Clin Chem Lab Med 2008;46(5):687–690 © 2008 by Walter de Gruyter • Berlin • New York. DOI 10.1515/CCLM.2008.131

Short Communication

Time to reconsider the clinical value of immunoglobulin G4 to foods?

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Abstract

Background: The usefulness of serum antibodies to common food antigens (immunoglobulin G4; IgG4) assay in management of patients suffering from food intolerance was assessed.

Methods: A total of 22 asymptomatic healthy subjects and 68 patients with symptoms referred for suspected food intolerance were studied. Serum IgG4 to 19 common foods was measured by an automated immunoassay.

Results: The area under the receiver operating characteristic curve was 0.92 (standard error 0.04) and, at a threshold value of 2.3 U/mL, the IgG4 determination had a sensitivity of 0.81, with a specificity of 0.87. With a pre-test probability of 5% and 20%, the post-test probability of having disease was found to be 24% and 61%, respectively, and 1.1% and 5% if the result was negative. Cohen's κ value (0.83) indicated a good agreement between symptoms and IgG4 concentrations.

Conclusion: Serum IgG4 assay may play a role in ruling out food intolerance, because of its satisfactory negative predictive value (0.99).

Clin Chem Lab Med 2008;46:687–90.

Keywords: diagnostic accuracy; exclusion diet; food intolerance; food-specific IgG4 antibody.

Food intolerance is recognised in up to one-fifth of the general population (1). Currently, the management of this condition consists of exclusion and reintroduction diets, with the subsequent confirmation of outcomes by means of the double-blind vs. placebo test. However, an approach involving the identification of the offending food(s) by dietary elimination and re-challenge can be cumbersome, and poor patient compliance can compromise its clinical efficacy. Because it is difficult to diagnose food intolerance, its true prevalence is unknown, but it has been estimated to occur in approximately 5% of the general population (2–4). Moreover, our understanding of the pathophysiology of food intolerance is incomplete, and this drawback is paralleled by a paucity of options available for the diagnostic work-up. Elevated values of serum IgG4 (immunoglobulin G4 subclass) antibodies to specific food antigens, before dietary exclusion, may prove useful in targeted dietary exclusion, obviating the need to exclude a large number of foods from the diet. We therefore investigated the appropriateness of using in vitro diagnostics for food intolerance based on food IgG4 determination in order to evaluate the potential role of this measurement method in patient management.

Serum IgG4 concentrations were evaluated in subjects classified with no symptoms associated with food ingestion and following a free diet without any restrictions (healthy controls). To assess the IgG4 concentrations in food intolerance, sera of patients classified with adverse food reactions were determined (study group). Although perceived food intolerance was not verified by a double-blind placebo controlled food challenge, subjects received a food-specific IgG4 antibody-guided exclusion diet for at least 2 months. At the end of the regimen period, all patients reported their symptoms and, if feasible, were given a repeat IgG4 assay.

Subjects in the control group, consisting of 22 asymptomatic healthy subjects (median age 39 years, range 25–55 years; 4 males and 18 females) recruited from the hospital staff, were asked specific questions about the absence of bowel symptoms, atopic dermatitis, bronchial asthma, headache related to food ingestion, pruritus without dermatitis, gastroenteritis and antibiotic consumption in the month prior to the start of the study, because transient alterations in gut permeability in healthy individuals, caused by various factors (gastroenteritis, antibiotics, altered microbial flora, stress), unlike in patients, may induce a transient IgG4 response to food antigens (5).

The study group consisted of 68 consecutive patients (median age 36 years, range 10–71 years; 26 males and 42 females) who were referred to our clinical service from September 2003 to January 2005 with symptoms, such as meteorism, diarrhoea, functional dyspepsia, food intake related headache. Clinical evaluation ruled out adverse reaction to lactose (evaluated by lactose breath test) or celiac disease (evaluated by anti-transglutaminase antibody), pathological gastrointestinal diseases and psychological disorders presenting with gastrointestinal symptoms.

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Received October 24, 2007; accepted January 28, 2008; previously published online February 26, 2008

Following the Helsinki II declaration, the design and execution of the experiment was thoroughly explained to the subjects, and informed consent was obtained from all subjects. The patients received a food-specific IgG4 antibody-guided exclusion diet for at least 2 months. In particular, they received a list of the foods they had been advised to eliminate and telephone contact details where they could contact personnel for further advice if necessary. Because 17 subjects either failed to follow the dietary indications or were not contactable, response to the regimen was established in 51 patients. A total of 19 patients, responding well to dietary exclusion after the first assay, underwent a second IgG4 assay test after the diet.

