Laboratory Tests for Total and Allergen-specific Immunoglobulin E

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ABSTRACT

The principles of measurement of IgE by enzyme-immunoassay are given. A Bayesian approach is taken for the clinical interpretation of serum total IgE. Application of Bayes’ Theorem to serum total IgE can significantly influence the probability of a clinical diagnosis of allergy.

There is a correlation between allergen-specific IgE measured by enzyme-immunoassay, by radioallergosorbent test and by skin tests for immediate hypersensitivity. However, there is a need to standardize the reagents used for allergy skin testing, as well as the reagents and methods used for the laboratory measurement of allergen-specific IgE.

INTRODUCTION

IgE is a principal immunological mediator of allergic diseases. Receptors on the membranes of mast cells and basophils bind the Fc region of IgE antibodies. Allergen-induced crosslinking of IgE in the plane of the membrane leads to Ca++-dependent degranulation by exocytosis, and the release of pharmacologically active mediators of allergic inflammation from the cells. Preformed mediators released from the granules include: histamine, various proteases, eosinophil chemotactic factor of anaphylaxis (ECF-A), heparin, and neutrophil chemotactic factor (NCF). Newly synthesized mediators which are derived from the cell membrane as a result of degranulation include: prostaglandin D2, the leukotrienes LTC4, LTD4 and LTE4, and platelet activating factor. The preformed and newly synthesized mediators produce allergic inflammation in the target tissues.

Because of the importance of antibodies of IgE isotype as mediators of allergic symptoms, we will consider tests for IgE as tools for the diagnosis of diseases caused by allergic inflammation.

MEASUREMENT OF IgE

Total and allergen-specific IgE can be detected and quantitated with precision and safety in the office laboratory by enzyme-immunoassay. The principle of the enzyme-immunoassay for total IgE is diagrammed in Figure 1. The sample to be tested for IgE is incubated with anti-IgE antibodies insolubilized on an immunosorbent surface. IgE in the sample is allowed to react with the insolubilized anti-IgE and excess IgE is washed away. The use of the solid-phase immunosorbent permits separation of bound from unbound IgE molecules by simple washing and aspiration. Following removal of unbound IgE molecules, anti-IgE conjugated to an enzyme is added and reacted with the IgE adsorbed to the immunosorbent. Excess reagents are washed away and the amount of enzyme adsorbed to the immunosorbent surface is determined by assay of bound enzymatic activity. Bound enzymatic activity is related to IgE by a standard curve.

The correlation between enzyme-immunoassay and radio-isotopic assay for total IgE is extremely high. For
example, the correlation coefficient for the regression analysis of the fluororallergosorbent test (FAST) and a sandwich radio-immunoassay for total IgE is greater than 0.97.5

The enzyme-immunoassay for allergen-specific IgE is similar to that for total IgE except that allergen, rather than anti-IgE antibody, is on the solid-phase immunosorbent (Figure 2).

**Figure 1.** Enzyme-immunoassay for total IgE.

**Figure 2.** Enzyme-immunoassay for allergen-specific IgE.

**BAYESIAN INTERPRETATION OF TOTAL IgE CONCENTRATION**

The test for serum total IgE can improve the estimate of the probability that a disease has an allergic basis. First, an estimate is made of the probability of allergy based on clinical criteria, including:

1. allergic and general medical history;
2. family history;
3. physical examination of the patient.

The estimate of the probability of allergy based on the above three factors is called a prior probability. After estimating the prior probability of allergy, the test for IgE is performed and the test result is used to correct to prior probability. The corrected prior probability is called a posterior probability. The posterior probability is calculated from Bayes' Theorem. Bayes' Theorem is useful for medical decision-making,6 and for other decision-making under conditions of uncertainty.7,8

Bayes' Theorem for the posterior probability is given below:

\[
\text{posterior probability} = \frac{\text{joint probability}}{\text{marginal probability}}
\]

where,

\[
\text{joint probability} = (\text{conditional probability})(\text{prior probability})
\]

and, the marginal probability is the sum of the joint probabilities.

