

HEP versus PNU Standardization of Allergen Extracts in Skin Prick Testing

A Comparative Randomized *in Vivo* Study

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During 1 year, all patients referred to an allergy outpatient clinic for adults were skin prick tested with a panel of standard allergen extracts from two manufacturers using different methods of standardization. One company referred to the histamine equivalent prick (HEP) and the other used the more traditional protein nitrogen units (PNU). Standard extracts and a five-fold dilution were tested. The results indicate that the ratio of concentration between two extracts of the same allergen should be measured by the absolute difference of the wheal diameters. We found significant differences between corresponding extracts from the two manufacturers.

Key words: allergen extracts; methods of standardization; skin prick test.

Accepted for publication 5 July 1985

The standardization of allergen extracts is a controversial subject. The HEP-system (HEP = histamine equivalent prick) has previously been recommended by Scandinavian authorities (1, 10). Some manufacturers are using their own interpretation of the HEP-system, and have labelled their products as being standardized in BU (biological units), or SQ (standardized quality), while others are referring to the more traditional weight/volume and PNU (protein nitrogen units).

In this study a panel of standard allergen extracts from two manufacturers using different methods of standardization were compared by routine skin prick testing of unselected adult patients.

MATERIAL AND METHODS

Patients and equipment

During 1983 a total of 318 adult patients referred consecutively to the allergy outpatient clinic at the county hospital in Hillerød, Den-

mark, participated in the study. The patients were tested with 10 different allergens, histamine and negative control. The extracts and their concentrations appear from Table 1.

Methods of allergen standardization

The two manufacturers have delivered the following descriptions of their methods of standardization:

The first company (1. Comp.) applied the HEP-system. By selecting at least 20 representative allergic patients for each allergen on the basis of case history, skin prick test, radio-allergosorbent test (RAST), and relevant provocation tests, the concentration of the extract giving the same wheal reaction as histaminedihydrochloride 1 mg/ml was found. This concentration was declared as 1 HEP (1, 10) and new batches were standardized to this concentration by comparative skin testing on at least 10 allergics. The concentration of major allergens in new extracts was further quantified and standardized against the reference

extract by quantitative immunoelectrophoresis (QIE) (9). Other allergenic components were controlled by crossed radio immunoelectrophoresis (CRIE) (8, 13), and the total activity was finally measured by means of RAST-inhibition.

The second company (2. Comp.) made extracts from source materials which were glycerinated (50 %) and buffered to retain activity. The solutions were subsequently sterilised by filtration. The concentration of allergen was controlled by protein nitrogen measurements to a level empirically found to be appropriate to the allergen, normally 5000 PNU/ml, except for Dermatophagoides where it is 1500 PNU/ml.

Technique

The skin prick tests were performed by two experienced nurses, whose skin testing reproducibility had been established previously (3). The test sites were placed at intervals of 15 mm on the volar side of the forearms in a straight line from approximately 5 cm below the elbow flexure to about 10 cm above the wrist. A strip of sticky tape numbered at 15-mm intervals was applied to the skin, and a drop of each extract was placed beside the respective numbers. Sterile disposable hypodermic needles 40 mm long and 0.8 mm in diameter (Yale Luer Lock no. 14) were used, and the point of the needle was directed through the drop at an angle of 60–70° to the skin, the needle point only catching the skin and lifting it, thus making a canal through which the extract could penetrate intracutaneously. The needle was then pulled out carefully in an inversely directed movement, and the skin blotted dry. The skin reactions were read after 15–20 min. The largest and smallest diameters of the wheal were measured using a transparent ruler, and the mean diameter recorded in mm.

Design

Skin prick test with standard allergen panel was performed on each patient in duplicate (once on each arm), and the patients were allocated to

eight groups for the testing as shown in Table 2.

Data analysis

Negative reactions to an allergen on both arms did not contain any information on possible differences between the extracts used. Therefore such double negative results were omitted in further calculations.

