with CVID.

**Methods:** Twenty-three patients with CVID were submitted to a standardized questionnaire to evaluate the prevalence of rhinitis and/or asthma; SPT with a standard aeroallergens battery; total serum IgE measurements and sIgE quantification for a common aeroallergens mixture and for allergens with positive SPT.

**Results:** SPT were positive for at least one aeroallergen in four patients with rhinitis, one of them with asthma complaints. Patients were sensitized to Dermatophagoides, storage mites, Parietaria and grass pollen. Three patients presented measurable total IgE (>2.0kU/L). Only one patient presented detectable (>0.35kU/L) sIgE to the common aeroallergens mixture and to Dermatophagoides mites.

**Conclusion:** We point out the presence of four patients with CVID and simultaneous rhinitis and/or asthma symptoms with positive sIgEs to aeroallergens. CVID includes a heterogenic set of patients, reflecting the underlying immunologic defect diversity, and should not be excluded in patients with respiratory allergy. The defect in the production of IgG, IgA and/or IgM may not always be accompanied by a total impairment in IgE-switch.

## BACKGROUND

Common variable immunodeficiency (CVID) is the most prevalent human primary immunodeficiency requiring medical attention, with an estimated prevalence of 1:25000 Europeans (1). Although recent emphasis has been put on increased incidence of autoimmune disorders and malignancies (2), recurrent bacterial infections, most frequently affecting upper and lower respiratory airways, dominate the clinical picture in the majority of patients with CVID. Allergy, as part of immune dysregulation in CVID, has been overlooked by both clinical and immunological studies. In contrast, a number of CVID patients present with clinical manifestations of rhinosinusitis and/or asthma, sometimes with clear seasonal pattern and good response to anti-histamines. Some of these patients report previous investigation for allergic respiratory disease, before CVID diagnosis, since uncontrolled respiratory allergic disease and predominantly humoral primary immunodeficiencies constitute concurrent differential diagnosis in cases of recurrent respiratory infections.

Although there have been a huge number of investigations into the nature of CVID since this syndrome was first recognized in 1953 (3), the primary cause of CVID has not been elucidated. Defects in B-cell phenotypic and functional maturation as well as survival have been observed, leading to a failure in early and/or late B cell differentiation with impaired secretion of immunoglobulins (4), the main phenotypic defect in CVID. However, it is not clear whether these B-cell defects are primary or are a consequence of the T cell abnormalities (5) that have been described, namely disturbances in T-cell subset composition, T-cell signalling and cytokine expression (5).

CVID diagnosis is based on impaired ability to produce specific antibodies after vaccination or natural exposure together with markedly reduced serum levels of IgG, IgA, and frequently IgM after exclusion of other causes of antibody deficiency (6, 7). Recently, CVID diagnosis has been questioned in presence of specific IgE (sIgE), either evaluated by skin prick tests (SPT) or peripheral blood quantifications, considering that those tests document isotype-switching ability and specific antibody production (8).

Given the lack of data published on this subject, we aimed to evaluate the prevalence of rhinitis and asthma symptoms and eventual IgE sensitizations within a group of CVID patients followed in our Department.

(1) Immunoallergology Department, Hospital de Santa Maria - rodrigoalves@portugalmail.pt

(2) Clinical Immunology Unit, Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa.

## MATERIAL AND METHODS

### Patients

Twenty-three patients were studied (7 men, 16 women, age  $40 \pm 16$  years). CVID was diagnosed in all patients according to European Society for Immunodeficiencies criteria: marked decrease in IgG associated with decreased IgM and/or IgA (2 SD below mean for age), onset of immunodeficiency after two years of age and absent isohemagglutinins and/or poor response to vaccines (6). Other causes for primary or secondary causes of antibody deficiency were excluded (6). Mean follow-up time since CVID diagnosis was  $7.3 \pm 5.9$  years.

All patients were under regular intravenous immunoglobulin (IVIG) substitution treatment with 300-500mg polyvalent IgG (Octagam™, Octapharma)/kg, each 3-4 weeks.

Informed written consent was obtained from all patients.

### Questionnaires

All patients were submitted to a standardized self-filling written questionnaire adapted from ISAAC study (9), in order to evaluate the prevalence and month distribution of rhinitis and/or asthma symptoms in the previous twelve months. Patients were also inquired concerning results of eventual previous allergological evaluations.

