

3. Skin tests for diagnosis of IgE-mediated allergy

The most important part of clinical diagnosis of specific atopic allergy (to a certain allergen) is the history of the patient, taken by an experienced allergist. Skin tests are used to confirm IgE-sensitization of the skin which is one possibility to support specific allergy diagnosis. Recently several position statements on skin testing have been published (3, 31).

3.1 DIAGNOSTIC CRITERIA

Skin testing means introduction into the superficial layer of (application on) the skin of allergenic material. Provided IgE-antibodies, with an idiotype corresponding to the allergen introduced, are attached to skin mast cells, mast cell mediators are released, causing an early (immediate)(EPR) and under certain circumstances also a late phase reaction (LPR)(1)(See Chapter 1). The latter is more often found when high concentrations of allergen are used for testing in highly sensitive individuals (3). In this chapter, emphasis will be put on EPR, since LPR is seen in only 8% of adults using a concentration suitable for routine diagnosis by SPT (16). However, LPR is more easily induced by ICT than by SPT, since ICT is $\approx 30\,000$ times more sensitive than SPT (3). This fact should be kept in mind in scientific studies, evaluating the influence of therapeutic measures on LPR.

3.1.1. Definitions

3.1.1.1. SENSITIVITY, SPECIFICITY, POSITIVE AND NEGATIVE PREDICTIVE VALUE AND EFFICIENCY OF SPT

As for all other diagnostic tests, the presence of a positive test does not always implicate clinical disease, and a negative test does not always de-

note absence of clinical allergy. The clinical meaning of results obtained by allergy skin testing highly depends on a number of factors, as mentioned under 3.2. Definitions of sensitivity, specificity, positive and negative predictive diagnostic value and efficiency are given in Table 1 (19). These terms can also be illustrated as in Fig. 1 (13). Results obtained under certain circumstances in a clinical trial, can only be applied to routine diagnostic work provided the same potency of allergen is used, the prevalence of disease is the same, the sample of patients subject to diagnostic work has the same characteristics as that studied in the trial etc.

3.1.1.2. SENSITIZATION VERSUS CLINICAL ALLERGY

Probably most individuals who get into contact with an appropriate protein antigen, capable of inducing an IgE response (an allergen) produce low amounts of allergen-specific IgE antibodies, i.e. they get sensitized. Atopic individuals are characterized by developing higher levels of idiotypic IgE antibodies, when exposed to very low doses of allergen (21), persisting for a longer period of time than in normals. However, all the children responding with IgE antibody production at early exposure and all adults exposed to high concentrations of allergen in their occupation, who also respond with IgE antibody production, do not show clinical allergy at contact with the allergen in question. Some develop clinical allergy later and in some the test may remain positive for years after clinical symptoms have disappeared. This phenomenon was described already in the thirties and forties by Carl Juhlin-Dannfeldt (24)(Fig. 2) who named the state of sensitization, without demonstrable symptoms, latent allergy. It has been studied in "modern time" e.g. by Horak (22).

Table 1

DEFINITIONS	
TP - <u>True positive</u> :	test positive and diseased, skin test and provocation test positive.
FP - <u>False positive</u> :	test positive but not diseased, skin test positive but provocation test negative.
TN - <u>True negative</u> :	test negative and not diseased, skin test and provocation test negative.
FN - <u>False negative</u> :	test negative but diseased, skin test negative but provocation test positive.

SENSITIVITY

True positive in percent of patients with disease

$$= \frac{TP}{TP + FN}$$

		Test		
		+	-	
Allergic	+	TP	FN	TP + FN
Non allergic	-	FP	TN	FP + TN
		TP + FP	FN + TN	TP + FP + TN + FN

SPECIFICITY

True negative in percent of patients without disease

$$= \frac{TN}{TN + FP}$$

		Test		
		+	-	
Allergic	+	TP	FN	TP + FN
Non allergic	-	FP	<u>TN</u>	<u>FP + TN</u>
		TP + FP	FN + TN	TP + FP + TN + FN

