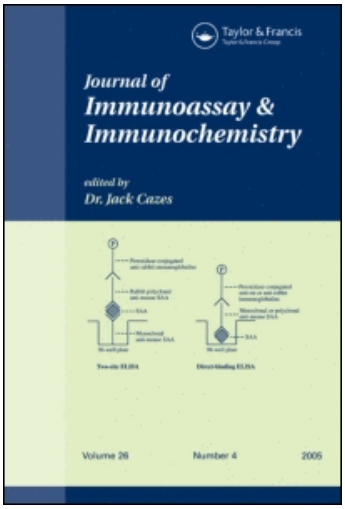

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Interference of *Dermatophagoides'* Specific Immunoglobulins G in the Quantification of Mite's Specific Immunoglobulins E

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Abstract: In order to investigate the interference of specific IgG in the quantification of specific IgE, using the ImmunoCAP 250® system, we studied, in parallel, the interference by total adsorption of interferent and the classical method of adding interferent increasingly. Furthermore, to evaluate if the interference is affected by different solid phases, total extract of *Dermatophagoides pteronyssinus* and recombinant allergens of *Dermatophagoides farinae* were used. The results showed a statistical significant interference by IgG in the quantification of the specific IgE, but neither analytical nor clinical significant interference were observed. Therefore, this analytical system provides an accurate method for determination of the specific IgE concentration contributing to the allergic disease diagnosis quality.

Keywords: Allergy testing, *Dermatophagoides farinae*, *Dermatophagoides pteronyssinus*, IgE, IgG, Interference

INTRODUCTION

Allergy is an important world-wide health problem. It affects a substantial proportion of the population and, for reasons that are poorly understood, the overall frequency of allergy is increasing.[1]

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Allergic reactions can express themselves in many different organs and in any age group. Besides the signals and symptoms that are present, mainly asthma, rhinoconjunctivitis, gastrointestinal symptoms, and characteristic skin lesions, allergic diseases also have a significant effect on the emotional and social health of patients and their families.[2] Thus, an accurate diagnosis and an adjusted therapeutic, contribute to improve patients life quality and to prevent life risk situations, as anaphylaxis. In this way, the use of standardized and reproducible methods can contribute to the quality of diagnosis of allergic disease.[3] *In vitro* measurement of total and specific IgE and IgG concentration against allergens provides critical information in clinical allergy.

Presently, the most used analytical system for the quantification of specific IgE and total specific IgG in the serum or plasma is the ImmunoCAP® system (Phadia, Uppsala, Sweden).[4] One of the problems identified concerning the ImmunoCAP® is the fact that it allows not only specific IgE linking to allergen, but also other isotype of immunoglobulins, such as specific IgG, that can be a source of immunological interference by means of solid phase allergen competitive blocking.[3,5] The strategy of presenting an excess of allergen linked to solid phase is an advantage that allows free-access of all specific IgE in the patient sample, no matter what quantity of specific IgG is present.[6,7] However, IgG can produce an important interference in laboratory diagnosis, since its concentrations are usually high and with variable avidity.

In order to assess potential interference of specific IgG in the quantification of mite's specific IgE, using the ImmunoCAP 250® system, we applied in parallel the interference study by total adsorption of interferent and classical method of increasingly adding interferent. Add to this, to evaluate if the interference is affected by different solid phases, total extract of *Dermatophagoides pteronyssinus* (d1) and recombinant allergens of *Dermatophagoides farinae* (Der f2) were used.

