

Short communication

The 3 mm skin prick test (SPT) threshold criterion is not reliable for *Tyrophagus putrescentiae*: the re-evaluation of SPT criterion to dust mites

Background: The mean wheal diameter ≥ 3 mm is the usual criterion for positive skin prick test (SPT) reaction to dust mites. The study assessed the accuracy of this SPT criterion with respect to specific IgE values of above $0.35 \text{ kU}_A/\text{l}$ (+ sIgE).

Methods: Specific IgE (ImmunoCAP, Pharmacia AB Diagnostics, Uppsala, Sweden) and standard SPT to *Dermatophagoides pteronyssinus* (DP) and *farinae* (DF), *Lepidoglyphus destructor* (LD) and *Tyrophagus putrescentiae* (TP) (ALK, Hørsholm, Denmark) were performed in a random sample of 457 subjects, of whom 273 men (mean age 35.3 ± 11.0 years) and 184 women (mean age 37.9 ± 9.5 years). Statistical analysis was performed using the chi-square test, regression analysis and discriminant analysis.

Results: When the mean wheal diameter of ≥ 3 mm was considered positive (+ SPT), the correlation between + SPT and + sIgE was 0.47 for DP ($P < 0.001$), 0.43 for DF ($P = 0.004$), 0.35 for LD ($P = 0.03$) and 0.37 for TP ($P = 0.014$). Regarding + sIgE, this SPT criterion has a specificity of 92.2% for DP, 82.3% for DF, 80.8% for LD and 70.1% for TP. When the value 4.5 mm was taken as the threshold for the positive SPT reaction to TP, specificity increased significantly from 70.1% to 86.4% ($\chi^2 = 32.04$, $P < 0.001$).

Conclusions: The 3 mm SPT threshold criterion is not reliable in evaluating sensitization to TP due to an insufficient specificity of the allergen extract to this mite. It is advisable either to re-evaluate the TP allergen extract or change the threshold criterion for positive SPT reaction to TP.

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Key words: allergen extract, dust mites, skin prick test, specific IgE, *Tyrophagus putrescentiae*.

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Accepted for publication 28 June 2002

The gold standard for determination of sensitivity to common inhalatory allergens is skin prick test (SPT) and/or the serum level of specific IgE antibodies (1). SPT is the method of choice because of its simplicity, safety and inexpensiveness. The usual criterion for positive SPT reaction is the mean wheal diameter (MD) of ≥ 3 mm (2–4) for all common inhalatory allergens (pollens, mites, moulds and animal danders). In clinical practice, SPT is used for screening, and if positive, it is followed by the IgE antibody measurement (1, 5). The interpretation of findings in diagnostic procedure and their application in the specific treatment is still difficult. In epidemiological studies, SPT is a major diagnostic tool for defining sensitization, but the criteria distinguishing positive from negative test results vary from study to study (most threshold values are at MD 2 mm or 3 mm). However, within each study, these values are unique for all tested allergens (3). Our earlier studies of sensitization to several dust mites in adult population from the continental regions of Croatia included SPT and IgE antibody measurements (6, 7). The results were evaluated using

the common criteria: MD ≥ 3 mm for positive SPT and $> 0.35 \text{ kU}_A/\text{l}$ for detectable IgE antibody. The subjects were tested for several dust mites species, and we noticed that their skin reactions (wheals) varied with the allergen, as well as the ability of positive SPT to detect IgE antibody in serum to the same allergen. The aim of this study was to evaluate the common SPT threshold criterion (MD 3 mm) with respect to IgE antibody values $> 0.35 \text{ kU}_A/\text{l}$ for pyroglyphid and nonpyroglyphid mites in adult subjects, in order to estimate the relative potency of SPT to detect measurable levels of IgE antibody specific to different mites.

