

6. Skin tests used for epidemiological studies

6.1. PURPOSE OF SKIN TESTING IN EPIDEMIOLOGICAL STUDIES

The purpose of skin testing in epidemiological studies has been to find out the prevalence of skin test positivity to common allergens in the general population or in groups of patients lacking a clinical history of atopy. In epidemiological follow up studies of samples of patients, skin tests have been used as a tool to study the development of sensitization and natural desensitization. It has also been suggested (51) that it might be valuable to advise symptom-free persons with positive skin tests, i.e. with latent allergy (41) that they may belong to a risk group which is at risk of developing atopic symptoms (58,51). Whether it is advisable to start prophylactic measures and avoidance of allergen exposure in these subjects should be further evaluated. As yet, the predictive value of positive skin tests is dubious. This is especially true for positive tests with pollen extracts.

6.2. NOMENCLATURE

Prevalence	the number (%) of patients with positive ST in the sample (population) studied
Incidence	the number of new cases with positive ST in the sample (population) studied
Cumulative incidence	the number of subjects in the sample (population) who have been ST positive during a given period of time
Atopic diseases	Diseases such as hayfever, asthma, urticaria, eczema, food allergy when based on IgE-antibody interaction

Atopic individual	A person prone to develop IgE mediated allergic disease when exposed to certain antigens (allergens)
Sensitization	Immunologic response with production of IgE antibodies, conceivably resulting in atopic disease
ED50	Median effective dose

6.3. ESTIMATES OF SENSITIZATION OF A SAMPLE (POPULATION)

6.3.1. Prevalence

Prevalence, i.e. the percentage of individuals showing a positive SPT to a certain allergen, or in some studies (30), two or several positive SPT's to any of the allergens tested has been used as a criterion of sensitization.

6.3.2. Median effective dose (ED50)

The median threshold concentration (with 95 % confidence interval) in the sample studied has been used as a measure of the sensitivity of that sample (8). This measure can be compared to the ED50 in other samples. In fact the Nordic Biological Unit 10 000 BU/ml (11,15,46) is the ED50 of patient samples referred to University Allergy outpatient Clinics (or similar).

6.4. INFLUENCE OF DIFFERENT FACTORS ON RESULTS

A number of factors influence the result of epidemiologic studies using skin tests for esti-

mation of the prevalence of sensitization: The cut off limit of the method; The technique employed and how it is performed; The precision of the method in the hands of the investigator (nurse, assistant); The composition and potency of allergenic extract employed.

6.4.1. *Cut off limit of the method - detection limit*

An SPT reaction is considered positive when the weal diameter is ≥ 3 mm in diameter ($= \geq 7$ mm²)(reviewed in 11). However, in some studies a cut off limit of ≥ 2 mm in diameter (3 mm²) has been used (34). Then the background response, i.e. the weal response to diluent in the same sample of patients should be carefully documented. The difference between 2 and 3 mm in mean diameter denotes a 10-fold difference in skin sensitivity (13).

6.4.2. *Cut off limit - number of positive SPT*

When the prevalence of sensitization is studied, then some investigators have used one positive SPT as an indication of atopy, others two or several positive tests. Haahtela and Jakomæki (30) found that the prevalence of atopy decreased from 49 to 34% when the limit for atopy was changed from one to two positive SPT.

6.4.3. *Precision of the method*

Precision of the SPT (and ICT) methods vary with technique (4,6,37), investigator / nurse / assistant performing the test (4,15). Therefore, the precision of the technique used in the hands of the investigator / nurse / assistant must be documented, preferably by performing at least duplicate tests with each test solution (or at least the reference substance)(11). Using the weal area as a measure of weal size, and transforming the data ($^{10}\log(\text{area mm}^2 + 1)$), the coefficient of variation (c.v. % = s.d. x 100 / mean) should be less than 40% (20% when the mean diameter is used). This limit seems reasonable, since 15% (7%) can

be achieved by skilled investigators (15), although much lower precision, i.e. higher c.v. %, has been recorded (15). This means that - 1 s.d. may not exceed 1.5 mm when the mean diameter is 4 mm (lower limit of the mean diameter 2.5 mm) and not 1.6 mm when the diameter is 5 mm (lower limit 3.5 mm). Similarly, - 2 s.d. = 1,7 and 1,9 mm, respectively (lower limit 2.3 and 3.1 mm, for 4 and 5 mm weals, respectively). This is a crucial point as those with a true weal diameter of 4 mm, all have allergen specific IgE attached to their skin MC. However, many weals will be recorded as negative despite the individuals are sensitized. Thus, differences in precision may account for differences in recorded prevalence between samples of patients with the same true prevalence.