Patients in Group 1–4 were tested with standard extracts on one arm and one 5-fold dilution of the same extracts on the other. The results on the two arms were compared by regression analyses (11) in the model:

$$DS = a + b \cdot DD + S$$

Table 1

The concentration of the allergen extracts used in the study

Extract	1. company	2. company
Neg. control	50 % glycerol	50 % glycerol
Histamine	1 and 5 mg/ml histaminechloride	2 and 10 mg/ml histaminephosphate
Birch	1 and 5 HEP	800 and 4000 PNU
Timothy	1 and 5 HEP	900 and 4500 PNU
Mugwort	1 and 5 HEP	1000 and 5000 PNU
Cat	1 and 5 HEP	900 and 4500 PNU
Dog	1 and 5 HEP	800 and 4000 PNU
Horse	1 and 5 HEP	800 and 4000 PNU
<i>Derm. pter.</i>	1 and 5 HEP	300 and 1500 PNU
House dust	1 and 5 HEP	600 and 3000 PNU
<i>Alternaria</i>	1 and 5 HEP	900 and 4500 PNU
<i>Cladosporium</i>	1 and 5 HEP	700 and 3500 PNU

Table 2

Distribution of patients in different groups

Group	Extract on right arm	Extract on left arm	No. of patients
1	1. comp. (1 HEP)	1. comp. (5 HEP)	29
2	1. comp. (5 HEP)	1. comp. (1 HEP)	44
3	2. comp. (diluted)	2. comp. (strong)	47
4	2. comp. (strong)	2. comp. (diluted)	43
5	1. comp. (1 HEP)	2. comp. (diluted)	42
6	2. comp. (diluted)	1. comp. (1 HEP)	31
7	1. comp. (5 HEP)	2. comp. (strong)	41
8	2. comp. (strong)	1. comp. (5 HEP)	41
Total			318

Diameter in mm of urticarial wheal

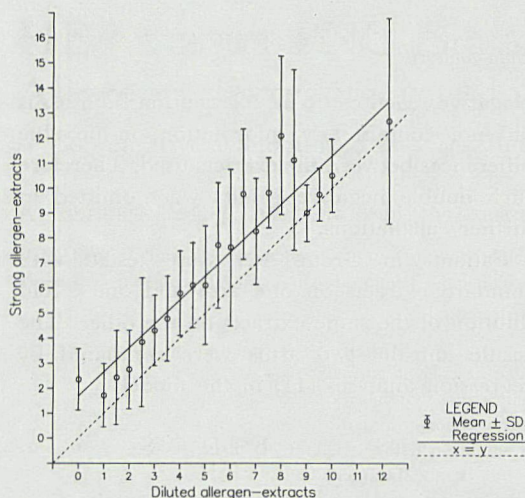


Fig. 1. The skin reactions to standard allergen extracts were compared to the reactions to 5-fold diluted extracts by regression analysis.

where DS is the diameter of the wheal using strong (undiluted) extracts, DD is the same diameter with diluted extracts, a and b are constants, and S is the residual variation. The results of the regression analyses were used to define how to measure the difference between corresponding extracts from the two manufacturers.

In Group 5-8 patients were tested with extracts from the two companies in concentrations thought to be comparable. The results were evaluated by Wilcoxon's rank sum test.

RESULTS

163 patients (Table 2: Group 1-4) were tested with one standard panel, and with a 5-fold dilution of the same extracts, thus examining 1630 pairs of skin pricks. In 602 pairs (37%) there was a measurable reaction to at least one of the two pricks in the pair. The reaction to the standard extract was compared with the reaction to the 5-fold diluted extract by regression analysis which resulted in the equation:

$$DS = 1.7 \text{ mm} + 0.96 \cdot DD$$

where DS is the wheal diameter of the reaction with standard extract, and DD the wheal diameter with a 5-fold diluted extract. $0.96 (\pm 0.03)$ is the slope ($\pm$ SD) of the regression line, and 1.7 mm the intercept (Fig. 1). The slope was not significantly different from 1.0 ($P > 0.2$). Assuming a slope of 1.0 changes the intercept to 1.6 mm, thus arriving at the equation:

$$DS = 1.6 \text{ mm} + DD$$

In consequence of this we decided to measure differences in concentrations of allergen by the differences in mm of the diameter of the wheals.