Material and methods

The study included a random sample of 457 adult subjects of both sexes from a continental urban region of Croatia: 273 men (mean age 35.3 ± 11.0 years) and 184 women (mean age 37.9 ± 9.5 years). They were recruited during preventive pre-employment examinations. None of the subjects had taken medications that might

interfere with SPT two weeks before skin testing (H1 and H2 antagonists, antidepressants). Corticosteroids were avoided for at least six weeks. Trained staff carried out the SPT on the lower aspect of the subjects' forearms using standard procedure (4, 8) using commercial extracts of *Dermatophagoides pteronyssinus* (10 HEP), *Dermatophagoides farinae* (10 HEP), *Lepidoglyphus destructor* (100 B.U) and *Tyrophagus putrescentiae* (100 B.U) (Pangramin, ALK-Abello, Denmark) and sterile lancets (ALK-Abello, Denmark). Histamine hydrochloride (1 mg/ml) and buffer solution were tested as controls of positive and negative skin reaction, respectively. The distance between test sites of histamine hydrochloride and of dust mite extracts was more than 4 cm (9). SPT results were recorded after 15 min. The mean wheal diameter (MD) was calculated according to the formula $(D + d)/2$, where D represents the largest longitudinal diameter and d its midpoint orthogonal diameter.

IgE antibody to *D. pteronyssinus*, *D. farinae*, *L. destructor* and *T. putrescentiae* was measured using the ImmunoCAP technology – UNI CAP 100 (Pharmacia AB Diagnostics, Uppsala, Sweden). A positive test was defined as values higher than 0.35 kU_A/l (10).

The statistical analysis was performed using statistical software package STATISTICA® (WINDOWS 5.0 A). The analysis included descriptive statistics, tables and banners, correlation matrices, Kolmogorov-Smirnov two-sample test, chi-square test, discriminant analysis and factor analysis. When parametric tests were used, the distribution was normalized by logarithmic transformation. Statistical significance was assumed at $P < 0.05$.

Results

We analyzed the ability of positive SPT defined as MD ≥ 3 mm to detect serum specific IgE antibody concentrations > 0.35 kU_A/l. The highest percentage of subjects with SPT reactions ≥ 3 mm and IgE antibody ≤ 0.35 kU_A/l was found for *T. putrescentiae* (73.2%), followed by *L. destructor* (68.1%), *D. pteronyssinus* (47.8%) and *D. farinae* (35.8%). The percentage of subjects with SPT reactions < 3 mm and IgE antibody > 0.35 kU_A/l was the highest for *D. pteronyssinus* (5.6%), followed by *D. farinae* (3.8%) and *L. destructor* (2.6%), whereas no subject showed such results for *T. putrescentiae*. The SPT threshold criterion of 3 mm has the specificity of 92.3% for *D. pteronyssinus*, 82.3% for *D. farinae*, 80.8% for *L. destructor* and 70.1% for *T. putrescentiae*. Figure 1 shows the mean skin reactions to dust mites in subjects with wheals ≥ 3 mm. The median value for *T. putrescentiae* is significantly higher than those for *L. destructor* (4.5 mm: 4 mm; $P = 0.02$).

The linear regression analysis of subjects with wheals ≥ 3 mm and IgE antibody > 0.35 kU_A/l (Fig. 2A–D) shows a mild to moderate degree of relationship between these two parameters (*D. pteronyssinus*, $r = 0.46$, $P < 0.001$; *D. farinae*, $r = 0.43$, $P = 0.004$; *L. destructor*, $r = 0.35$, $P = 0.03$; *T. putrescentiae*, $r = 0.37$, $P = 0.014$). Table 1 shows the results of a discriminant analysis of different threshold values for SPT reactions with respect to the IgE antibody values > 0.35 kU_A/l. If 4.5 mm is taken as a threshold value for positive SPT reaction to *T. putrescentiae*, a significant increase in the specificity of the SPT to this mite is achieved, that is, from 70.1% to

