

Skin testing

The safety of skin tests and the information obtained from using different methods and concentrations of allergen

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In this issue of *Allergy*, Niemeijer et al. (8, 9) discuss the most suitable concentrations of allergen for skin testing. They used a positive RAST as an indicator of sensitization and clinical history (CH) as an indicator of clinical allergy.

The basis for documentation of diagnostic efficiency must be standardization of all materials and procedures. Since both skin test methods and the composition and total allergenic potency of extracts are essential, data given in the papers by Niemeijer et al. (8, 9) are valid only in situations when the same methods and materials are used. Only then is it possible for others to draw conclusions on the degree of sensitization and allergic disease in patients tested in the routine. Therefore, methods and materials used must be well defined to ensure that results obtained can be utilized by readers.

Skin test methods

There are several methods of both intradermal skin testing and skin prick testing. Niemeijer et al. (8, 9) used the skin prick test method as well as the method of intracutaneous skin testing recommended by the European Academy of Allergy and Clinical Immunology (EAACI) (1).

Allergens and reference substances

Niemeijer et al. (8, 9) also used well-standardized extracts with known potency according to IUIS guidelines (7). They give the biologic potency according to the Nordic Guidelines (10). Furthermore, they give the content of major allergens. However, they refer to histamine references not usually employed in other parts of Europe; that is, histamine phosphate (base) 0.01 mg/ml for intracutaneous skin testing and histamine dihydrochloride (base) 3 mg/ml for skin prick testing (9). This decreases the value of their results.

Sensitization

Niemeijer et al. (8, 9) chose to define sensitization as a positive RAST test, i.e. Phadebas RAST class ≤ 1 . It is true that an *in vitro* test for IgE, with high specificity for IgE, documents IgE sensitization. However, this is hazardous, since the same manufacturer has introduced the CAP system, which is about three times more sensitive than Phadebas RAST; therefore, it should detect circulating IgE against allergens in many more patients. Then, the cutoff limit used in this trial, 0.35 PRU/ml with Phadebas RAST, should be changed to 1.1 PRU/ml in clinics using the CAP system and to other, undefined values when they use

tests of other manufacturers. In their approach, Niemeijer et al. exclude the possibilities that more sensitive *in vitro* tests may be developed in the future and that positive skin prick tests with higher concentrations than those correlated to a positive *in vitro* IgE test indicate specific IgE on mast cells in the skin.

Clinical allergy

The definition of allergic disease is more controversial. Niemeijer et al. (8) used CH as an indicator of clinically important allergy. The definition of clinical allergy on the basis of CH is sometimes easy, as in allergy to birch or olive pollen, which have well-defined seasons. However, there is, to my knowledge, no criterion to define mite allergy other than perennial symptoms which worsen during autumn and winter; that is, a rather nonspecific CH combined with a positive diagnostic test. In northern Europe, mite allergy CH is similar to that of animal dander allergy. Most persons sensitized to dog and cat dander show symptoms not at direct contact, but after prolonged exposure, and they are therefore often unaware of the importance of animal danders for their symptoms. They also show increasing symptoms during the autumn, when returning to school or job after vacations; that is, a period without exposure to animal danders. Thus, it is obviously very difficult to distinguish between clinical allergy to mites and other environmental allergens by CH. Therefore, the results of Niemeijer et al. must be treated with caution. However, at the moment, there are no better criteria for the definition of clinical allergy caused by environmental allergens.

Sensitivity and specificity

Niemeijer et al. (8, 9) calculate the concentration of allergen giving the optimum concentration for sensitivity and specificity in combination. However, many investigators prefer the concentration of allergen of maximum sensitivity; that is, the concentration which does not exclude any patient sensitized. Then other methods or "clinical judgment" is used to distinguish between patients who are only sensitized and those with clinical allergy. Personally, I prefer the latter approach. The concentration giving a high sensitivity is probably about 100 times higher than that found by Niemeijer et al., since there is a 10^4 difference of magnitude in skin sensitivity between the most and the least sensitive patient in a university hospital allergy clinic or similar clinic with referred patients (2). This agrees with preliminary results from our group, since we found 1 million BU/ml to have the highest sensitivity in skin prick testing (3). Clinicians must know how disease (al-

lergy) has been defined, when deciding upon cutoff limits for skin tests in their practices.