6.4.4. *Technique and performance*

It has been demonstrated that the size of the weal differs between techniques in the hands of the same investigator (4,6), i.e. a technique that induces smaller weals, gives a lower prevalence than does a technique that induces larger weals (6,47). The technique is of greatest importance in patients with a skin sensitivity at the border of positivity. However, differences in performance between investigators using the same technique and test solution should also be considered. Differences in weal size as large as 3.5 versus 7 mm in mean diameter when using the same allergen solution, have been registered (4,11).

6.4.5. *Potency and composition of extract*

The higher the potency of the allergenic extract used, the more patients will show a positive reaction, i.e. sensitization (12,18)(Fig. 1 and 3, Chapter 3). It has been documented in biological standardization trials that patients attending a University Allergy Outpatient Clinic, all will get a positive reaction with 100 000 BU/ml(15-17). When the same extract was used in a diagnostic trial using subjects from the general population, i.e. including also individuals only slightly allergic, then 1 mill. BU/ml was needed to diag-

nose all patients, whereas 10 000 BU/ml showed a high specificity (18). These examples clearly demonstrate the necessity of using allergenic preparations with known potency, relevant to the question raised in the study.

When an allergenic preparation with only one component, to which some people are highly allergic, is used for diagnosis, only some of the patients clinically sensitive to the allergen will give a positive test. Thus, 98 % of dog sensitive patients were positive when SPT was performed with a well characterized dog allergen preparation (50) prepared from the best allergen source material, dog dander, whereas only $\approx 20\%$ were positive with one of the allergens of dog dander, dog serum albumin (12,44).

6.4.6. *Prevalence of clinical allergy*

When the aim of an epidemiological investigation is to study the prevalence of sensitization, then it is recommended to use an extract with defined and high potency and relevant composition. Provided the aim permits it, one major allergen can be used. When the aim is to study the prevalence of clinical allergy, then the concentration used should be documented regarding its diagnostic capacity in a suitable sample of patients, preferably with characteristics similar to those of the sample studied in the epidemiological study. However, the prevalence of sensitization and/or clinical allergy is often higher in samples studied in diagnostic trials, than in epidemiological studies, because many patients are needed when the prevalence of disease is low (as for example in the general population). Then, corrections must be made for the lower prevalence of atopic disease, otherwise many individuals without disease will be diagnosed ("false positives", patients with latent allergy (41)).

6.5. PREVALENCE OF SENSITIZATION TO COMMON ALLERGENS AND ATOPY, RESPECTIVELY

6.5.1. *General considerations*

The prevalence of positive skin tests to defined allergens and of atopy defined e.g. as two

positive skin tests using a battery of allergen extracts known to be of importance in the region, has been extensively studied (1-3,5,7-10,19-36,38-43,52)(Table 1). The quality of allergens employed was unsatisfactory in most, but not all studies, the potency was ill defined and skin test techniques and criteria for positivity varied between studies. Therefore, comparison of results between these studies is difficult. The prevalence of positive skin tests varied considerably i.e. from 19 to 70 %. These differences do probably not reflect differences in prevalence, but rather variation in technique, potency etc., which highly stresses the need for new studies with defined materials, techniques, documented precision etc. Extracts should be stored freeze-dried for follow up studies and for calibration against standards with known potency and composition, or for comparison with extracts used by other investigators or extracts used in the marketplace.

6.5.2. *Variation with age*

Sensitization varies with age (34,35,45,48,51). Sensitization to foods is common in infants and young children, but often disappears within a few years (34,51), whereas sensitization to inhalants is more common in older children and adults (34,35,51). The skin reactivity seems to be more pronounced in older children and young adults than during the two first years of life (45,48,51). Skassa-Brociek et al (48) found a lower sensitivity to histamine in young and elderly individuals. However, Stucky et al (49) found that individuals with skin sensitivity to many allergens had a higher skin sensitivity to histamine. It is well known that the number of patients with atopic skin sensitization is lower in young children and the elderly. Therefore, the difference in reactivity to histamine may be an expression of the degree of allergen sensitization, varying with age. This interpretation is favoured by the findings that children with sensitization to foods showed similar skin reactivity to histamine irrespective of age (14).

