Comparison between the Acarex R test and a Der p 1 ELISA for the detection of house-dust mites in the homes of asthma sufferers

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Abstract **Exposure to house-dust mite allergens in early childhood is an important determinant of the subsequent development of asthma. The Acarex R semi-quantitative test (Noristan) is marketed for use in patients' homes to assess mite levels in house dust. In order to evaluate the reliability of the test in a coastal area where house-dust mites are known to be prevalent, house-dust mite levels were estimated in 119 dust samples obtained from the homes of asthmatic children in a comparative study, by means of the Acarex R test and a Der p 1 enzyme-linked immunosorbent assay (ELISA). A linear regression of the 4 Acarex classes against log Der p 1 revealed a significant correlation (P = 0,0001) but there was a poor correlation between low Acarex R scores and the Der p 1 allergen levels determined by ELISA. Acarex R scores of 2 and 3 were usually associated with Der p 1 levels greater than 10 μg/g dust.**

Our studies indicate that the Acarex R test will identify high levels of mite allergens. Although its application may be limited in coastal areas such as the Cape Peninsula, it may be more useful inland, in climates where house-dust mites are not commonly encountered in all homes.

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House-dust mites are important allergens in the pathogenesis of eczema, asthma and allergic rhinitis.[1,2] Determination of mite allergen levels within the patient's home is important in the assessment of both exposure to allergens and the efficacy of mite avoidance measures employed by mite-sensitive individuals. Since exposure to house-dust mite allergens in early infancy is an important determinant of the development of asthma,[3] it is important to initiate mite avoidance measures early. Until recently, the only method of assessing mite infestation was to count the mites obtained from a dust sample with a light microscope. This method is laborious and time-consuming, it requires acarological expertise and does not give an accurate estimation of the allergen load.

The development of enzyme-linked immunosorbent assays (ELISAs) that make use of monoclonal antibodies directed against the major mite allergens has enabled accurate determination of allergen exposure,

and the expression of results as micrograms of group 1 mite allergen per gram of dust is regarded as the best-validated index of exposure. These assays are not commercially available as yet and are used mainly by research laboratories.[4] The Acarex R test is a simple semi-quantitative dipstick test for guanine and can be used by the patient at home.[5-7] Since guanine is the major component of the nitrogenous excreta of mites, its measurement is an indirect estimation of mite allergen level. The mite allergen content of dust is estimated on the basis of the colour change of the guanine azodye formed by an aromatic diazonium compound in the presence of guanine. In order to validate this test in Cape Town, where we have previously observed high levels of mite allergens with the Der p 1 ELISA,[8] we compared the Acarex R test (Noristan) with measurements of Der p 1 antigen (group 1 antigen of *Dermatophagoides pteronyssinus*).

Methods

Dust samples from the homes of children attending Red Cross Children's Hospital Allergy Clinic were studied. Dust was collected from carpets, and the upper surfaces of the mattresses of asthmatic children whose sensitivity to house-dust mites had been confirmed via skin tests or radio-allergosorbent tests (RASTs). Areas of 1 m^2 of the carpet and 2 m^2 of the upper surface of the mattress were vacuumed for 2 minutes each and the dust sample sieved through an 0,3 mm mesh to obtain fine dust. Dust samples were stored at –20°C until assayed with a Der p 1 ELISA.[4] The Acarex R test was performed on the dust samples according to the manufacturer's instructions. Colour reactions obtained with the Acarex R test were classified by means of a scoring system proposed by the manufacturers:

Score 0 — negative (± 0,01 mg guanine) — no reaction

Score 1 — slightly positive (± 0,6 mg guanine) — yellow

Score 2 — moderately positive (± 2,5 mg guanine) — orange

Score 3 — strongly positive (± 10 mg guanine) — red.

Differences in the levels of Der p 1 allergen obtained in the Acarex score categories were evaluated by means of analysis of variance, and Acarex scores and Der p 1 levels were correlated by means of linear regression. The ability of the Acarex test to identify Der p 1 levels above and below 10 μg/1 g dust (the published threshold level for the development of asthma attacks[9]) was analysed.

Permission to collect dust samples was obtained from the parents of the children. The study was reviewed and passed by the Ethics Review Committee of the University of Cape Town Medical School.

Results

One hundred and nineteen dust samples were analysed. The mean values of Der p 1, standard deviations and 95% confidence intervals are shown in Table I. Comparison of the mean Der p 1 level (μg/g) for each Acarex R score (for guanine) demonstrates a four-fold difference between scores 0 and 1, a 2-fold difference between scores 1 and 2 and an almost 2,5-fold difference between scores 1 and 3 (Fig. 1). Although the data for all categories varied widely and were not normally distributed (Table I), high Der p 1 levels were generally associated with a score of 3. The confidence intervals were fairly large with minimal overlapping between scores 2 and 3. Analyses of variance demonstrated significant differences between the four scores ($f = 16,6$; $P = 0,0001$). Pairwise comparisons by means of Duncan's multiple range testing demonstrated significant differences between scores 1 and 3, scores 0 and 3 and scores 0 and 2 but not between scores 2 and 3.