+ PREDICTIVE VALUE

Patients with disease in percent of test positive individuals

$$= \frac{TP}{TP + FP}$$

		Test		
		+	-	
Allergic	+	<u>TP</u>	FN	TP + FN
Non allergic	-	FP	TN	FP + TN
		<u>TP + FP</u>	FN + TN	TP + FP + TN + FN

- PREDICTIVE VALUE

Patients without disease in percent of test negative individuals

$$= \frac{TN}{FN + TN}$$

		Test		
		+	-	
Allergic	+	TP	FN	TP + FN
Non allergic	-	FP	TN	FP + TN
		TP + FP	FN + TN	TP + FP + TN + FN

EFFICIENCY

Tested individuals correctly classified by the test in percent of all tested

$$= \frac{TP + TN}{TP + FP + TN + FN}$$

		Test		
		+	-	
Allergic	+	<u>TP</u>	FN	TP + FN
Non allergic	-	FP	<u>TN</u>	FP + TN
		TP + FP	FN + TN	<u>TP + FP + TN + FN</u>

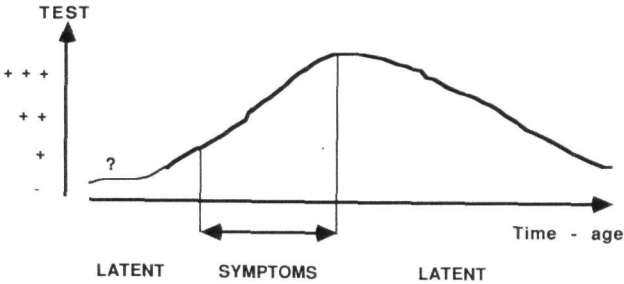
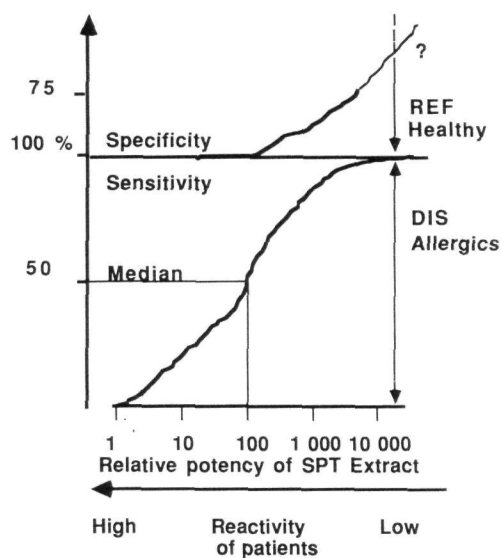


Figure 2. Patients are said to be latent allergic when they have a positive test (skin test) without any symptoms at contact. Latent allergy may precede, but also follow, clinical allergy. It probably also appears without clinical allergy, i.e. sensitization that disappears without clinical symptoms.

originating from one of several investigated concentrations (classes of weal sizes, when one concentration of allergen is used). This system makes it possible for readers of papers (later users of diagnostic tests like SPT or ICT) to understand the difference in diagnostic value when changing the cut off, as measured by allergen concentration or weal size.

3.2. FACTORS INFLUENCING THE RESULT

As mentioned above, a number of factors are of importance for the diagnostic information obtained by skin tests. These are: Definition of disease (allergy to the allergen under investigation); Potency of allergenic extract; Skin prick test technique and prevalence of disease (allergy) in the sample studied. When test techniques used by investigators and how they defined disease was well described, then their results can be applied to routine diagnosis, provided the same technique and diagnostic criteria are used by practising doctors. The degree of exposure to the allergen in question is of importance for the prevalence of allergy and in the case of continuous exposure with ongoing allergic inflammation also for the outcome of diagnostic tests, especially provocation tests.

3.2.1. Definition of disease

Definition of criteria for positive diagnosis.