Table 1

Prevalence of immediate skin test reactivity in “normal” subjects and in general populations according to selected studies

Country and study	Number, type and age of subjects	Skin test method	Number of al- lergens tested	Criteria for a positive reaction (weal mm) reactions (one or more)(%)	Prevalence of positive
<i>USA</i>					
Grow and Herman (1936)	150 normals 20-30 yrs	ic.	13	5	55.5
Curran and Gold- man (1961)	100 normals 16 and over	scratch ic.	9 5-10	weal and flare	9
Lindblad and Farr (1961)	100 normals 16-81	ic.	5 1:100 1:10 1:1	5	4 24 71
Hagy and Set- tipane (1969)	1243 students 16-20	scratch	15	weal and erythema	30.9
Barbee et al. (1976)	3102 general population	prick	5	1	34
Brown et al. (1979)	307 allergic and non-allergic 9-78	prick	14	1 5	68.7 47.9
Friedhoff et al. (1981)	406 factory workers	prick	variety of crude extr.		♀ 7 ♂ 29
Barke, R. et al. (1987)	1333 cohort > 3 y. followed 8.1 y.	prick	5 W/w	> contr.	init. 39.1 % follow up 50.7 %
Gergen, P. et al. (1987)	16.204 6-74	prick	8	erythema > 10.5 mm =	20.2 %
<i>Italy</i>					
Corbo, G. et al. (1988)	295 11-14 y.	prick	4	2.5 mm ²	♀ 18 % ♂ 32 %
<i>Canada</i>					
Chang-Yeung, M. et al. (1985)	3353 workers 3 diff. groups	prick	common allergens		22.4
<i>United Kingdom</i>					
Herxheimer et al. (1954)	100 normals 5-75	prick	12	3	34
Greenberg et al. (1970)	121 factory workers	prick	7	1	21
Godfrey and Griffiths (1976)	303 schoolchild- ren, 8-14	prick	2	3	33.7
D'Souza and Da- vies (1977)	1267 general po- pulations, 45-70	prick	4	1	24.3
How et al. (1978)	288 factory workers	prick	3	1	38.8

Table 1 continued

Prevalence of immediate skin test reactivity in "normal" subjects and in general populations according to selected studies

Country and study	Number, type and age of subjects	Skin test method	Number of al- lergens tested	Criteria for a positive reaction (weal mm) reactions (one or more)(%)	Prevalence of positive
<i>Australia and Papua New Guinea</i>					
Andersson (1979)	335 general population	prick	11	3	19
Woolcock et al (1978)	505 general po- pulation, 20-69	prick	8	2	44.3
<i>Denmark</i>					
Johnson and Mygind (1978)	127 medical students, 21-35	prick	21	3	29.9
<i>Hungary</i>					
Cserhati et al. (1983)	300 negat. fam. hist.	prick	comm. allergens	> contr.	64 %
<i>Finland</i>					
Haahtela (1979)	218 adoles- cents, 14-16	prick	8	3	31
Haahtela and Jokela (1979)	295 conscripts, 18-19	prick	16	3	50
Haahtela et al. (1980)	708 adoles- cents, 15-17	prick	16	3	49
Haahtela (1980) (thesis)	1217 young people, 14-19	prick	16	3	46
Haahtela (1988)	1640 male smokers 50-69	prick	7	3	26 1 10 2

6.5.3. Prevalence of clinical allergy

As mentioned in Chapter 3, many patients show positive skin tests, provided the potency of allergens used is high, i.e. they are sensitized but not clinically allergic, they have latent allergy (41). Van Asperen et al (51) showed that those with clinical allergy were more sensitive than those without clinical symptoms at contact with the allergen in question. Horak (38) followed a group of children with positive skin tests but without clinical symptoms. He found that over a period of 5 years, more than 50 % acquired rhino-conjunctivitis and an additional 5 % asthma. The prognostic value of such data is high, provided standardized allergens with known potency are used, i.e. the results can be utilized by practicing allergists/pediatricians.