EXPERIMENTAL

Reagents and Equipment

Total IgG was measured on a Beckman Coulter Immage® Nefelometer using Beckman Coulter reagents. Controls were performed with Immunoassay Plus, from BIO RAD Laboratories. Specific IgG was measured using a UniCAP100® (Phadia, Uppsala, Sweden), with UniCAP 100 reagents and controls. Specific IgE was determined on a ImmunoCAP 250® (Phadia, Uppsala, Sweden), using ImmunoCAP 250® reagents and controls. Specific IgE and IgG had been quantified using solid fase (ImmunoCAP) with *Dermatophagoides pteronyssinus* total

extract. Because recombinant allergens of *D. pteronyssinus*, linked to a solid phase for use in the ImmunoCAP 250® are not commercially available, *Dermatophagoides farinae* recombinant allergens (Der f2) were used, since different works indicate the existence of a structural homology of the Der p2 and Der f2 allergens of about 88%.[8–10]

Other reagents used were: Eurosorb IgG/RF absorbent (Euroimmun, Medizinische Labordiagnostika, AG), total IgE diluent (Phadia Uppsala, Sweden), rabbit serum immunized with d1 (this serum had 1 mg/mL of anti d1 IgG and none IgE) and non immunized rabbit serum (Phadia Uppsala, Sweden), PBS solution (Phosphate Buffered Saline), Na_2HPO_4, NaH_2PO_4, NaCl, from BIO RAD Laboratories.

Samples

Serum samples from 50 patients with ages between 3 and 56 years old were obtained by venipuncture in accordance with the established in the Portuguese laws about ethical management of products from human source. Inclusion criteria were allergy to Dp confirmed clinically and by skin prick tests, and a specific serum IgE levels above 3.5 kUA/L. The exclusion criteria were pregnancy, local or systemic disease, systemic medication usage and immuno-therapy in the last 5 years, since we want to investigate if in the diagnostic phase, the patients natural IgG are sufficient to cause interference, leading to a poorly diagnosis of allergic disease.

Total IgG Adsorption

The samples were diluted 1/5 with the total IgE diluent and then submitted to a second dilution 1/2 with the Eurosorb IgG/RF absorbent. After a 15 minutes period of rest, the samples were centrifuged 5 minutes at 2000 rpm. The supernatant was removed and the *Dermatophagoides* specific IgE was quantified by the immunofluorenzimatic method (FEIA) using d1 and Der f2. To exclude any problem related with this process, the total IgG was quantified by kinetic nefelometry after the adsorption procedure.

Increasingly Adding Interferent

To study the influence of d1 specific IgG addition to human serum samples, eight samples with different concentrations of d1 specific IgE were used.

To exclude the precipitation of immune complexes we avoided addition of neat rabbit serum to human samples. An initial dilution 1/10 in PBS of serum from rabbit immunized with d1 was made to obtain

anti-d1 serum (A). A same dilution of serum from nonimmunized rabbit was also performed to obtain 0-serum (B). Finally, four different dilution series of anti-d1 serum (A) in 0-serum (B) were prepared.

In order to keep constant the rabbit serum proteins concentration in the mixture with human samples, 50 μL from these dilutions were added to 150 μL of the serum samples to be tested. As a control for this procedure, serum from the nonimmunized rabbit was diluted in PBS solution (1/10, 1/20, 1/40, 1/80, and 1/160). Then, 50 μL of each one of the dilutions were mixed with 150 μL of serum from two patients.

Determination of the Interference

The protocol advised by the Spanish Society of Clinical Chemistry and Molecular Pathology (SEQC) and also used by International Union of Pure and Applied Chemistry (IUPAC) was applied for calculation of interference. This protocol considers that there is a statistical significant interference if the confidence interval did not include the zero. In this case, the interference must be quantified. If the confidence interval includes the zero, it can not be consider that the interference is different than zero and therefore, no statistical significant interference was considered. Interference can be analytically significant, with a 99,86% confidence level, when the difference between calculated medians is superior to three times the standard deviation found in a precision study of the method, that is, when a mathematically significant effect is out of the imprecision limits of the analytical method. Finally, interference is considered clinically significant when the value of the statistical interference is superior to half of the biological intraindividual coefficient of variation.

In order to calculate the interference of total IgG in the quantification of specific IgE anti *Dermatophagoides* using d1 and Der f2, we used the following formula:

Interference = Experimental concentration of the studied constituent/Theoretical concentration or Initial concentration.