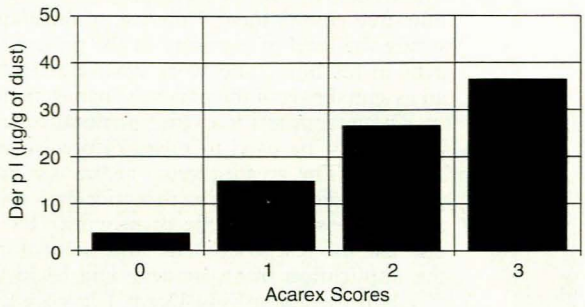

FIG. 1.
Mean Der p 1 levels (μg/g) in dust sample scoring 0 - 3 on the Acarex test.

TABLE I.
Results — mean Der p 1 levels v. Acarex scores

		Der p 1 (μg/g of dust)	
Acarex	No.	Mean (±SD)	95% CI
0	3	3,83 ± 2,86	0,18 – 7,04
1	57	14,96 ± 14,07	11,31 – 18,61
2	27	25,73 ± 15,92	19,725 – 31,74
3	32	36,06 ± 14,36	31,082 – 41,04

The linear regression of the 4 classes against log Der p 1 revealed a significant correlation ($P = 0,0001$).

Three dust samples contained less than 2 μg/Der p 1/g of dust on ELISA. Three dust samples scored 0 on the Acarex R test, and these contained less than 10 μg/Der p 1/g of dust on ELISA. The frequency of samples with Acarex scores 1 - 3, with high (> 10 μg Der p 1/g) and moderate (between 2 and 1 μg Der p 1/g) Der p 1 levels, is shown in Table II. Differences between scores 1 and 3 ($\chi^2 = 20,3$; $P < 0,0001$) and between scores 1 and 2 ($\chi^2 = 9,62$; $P = 0,002$) were significant but there was no significant difference between scores 2 and 3. With an Acarex score of 1, the frequency of samples with moderate levels and high levels of Der p 1 allergens was not significantly different (Table II).

TABLE II.
Frequency (%) of threshold values for different Acarex scores

	Der p 1 (μg/g of dust)	
Acarex scores	< 10	> 10
1	54,39	45,61
2	18,52	81,48
3	6,25	93,75

Discussion

The relationship between exposure to house-dust mites and clinical disease is now well recognised in sophisticated and developing countries. In the highlands of Papua New Guinea, Dowse[10] showed that exposure to house-dust mites increased with the introduction of blankets and that exposure correlated with an increase in symptoms of asthma. In South Africa we have also observed an increase in the incidence of asthma when rural people move to coastal areas where house-dust mites thrive in the warm humid environment. Prolonged exposure to house-dust mite allergens is associated with an increase in bronchial hyperreactivity and renders the

asthmatic more susceptible to the spasmogenic effects of other triggers such as cold air, exercise and viral infection. Since mite-allergic asthmatic subjects experience a significant reduction in their nonspecific bronchial hyperreactivity and in their symptoms when placed in a mite-free environment,[11] the use of techniques for measuring the level of exposure of the patient to mite allergens in his home and work environment have become an essential part of the patient's management.

The Acarex test is a semi-quantitative test and therefore cannot be used to measure precise levels of mite allergens. The greatest value of the test lies in its simplicity. Mite-sensitive asthmatics can use the test to assess exposure at home or at work. The patient can also use the test to evaluate mite control measures and the application of acaricides. The ELISA is the gold standard for quantifying Der p 1 levels and, at present, is only available to research laboratories. Since threshold levels of mite exposure have been determined for Der p 1 allergens, we considered it important to investigate the degree of correlation between Acarex scores and Der p 1 levels. We encountered a wide variation in Der p 1 levels for the different Acarex scores. There were insufficient low levels of Der p 1 and few samples with scores of 0 to analyse the significance of an Acarex score of 0. Our results also indicate that a score of 1 is inconclusive and cannot be used to differentiate between high or low values. However, scores of 2 and 3 were frequently and significantly associated with Der p 1 levels greater than 10 μg/g of dust (81,48% and 93,75% respectively). Scores of 2 and 3 can therefore be used to identify the homes of asthmatics with very high mite contamination in whom rigorous house-dust mite control measures are indicated. We have studied the data in relation to the published threshold values of Der p 1 allergen that result in sensitisation or the development of acute asthma attacks.[9] It has been proposed that levels above 2 μg Der p 1/g of dust constitute a risk factor for allergic sensitisation and that Der p 1 levels of more than 10 μg/g of dust constitute a major risk factor for the development of acute attacks in mite-sensitive asthmatics. The Acarex R test will therefore also identify work environments which are likely to be unsuitable for mite-sensitive asthmatics and may be used to evaluate the efficacy of dust mite control measures in the work environment as well.