The conditional probability is the probability of an observed IgE concentration in either the allergic or non-allergic state. The conditional probability can be calculated from the frequency distributions of IgE concentration, derived from the cumulative frequencies of IgE concentration reported by Marsh et al.9 Because the cumulative frequencies were normalized to 100 by Marsh et al., the conditional probability is the relative frequency divided by 100. The conditional probabilities for various concentrations of IgE in the allergic and non-allergic states are given in Table I. For example,
### Table I

<table>
<thead>
<tr>
<th>IgE Concentration↓†‡</th>
<th>Non-Allergic State†</th>
<th>Allergic State†</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
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<td>.00</td>
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<tr>
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<td>.01</td>
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<td>.01</td>
</tr>
<tr>
<td>12800</td>
<td>.00</td>
<td>.01</td>
</tr>
</tbody>
</table>

† The conditional probability of an IgE concentration in either the non-allergic or allergic state is the normalized relative frequency of that concentration, which was calculated from the cumulative frequencies reported by Marsh et al.9

‡ IgE concentration is in International Units per ml.

The conditional probability of an IgE concentration of 12.5 IU/ml, given the allergic state, is equal to 0.01. The conditional probability of an IgE concentration of 800 IU/ml, given the non-allergic state, is equal to 0.02.

The joint probability is the conditional probability multiplied by the prior probability. Thus, the joint probability is the probability of the patient being allergic, as estimated clinically, and also having a certain IgE concentration, as determined by the test for total IgE. The marginal probability is the sum of the joint probabilities for the allergic and non-allergic states, at any observed IgE concentration.

That the result of the assay for serum total IgE can have a profound effect on the diagnosis of the likelihood of allergy in a given patient is seen from the posterior probabilities given in Table II. The posterior probabilities for allergy were calculated from Bayes' Theorem, using the conditional probabilities from Table I, for various clinical estimates of the prior probabilities. Suppose the clinical estimate of the chance of allergy is only 10%, corresponding to a prior probability of 0.10. A serum total IgE of 800 IU/ml would raise that estimate to 42%. An IgE concentration of 1600 IU/ml, or higher, would make the diagnosis of allergy virtually certain, provided that non-allergic diseases which are associated with elevated IgE concentrations are ruled out. Some non-allergic diseases associated with high IgE include schistosomiasis, trichinosis, Hodgkin's disease, graft versus host disease, IgE myeloma and Kawasaki's disease. It usually is not difficult to rule out a non-allergic basis for a hyper-IgE state.

Suppose the prior probability of allergy is only 0.3, corresponding to more than two to one odds against the diagnosis of allergy. A serum total IgE of 800 IU/ml reverses those odds to more than two to one in favor of allergy (Table II).

Suppose the allergist is clinically unable to distinguish between allergy and non-allergy in a particular patient (prior probability = 0.5), i.e., the odds are 50% in favor of the diagnosis of allergy and 50% against. An IgE concentration below 100 IU/ml shifts the odds in favor of diagnosing other than allergy, while a concentration much greater than 100 IU/ml makes the diagnosis of allergy highly likely (Table II).

Even if the allergist were 70% certain of a diagnosis of allergy (prior probability equals 0.7) on the basis of the clinical impression and other data, an IgE of 12.5 IU/ml would reduce the probability of allergy to 0.44, which is greater than even odds against the diagnosis (Table II). In contrast, the result of the serum total IgE assay has little effect on the diagnosis of allergy if the physician is 90% certain of that diagnosis.

The results given in Table II show that:

(1) the posterior probability for the diagnosis of allergy

<table>
<thead>
<tr>
<th>IgE Concentration†‡</th>
<th>Prior Probability†</th>
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</thead>
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<td>12.5</td>
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<td>.04 .13 .25 .44 .75</td>
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<tr>
<td>800</td>
<td>.09 .28 .47 .68 .89</td>
</tr>
<tr>
<td>1600</td>
<td>.42 .74 .87 .94 .98</td>
</tr>
<tr>
<td>3200</td>
<td>1.00 1.00 1.00 1.00 1.00</td>
</tr>
</tbody>
</table>

† The prior probability is the clinical estimate of the probability of allergic disease, prior to obtaining the result of the assay for serum total IgE. The numbers in the table are the posterior probabilities of allergic disease, calculated from Bayes' Theorem, using conditional probabilities from Table I.

‡ IgE concentration is in International Units per ml.
is greatly influenced by the prior probability of allergy, as determined from the clinical diagnosis; (2) a high IgE concentration increases the likelihood of allergy, especially if allergy is initially thought to be unlikely; and (3) a low IgE decreases the likelihood of allergy, but cannot override the effect of a very strong clinical impression in favor of the diagnosis of allergic disease.