Thus,

a. For d1, Interference = d1 specific IgE after total IgG adsorption/d1 specific IgE
b. For Der f2, Interference = Der f2 specific IgE after total IgG adsorption/Der f2 specific IgE

Method Precision

To calculate the average of intra assay coefficient of variation, we used three samples that had been tested five times in the same run, and using the same lot of reagents (Table 1).

Table 1. d1 specific IgE concentrations in determination of the method's precision

Sample	d1 specific IgE concentration (kUA/L)					Mean (kUA/L)	Standard deviation
1	57.1	53.5	56.4	51.6	59.4	55.6	3.07
2	1.52	1.38	1.54	1.54	1.56	1.51	0.07
3	2.29	2.45	2.27	2.44	2.38	2.37	0.08
Intra assay mean value of variation							1.07

Statistical Analysis

Data analysis was performed using Microsoft Excel 2003 and SPSS version 15.0 for Windows. Within group (before/after adsorption of total IgG) analyses were performed with the two-tailed Wilcoxon matched-pairs signed-rank test. Within group (before/after addition of d1 specific IgG) analyses were performed with the Friedman test. The level of statistical significance was considered to be $p < 0.05$.

RESULTS

Concentration of Specific IgE Anti d1 Before and After Total IgG Adsorption

After total IgG adsorption, one of the 50 samples still had measurable quantity of total IgG (lower detection limit: 33.3 mg/dL) and for this reason was excluded from the statistical treatment (Figure 1).

The comparative analysis of d1 specific IgE, showed a statistically significant increase ($p < 0.05$) in the concentration after IgG adsorption (Figure 2a). From 49 samples, 43 (87.8%) showed an increase in the specific IgE concentration; 5 samples showed a reduction (10.2%) and only one (2.0%) did not undergone any change in the specific IgE concentration.

Concentration of Der f2 Specific IgE Before and After Total IgG Adsorption

The comparative analysis of the Der f2 specific IgE, before and after the adsorption of total IgG, showed a statistically significant reduction

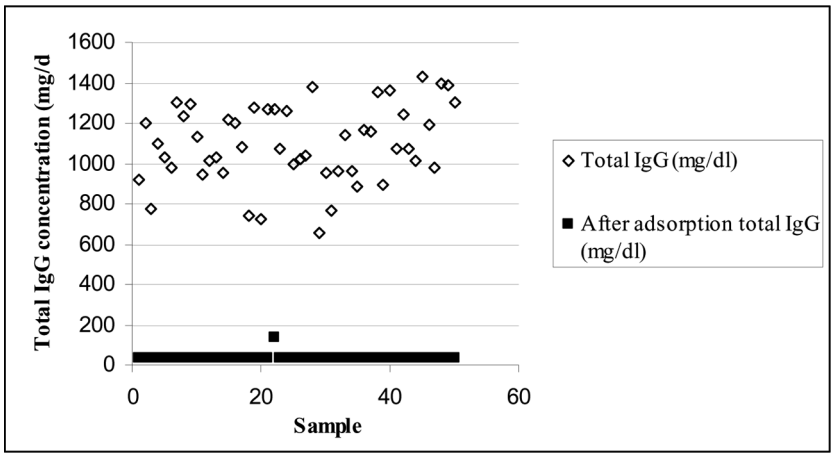

Figure 1. Total IgG concentrations before and after total IgG adsorption. The lower limit of detection was 33,3 mg/dl. The sample with measurable IgG after total IgG adsorption was excluded from the statistical treatment.

($p < 0.05$) in the concentration of specific IgE after total IgG adsorption (Figure 2b). From 49 samples, 14 (28.6%) showed an increase in Der f2 specific IgE concentration, after total IgG adsorption.