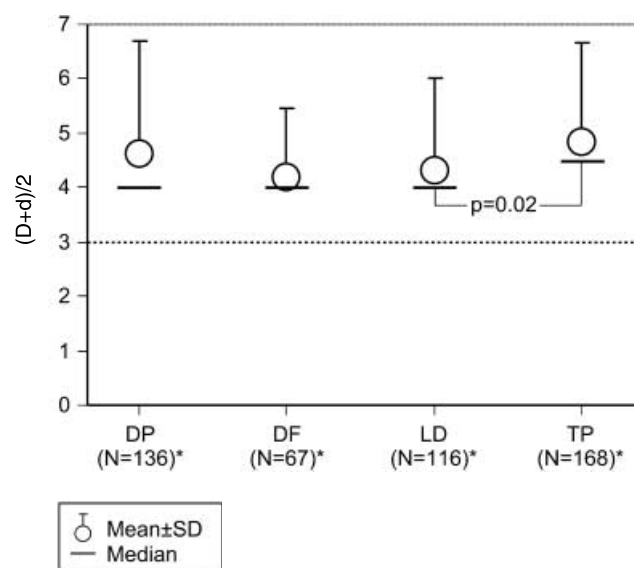


Figure 1. Skin reactivity (urtica's mean and median) to dust mites *Dermatophagoides pteronyssinus* (DP), *Dermatophagoides farinae* (DF), *Lepidoglyphus destructor* (LD) and *Tyrophagus putrescentiae* (TP) in subjects with SPT reaction ≥ 3 mm (Kolmogorov-Smirnov two sample test). *Number of subjects with SPT reaction ≥ 3 mm in total sample ($N = 457$).

86.4% ($\chi^2 = 32.04$, $P < 0.001$), maintaining sensitivity at 77.8%.

Discussion and conclusions

Generally it is expected that a positive SPT reaction would be determined by the presence of specific IgE antibodies in the skin developed after environmental exposure to mite allergens. Mite allergen extracts for SPT are usually prepared from laboratory mite cultures and contain bodies of adult mites, eggs, larvae, faecal particles as well as mite decomposition material and contaminants from the culture medium (11). Therefore, positive SPT reaction can also occur because of the nonallergenic compounds that induce nonspecific reactions, either directly (as histamine) or indirectly (through triggering histamine release) falsely rendering the results positive (1, 12). Furthermore, literature abounds with evidence of cross-reactivity between various dust mite species (that is, between various pyroglyphid mite species as well as between pyroglyphid and nonpyroglyphid mites). The cross-reactivity is due to the protein homology of different mite allergens (13) and can cause positive SPT. Recent studies suggest similarities between the tropomyosin of house dust mites (Der 10) and other invertebrates. These lead to cross-reactivity between dust mites and cockroaches, shrimps, some nematodes (*Anisakis simplex*) and snails (14).

This, and our earlier studies of pyroglyphid and nonpyroglyphid mite sensitization markers (5, 6) showed

