

Comparison between *Der p I* plus *Der f I* content determinations and guanine measurements in 239 house dust samples

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The purpose of this study was to confirm the correlation between the mite allergenicity of HDSs and their actual guanine contents and to assess the reliability of a semiquantitative test (Acarex test, distributed by Fisons Corp., Bedford, Mass.) when it is performed alone. We compared a large number of HDSs by three methods: Acarex-test, quantitative guanine measurements, and *Der p I* plus *Der f I* determinations.

Two hundred thirty-nine samples were studied; 129 were collected under standardized conditions by the same technician in the homes of patients with asthma; 110 HDSs were collected by patients themselves or by healthy individuals with their own vacuum cleaner. HDSs were sieved through a 0.3 mm mesh screen to obtain a fine dust. Guanine measurements (Acarex test) and quantitative guanine determinations are based on a chemical diazo assay.¹ Acarex test results are expressed semiquantitatively in four classes for which the corresponding guanine contents are as follows: 0 (yellow), a guanine content <600 µg/gm of dust; + (light red), a guanine content between 600 and 2500 µg/gm of dust; ++ (red), a guanine content between 2500 and 10,000 µg/gm of dust; and +++ (dark red), a guanine content of at least 10,000 µg/gm of dust.

Quantitative guanine determinations were expressed in micrograms per gram of house dust. A two-site MAb ELISA was used to determine group I al-

Abbreviations used

HDS:	House dust samples
CV:	Coefficient of variation
MAb:	Monoclonal antibody
Dp:	<i>Dermatophagoides pteronyssinus</i>
Df:	<i>Dermatophagoides farinae</i>

lergens (MAb 5H8 specific for *Der p I* and MAb 6A8 specific for *Der f I*).² Results were expressed as micrograms of *Der p I* and *Der f I* per gram of dust.

Comparison of the semiquantitative test versus the quantitative guanine assay, as well as comparison of the semiquantitative test versus the quantitative *Der p I* plus *Der f I* HDS contents were performed with a nonparametric test adapted for the case of ordered alternative hypotheses of the form $\mu_1 \leq \mu_2 \leq \dots \leq \mu_k$ with at least one strict inequality.³ Quantitative relationships between guanine content and major group I mite-allergen contents were investigated by means of regression analysis.

RESULTS

Comparison between semiquantitative and quantitative guanine determinations

Semiquantitative and quantitative guanine determinations demonstrated that guanine concentration intervals for the classes proposed by the manufacturer were, for Acarex class 0 (N = 15), from 281 to 3025 µg/gm of dust (mean, 1278.7; SEM, 181.6); for class + (N = 106), 550 to 4912 µg/gm of dust (mean, 1927.2; SEM, 74.2); for class ++ (N = 86), from 1900 to 15625 µg/gm of dust (mean, 5102.4; SEM, 244.2); and for class +++ (N = 32), from 7750 to 31750 µg/gm of dust (mean, 16218.5; SEM, 1158.3). Statistical comparison³ revealed a significant ordered difference between each class and guanine

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content ($\epsilon(U) = 14.8$; $p < 0.0001$). The threshold for the quantitative guanine determination assay was $100 \mu\text{g/gm}$ of dust. The specificity was excellent; interassay CV ranged from 3% to 15%, and the intra-assay CV was 7.1%.

Der p I and *Der f I* HDS contents and quantitative guanine determinations

The levels of mite allergens *Der p I* plus *Der f I* demonstrated large variations, from 0.03 to $481 \mu\text{g/gm}$ of dust for mattresses and from 0.3 to $93.5 \mu\text{g/gm}$ of dust for other samples. Assay sensitivity was 55 ng/ml for *Der p I* and 7.5 ng/ml for *Der f I*, respectively. Interassay CV ranged from 5% to 20%, and the intra-assay CV was 4%. The graphic representation of quantitative guanine values and the corresponding *Der p I* and *Der f I* contents are illustrated in Fig. 1. These results indicate a significant correlation ($r = 0.76$; $N = 239$). No significant variations among correlations were found regardless of the sampling sites, and the collecting methods ($r = 0.80$, $N = 129$ for technician; $r = 0.80$, $N = 110$ for patients or healthy individuals).

