

Prediction of mite allergen levels by guanine measurements in house-dust samples

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We studied the sensitivity and specificity of guanine environmental tests in the evaluation of the two mite allergen levels, i.e. 2 µg/g and 10 µg/g of *Der p* I and *Der f* I, considered to be risk factors for sensitization or for the development of acute asthma. We examined 239 house-dust samples for *Der p* I and *Der f* I levels (ELISA) and guanine contents (semiquantitative guanine test and quantitative assays). All house-dust samples with class 2 or 3 guanine tests contained more than 2 µg/g of *Der p* I and *Der f* I. The probability that house-dust samples of class 2 contained more than 10 µg/g of mite allergens was 88%; it was 100% for house-dust samples of class 3. The probability that a house-dust sample of class 0 contained less than 2 µg/g of mite allergen was 87%. For each level of mite allergen, a ROC curve was constructed with the true positive rates and the false positive rates calculated by different cutoffs of guanine concentration. The cutoff point which gave the best compromise between sensitivity (76%) and specificity (89%) was 2100 µg/g for the threshold of 2 µg/g of *Der p* I and *Der f* I. For detection of a mite allergen > 10 µg, a guanine content of 3000 µg/g gave the best compromise between sensitivity (86%) and specificity (93%). In conclusion, the guanine test represents a satisfactory environmental test, inexpensive and simple, for predicting mite allergen levels. Semiquantitative and quantitative guanine measurements are more accurate in predicting an exposure level of 10 µg/g than of the level 2 µg/g of *Der p* I and *Der f* I.

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The contribution of the mites *Dermatophagoides pteronyssinus* and *D. farinae* to the allergenicity of house dust is an important indoor environmental factor both in the sensitization of atopic subjects and in generating the asthmatic attacks of dust-sensitive patients.

In the past years, new assays have been developed to assess mite levels. Besides immunologic methods, mainly ELISA (7), chemical assays of guanine (1) have been proposed to quantify mite allergens in house dust. Guanine, a metabolic excretion product of chelicerate arthropods, is used as a marker for mite feces; the latter contain the major allergens from house-dust mites (*Der p* I from *D. pteronyssinus* and *Der f* I from *D. farinae*: group I mite allergens). A good overall correlation has been demonstrated between guanine determinations and group I mite allergen (*Der p* I and *Der f* I) levels (3, 5, 14). Up to now, the predictive value of guanine determinations in estimating the level of mite allergens has not been studied in detail.

The purpose of this study was to determine the sensitivity (Se) and specificity (Sp) of the guanine determinations in the evaluation of the two thresholds of mite allergen levels (i.e., 2 and 10 µg of *Der p* I and *Der f* I per g of dust, respectively) considered to be risk factors for sensitization (4, 12) or for the development of acute asthma (10).

Material and methods

We collected 239 house-dust samples by vacuum cleaning under standardized conditions (9) from mattresses, carpets, or upholsteries in homes located in Strasbourg (France). The samples were stored at 4°C in sealed plastic bags until the raw dust was sieved through a 0.3-mm-mesh screen to obtain a fine dust.

Mite group I measurements. Measurements were performed on aqueous dust extracts by a two-site mon-

oclonal antibody ELISA (3, 8). Results were expressed as μg of *Der p* I and *Der f* I per g of fine dust.

Guanine determinations. Guanine determinations were performed on dust samples by the guanine test Acarex® (1) or the spectrophotometric method (6). Acarex-test results were expressed in four upgrading classes (0–3). Quantitative assay results were expressed in μg of guanine per g of fine dust.

Statistical analysis. Determination of the ability of guanine determinations to predict mite allergen levels, as defined by *Der p* I and *Der f* I levels in house-dust samples, was performed by calculating Se and Sp indices (2). Analyses were done in order to situate mite allergen levels with respect to those considered to be risk factors in mite allergic diseases: 2 and 10 μg of *Der p* I and *Der f* I/g of dust. Se corresponded to the true positive (TP) rate (i.e., TP/(TP + FN)). TP represented dust samples with > 2 or > 10 μg of *Der p* I and *Der f* I per g dust, and a positive guanine content; FN (false negative) represented dust samples with > 2 or > 10 μg of *Der p* I and *Der f* I per g dust and a negative guanine content; Sp corresponded to the true negative (TN) rate (i.e., TN/(TN + (false positive) FP)); TN to dust samples < 2 or < 10 μg of *Der p* I and *Der f* I per g dust, and a negative guanine content; and FP to dust samples < 2 or < 10 μg of *Der p* I and *Der f* I per g dust, and a positive guanine content. For the guanine-test, Se and Sp were calculated with different thresholds of guanine positivity (i.e., from class 1 to class 3). For quantitative guanine determinations, the TP and FP rates were calculated with different cutoffs of guanine concentration (at 300 μg intervals) and plotted to construct receiver operating characteristic (ROC) curves (11). This kind of curve allows one to determine the optimum level of guanine concentration, giving the best compromise between Se and Sp for the prediction of a given level of *Der p* I and *Der f* I.