155 patients (Table 2: group 5-8) were tested with extracts from two different manufacturers. The results are summarized in Table 3.

Different preparations of histamine were used by the two companies (Table 1). The concentrations of histamine, however, were very similar: 1 mg/ml of histaminedihydrochloride = 5.43 mmol of histamine, and 2 mg/ml of histaminephosphate = 6.15 mmol of histamine, and no difference could be detected in prick test ($P = 0.472$) (Table 3).

Significant differences in size of wheal were observed for birch, mugwort, cat, dog, *Dermatophagoides pteronyssinus*, and house dust, and for the extracts of cat and dog the differences in the mean diameter were greater

Table 3

Results of routine skin prick testing with allergen extracts from two different companies (1. and 2. comp.). Negative reactions to the extracts from both companies were removed before calculating "No. of patients" and "Mean diameter"

Extract	No. of patients	Mean diameter		Wilcoxon rank test <i>P</i>
		1. comp.	2. comp.	
Histamine	155	5.35 mm	5.38 mm	0.472
Birch	59	3.51 mm	5.08 mm	< 0.001
Timothy	58	4.37 mm	4.84 mm	0.153
Mugwort	61	3.48 mm	2.95 mm	0.041
Cat	64	4.78 mm	2.35 mm	< 0.001
Dog	73	3.55 mm	1.20 mm	< 0.001
Horse	42	3.16 mm	2.60 mm	0.122
<i>Derm. pter.</i>	72	3.01 mm	2.56 mm	0.042
House dust	89	3.80 mm	2.69 mm	< 0.001
<i>Alternaria</i>	31	2.43 mm	2.11 mm	0.472
<i>Cladosporium</i>	9	2.83 mm	1.55 mm	0.441

than 1.6 mm (cat: 2.43 mm; dog: 2.35 mm), indicating a more than 5-fold difference between these extracts from the two manufacturers.

DISCUSSION

The results demonstrate considerable differences between the products of the two companies, although for four of the 10 allergens the differences did not reach significance at the 5 % level. However, as far as the horse and the two mould extracts are concerned only few patients demonstrated a measurable reaction, and the statistically insignificant differences may therefore very well cover up considerable real differences between the products (type II error).

Thus, a reasonable similarity was found only for the extracts of grass pollen, and this can probably be explained by the fact that grass pollen is the allergen from which an acceptable extract can most easily be produced. However, both manufacturers buy the raw materials for pollen extracts from the same company (Allergon, Sweden), while the raw materials for the other extracts, coming from different sources, are probably more or less incommensurable as regards the total content and ratio of different major and minor allergens.

Since no information on batch to batch variation and decay of allergenicity has been made available, it is not known to what extent the difference between the two panels could be ascribed to such factors.

Some investigators (2, 4) make the assumption that differences in allergen concentration are reflected by proportional differences in wheal diameter. However, Björkstén et al. (2) mention that this relationship may not hold, and our data clearly disprove the assumption of proportionality in so far as the intercept of the regression analysis of Fig. 1 was not zero. Our data fit nicely into a model where proportional differences in concentration are measured by absolute differences in the mean wheal diameters. This is in agreement with Turkeltaub et al. (12), who, however, measured erythema - not wheals.

Standardization of allergen extracts remains

an important, very complex and, for obvious reasons, controversial subject of clinical allergy. There is a need for international standards probably based on both *in vivo* and *in vitro* methods (5-7, 14).

ACKNOWLEDGEMENTS

Henning Løwenstein and colleagues at the Protein Laboratory, Copenhagen for valuable discussions, and Dome/Hollister-Stier and Allergologisk Laboratorium, Copenhagen for generous financial support.

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