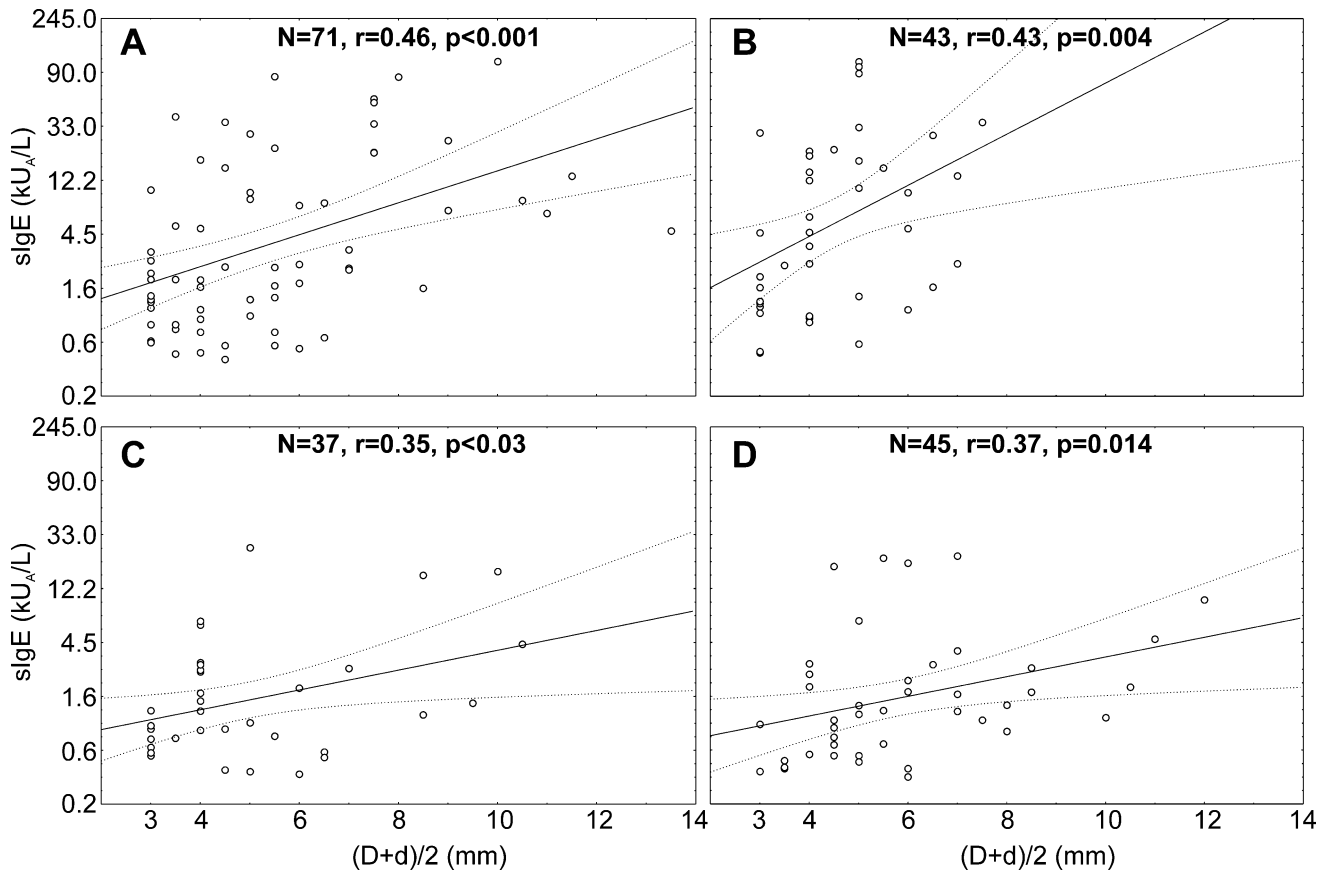


Figure 2. Linear regression analysis of relationship between specific IgE > 0.35 kU_A/L and SPT reaction ≥ 3 mm to *Dermatophagoides pteronyssinus* (A), *Dermatophagoides farinae* (B), *Lepidoglyphus destructor* (C) and *Tyrophagus putrescentiae* (D).

Table 1. Sensitivity and specificity of skin prick test reactions according to different cut-off values to determine raised levels of specific IgE (> 0.35 kU_A/L)

Dust mite		Cut-off 3 mm	Cut-off 4.5 mm	Cut-off 6.5 mm
<i>Dermatophagoides pteronyssinus</i>	sensitivity (%)	79.6	37.0	9.3
	specificity (%)	92.2	99.3	99.7
<i>Dermatophagoides farinae</i>	sensitivity (%)	79.8	46.1	22.5
	specificity (%)	82.3	96.5	99.2
<i>Lepidoglyphus destructor</i>	sensitivity (%)	80.4	34.6	17.4
	specificity (%)	80.8	94.8	98.8
<i>Tyrophagus putrescentiae</i>	sensitivity (%)	100.0	77.8	31.1
	specificity (%)	70.1	86.4	95.9

unexpectedly high prevalence of positive SPT (MD ≥ 3 mm) to *T. putrescentiae* with the greatest mean skin reaction in respect to other tested mites. The positive SPT results to *T. putrescentiae* showed lower ability to detect increased serum IgE antibody than for other mites. Although it can represent a certain level of cross-sensitization between pyroglyphid and nonpyroglyphid mites, cross-reactivity cannot account for all founded differences in SPT reactions to dust mites allergens in our study. It is more likely that they are due

to nonspecific skin reactions to the allergen extract of *T. putrescentiae*.

In conclusion, our results suggest that the criterion using the 3 mm wheal diameter as the threshold for a positive SPT is not reliable enough in detecting measurable levels of IgE antibody specific to *T. putrescentiae*. Using this SPT criterion for epidemiological and preventive medicine (for example, pre-employment examinations) purposes will result in overestimated sensitization to *T. putrescentiae* (15). In clinical practice, it will lead to unnecessary IgE antibody measurements and increased healthcare costs. According to the results of this study, we recommend re-evaluation of the *T. putrescentiae* commercial allergen extract due to its insufficient specificity. Alternatively, we suggest that the threshold value for establishing positive SPT reaction to *T. putrescentiae* should be 4.5 mm. This threshold value has achieved better specificity of SPT, maintaining satisfactory sensitivity. Its application can reduce the number of IgE antibody measurements for about 30% and so the relative cost of diagnostic procedure. However, the very high variation of a SPT (16) should also be taken into consideration when used for diagnosis of IgE-mediated allergy (17).

