

Skin-prick test preparations of *Dermatophagoides pteronyssinus* for prediction of a positive response to provocation testing

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Summary

Skin-prick testing (SPT) with allergen is widely used in the investigation of respiratory disease, yet its relationship to the results of provocation testing of the nose or bronchus remains obscure. We have studied this relationship by determining the concentration of *Dermatophagoides pteronyssinus* extract and the associated weal size which resulted in the lowest 'false positive' (FP) and highest 'true positive' (TP) rates when compared to the results of bronchial and nasal provocation testing. Subjects studied included those with both positive and negative bronchial or nasal provocation tests. For each of a series of concentrations of *D. pteronyssinus* extract, standardized in relation to content of Der pI antigen, receiver operating characteristic (ROC) curves of TP against FP rates were constructed. Optimal diagnostic concentrations for SPT were $0.112-1.122 \times 10^6$ IU of Der pI ml⁻¹. However, at the lower end of this range the optimum weal areas for a positive test with the *D. pteronyssinus* extract were very small (<5 mm²). More appropriate concentrations for use in clinical practice were $0.56-1.122 \times 10^6$ IU Der pI ml⁻¹ since at these concentrations more easily measurable weal areas of 5-10 mm² (2.6-3.6 mm diameter) were almost equally predictive of a positive provocation test.

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Introduction

Skin-prick testing with allergen extracts plays an important role in the investigation of patients with respiratory allergy. However, the clinical significance of a positive response to skin-prick testing and its relationship to the aetiology of respiratory disease is still unclear. The immediate reaction to skin-prick testing is essentially an immunological indicator of the patient's capacity to recognize a particular allergen due to the presence of skin sensitizing IgE antibody. It is uncertain whether exposure of the respiratory tract to the same allergen will result in disease.

A positive nasal or bronchial provocation test indicates that inhalation of a particular allergen can cause an

immediate hypersensitivity reaction, though its relevance to the natural occurrence of asthma or rhinitis is less clear. However, since skin-prick testing is a more simple and less time consuming investigation which requires no specialized facilities, the relationship between responsiveness to inhaled allergen and cutaneous sensitivity to the same allergen is of interest. Although it has been shown that there is a broad correlation between the weal diameter and the likelihood that bronchial provocation with the same allergen will be positive, as many as 30% of patients with a positive history of asthma provoked by house dust, but with negative skin tests, may exhibit a positive response when the allergen is inhaled during bronchial provocation testing [1]. Possible explanations for this anomaly include the concentration of allergen used in the skin-prick test and the weal area considered to constitute a positive test. The purpose of this study was to examine

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the relationship of the weal size, following skin-prick testing with various concentrations of allergen, to the results of specific provocation testing.

A clinical investigation such as skin-prick testing can be compared to a radar system, in which the output of some receiver (the test result) must be interpreted as indicating the presence or absence of a meaningful signal (clinical allergy). In the same way that a radar operator must use the peaks on the screen to decide whether or not a missile or aircraft is present the physician uses a test result to decide whether or not a patient has a particular clinical condition. The same theory that is used for the study and interpretation of radar signals can be applied to the evaluation of clinical tests as described by Robertson and Zweig [2]. Our aim in this study was to improve the diagnostic precision of the skin-prick test by applying this theory to the ability of the test to identify individuals with positive nasal or bronchial provocation tests.

Subjects and methods

Subjects

A group of 45 patients referred to St Bartholomew's Hospital, London for investigation of allergic respiratory symptoms took part in the study. All subjects gave informed consent to the study. Those who were receiving treatment with antihistamines, or either oral or inhaled steroids were excluded from the study as were those exhibiting dermatographism. All subjects were required to undergo bronchial or nasal provocation testing as appropriate, with house dust mite (*Dermatophagoides pteronyssinus*) extract irrespective of whether this was suspected of being a causal factor in their asthma or rhinitis by the referring physician. We therefore aimed to include those subjects with a positive and also those with a negative response to inhalation testing. All individuals undergoing bronchial provocation testing (BPT) were capable of a forced expiratory volume in 1 sec (FEV₁) of >70% of that predicted for their age and height immediately before bronchial provocation testing, when inhaled bronchodilator therapy had been omitted for the previous 6 hr.

Bronchial provocation testing

The technique of bronchial provocation testing was that recommended by Chai *et al.* [3]. Phenol saline (0.4% phenol, 0.275% sodium bicarbonate in 0.5% saline) was used as a control solution for inhalation and as a diluent for allergen extracts. Freeze-dried allergen extracts were diluted to concentrations of 1:500, 1:1000, 1:5000 and 1:10 000 (w/v). All solutions were administered via a De Vilbiss 646 nebulizer (De Vilbiss Health Care U.K.