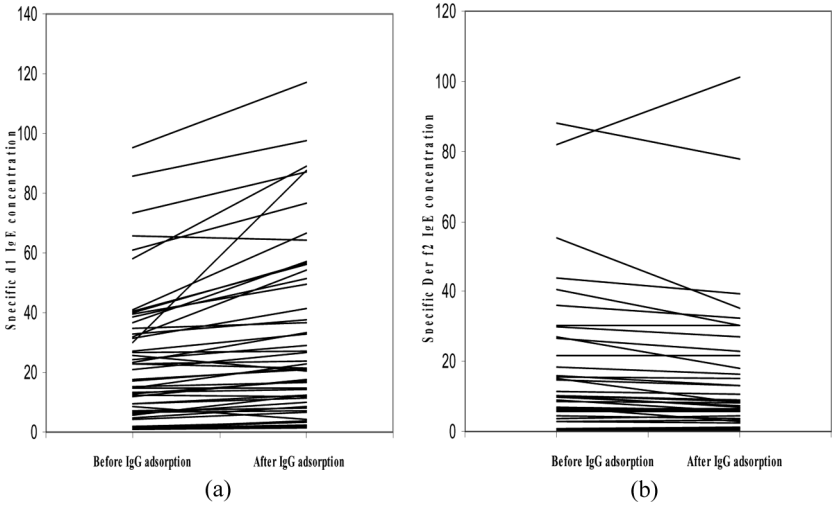

Figure 2. Comparative analysis of d1 (a) and Der f2 (b) specific IgE before and after total IgG adsorption. It was found a statistically significant increase ($p < 0.01$) in the concentration of d1 specific IgE and a statistically significant decrease ($p < 0.01$) in the concentration of Der f2 specific IgE after IgG adsorption.

Concentration of d1 and Der f2 Specific IgE After Addition of Rabbit Serum with d1 Specific IgG

The next step was to establish if the statistical interference was dependent on the interferent concentration, by adding different concentrations of interferent to different samples. Five dilutions of d1 specific IgG were prepared (1/10, 1/20, 1/40, 1/80, and 1/160) and were tested in eight patient samples for d1 and Der f2 specific IgE. As a control, we used samples treated in the same way but using rabbit serum without the interferent (Table 1).

The results showed no significant statistical difference ($p = 0{,}578$) in the d1 specific IgE values before and after the addition of d1 specific IgG (Fig. 3a). On the other hand, it was observed a significant increment ($p < 0.05$) in Der f2 specific IgE with the increase of d1 specific IgG dilution (Figure 3b). To investigate if this increment was due to the method imprecision, the average and the standard deviation of each

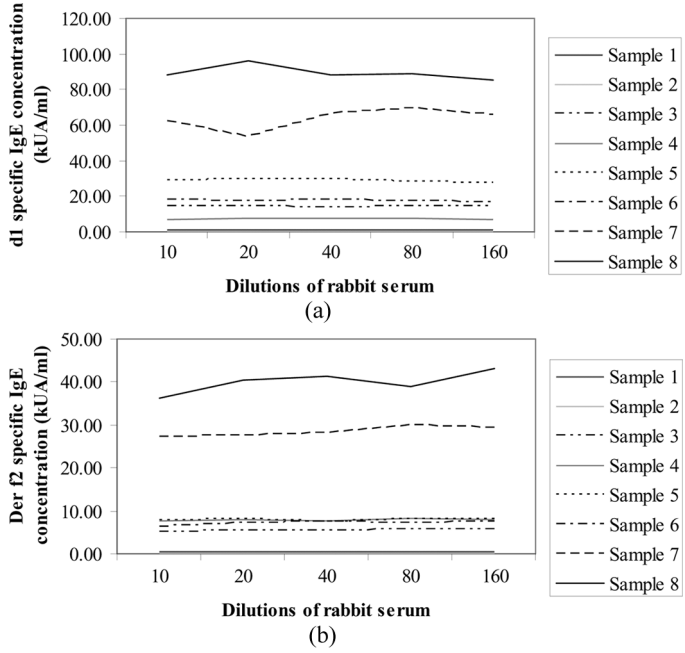

Figure 3. Comparison of d1 (a) and Der f2 (b) specific IgE concentrations in eight samples after addition of different dilutions (1/10, 1/20, 1/40, 1/80 and 1/160) of rabbit serum with d1 specific IgG. No significant statistical differences ($p = 0.578$) in the d1 specific IgE values (a) were observed after the addition of d1 specific IgG, but a significant ($p < 0.05$)increase in Der f2 specific IgE (b) was detected with the increase of d1 specific IgG dilution.