6.6. RECOMMENDATIONS FOR FUTURE EPIDEMIOLOGICAL STUDIES

The skin prick test, scratch and ICT methods are all possible to use for epidemiological studies. However, in epidemiological investigations in which a large number of individuals are studied, and skin tests are used as a diagnostic tool, the test method should be reproducible and easy to perform. The recording of skin reactions (weal size) should be simple and it should be easy to obtain an estimate of skin sensitivity. Due to its simplicity, safety and painlessness in relation to the ICT method and higher precision in relation to the scratch test method, the SPT method should be preferred in epidemiological studies. Among SPT methods, the standardized method using a needle of the fsterballe type should be preferred (See Chap-

ter 2 and 3). Allergenic preparations used in epidemiologic studies should fulfil the criteria given in Chapter 2. Preferably, the diagnostic value of such extracts should be documented in a sample/population with known prevalence of disease (disease defined as positive history, positive challenge test at a certain concentration etc.) and including all patients in the general population with clinical allergy to the allergen in question.

Future epidemiological studies using skin tests should

- be performed with the SPT/puncture technique and a needle of the Østerballe type,
- use allergen extracts which are freeze-dried and at least one vial should be stored frozen for future comparison with later developed standards,
- use allergens known to be of importance or probably of importance in the region, with a composition reflecting that of the naturally occurring allergen source material, or for some purposes, well defined major allergens
- have a diagnostic capacity of the concentration chosen documented in relation to clinical allergy, when the possible clinical relevance of an allergen is addressed
- be documented regarding the precision of the technique of the investigator/assistant, preferably by performing tests in duplicate with each test solution,
- be performed at a defined time of the year and time of the day (See Chapter 2)
- have weal sizes documented in a permanent manner (See Chapter 2),
- use ≥ 3 mm diameter as cut off limit for weal size and the presentation of distribution of sensitivities in the sample should be by weal area and/or mean diameter, complemented with the corresponding end point concentration, estimated by an equation using the mean slope for allergen (0.4 for area, 0.2 for diameter when the mean diameter is used as measure of weal size):

$$\text{End point conc in e.g. IU} = \text{Tested conc} \times [3/\text{actual mean weal diameter}]^5$$

where 3 denotes the cut off limit for weal diameter and 5 is derived from $1/b$ (b slope; bestimated on the basis of weal diameters = 0.2).

The above mentioned guidelines should be followed. If it is not possible to follow them in all details, the reason should be given and commented on.

6.7 REFERENCES

1. Andersson H: The epidemiological and allergic features of asthma in the New Guinea Highlands. *Clin Allergy* 4, 171-180, 1974.
2. Barbee R, Kaltenborn W, Lebowitz M, Burrows B: Longitudinal changes in allergen skin test reactivity in a community population sample. *J Allergy Clin Immunol* 79, 16-24, 1987.
3. Barbee R, Lebowitz M, Thompson H, Burrows B: Immediate skin test reactivity in a general population sample. *Ann Intern Med* 84, 129-133, 1976.
4. Basomba A, Sastre A, Pelaez A, et al.: Standardization of the skin prick test. A comparative study of three methods. *Allergy* 40, 395-399, 1985.
5. Bousquet J, Skassa-Brociek W, Dreborg S, Segalen C, Michel FB: Precision of skin prick tests using nine methods. *J Allergy Clin Immunol* (in press).
6. Brown W, Halonen M, Kalterborn W, Barbee R: The relationship on respiratory allergy, skin test reactivity, and serum IgE in a community population sample. *J Allergy Clin Immunol* 63, 328-335, 1979.
7. Chan-Yeung M, Vedal S, Lam S, Emerson D: Immediate skin reactivity and it's relationship to age, sex, smoking and occupational exposure. *Arch Environ Health* 40, 53-57, 1985.
8. Corbo G, Foresi A, Morandini S, Valente S, Mottoli S, Ciappi G: Probit analysis applied to the allergen dose-response curve: a method for epidemiological surveys. *J Allergy Clin Immunol* 81, 41-47, 1988.
9. Cserhati E, Kiss A, Mezei G: Positive skin prick tests of immediate type of non-allergic children. *Acta Ppdiatric Hung* 24, 189-0, 1983.
10. Curran W, Goldman G: The incidence of immediately reacting skin tests in a "normal" adult population. *Ann Intern Med* 55, 777-783, 1961.
11. Dreborg S: The skin prick test - an update. *Folia Allergol Immunol* 35, 3-11, 1988.
12. Dreborg S: The skin prick test in diagnosis of food allergy. *Ann Allergy* (in press).
13. Dreborg S: The skin prick test in diagnosis of atopic allergy. In LaRosa M (eds.): *Proceedings of the 50 Congresso dell Associazione per la prevenzione delle allergopatie infantili* (in press).
14. Dreborg S: The skin prick test. Methodological studies and clinical applications, pp. 1-148. Linköping University Medical dissertation No 239, 1987.
15. Dreborg S, Basomba A, Belin L, et al.: Biological equilibration of allergenic preparations. Methodological aspects and reproducibility. *Clin Allergy* 17, 537-550, 1987.
16. Dreborg S, Basomba A, Einarsson R: Sensitivity to *Parietaria officinalis* and *Parietaria judaica* pollen aller-