Ltd, Feltham, U.K.) attached to a Rosenthal-French dosimeter (John Hopkins University, Baltimore, U.S.A.). The airway response to provocation was monitored by measurement of the FEV₁ using a Vitalograph dry bellows spirometer (Vitalograph, Buckingham, U.K.). Provocation testing was commenced with five inhalations of saline control solution and the FEV₁ recorded after 30 sec, 5 and 10 min. In the absence of a significant fall (>15%) in the FEV₁ from pre-challenge levels testing was continued with increasing concentrations of allergen until a fall in FEV₁ of 20%, compared to the lowest post-saline values had been achieved, or the highest concentration of allergen available (1:500 (w/v)) had been administered. Subjects who did not achieve a fall of $\geq 20\%$ in FEV₁ during provocation testing were classified as negative responders for the purposes of this study. Those whose FEV₁ fell by $\geq 20\%$ in response to any of the allergen concentrations tested were classified as positive on bronchial provocation testing.

Nasal provocation testing

The technique of nasal provocation testing was based on that described by Britton *et al.* [4] using a technique of passive anterior rhinomanometry. Phenol saline was used as a control solution. Allergen extracts were prepared as described for bronchial testing. All solutions were administered in a volume of 100 μ l which was nebulized into one nostril only at an airflow rate of 7 l min⁻¹ (116.7 ml sec⁻¹) using a modified air brush (Humbrol, Hull, U.K.). Subjects were instructed to hold their breath during the nebulization to minimize exposure of the lower airways to allergen. The response of the nasal airways to provocation was monitored by measurements of nasal airways resistance (NAR) using a nasal airways resistance tester (PK Morgan Ltd, Chatham, U.K.), which allows recordings of NAR to be taken from each nostril separately at a flow rate of 3 l min⁻¹ (50 ml sec⁻¹). Nasal airways resistance was measured before nasal provocation, and at 2, 5, 10 and 15 min after instillation of the control solution and each concentration of *D. pteronyssinus*. Five measurements of NAR (kPa l⁻¹ sec) were recorded in each nostril at every time point. Following instillation of control solution, testing was continued with increasing concentrations of allergen until NAR in the challenged nostril rose by 300% from the highest post-saline value, or the highest allergen concentration had been administered. Subjects who did not achieve a rise of $\geq 300\%$ during provocation testing were classified as negative responders. Those whose NAR in the challenged nostril rose by $\geq 300\%$ in response to any of the allergen concentrations tested were classified as positive on provocation testing for the purposes of this study.

Table 1. Concentrations and *Der pI* content of allergen extracts used in the study

Concentration of extract (w/v)	<i>Der pI</i> content (IU ml ⁻¹)
1:100	5.610×10^6 *
1:500	1.122×10^6
1:1000	0.561×10^6
1:5000	0.122×10^6
1:10 000	0.059×10^6
1:50 000	0.013×10^6

* Freeze dried allergen extract was initially re-constituted to a 1% (1:100, w/v) solution. This concentration formed the stock solution for preparation of test solutions and was not used in subject testing.

A fall in FEV₁ of 20% during bronchial provocation testing was not assumed to equate to a rise in NAR of 300% during nasal provocation testing. The values chosen to indicate a positive response in the two organs were those which are considered positive in subjects routinely referred for testing from our Allergy Clinic at St Bartholomew's Hospital.

Skin-prick testing

Skin-prick testing was performed in duplicate on the volar surface of the forearm using the modified skin-prick test technique [5]. Allergen extracts were diluted in phenol saline to provide concentrations identical to those used for bronchial provocation testing. Weal areas were recorded 12 min after testing by outlining using a ballpoint pen and transferring the outline to paper using adhesive tape. Areas were then measured using an electronic planimeter and the mean of each pair of weal reactions used in the analysis of results.

Allergen extracts

All allergen solutions used were based on an aqueous extract of *D. pteronyssinus*, freeze-dried into glass vials as previously described by Hordle *et al.* [6]. The *Der pI* antigen content of extract was determined by incubating dilutions of the extract or of the International Standard of *D. pteronyssinus* [7] (National Institute of Biological Standardization and Control, U.K.) with ¹²⁵I-labelled *Der pI* [8] and a 1:10 000 dilution of serum from a rabbit immunized with *D. pteronyssinus* in Freund's complete adjuvant. Bound and labelled *Der pI* was purified by incubating with Sepharose protein antigen A (Pharmacia,

Milton Keynes, U.K.), which was then isolated by centrifuging and washing. Relative potencies were obtained using parallel line bioassay statistics [9] and the results expressed as *Der pI* international units (IU *Der pI*). The relative potency of freeze-dried extract was found to be 6.6×10^6 IU *Der pI*. From this value the potencies of the allergen solutions used in the study were calculated (Table 1).