Results

Der p I and *Der f* I contents in house-dust samples

Concentrations of the major mite allergens *Der p* I and *Der f* I measured in 239 house-dust samples varied from 0.03 to 481 $\mu\text{g}/\text{g}$ dust. Considering that *Der p* I and *Der f* I levels represent a risk factor for sensitization (i.e., > 2 $\mu\text{g}/\text{g}$ of dust) or for development of asthma (i.e., > 10 $\mu\text{g}/\text{g}$ of dust), we observed that 80% of house-dust samples tested contained more than 2 μg of mite allergen and 54% more than 10 μg .

Table 1. Distribution of guanine-test in house-dust samples by their *Der p* I and *Der f* I contents

Guanine-test classes	Number of house-dust samples with a <i>Der p</i> I and <i>Der f</i> I concentration			
	< 2 $\mu\text{g}/\text{g}$	> 2 $\mu\text{g}/\text{g}$	< 10 $\mu\text{g}/\text{g}$	> 10 $\mu\text{g}/\text{g}$
Class 0	13	2	14	1
Class 1	34	72	86	20
Class 2	—	86	10	76
Class 3	—	32	—	32
Total	47	192	110	129

Semiquantitative guanine test: sensitivity, specificity

The distribution of the guanine-test classes (0–3) in house-dust samples according to their *Der p* I and *Der f* I levels is given in Table 1. With these data, Se and Sp were calculated, and are reported in Table 2.

When the positive threshold of guanine was set at class 1, the sensitivity of the guanine test for detecting house-dust samples with > 2 or > 10 $\mu\text{g}/\text{g}$ *Der p* I and *Der f* I per g dust was 98 and 99%, respectively, indicating respective FN rates of 2 and 1%. The respective specificities (i.e., 28 and 12.7%) were low because of the presence of FP responses, all of which were class 1 for the level 2 $\mu\text{g}/\text{g}$ *Der p* I and *Der f* I, and 89% were class 1 for the level 10 $\mu\text{g}/\text{g}$ *Der p* I and *Der f* I. The negative predictive value of the guanine test (i.e., probability that house-dust samples of class 0 contain less than 2 μg of mite allergen) is 87%.

When the positivity was set at class 2, the specificity reached 100% at the expense of a loss in sensitivity in detecting house-dust samples with more than 2 $\mu\text{g}/\text{g}$ of *Der p* I and *Der f* I. This threshold (class 2) gave the best compromise between an acceptable FP fraction (FP = 9%) and the highest sensitivity (84%) for identifying house-dust samples with more than 10 $\mu\text{g}/\text{g}$ of *Der p* I and *Der f* I. When the threshold was set at class 3, 100% of house-dust samples with a positive guanine content corre-

Table 2. Sensitivity and specificity of guanine test with different positive guanine thresholds (classes 1–3) for identifying mite allergen exposures > 2 or 10 $\mu\text{g}/\text{g}$ of *Der p* I and *Der f* I

Positive threshold of guanine-test class	<i>Der p</i> I and <i>Der f</i> I level detected in dust samples			
	> 2 $\mu\text{g}/\text{g}$		> 10 $\mu\text{g}/\text{g}$	
	Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)
≥ 1	98	28	99	12.7
≥ 2	61	100	84	91
3	17	100	25	100

sponded to a mite allergen level $> 10 \mu\text{g/g}$. The positive predictive value of the guanine test (or probability that house-dust samples of classes 2 and 3 contain more than $2 \mu\text{g}$ of *Der p* I and *Der f* I) is 100%; the probability that house-dust samples of class 2 contain more than $10 \mu\text{g}$ of mite allergen is 88%; it is 100% for house-dust samples of class 3.

Quantitative guanine determination: ROC curve analysis

Guanine was found in all house-dust samples. ROC curves constructed from the various guanine contents (i.e., with increases of $300 \mu\text{g/g}$) are shown in Fig. 1. The two curves showed that 100% of house-dust samples with less than or equal to 900 and less than or equal to $1200 \mu\text{g/g}$ of guanine contained less than 2 or $10 \mu\text{g}$ of *Der p* I and *Der f* I, respectively. For the identification of house-dust samples with more than $2 \mu\text{g}$ of *Der p* I and *Der f* I (Fig. 1A), the guanine cutoff point which gave the best compromise between Se and Sp was $2100 \mu\text{g/g}$, with an FP rate of 11% and a sensitivity of 76%. Moreover, 100% of house-dust samples with over $3300 \mu\text{g/g}$ of guanine contained more than $2 \mu\text{g}$ of *Der p* I and *Der f* I (Sp = 100%). ROC curve analysis indicated that for detection of a mite allergen level $> 10 \mu\text{g}$ of *Der p* I and *Der f* I (Fig. 1B), a guanine content of $3000 \mu\text{g/g}$ of dust gave the best compromise between the lowest FP fraction (7%) and the highest sensitivity (86%); the specificity reached 100% for house-dust samples with more than $4200 \mu\text{g}$ of guanine.