3.2.1.1. HISTORY

The diagnosis of allergy to a certain allergen is mainly based on the clinical history. The history is

Figure 1. The higher the concentration of allergen used for skin testing (high to the right on the x-axis), the more patients will respond with a weal and flare reaction. 100 % sensitivity and specificity are illustrated by the horizontal line. To the left, specificity is high, the more to the right the lower the specificity and higher the sensitivity. At the concentration inducing a positive weal (≥ 3 mm in diameter) in all patients sensitive to the allergen investigated, sensitivity is 100 %. There is no need to further increase the allergen concentration. The two most important concentrations are those giving 100 % specificity and 100 % sensitivity (or close to it).

3.1.2. Suitable methods for illustration of diagnostic results

The sensitivity and specificity etc are most often given as a percentage based on cross tables as illustrated in Table 1 (19). Then only one concentration of allergen or weal size is used. However, by using the S-curve shown in Fig. 1, it is possible to illustrate the sensitivity and specificity using different concentrations, when end point titration is used, or weal sizes, when only one concentration of allergen is used. The most informative system for presentation of results of diagnostic test results in relation to disease, is that proposed by Gerhardt and Keller (20), which is illustrated by a principle drawing in Fig. 3a and an example in figure 3b (16). That system allows not only illustration of sensitivity, specificity and the other diagnostic parameters at one concentration but at several different concentrations, i.e. original data and results from several cross-tables, each one

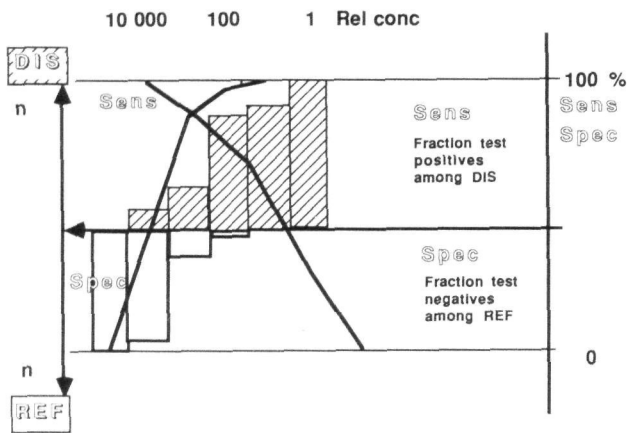


Figure 3 a. The system of Gerhardt and Keller (20). The number of individuals (left scale) with disease (allergy) are given by an histogram above the horizontal line, those without allergy, i.e. reference persons, below the line. For each class (positive ST at a given concentration) or weal size (log transformed), sensitivity and specificity can be calculated and plotted (right scale). In most situations a high sensitivity or a high specificity is preferred.

obvious, when patients get symptoms at direct contact with the allergen, e.g. animals, or when symptoms are only present during a short and well defined pollen season, e.g. the birch or plane pollen seasons. Sensitive patients exposed to low doses of allergen or patients who are only slightly sensitive and continuously exposed to high doses of allergen, do not give a clear history. Therefore, diagnostic tests must be used to support the history.

3.2.1.2. PROVOCATION TESTS

Since long, these tests have been regarded as the ultimate answer as to whether a patient is allergic or not. However, provocation tests are not physiologic, as high doses of allergen are administered during a short period of time (extract, pollens/spores or freeze-dried powdered allergen), whereas patients often are exposed to much lower doses over a long period of time. As yet, there has not been any agreement as to which doses of allergen should be used. The higher the dose, the more patients react (16). Probably, the most relevant tests are performed with pollen grains in naturally occurring doses (1 - 10 000 pollens per test) (4,8,10,30) or with powdered allergenic material (30). When conjunctival and bronchial provoca-