Receiver operating characteristic curves

For most clinical investigations there is an overlap in test results from patients who suffer from a particular condition and those without that condition. Each decision by the physician in the interpretation of a test result can be categorized as:

- (i) true positive (correctly identifying a patient with disease);
- (ii) true negative (correctly identifying a patient who does not have disease);
- (iii) false positive (suspecting disease in a healthy patient); and
- (iv) false negative ('missing' the diagnosis in a patient with disease)

For example, in the Allergy Clinic physicians may use the size of the skin weal to a particular allergen to help decide whether that allergen is clinically relevant to a patient's condition. If the physician believes that even a very small skin response indicates clinical relevance he will 'miss' relatively few cases where that allergen is truly important. However, he will also wrongly identify many patients for whom the allergen is not important, i.e. he will have a very high 'false positive' rate. In this case sensitivity will be high but specificity low. Raising his decision level by increasing the size of weal to be considered relevant, specificity can be increased but sensitivity will decrease (some cases will be missed).

The best decision level would be that which combines the highest possible sensitivity with the highest possible specificity, represented by a 100% true positive rate and a 0% false negative rate. In practice this degree of precision is not usually attainable. However, it is possible to determine the optimum decision level by using a plot known as the receiver operating characteristic (ROC) curve, which plots true positive (TP) rate as a function of the false positive (FP) rate [2]. Receiver operating characteristic curves are constructed by plotting TP and FP rates at a variety of decision levels (in our case skin weal areas).

For each skin-prick test weal area, or decision level, TP and FP rates were calculated to represent the correct or incorrect prediction of a nasal or bronchial response (the response to provocation testing being taken to represent the 'true diagnosis' for the purposes of this study).

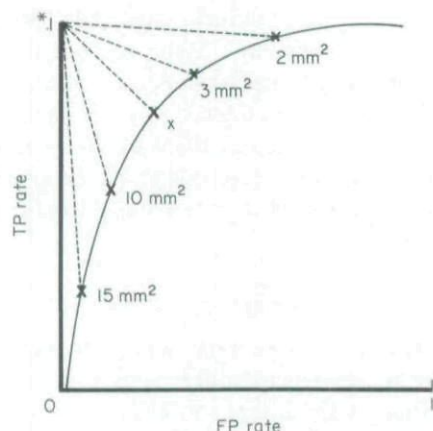


Fig. 1. Specimen receiver operating characteristic curve showing the decision level (x) where the curve most closely approaches the ideal point (*) giving the best combination of a high true positive (TP) rate and low false positive (FP) rate.

	Provocation positive	Provocation negative	
SPT positive	a	b	$TP = \frac{a}{a+c}$
SPT negative	c	d	$FF = \frac{b}{b+d}$

A decision level of 6 mm² means that all skin weals with an area of ≥ 6 mm² were considered to be positive (SPT positive). On the basis of the TP and FP rates obtained at each decision level ROC curves were constructed for each of a series of allergen concentrations [2,10–12].

A theoretical example of an ROC curve is shown in Fig. 1. The decision level which discriminates most efficiently between patients having a positive or a negative response to bronchial provocation testing is that point which most closely approaches the ideal point where there are 100% true positive predictions (TP=1) and no false positives (FP=0). At this point the test shows a perfect sensitivity, correctly identifying all patients with a positive BPT, and a high specificity as indicated by a low false positive rate. In Fig. 1 the greatest diagnostic efficiency would therefore be obtained by considering all skin weals of $\geq x$ mm² in area as positive.

Results

Provocation testing

On the basis of the criteria outlined above, 21 of the 45 subjects included in the study were found to have a positive response to provocation testing; seven of the 19 subjects who underwent nasal provocation testing, and 14 of the 26 undergoing bronchial provocation testing.