### Prick tests

All patients underwent skin prick tests (SPT) to those aeroallergens that most frequently induce clinically relevant sensitizations in Southern Continental Portugal: *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, *Lepidoglyphus destructor*, *Euroglyphus maynei*, *Glycyphagus domesticus*, *Acarus siro*, *Blomia tropicalis*, *Tyrophagus putrescentiae*, *cat dander*, *dog dander*, *Alternaria alternata*, *Aspergillus fumigatus*, *Cladosporium herbarum*, *Lolium perene*, *Dactylis glomerata*, *Parietaria judaica*, *Artemisia vulgaris*, *Olea europea*, *Plantago lanceolata* and *latex*. Commercial extracts (BIAL-Aristegui, Spain) were used. Tests were performed according to EAACI guidelines

(10). Saline was used as a negative control and 10mg/ml histamine phosphate as a positive control. Results were read after 15 min. Reactions were considered to be positive if the largest diameter of the wheal was 3 mm over the negative control.

### Total and specific IgE

A 3 mL sample of peripheral blood in 0.05% EDTA was collected from all patients, prior to IVIG substitution. Total serum IgE and specific IgE (sIgE) for a common aeroallergens mixture were determined in all patients, respectively with ImmunoCap Total IgE™ and Phadiatop™ (Phadia, Sweden), according to manufacturer's instructions. Specific IgEs, determined by ImmunoCAP 100® immunoassay system (Phadia, Sweden), were performed in conformity with the positivity of SPT in each patient.

Total IgE quantification was performed in five randomly selected Octagam™ samples. IgE level was not detectable ( $<0.15$  kU/L) in any sample.

## RESULTS

According to the questionnaire, 16/23 patients (69.6%) had rhinitis symptoms and 9/23 patients (39.1%) had asthma symptoms in the previous twelve months. Skin prick tests were positive to at least one aeroallergen in 4/23 patients (17.4%), all of them reporting recent symptoms of rhinitis and one also reporting asthma complaints in the previous twelve months. Positive results of SPT and/or detectable total or sIgE are detailed in table 1. Sensitizations were found to *Dermatophagoides* and storage mites, *Parietaria* and grass pollens and patients sensitized to pollens repor-

| Patient (gender/age) | Rhinitis/asthma symptoms | Positive SPT (mm)                                   | Total IgE (kU/L) | Phadiatop™ | sIgE (kU/L)         |
|----------------------|--------------------------|-----------------------------------------------------|------------------|------------|---------------------|
| F/31                 | Rhinitis                 | Dpt:10x8<br>Df:9x7                                  | 4.31             | negative   | <0.35               |
| F/28                 | Rhinitis                 | Dpt:5x6<br>Df:5x5                                   | <2.0             | negative   | <0.35               |
| M/69                 | Rhinitis                 | Lep:5x4<br>Gly:5x5<br>Eur:4x6<br>Lol:6x4<br>Dac:5x4 | <2.0             | negative   | <0.35               |
| F/35                 | rhinitis and asthma      | Dpt:9x5<br>Df:4x4<br>Par:4x3                        | 3.49             | positive   | Dpt:0.89<br>Df:0.46 |
| M/33                 | Rhinitis                 | negative                                            | 3.95             | negative   | Not done            |

**Table 1:** Patients with positive SPT or total IgE  $>2.0$  kU/L.

F-female, M-male, Dpt-*Dermatophagoides pteronyssinus*, Df-*Dermatophagoides farinae*, Eur-*Euroglyphus maynei*, Gly-*Glycyphagus domesticus*, Lep-*Lepidoglyphus destructor*, Lol-*Lolium perene*, Dac-*Dactylis glomerata*, Par-*Parietaria judaica*

ted seasonal worsening. Additionally, three patients with negative SPT referred previous positivity of SPT in the past, including two patients with rhinitis and one with rhinitis and asthma.

Regarding serum total IgE, 3/23 patients (13.0%) presented measurable low range levels ( $>2.0\text{kU/L}$ ), namely  $4.31\text{kU/L}$ ,  $3.95\text{kU/L}$  and  $3.49\text{kU/L}$ , all of them with clinical manifestations of rhinitis and/or asthma. Specific IgE to the common aeroallergens mixture (Phadiatop™ - Phadia) was positive in only one patient, who was also the only one with detectable ( $>0.35\text{kU/L}$ ) sIgE to aeroallergens (*Dermatophagoides pteronyssinus*  $0.89\text{kU/L}$  and *Dermatophagoides farinae*  $0.46\text{kU/L}$ ).