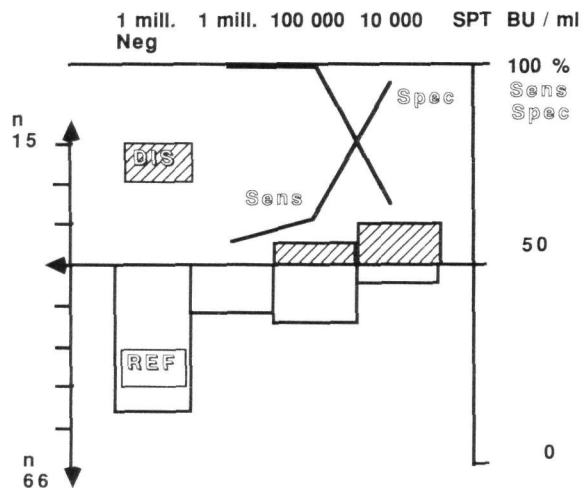


Figure 3 b. An example taken from reference (16). With dog allergy defined as symptoms at direct contact, SPT showed a high sensitivity at 100 000 BU/ml but still higher at 1 mill. BU/ml and the specificity was high at 10 000 BU/ml.

tion tests are performed in samples of patients with clinical sensitivity, then the distribution of threshold concentrations (concentration giving a positive test result at titration) has a similar S-shaped distribution as found for skin sensitivity (10). The difference in sensitivity between the most and the least sensitive patient in a sample is about the same as for SPT, i.e. 4 powers of ten (13-15,18).

It is well known that e.g. during the pollen season, patients have more symptoms during the last part of the season than in the beginning of the season, although pollen counts are similar. This has been explained by priming (10) of the shock organ by continuous pollen load for several weeks, that makes the shock organ more sensitive. This phenomenon is probably due to cumulation of late responses, which persist for several days. Thus, one pollen peak influences symptoms in patients with pollinosis for about one week (9,35). Many patients are continuously exposed to allergens, e.g. mite or animal dander allergens, and are therefore primed. Such patients will probably react to lower concentrations of allergen than non-primed individuals. It has also been shown that the hyperreactivity and sensitivity to allergen increases during the pollen season (5,6). Therefore, no definite concentration can be given that can be generally recommended as the highest relevant for clinical allergy, i.e. results from provocation tests can not be used as the golden standard for discrimination between clinical and latent allergy. This also applies to repeated low dose

provocation tests (23) as the way allergen is applied never can mimic the natural often continuous exposure to environmental allergenic particles.

3.2.1.3. OTHER CRITERIA

Patient's symptom scores correlating to exposure, e.g. pollen or mould spore counts, have been used to define patients with a true association of symptoms to the allergen in question (27,34). However, such complicated procedures are not possible to use in routine diagnosis and therefore only relevant for scientific studies aiming at increasing our knowledge about the clinical symptomatology of allergy to allergens without defined clinical picture or to define patient populations under study.

3.2.1.4 COMBINATION OF CRITERIA

The relevance of a certain less defined diagnostic criterion, does not increase because it is combined with another ill defined criterion. One possibility might be to define a sub-population of patients, obviously clinically allergic to the allergen, investigate which concentration that has the highest sensitivity in challenge testing, i.e. diagnoses most of these obviously sensitive patients by a certain challenge method, and then use this concentration for challenge testing of patients with the same specific allergy, but with less defined clinical history (16).

3.2.2. *Potency of allergenic preparations*

It has been calculated (36) that, to obtain the same response with ICT as with SPT, 30 000 times more diluted extracts can be used. Therefore, ICT should be used when extracts are available in low concentrations or the aim is to trace patients with low degrees of sensitization.

As illustrated in Fig. 1 and 3b, allergenic preparations with low potency does not diagnose all patients with clinical allergy (according to the definition of disease). In biological standardization trials, consecutive patients attending a University allergy department were included. The

sensitivity of these patients differed four powers of ten (13,16). To diagnose the least sensitive patient in such a sample, 100 000 BU/ml (See Chapter 4) should be used. To diagnose all patients in a community with clinical allergy to the same allergen but not with symptoms so severe that patients were referred to a University allergy clinic, a still higher concentration of allergen was needed (16). Actually, it was found that a 10 times higher concentration of allergen (1 mill. BU/ml) was needed to diagnose all patients (high sensitivity), whereas a ten times lower concentration (10 000 BU/ml) was sufficient to obtain a high specificity (16).