References

1. EUROPEAN ALLERGY WHITE PAPER. Allergic Diseases as a Public Health Problem. Brussels: UCB Institute of Allergy, 1997, .
2. MALLING HJ. Methods of skin testing. In: DREBORG, S, FREW, A, eds. Position Paper. Allergen Standardisation and Skin Tests. Allergy 1993;**48**(Suppl. 14): 55–56.
3. HAAHTELA T. Skin tests used for epidemiologic studies. In: DREBORG, S, FREW, A, eds. Position Paper. Allergen Standardisation and Skin Tests. Allergy 1993;**48**(Suppl. 14): 76–80.
4. DEMOLY P, MICHEL FB, et al. In vivo methods for study of allergy: skin tests, techniques and interpretation. In: MIDDLETON, E, REED, CE, ELLIS, EF, ADKINSON, NF, YUNGINGER, JW, BUSSE, WW, eds. Allergy. Principles and Practice, 5th edn. St. Louis: Mosby Year Book 1998, 430–439.
5. BOUSQUET J, LOCKEY RF, MALLING HJ, eds. WHO. Position Paper. Allergen Immunotherapy: Therapeutic Vaccines for Allergic Diseases. Allergy 1998;**53**(Suppl. 44).
6. KANCELJAK-MACAN B, MACAN J, BUNETA L, MILKOVIĆ -KRAUS S. Sensitisation to non-pyroglyphid mites in urban population of Croatia. Croatian Med J 2000;**41**:54–57.
7. MACAN J, KANCELJAK-MACAN B, ŽUŠKIN E, MILKOVIĆ -KRAUS S. Sensitization to storage mites in urban working environment. Arh Hig Rada Toksikol 1998;**49**:27–32.
8. DREBORG S, ed. Skin tests used in type I allergy testing: Position paper. Allergy 1989;**44**(Suppl. 10):22–59.
9. TERHO EO, HUSMAN K, KIVEKAS J, RIIHIMAKI H. Histamin control affects the weal produced by the adjacent diluent control in skin prick tests. Allergy 1989;**44**:30–32.
10. AXEN R, DREVIN H, KOBER A, YMAN L. A new laboratory diagnostic system applied to allergy testing. In: JOHANSSON, SGO, ed. Proceedings of a Clinical Workshop "Ige Antibodies and the Pharmacia CAP System in Allergy Diagnosis. Uppsala, Sweden: Pharmacia Publication 1988, 3–5.
11. IPSEN H, LARSEN JN, NIEMEIJER NR, et al. Allergenic extracts. In: MIDDLETON, E, REED, CE, ELLIS, EF, ADKINSON, NF, YUNGINGER, JW, BUSSE, WW, eds. Allergy. Principles and Practice, 5th edn. St. Louis: Mosby Year Book 1998, 404–416.
12. WITTEMAN AM, STAPEL SO, PERDOK GJ, et al. The relationship between RAST and skin test results in patients with asthma nad rhinitis: a quantitative study with purified major allergens. J Allergy Clin Immunol 1996;**97**:16–25.
13. THOMAS WR, SMITH W. An update on allergens. House-dust mite allergens. Allergy 1998;**53**:821–832.
14. SIDENIUS KE, HALLAS TE, POULSEN LK, MOSBECH H. Allergen cross-reactivity between house dust mites and other invertebrates. Allergy 2001;**56**:723–733.
15. KANCELJAK-MACAN B, MACAN J, PLAVEC D, et al. Pre-employment screening for atopic respiratory and skin disorders. In: BASOMBA, A, HERNANDEZ, F DE ROJAS, MD, eds. XVI. European Congress of Allergology and Clinical Immunology, EAACI 95, Madrid: Proceedings II. Monduzzi Editore, Bologna, 1995, 893–897.
16. VOHLONEN I, TERHO EO, KOIVIKKO A, VANTO T, HOLMÉN A, HEINONEN OP. Reproducibility of the skin prick test. Allergy 1989;**44**:525–531.
17. JOHANSSON SGO, HOURIHANE J O'B, BOUSQUET J, et al. A revised nomenclature for allergy. An EAACI position statement from the EAACI nomenclature task force. Allergy 2001;**56**:813–824.