ALLERGEN-SPECIFIC IgE

Correlation Between GIST and RAST

The enzyme-immunoassay for total IgE has been adapted to allergen-specific IgE (Figure 2). We developed an enzyme-immunoassay for allergen-specific IgE based upon covalent conjugates of galactosidase.\textsuperscript{11} Figure 3 shows ragweed-specific IgE measured by the galactosidase immunosorbent test (GIST) and by the radioallergosorbent (RAST). The correlation coefficient for the regression of the GIST on the RAST is 0.97 ($p < 0.01$).

Correlation Between FAST and RAST

The data of Seltzer et al.\textsuperscript{12} comparing the fluorocallergosorbent test (FAST) with the RAST, were analyzed by linear regression. To accomplish this analysis, the results reported by Seltzer et al. as a frequency histogram for FAST class and modified RAST class were converted to individual data points. Linear regression analysis was conducted on the individual data points, which numbered 436, corresponding to 436 sera tested with 94 allergens. The regression line obtained for the data of Seltzer et al. was:

\[ y = 0.788 + 0.628x \]

where $y$ is the allergen-specific IgE FAST class and $x$ is the modified RAST class.

The correlation coefficient relating the allergen-specific FAST and RAST exact class was 0.67. This correlation is statistically significant ($p < 0.01$), for the 434 degrees of freedom associated with the 436 serum samples, but does not fully reflect the potential power and elegance of both the FAST and RAST technologies.

The correlation between FAST and RAST may be improved by standardization of allergens used to prepare the immunosorbents. In addition, the FAST and RAST use different types of immunosorbent. Insolubilization can be achieved through either covalent or non-covalent linkage of the allergen to the surface of the solid phase. The RAST utilizes allergens which have been covalently linked to a cellulose immunosorbent by the CNBr method.\textsuperscript{13,14} The FAST utilizes allergens which have been physically adsorbed by non-covalent bonding to a plastic immunosorbent surface by a proprietary method. Lack of allergen standardization, and the different methods of insolubilization, may partially explain disparate results between the FAST and the RAST for allergen-specific IgE antibodies.

Correlation Between FAST and Skin Tests

The correlation between the IgE-specific FAST and the skin test was determined for short ragweed al- lergen from the data of Tsay and Halpern.\textsuperscript{5} In this study, skin testing by end-point dilution was compared with FAST class in 20 subjects with differing degrees of sensitivity. Figure 4 shows the data of Tsay and Halpern, analyzed by statistical regression. The correlation between skin test and allergen-specific IgE FAST was extremely high ($r = 0.95$).

Seltzer et al.\textsuperscript{12} compared allergen-specific FAST with skin tests in eight subjects, using 60 allergens. Regression analysis was carried out on the data of Seltzer et al., after converting the published frequency histograms to individual data points. The data of Seltzer et al. yielded the following regression equation:

\[ y = 0.514 + 0.562x \]
where \( y \) is the allergen-specific IgE FAST class and \( x \) is the class score of the prick puncture skin test. There were 253 classified data points in the data of Seltzer et al., with a correlation coefficient of 0.59. This correlation is significant for 251 degrees of freedom (\( p < 0.01 \)).

**Comments on Laboratory Measurement of Allergen-Specific IgE Antibodies**

Laboratory assays for allergen-specific IgE antibodies have great potential and promise. The assays are simple, safe, and convenient. High correlation between enzyme-immunoassay and radioimmunoassay for allergen-specific IgE can be achieved at least for single allergens (Figure 3). In a published study of 436 sera tested with 94 allergens, identical classes were obtained in 42% of the cases, and a total of 81% of the tests differed by one class or less. The laboratory methods for the measurement of allergen-specific IgE antibodies must be further standardized before these methods can be used clinically.

High correlation between a laboratory assay for allergen-specific IgE and skin test can be achieved, at least for single allergens (Figure 4). In a published study which used 60 allergens, identical classes were obtained in 54% of the cases, and 86% of the results were within one class difference.

There is a need to standardize the reagents used for skin testing, as well as the reagents and methods used for the laboratory measurement of allergen-specific IgE. Published data should be ungrouped, to facilitate statistical analyses and standardization of laboratory tests for allergen-specific IgE.

**REFERENCES**