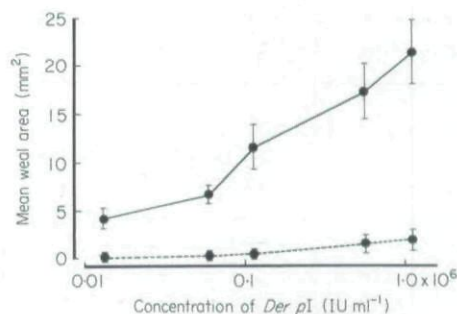


Fig. 2. Mean weal areas of skin-prick test responses to increasing concentrations of *D. pteronyssinus* extract in subjects with a positive (●—●) or negative (●---●) response to bronchial provocation testing.

Skin-prick testing

The results of skin-prick testing are displayed in Fig. 2. A well defined dose-response relationship was found in subjects who showed a positive response to provocation testing. At the highest concentration of allergen used, 1.122×10^6 IU *Der pI* ml⁻¹, weal areas ranged from 0–58 mm² with a mean weal area of 21.4 mm². A dose-response relationship was also observed in subjects negative to bronchial provocation testing, although the resulting curve was quite shallow (indicating a much lower level of response in those subjects) with a mean weal area of 3.28 mm² being elicited by the highest allergen concentration tested.

Receiver operating characteristic curves were constructed for each concentration of allergen used (examples are shown in Fig. 3) and compared. The optimal diagnostic concentrations of the extract of *D. pteronyssinus* were determined by measuring distances from the ideal point on the ROC curves. These distances have been plotted for each decision level on each ROC curve and the results are displayed in Fig. 4. We were unable to identify any decision level at any allergen concentration which gave perfect agreement between BPT, NPT and SPT (i.e. passed through the ideal point). At lower allergen concentrations of 0.06 and 0.11×10^6 IU *Der pI* ml⁻¹, diagnostic efficiency was high only at very small weal areas ≤ 1 mm². At these concentrations even a cut-off point of 4 mm² (a diameter of 1.13 mm) would not identify all subjects with a positive provocation test. The concentrations which gave optimal diagnostic efficiency over a wide range of weal sizes were 0.56 and 1.122×10^6 IU *Der pI* ml⁻¹. At these concentrations all decision levels (weal areas) between 1 mm² and 10 mm² (corresponding weal diameters 1.1–3.6 mm) were equally predictive of the response to allergen inhalation. Only when a larger weal size of >10 mm² was adopted as the criterion for a

Fig. 3. Receiver operating characteristic curves showing the relationship between true positive rates (TP) and false positive rates (FP) for different weal areas (5 mm² intervals) at two different concentrations of *D. pteronyssinus* extract. (a) *Der pI* concentration 1.12×10^6 IU ml⁻¹ and (b) *Der pI* concentration 0.122×10^6 IU ml⁻¹.

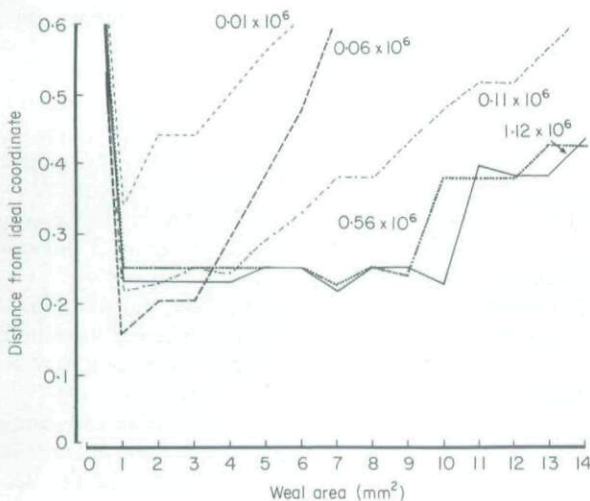
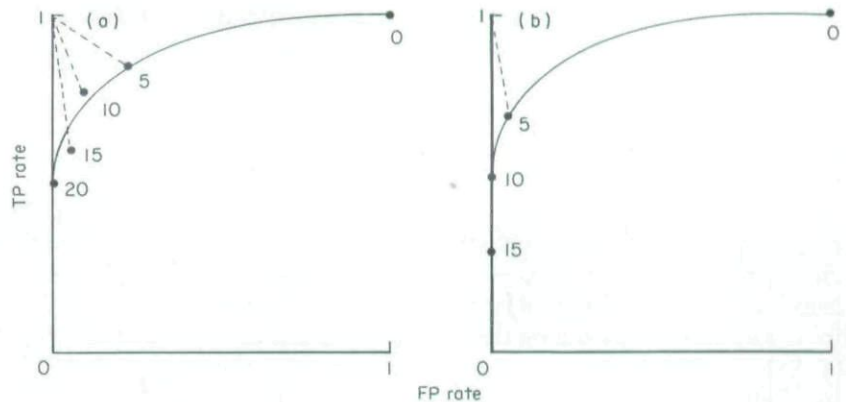