- gens in a Spanish population. *Allergol Immunopathol* 14, 499-508, 1986.
17. Dreborg S, Belin L, Eriksson NE, et al.: Results of biological standardization with standardized allergen preparations. *Allergy* 42, 109-116, 1987.
 18. Dreborg S, Björkstén B, Kjellman N IM, Rimås M: How to define allergy to dog? and what's the optimal concentration for diagnosis of allergy to dog by skin prick test. *Allergy* (submitted)
 19. D'Souza M, Davies R: The distribution of allergic disorders and atopy in the community and their relationship to total levels of serum IgE antibody. *Am Rev Respir Dis* 115, 211, 1977.
 20. Freidhoff L, Meyers D, Bias W: A genetic-epidemiological study of human immune responsiveness to allergens in an industrial population. *Am J Med Genet* 9, 323-340, 1981.
 21. Godfrey R, Griffiths M: The prevalence of immediate positive skin tests to *Dermatophagoides pteronyssinus* and grass pollen in school children. *Clin Allergy* 6, 79-80, 1976.
 22. Greenberg M, Milne J, Watt A: Survey of workers exposed to dusts containing derivatives of *Bacillus subtilis*. *Br Med J* 2, 629-633, 1970.
 23. Glegen P, Turkeltaub PC, Kovar M: The prevalence of allergic skin tests reactivity to eight common aeroallergens in the U.S. population. *J Allergy Clin Immunol* 80, 669-679, 1987.
 24. Grow M, Herman N: Intracutaneous tests in normal individuals. *J Allergy* 7, 108-110, 1936.
 25. Haahetela T: (personal communication).
 26. Haahetela T: Allergy in young people. Thesis Medical Faculty of the University of Helsinki, 1986.
 27. Haahetela T: The prevalence of allergic conditions and immediate skin test reactions among Finnish adolescents. *Clin Allergy* 9, 53-60, 1979.
 28. Haahetela T, Björkstén F, Heiskala M, Suoniemi I: Skin prick test reactivity to common allergens in Finnish adolescents. *Allergy* 35, 425-431, 1980.
 29. Haahetela T, Heiskala M, Suoniemi I: Allergic disorders and immediate skin test reactivity in Finnish adolescents. *Allergy* 35, 433-441, 1980.
 30. Haahetela T, Jaakomäki I: Relationship of allergen, specific IgE antibodies, skin prick test and allergic disorders in unselected adolescents. *Allergy* 36, 251-256, 1981.
 31. Haahetela T, Jokela H: Asthma and allergy in Finnish conscripts. *Allergy* 34, 413-420, 1979.
 32. Hagy W, Settupane G: Bronchial asthma, allergic rhinitis, and allergy skin tests among college students. *J Allergy* 44, 323-0, 1969.
 33. Hagy W, Settupane G: Risk factors for developing asthma and allergic rhinitis. *J Allergy Clin Immunol* 58, 330-336, 1976.
 34. Hattevig G, Johansson SGO, Björkstén B: Clinical symptoms and IgE responses to common food proteins in atopic and healthy children. *Clin Allergy* 14, 551-559, 1984.
 35. Hattevig G, Kjellman B, Björkstén B: Clinical symptoms and IgE responses to common food proteins and inhalants in the first 7 years of life. *Clin Allergy* 17, 571-578, 1987.
 36. Herxheimer H, McInro P, Sutton K, Utidjian HL, Utidjian HMD: The evaluation of skin tests in respiratory allergy. *Acta Allergol* 7, 380-396, 1954.