3.2.3. *Skin test technique*

When ICT is used, then the technique used is of little importance (3) provided recommended procedures are used. However, when the SPT method is used, it is of great importance (2) that a constant technique is used in the routine and that it is sufficiently documented when scientific trials are performed (13,28). Probably the pressure and the time during which this pressure is applied are the two factors which are most important. Therefore, no recommendations regarding allergen concentrations etc given by authors of scientific papers are possible to apply to routine diagnostic work, if not the same technique is used and the same precision is obtained in routine, as was achieved by the author of the scientific paper. This fact points on the need to document the technique, the precision and the size of the weal obtained with a well established reference substance such as histamine HCl 10 mg/ml (13,15,28, 37) or codein phosphate 9 mg/ml (7) in all scientific papers using diagnostic tests.

3.2.4. *Prevalence of allergy*

Diagnostic parameters such as sensitivity and specificity are most often investigated in patients attending hospitals, mainly University allergy outpatient clinics. Most often the prevalence in such studies is 40-60%. In the population in general, there is a prevalence of different types of

allergies of a few - 20 % (See Chapter 6). If the same cut off limit (weal size or allergen concentration) is used when the test is applied to patients in the general populations with a low prevalence of that allergy, there will be more false positive, and less false negative results, provided the degree of sensitivity in individuals in the general population who are sensitive to the allergen in question, is similar to patients investigated in the trial. However, in practice it has been found that there are patients in the general population, who are clinically sensitive but to a lesser degree than patients referred to Universities (13,16). The problem is illustrated in Fig. 5. The sum of these effects was, that a ten times higher concentration should be used to obtain a high sensitivity with SPT in "the general population" (16).

3.2.5. Sensitization versus correlation to clinical allergy

From the discussion above it comes that positive skin tests indicate presence of specific IgE-antibodies on mast cells in the skin, i.e. the body has reacted with formation of allergen-specific homocytotropic antibodies attached to type 1 IgE Fc-receptors on MC, but that only part of the individuals with such sensitization complain of allergic symptoms at contact with the allergen in question. There exists no definite limit as measured by skin sensitivity between clinically sensitive and non-sensitive individuals with latent allergy (24), as illustrated in Fig. 4b.

3.3. RECOMMENDATIONS FOR SCIENTIFIC INVESTIGATIONS

Skin prick tests with a needle of the Østerballe type, standardized allergenic extracts, preferably with known potency in relation to International Standard Preparations (IU/ml)(33) and acknowledged Biological Units (Nordic BU/ml (32) or FDA AU/ml (36)) or any biological unit, provided the potency is also given in IU/ml, should be used (alternatively the same type of needle with dry standardized extract). At least duplicate, if possible quadruplicate, tests should be performed with each test solution, to avoid that patients fulfilling the diagnostic criteria used in the trial

are not diagnosed by the test because of accidental negative tests, due to the fact that the precision is bad in some centres (13). Weal (and erythema) contours should be encircled with a fine pen and the contours transferred to a record sheet for permanent storage. The mean of the longest and the midpoint orthogonal diameters (mean diameter) or the area should be used as a measure of weal (erythema) size, and ≥ 3 mm in diameter used as cut off limit for positivity (3,13,25)(See further Chapter 2).

The ICT method is preferred to SPT for diagnostic studies by some investigators (31). However, the clinical relevance of concentrations used should be documented.

3.4. RECOMMENDATIONS FOR ROUTINE USE

In routine the SPT method should be preferred to the ICT method, because it causes less pain and the lower risk of systemic reactions. Needles of the Østerballe type should be used in combination with standardized allergenic extracts (or coated with such extract) of known and clinically documented potency, i.e. the clinical value of a positive or negative diagnostic test is known. Furthermore, to allow continuous supervision of test technique/precision and assure patients with clinical allergy to be diagnosed, tests should be performed in duplicate. Results should be registered and evaluated according to guidelines given in Chapter 2.

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