Discussion

Mite allergen exposure should now be considered to be a risk factor in allergic respiratory diseases (10, 12) and a factor influencing the degree of sensitization of children and young adults (4). It has been proposed that the concentrations of major mite allergens such as *Der p* I in house-dust samples could serve as a good index of mite exposure (13). Determination of guanine, a marker for the presence of mites in house dust, is based on chemical methods and is easier to perform in clinical practice than measurements of group I allergens.

The concordance between guanine content and *Der p* I and *Der f* I allergen concentrations has been established by nonparametric tests and regression analysis (3, 5, 14). We therefore decided to study the association between the *Der p* I and *Der f* I threshold levels of clinical relevance and the guanine contents of house-dust samples by calculating indices of sensitivity and specificity and by using ROC analysis to predict diagnostic validity. Changing the threshold of guanine positivity (i.e., expressed as guanine-test classes or guanine concentration) allows one to evaluate the correct or incorrect predictions of a given *Der p* I and *Der f* I level at a variety of decision levels. An accurate prediction gives few FN responses (i.e., high sensitivity) and few FP responses (i.e., high specificity).

Our results show that a negative response with the guanine test (class 0) or a guanine content less than or equal to $900 \mu\text{g/g}$ of dust, as defined by ROC curves, correctly identifies HDS with $< 2 \mu\text{g/g}$ *Der p*

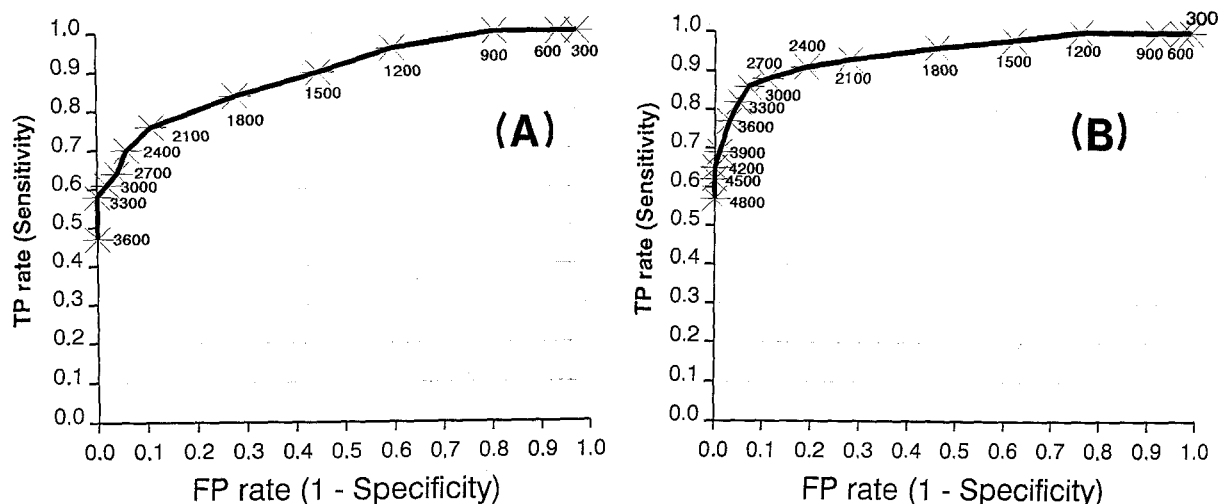


Fig. 1. Receiver operating characteristic curves: true positive rate (TP) and false positive rate (FP) for different guanine concentrations (300- μg intervals) at two mite allergen levels: A. $22 \mu\text{g/g}$ of *Der p* I and *Der f* I; B. $10 \mu\text{g/g}$ of *Der p* I and *Der f* I.

I and *Derf* I. In these cases, mite allergen elimination procedures are not mandatory in a patient's household. This decision could be dangerous, for the FN responses lead to an underestimation of the presence of group I allergens. Actually, the FN rate within class 0 was insignificant. On the other hand, mite allergen avoidance measures should be introduced when the guanine test is class 2 or 3, or if the guanine content is $> 3300 \mu\text{g/g}$. Only class 2 gave few FP responses, and the avoidance procedures will be useful in most cases. A class 1 guanine test will be more difficult to interpret. Two-thirds of the class 1 dust samples contained more than $2 \mu\text{g/g}$ *Der p* I and *Der f* I; these were clinically significant mite allergen concentrations. The others were FP responses overestimating levels of these mite allergens, although they could indicate the presence of other mite species only partly related antigenically to *Der p* I and *Der f* I allergens, such as *D. microceras*, *Euroglyphus manei*, and storage mites (15). A class 1 response with the guanine test needs verification on a second house-dust sample by quantitative assays before clinical decision. These assays are based on a colorimetric method which requires only conventional laboratory equipment and commercial chemical reagents.

In conclusion, the semiquantitative guanine test and quantitative guanine determination represent satisfactory environmental tests to predict house-dust mite allergen levels which are considered to be risk factors. The guanine test has the advantage of being a simple and inexpensive test which can be performed by clinicians or patients themselves at home. It can be used to locate the source of mite allergens or to evaluate the efficacy of avoidance measures.

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