The value of reference substances

Niemeijer et al. (8, 9) use a ratio between the wheal response to allergen and histamine, as recommended by the Nordic Committee on Allergen Standardization some years ago. Such a ratio depends on several factors, such as the concentration of histamine and allergen used and the degree of atopy in the population investigated. Stucky et al. (12) found that the histamine wheal was larger in patients with a higher degree of atopy as measured by the number of positive skin tests and the level of total IgE. Whether reactions to allergens increase to the same extent in patients with many other positive tests or a high IgE level, as do reactions to histamine, is difficult to determine, since skin reactivity must be investigated in patients with similar degree of allergy to the allergen studied and with varying levels of total IgE or numbers of positive skin tests. Methods for determination of these variables are correlated. However, on the basis of this knowledge, reference to histamine or any other reference substance can no longer be recommended, except for biologic standardization. Instead we should recommend documentation of the technique used for skin testing by giving the allergen concentration and the mean histamine wheal size, with the histamine concentration recommended for trials and general use; that is, histamine dihydrochloride 10 mg/ml (6.3 mg histamine base/ml) (3). In clinical trials and epidemiologic studies, an independent measure of the degree of atopy should be used, as by giving the median of total IgE or, even better, allergen-specific IgE with a well-established method, to ensure that differences found by different investigators are not due to differences in skin test technique. Furthermore, at least duplicate skin tests should be used, to allow calculation of the precision of the test technique employed, since, if the skin test technique is not precise, positive tests may be overlooked because of accidentally negative tests, and therefore the prevalence found will be lower than the "true" prevalence.

Safety

Some years ago, intradermal skin testing was always performed by skin titration. In the last decade, routine skin testing has been more or less exclusively performed by skin prick testing with one concentration of allergen. At the same time, some investigators still use intradermal skin testing even when testing with new substances of unknown allergenic potency, ignoring the old experience that severe general reactions may occur (5, 6). In the case of unknown po-

tency of the test substance, extremely low concentrations are used for the initial test (1). The risk for severe general reactions has increased because investigators have lost their former respect for skin testing during the last decades when skin prick tests have become predominant, and because pure proteins can be produced in large quantities, making it possible to use concentrations which are extremely high.

Recently, anaphylaxis after intradermal testing with phytohemagglutinin (PHA) was reported in this journal by Schiavino et al. (11). Their report was the first on anaphylaxis after intradermal injection of PHA. The patient had no known allergy to the substance, 1 mg/ml of which had been used for intradermal skin tests, and the intention was not to test for IgE-mediated allergy. However, that situation is typical of most accidents. Niemeijer et al. (8) found that 30 BU/ml was safe and best suited for diagnosis according to their criteria. One BU corresponds roughly to 1 ng of major allergen (4). Thus, severe general reactions from intradermal skin testing can be avoided by using concentrations of substances with unknown allergenic properties of 30 ng/ml, according to Niemeijer et al. From this calculation it follows that the injected dose of PHA (11) was approximately 30 000 times too high if PHA was expected to react as an allergen.

Alternatively, a titration starting at this level can be recommended, or a pretest with the same allergen by skin prick and a higher concentration, such as 10 000 BU/ml (10 µg/ml), continuing with an intradermal skin test with the same concentration if the skin prick test is negative. Titration by skin prick testing can be performed as a safety measure. The increase in wheal size when the allergen concentration is increased 10 times is 1.5 times; that is, from 3 mm in diameter to 4.5, 6.8, 10.3, 15.2, etc. Thus, if the initial test is negative, that is, less than 3 mm in diameter, and if four 10-fold concentrations are then added, no wheal will be larger than 15 mm in diameter, posing no risk to the patient.

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