## DISCUSSION

In our population of CVID patients, we found rhinitis and asthma symptoms prevalence in the previous twelve months quite above national estimative for the general population, respectively 69.6% and 36.1%. The prevalence of rhinitis among 6859 patients attending primary care centres in Portugal was recently estimated as 26.1% (11) and asthma prevalence was estimated as 10% in the general Portuguese population (12).

Other factors, besides allergy, may be of major importance in the aetiology of rhinitis in patients with CVID, namely viral and bacterial nasal and sinus infections. Moreover, asthma may be easily overestimated by questionnaire method as we applied. Obstructive pattern has been reported in patients with CVID although reversibility and hyper-reactivity were not always evaluated, therefore failing spirometric criteria for rigorous asthma diagnosis (13,14,15).

As opposed to other primary immunodeficiencies where class-switch recombination to all immunoglobulin isotypes is affected, CVID is characterized by variability in the degree and type of hypogammaglobulinemia. Piqueras *et al* evaluated immunoglobulin isotype production in patients with CVID (16). Isotype expression was quite heterogeneous and there was a graduation in isotype mRNA transcript expression  $\text{IgM} > \text{IgG3} > \text{IgG1} > \text{IgG2} > \text{IgA1} > \text{IgA2} > \text{IgG4} > \text{IgE}$ . When analyzing isotype switching within naïve/memory subgroups, there were no clear differences or any correlation with serum immunoglobulin level at the time of diagnosis.

We found three patients with measurable total IgE, which was most probably self produced, as IVIG that is administered to the patients is not supposed to contain IgE, in agreement with our findings in five randomly selected IVIG samples.

We further demonstrated the production of sIgE *in vivo* and/or *in vitro* in patients with clearly defined

CVID and rhinitis and/or asthma symptoms. Four patients presented positive SPT to aeroallergens, all of them with clinical symptoms of allergic respiratory disease, one of which with positive sIgE measurements. Pope *et al* also reported positive SPT associated with clinical symptoms of allergy in five patients with CVID, although CVID diagnostic criteria were not mentioned in that study (17). Different international guidelines accept that specific antibody production capacity may not be totally abolished in CVID (6,7). Emphasis is put on patient's impaired ability to achieve protective levels of antibodies in response to vaccines, as compared to healthy controls, rather than on absolute absence of specific antibodies (18,19). Likewise, we think that positive SPT or serum sIgE to aeroallergens are not contradictory to CVID diagnosis.

Only one patient presented detectable sIgE, although SPT were clearly positive in other three patients. *In vitro* detection of sIgEs was possibly biased by low/undetectable total IgE. Lowering sIgE detection limit might improve substantially the sensitivity of *in vitro* allergy diagnosis in CVID. Additionally, three patients with negative SPT reported positive SPT to aeroallergens in the past. This variation might be discussed as eventually being part of a progressive decrease of specific antibody synthesis ability in CVID, as these patients appear to have a loss of normal antibody synthesis that coincides with declining levels of Igs, particularly IgG and IgA (20). In our patients it is difficult to establish whether IgE sensitization preceded the development of CVID, since they had not been previously submitted to SPT.

Investigation on the possible genetic basis of CVID, currently ongoing, may soon give important contribution to clarifying immune and clinical heterogeneity of this syndrome. In this context, mutations in the TNF-receptor family member transmembrane activator and calcium-modulator and cyclophilin ligand interactor (TACI), were found to be present in 10-20% patients with CVID (1). TACI has been involved in the regulation of IgE-switch in an alternative T-independent pathway (21), which might condition allergic diseases expression in CVID.

In summary, we strengthen that CVID and respiratory allergy diagnosis should not exclude each other. Moreover, allergy should alternatively be regarded as part of the immune dysregulation in CVID population and epidemiological studies should address this association. Allergological evaluation might be useful in CVID patient's follow-up, allowing correct diagnosis, eventual allergens avoidance strategies and pharmacological treatment, in selected cases.

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