Serum IgG4 antibody concentrations to common foods, including milk, egg white, wheat, casein, rice, yeast, potatoes, peanuts, cod fish, chicken, lamb, beef, pork, tomatoes, carrots, onions, apples, bananas and soy beans, were measured. Blood samples were left to stand for 20–30 min before being centrifuged at 3000 cycles/min (1300×*g*) for 15 min. The serum was separated and frozen at −20°C for subsequent analysis. Samples were processed using a commercially available immunoassay (Enea Specific IgG4, BioAllergy International, Trieste, Italy). The antibody titers were expressed as U/mL and the measured range was between 0 U/mL and 30 U/mL. Inter- and intra-assay variation coefficients ranged from 35% (mean 10.5 U/mL) to 23% (mean 27.00 U/mL), with some further differences among allergens.

The mean difference between the two study groups was analyzed using the unequal variance t-test. A p-value <0.05 was considered statistically significant.

Receiver operating characteristic (ROC) curve analysis (6) defined the IgG4 cut-off with the best diagnostic sensitivity and specificity and the highest diagnostic power to discriminate between patients classified with adverse food reaction and control subjects. In view of the difficulty in diagnosing food intolerance, we defined as true positives the cases in which patients reported a dramatic reduction in all the symptoms over 2 months following the food-specific IgG4 antibody-guided exclusion diet.

Yet, also in view of the known diagnostic difficulties, the true prevalence of food intolerance remains unknown. We therefore calculated the accuracy measurements of the test in two different populations: patients referred to general practitioners and those referred to allergy specialists. In the former category, the prevalence of subjects reporting a complaint following the ingestion of a particular food was estimated at approximately 5%; in the second category, the estimated prevalence increased to approximately 20%, because patients had already been screened for food protein-induced enteropathy, such as celiac disease, or for lactose deficiency.

Agreement between categorical data was measured by Cohen's κ, the chance corrected proportional agreement (7). Although no objective criteria were available for judging intermediate values, κ is often considered to provide agreement, which is poor if <0.2, fair if $0.21<\kappa<0.40$, moderate if $0.41<\kappa<0.60$, substantial if $0.61<\kappa<0.80$ and good if $\kappa>0.80$.

IgG4 concentrations to milk, egg white, wheat, rice, pork, tomatoes, apples and bananas were significantly ($p<0.001$) lower in the control subjects than in patients classified with adverse food reaction. In particular, egg white and cow's milk specific IgG4 (the most frequently represented antibodies in our population) were 0.8±0.3 and 3.1±2.1 U/mL [mean and standard error (SE)] in the control group, respectively. In the patient group, IgG4 values were 11.9±1.7 and 13.7±1.6 U/mL, respectively (Figure 1).

Figure 2 shows the results of the ROC curves analysis for the cut-off value. The area under the ROC curve (AUC) of IgG4 specific for cow's milk (Figure 2, AUC in bold font) was 0.89 (SE 0.06) and, at a threshold value of 2.8 U/mL, IgG4 determination had a sensitivity of 0.83, with a specificity of 0.92. When comparing controls with patients classified with adverse reaction to egg white, the AUC (Figure 2, broken line) was 0.87 (SE 0.07), and at a threshold value of 2.0 U/mL, IgG4 determination showed a sensitivity of 0.73, with a specificity of 0.83.

When prevalence was considered 5% (current estimate), the positive predictive value (PPV) was low (0.35 for cow's milk IgG4; 0.25 for egg white IgG4) and the relative number of false positives high, although both sensitivity and specificity were good. The negative predictive values (NPV) were 0.99. As expected, on increasing pre-test probability value to 20%, PPV

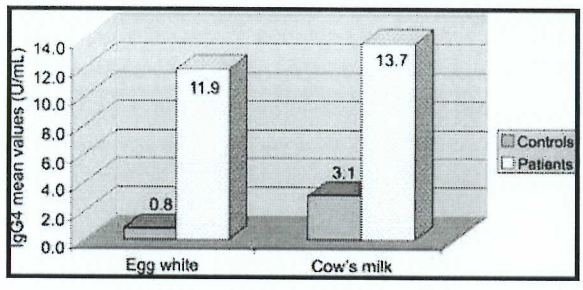

Figure 1 Egg white and cow's milk specific serum IgG4 mean values (U/mL) in control and patient groups.