Table 2. Medians of d1 and Der f2 specific IgE before and after the total IgG adsorption

	Median
d1 specific IgE	19,20
d1 specific IgE after adsorption of total IgG	21,45
Der f2 specific IgE	6,71
Der f2 specific IgE after adsorption of total IgG	6,00
Intra assay mean value of variation of	1,07
Difference between medians in d1	2,25
Difference between medians in Der f2	0,71

sample was calculated. The obtained standard deviation average (average value of variation) was 0.55. On the other hand, the method's precision test gave us an intra assay average value of variation of 1.07 (Table 1). As this value was superior of the average value of variation found in the addition assay (0.55), the increased specific IgE concentration was not considered significant.

When non immunized rabbit serum was used, no significant differences ($p = 0.525$ for d1, and $p = 0.592$ for Der f2) were detected in the specific IgE concentration before and after the d1 specific IgG addition procedure.

To verify if the statistical interference found in the total IgG adsorption study was analytically significant, we have calculated the median of anti d1 and Der f2 specific IgE before and after total IgG adsorption (Table 2). Since the difference between medians for d1, as for Der f2, is less than 3.21 (three times the standard deviation found in a precision study of the method, with a 99,86% confidence level), analytically significant interference of specific IgG in the quantification of mite specific IgE was not observed. Clinically significant interference was not also detected because this would only occur if there was analytically significant interference.

DISCUSSION

Allergic disease is a worldwide increasing health problem, so it is advisable to have an accurate and well done diagnosis to define the best therapeutic.[11] The use of standardized and reproducible methods can contribute to the quality of diagnosis of allergic disease.[3] One of the problems identified, concerning one of the most used system, the ImmunoCAP 250®, is the fact that it allows not only specific IgE linking to allergen, but also other isotype of immunoglobulins, such as specific IgG. This can be a source of immunological interference by means of

solid phase allergen competitive blocking. Although the strategy of presenting an excess of allergen linked to solid phase is an advantage to allows free-access of all specific IgE existing in the patient sample, a possible interference of IgG can occur due to its high concentration and variable avidity.[3,6,12] In this way, the aim of this work was to verify if the IgG is, in fact, an important source of interference in this method.

The results showed a statistically significant alteration ($p < 0.05$) in the specific IgE concentrations, using either d1 or Der f2, after the adsorption of total IgG. We found an increase in the specific IgE concentration in 87,8% of the studied samples when d1 was used as solid phase. Surprisingly, it was observed a reduction in Der f2 specific IgE concentrations in 55,1% of the studied samples, after the total IgG adsorption. This result can be explained by a matrix effect and can be related with the adsorption protocol since the sample is diluted 1/10 and then one of the sample constituents was removed by addition of an absorbent. On the other hand, this effect was not observed when we used d1, because in this case the interference of specific IgG can be enough to overlap this matrix effect.

The results of the addition of different rabbit d1 specific IgG concentrations, suggests that the statistical interference found in the adsorption study, is independent of interferent concentration. In spite of the observed significant statistical interference, it can not be considered neither as a significant analytically interference, nor as a significant clinically interference.

This work supports the idea that in analysed method the strategy of presenting an excess of allergen linked to solid phase, really allows free-access of all specific IgE present in the patient sample, independently of the specific IgG concentration. The reliability of this method can be considered a valuable tool for determination of the specific IgE concentration and therefore can contribute to the correct allergic disease diagnosis.

ABBREVIATIONS

IgE, Immunoglobulin E; IgG, Immunoglobulin G; d1, total extract of *Dermatophagoides pteronyssinus*; Der f2, recombinant allergen of *Dermatophagoides farinae*; FEIA, immunofluorenzimatic method; Dp, *Dermatophagoides pteronyssinus*; Df, *Dermatophagoides farinae*; SEQC, Spanish Society of Clinical Chemistry and Molecular Pathology

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