With ELISA technology it is now possible to quantify the efficacy of house-dust mite control measures and as a result, certain previously recommended techniques, such as vacuuming, are now known to be ineffective. Even with the enthusiastic use of vacuum cleaners, live mites are not eliminated from carpets and bedding because they generally live deep in fabric, well away from the surface.

Acaricidal cleaning has resulted in a significant improvement in the management of mite-allergy diseases. Compared with the replacement of home textiles, this form of treatment has recently been shown by Kniest *et al.*[12] to be less expensive and more effective. In view of the fact that the Acarex R test is not reliable at low Der p 1 levels, it will not be useful in monitoring the efficacy of house-dust mite elimination strategies.

A number of acaricides are now being marketed in South Africa but some are expensive to apply. Cumulative data from our studies in the Cape Peninsula suggest that in the western Cape, the homes of most asthmatics will require intensive mite avoidance strategies. Although one would expect a similar level of Der p 1 along coastal areas, recent measurements of samples from the Durban area (unpublished data) show that not all homes are infested with house-dust mites. Inland, where it is drier, and mite infestation is much lower actual mite exposure in the patient's home should be quantified for sensitive asthmatics, prior to the undertaking of rigorous and expensive mite control protocols. Our studies have shown that the Acarex R test will identify high levels in the homes in which mite control measures are required, but that the test is more likely to be useful in areas where house-dust mite infestation is not present in every home. However, since actual levels of Der p 1 in different parts of South Africa have only been determined in a few areas, further studies are required in the major climatic regions.

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REFERENCES

1. Van Niekerk CH, Shore SC, Weinberg EG. The house dust mite and childhood asthma in the Cape Peninsula. *S Afr Med J* 1977; **52:** 74-75.
2. Potter PC, Berman P, Toerien A, Malherbe D, Weinberg EG. Clinical significance of allergen identification in the western Cape. *S Afr Med J* 1991; **79:** 80-84.
3. Sporik R, Holgate ST, Platts-Mills TAE, Cogswell JJ. Exposure to house dust mite allergen Der p 1 and the development of asthma in childhood. *New Engl J Med* 1990; **323:** 502-507.
4. Luczynska CM, Arruda LK, Platts-Mills TAE, Miller JD, Lopez M, Chapman MD. A two-site monoclonal antibody ELISA for the quantification of the major *Dermatophagoides* spp. allergens Der p 1 and Der f 1. *J Immunol Methods* 1989; **118:** 227-235.
5. Van Bronswijk JEMK, Birschoff E, Schurmacher W, Berren L, Schaber G. A rapid house dust allergen test: preliminary results. *J Med Entomol* 1986; **23:** 217-218.
6. Van Bronswijk JEMK, Birschoff E, Schurmacher W, Kniest FM. Evaluating mite (Acari) allergenicity of house dust by guanine quantification. *J Med Entomol* 1989; **26:** 55-59.
7. Van der Brempt H, Haddi E, Michel-Nguyen A, *et al.* Comparison of the Acarex test with monoclonal antibodies for the quantification of mite allergens. *J Allergy Clin Immunol* 1991; **87:** 130-132.
8. Manjra A, Berman D, Toerien A, Weinberg EG, Potter PC. The effect of a single treatment of an acaricide (Acarosan) and a detergent (Metsan) on *Dermatophagoides pteronyssinus* (Der p 1) allergen levels and bronchial hyperreactivity. *S Afr Med J* 1994; **84** (in press).
9. Platts-Mills TAE, De Weck AL. Dust mite allergens and asthma — a worldwide problem. *J Allergy Clin Immunol* 1989; **83:** 416-427.
10. Dowse GK, Turner KJ, Stewart GA, Alpers MP, Woodcock AJ. The association between *Dermatophagoides* mites and increasing prevalence of asthma in village communities within the Papua New Guinea highlands. *J Allergy Clin Immunol* 1985; **75:** 75-83.
11. Platts-Mills TAE, Mitchell EB, Norte P, Touer EK, Moszoro H, Wilkins SR. Reduction of bronchial hyperreactivity during prolonged allergen avoidance. *Lancet* 1982; **2:** 675-678.
12. Kniest FM, Wolfs BJ, Vos H, *et al.* Mechanisms and patient compliance of dust mite avoidance regimens in dwellings of mite-allergic rhinitis patients. *Clin Exp Allergy* 1992; **22:** 681-689.