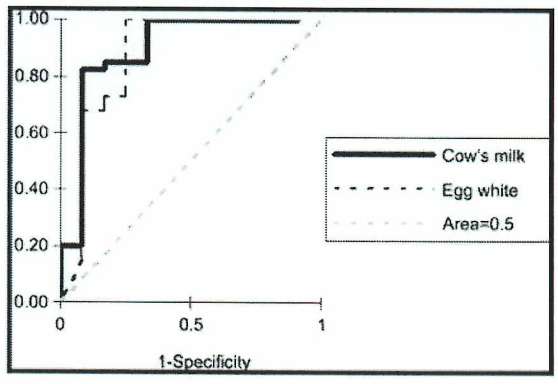

Figure 2 ROC curves for IgG4, in bold font and broken line, when comparing controls with patients whose symptoms resolved following milk or egg white exclusion diet, respectively.

also increased to 0.72 and 0.61, respectively, and NPV maintained the value of 0.95.

The likelihood ratio was calculated to estimate the extent to which the test result would change the odds of having intolerance, and, for a positive value (LR+) of IgG4 measurement it was 10.38 for cow's milk and 6.23 for egg white, meaning that the odds of having the syndrome increased ten- or six-fold, respectively, when the test is positive.

The likelihood ratio for a negative value (LR–) in IgG4 measurement was 0.18 for cow's milk IgG4 and 0.22 for egg white, meaning that the odds of having the syndrome decreased by 0.05 when the test is negative.

When the diagnostic test result was positive, the post-test probability was 35% (milk), 25% (egg white), and 72% (milk) and 61% (egg white) with a pre-test probability of 5% and 20%, respectively. With a negative result, the post-test probability was 1% (milk and egg white) and 4%–5% (milk and egg white) with a pre-test probability of 5% and 20%, respectively (Figures 3 and 4).

Cohen's κ value, an index of agreement between symptoms and IgG4 concentrations, was found to be 0.83.

The exclusion diet and the double-blind food challenges are currently considered the best available methods of identifying food to which patients are intolerant. However, a biomarker that could accurately diagnose symptomatic food intolerance would greatly facilitate clinical practice. The results of our study, showing a significant difference between the control and patient groups suggest that IgG4 determination may play a role in differentiating subjects

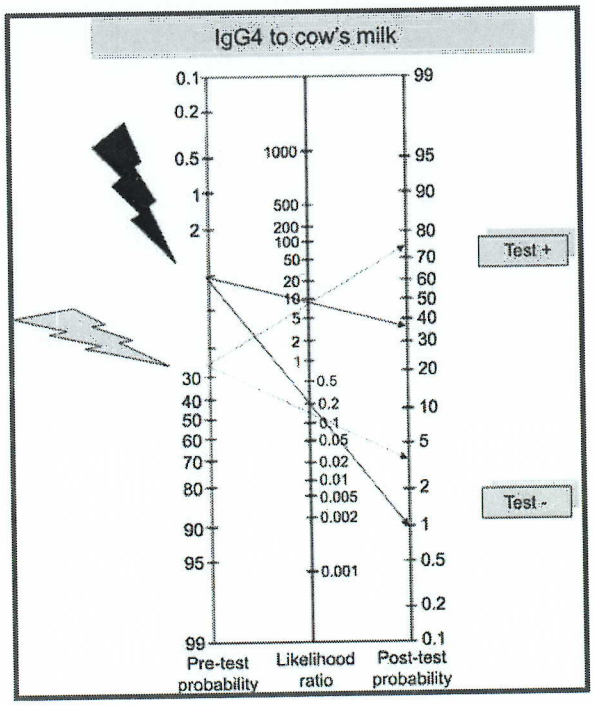

Figure 3 Pre- and post-test probability of IgG4 to cow's milk determination.