 37. Holgersson M, Ståhlenheim G, Dreborg S: The precision of skin prick test with Phazet TNM, the Østerballe needle and the bifurcated needle. *Allergy* 40, Suppl. 4, 64-65, 1985.
 38. Horak F: Manifestation of allergic rhinitis in latent-sensitized patients. A prospective study. *Arch Otorhinolaryngol* 242, 242-249, 1985.
 39. How M, Goodwin B, Juniper C, Kinshott A: Comparative serological and clinical findings in subjects exposed to environmental allergens. *Clin Allergy* 8, 347-360, 1978.
 40. Johnsen N, Mygind N: Incidence of latent and clinical respiratory tract allergy in medical students (Danish). *Ugeskr Læger* 140, 596-600, 1978.
 41. Juhlin-Dannfelt C: About the occurrence of various forms of pollen allergy in Sweden. *Acta Med Scand* 26, 563-577, 1948.
 42. Kjellman N IM, Dreborg S, Fæltch-Magnusson K: Prick test with Phazet compared with allergen extracts, serum IgE and history in 314 adults. *Allergy* 43, 277-288, 1988.
 43. Lindblad J, Farr R: The incidence of positive intradermal reactions and the demonstration of skin sensitizing antibody to extracts of ragweed and dust in humans without history of rhinitis or asthma. *J Allergy* 32, 392-400, 1961.
 44. Lindgren S, Belin L, Dreborg S, Einarsson R, Pålman I: Breed specific dog dandruff allergy. *J Allergy Clin Immunol* 82, 196-204, 1988.
 45. Menardo J L, Bousquet J, Rodère M, Astruc J, Michel FB: Skin test reactivity in infancy. *J Allergy Clin Immunol* 75, 646-651, 1985.
 46. Nordic Council on Medicines: Registration of allergenic preparations. Nordic guidelines. pp. 1-34. Uppsala, NLN Publications, No 23, 2nd ed, 1989.
 47. Østerballe O, Weeke B: A new lancet for skin prick testing. *Allergy* 34, 209-212, 1979.
 48. Skassa-Brociek W, Manderscheid J C, Michel FB: Skin test reactivity to histamine from infancy to old age. *J Allergy Clin Immunol* 80, 711-715, 1987.
 49. Stucky MS, Witt CS, Schmitt LH, et al.: Histamine sensitivity influences reactivity to allergens. *J Allergy Clin Immunol* 75, 373-376, 1985.
 50. Uhlin T, Reuterby J, Einarsson R: Antigenic/allergenic composition of poodle/ alsatian dandruff extract. *Allergy* 39, 125-133, 1984.
 51. Van Asperen PP, Kemp AS, Mellis CM: Skin test reactivity and clinical allergen sensitivity in infancy. *J Allergy Clin Immunol* 73, 381-386, 1984.
 52. Woolcock A, Colman M, Jones M: Atopy and bronchial reactivity in Australian and Melanesian populations. *Clin Allergy* 8, 155-164, 1978.

49. Stucky MS, Witt CS, Schmitt LH, et al.: Histamine sensitivity influences reactivity to allergens. *J Allergy Clin Immunol* 75, 373-376, 1985.
50. Uhlin T, Reuterby J, Einarsson R: Antigenic/allergenic composition of poodle/ alsatian dandruff extract. *Allergy* 39, 125-133, 1984.
51. Van Asperen PP, Kemp AS, Mellis CM: Skin test reactivity and clinical allergen sensitivity in infancy. *J Allergy Clin Immunol* 73, 381-386, 1984.
52. Woolcock A, Colman M, Jones M: Atopy and bronchial reactivity in Australian and Melanesian populations. *Clin Allergy* 8, 155-164, 1978.

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