Fig. 4. Distances from the ideal coordinate on the receiver operating characteristic curve for decision levels of increasing weal areas using each concentration of allergen extract (*Der pI* content is indicated in IU ml⁻¹).

positive skin test did diagnostic efficiency begin to fall away, 'missing' individuals who were positive on provocation testing but had a weal area in response to these concentrations of < 10 mm² (2.6–3.6 mm diameter). Any individuals who had a skin weal of ≥ 2.6 mm in diameter in response to either of these allergen concentrations had the highest probability of having a positive provocation test.

Discussion

There is little information in the literature concerning the relationship between skin-prick tests and nasal provocation testing, but the relationship between skin-prick test responses and response to bronchial provocation testing has been widely studied. There is some evidence that the

degree of bronchoconstriction caused by allergen inhalation depends not only on sensitivity to that allergen, as expressed by cutaneous reactions, but also on the degree of non-specific hyperresponsiveness of the airways [13]. Cockcroft *et al.* [14] have shown that skin-prick testing with an allergen concentration which, when inhaled, causes a fall in FEV₁ of 20% produces weal diameters which show a significant negative correlation with the level of airway responsiveness to histamine, i.e. individuals who were most sensitive to histamine inhalation had the smallest allergen-induced skin weals. This may indicate that when airway responsiveness is high only a mild allergic reaction in the airways is needed to trigger bronchoconstriction and vice versa. These authors suggested that the use of this relationship would make it possible to predict the concentration of allergen extract which should elicit an immediate reaction on bronchial provocation testing from knowledge of skin weal size and the degree of histamine reactivity. This relationship is potentially useful in relating skin weal responses to events in the airways provided that the degree of non-specific airway responsiveness is known. In practice, in situations where allergen inhalation testing is not possible, assessment of non-specific airway responsiveness by bronchial provocation testing with pharmacological agents may be equally impractical.

The present study has shown that it is possible to determine an optimal diagnostic concentration of a standardized allergen extract for skin-prick testing, and to specify decision levels in respect of weal sizes produced which are most likely to be predictive of a positive response to allergen provocation testing with prior knowledge of non-specific hyperresponsiveness. The tolerance of decision levels above which a skin-prick test is considered positive is high when the stronger concentrations of 0.56 or 1.122×10^6 IU *Der pI* ml⁻¹ are used, any decision level (weal area) in the range 1–10 mm² (corresponding weal diameter 1.1–3.6 mm) being equally useful.

Little information has been published about methods for determining the clinical value of any diagnostic test. An ROC curve analysis is one method by which this determination can be attempted. The information generated from ROC curve analysis depends on calculation of true positive and false positive rates in relation to the true diagnosis. In assessing any test by this method two groups of patients are therefore required—those whose true diagnosis is positive and those whose true diagnosis is negative. The usefulness of the information obtained from the analysis depends on the clinical appropriateness of division of patients into these two groups. We have chosen to relate the results of skin-prick testing to those of bronchial or nasal provocation testing, allowing a clear separation of patients into responders and non-responders. However, the clinical significance of a positive response to provocation testing remains obscure. For example, Townley *et al.* [15] have reported that at an antigen dose of 10 000 protein nitrogen units 11 out of 14 hay fever sufferers who had never previously wheezed had a positive bronchial challenge, indicating that this dose of allergen was too high to be clinically relevant to lower airways responses in these patients.

The real value of any test, including skin-prick testing, lies in its contribution to patient management. The analysis described in this paper relates the results of skin-prick testing purely to the results of provocation testing, and does not directly relate skin-prick testing to symptomatology. However, it does provide a method which allows us to maximize the chance of correctly identifying patients in whom inhalation of a specific allergen will **not** cause bronchoconstriction or rhinitic symptoms, and which is therefore less likely to be involved in the aetiology of their disease.

In conclusion, we suggest that comparison of diagnostic techniques by means of a modified ROC curve analysis allows an objective determination of optimal diagnostic concentrations of skin-prick test materials for prediction of a positive response to provocation tests in another target organ such as the nose or lungs. Further work is required to extend our experiments to studies of the nose and lung individually, to other allergen extracts and to other populations. In addition a prospective study in a similar population is now required to confirm that responses to provocation testing predicted using optimal concentrations established in this study correlate with the objective results of inhalation testing.

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