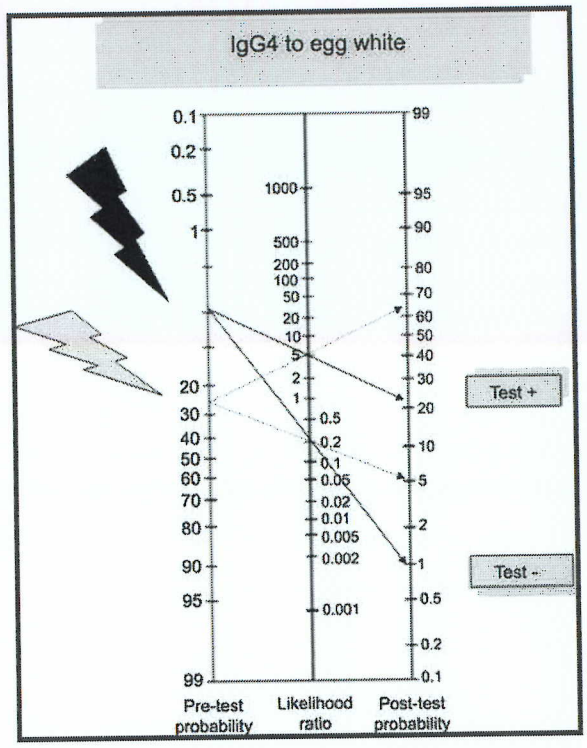

Figure 4 Pre- and post-test probability of IgG4 to egg white determination.

with from those without food intolerance. In particular, the threshold value selected provides the best diagnostic sensitivity and specificity for IgG4 determination, thus it appears to be an assay with a good diagnostic accuracy. However, sensitivity and specificity do not solve the problems of the prevalence of the condition in different populations worldwide. Therefore, the IgG4 predictive value was calculated, providing a low PPV in the group with a prevalence of 5%. Thus, in screening the general population, many individuals with false positive test results would be obtained. On the other hand, the NPV was very good, showing that the condition can be ruled out if the screening result is negative. As expected, on increasing pre-test probability value to 20%, the PPV increased accordingly and the NPV value was maintained; this means that the test can be used to rule out the condition. Moreover, we quantified the probability of a patient having the disease by considering the post-test odds after measuring serum IgG4 and a negative result appears to have a greater impact than a positive test result, in particular with a prevalence of 5%. This confirms that the test is better at ruling out food intolerance than ruling it in, thus sparing the patient an unnecessarily restrictive diet and allowing appropriate treatment to be promptly initiated. Furthermore, an evaluation was made of the agreement between IgG4 concentrations and clinical data by assessing the patient's response (based on the patients' symptoms and IgG4 variation) to food-specific IgG4 antibody-guided exclusion diet over 2 months. Cohen's κ value indicated a good agreement. The response after 2 months was, in fact,

encouraging, with symptoms resolving in 78.5% of subjects. The IgG4 results following the second assay, available in 19 subjects, showed that IgG4 values decreased after 2 months of diet in 89.5% of these patients.

The present study has several limitations: a) the number of control subjects was small; b) the value for decreased IgG4 after the exclusion diet should have been evaluated in a larger number of patients in a prospective trial; and c) food responsible for symptoms was reintroduced in only a few cases for both ethical and organizational reasons. However, our preliminary data suggest that serum IgG4 could accurately rule out symptomatic food intolerance and would greatly facilitate clinical practice. However, the non-satisfactory PPV does not allow clinicians to use it as a definitive confirmatory tool. Further studies should confirm these data in a more representative number of patients and controls.

References

1. Young E, Stoneham MD, Petruckevitch A. A population study of food intolerance. Lancet 1994;343:1127–30.
2. Woods RK, Abramson M, Bailey M, Walters EH. International prevalences of reported food allergies and intolerances. Comparison arising from the European Community Respiratory Health Survey (ECRHS) 1991–1994). Eur J Clin Nutr 2001;55:298–304.
3. Crowe SE, Perdue MH. Gastrointestinal food hypersensitivity: basic mechanism of pathophysiology. Gastroenterology 1992;103:1075–95.
4. Shanaham F. Food allergy: fact, fiction, and fatality. Gastroenterology 1993;104:1229–31.
5. Zar S, Benson MJ, Kumar D. Food-specific serum IgG4 and IgE titers to common food antigens in irritable bowel syndrome. Am J Gastroenterol 2005;100:1550–7.
6. Astute. Statistics add-in for Microsoft Excel. DDU software ver. 1.5. Leeds, UK: The University of Leeds, Old Medical School, 1995.
7. Petrie A, Sabin C. Medical statistics at a glance, 2nd ed. Oxford: Blackwell Science Ltd., 2000.