Comparison between Acarex classes and *Der p I* plus *Der f I* HDS contents

A broad range of *Der p I* plus *Der f I* values was found inside each Acarex class. The mean *Der p I* plus *Der f I* values were, for class 0, from 0.125 to $11.5 \mu\text{g/gm}$ (mean, $1.7 \mu\text{g/gm}$; SEM, 3.03), for class +, 0.03 to $25.5 \mu\text{g/gm}$ (mean, $5.7 \mu\text{g/gm}$; SEM, 0.5), for class ++, from 3.9 to $102.3 \mu\text{g/gm}$ (mean, $35.2 \mu\text{g/gm}$; SEM, 2.68), for class +++, from 24.1 to $481 \mu\text{g/gm}$ (mean, $131.3 \mu\text{g/gm}$; SEM, 20.4). Statistical comparison³ revealed a significant ordered difference between each class and *Der p I* plus *Der f I* content ($\epsilon(U) = 14.1$; $p < 0.0001$).

DISCUSSION

In this study we found a good correlation between the quantitative guanine determinations and the group I allergens from *Dp* and *Df*, confirming previous results obtained by Van Bronswijk et al.⁴ We had previously performed studies on the relationship between guanine determination and mite allergenicity^{5,6} on a limited number of samples with cutaneous tests, RAST-inhibition experiments, and Carlie analysis. Discrepancies observed for some samples that contained more guanine than would be expected from their mite allergenicity could be due to the fact that measurements of group I allergens may underestimate the allergen content of samples containing *Dermatophagoides microceras*, *Euroglyphus maynei*, and storage mites.

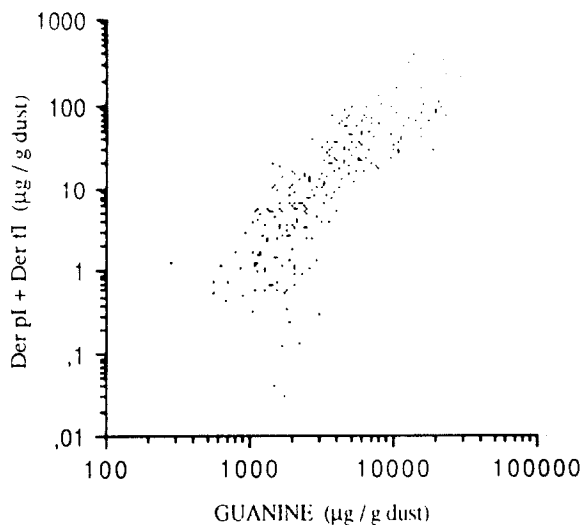


FIG. 1. Correlation between the quantitative guanine values and the *Der p I* plus *Der f I* contents.

The overlap between different Acarex test classes in terms of actual guanine contents could result from the heterogeneity of HD samples; one part of a given sample is used to perform the Acarex test and another part to prepare the extract for quantitative guanine determination. Moreover, some discrepancies between Acarex test classes and guanine content may be partly due to subjective readings of the colorimetric scale. Our results also demonstrated that for class 0, guanine content was greater than the upper limit indicated by the manufacturer. The broad range of *Der p I* and *Der f I* values found for each Acarex test class confirm the results obtained by Lau et al.⁷ and Vervloet et al.⁸ who compared the Acarex test with measurements of *Der p I* plus *Der f I* by an ELISA test. Both author groups found a good overall correlation between the two methods but indicated that there was only a clear-cut difference in *Der p I* and *Der f I* contents between low (0 and +) and high (++++) Acarex classes. A significant point of our own study was also the absence of overlap between class 0 and + and class +++. With respect to levels considered to represent a risk factor for sensitization (i.e., $2 \mu\text{g/gm}$ of dust) or for development of asthma (i.e., $10 \mu\text{g/gm}$ of dust), it should be noted that only class 0 had mean *Der p I* plus *Der f I* values $< 2 \mu\text{g/gm}$ and that classes ++ and +++ had mean values of *Der p I* plus *Der f I* much higher than $10 \mu\text{g/gm}$.

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Urticaria from caffeine

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Caffeine is a naturally occurring biologic amine found in foods, particularly beverages (coffee, tea, cola drinks, and chocolate). Chemically, it is a methylxanthine, a potent pharmacologic agent, closely related to theophylline and theobromine. Caffeine is the most popular and widely used stimulant drug in the world; it causes habituation and has widespread pharmacologic actions that include the stimulation of the central nervous system and the heart and increased output of gastric acid and urine, as well as a moderate bronchodilator effect.¹ A cup of coffee contains between approximately 50 and 150 mg of caffeine, and the pharmacologically active dose is about 200 mg.

We report a patient who developed urticaria after the ingestion of caffeine-containing beverages.

CASE REPORT

A 53-year-old man, with personal and family history of atopy (hay fever), referred to acute episodes of urticaria after ingestion of coffee, tea, cola drinks, and, eventually,

with chocolate. This "sensitivity" had begun 15 years previously, when, 20 minutes after ingestion of a cup of coffee, he developed itching, erythema, and wheals on his face, neck, and upper trunk. These symptoms reappeared with the same latency and in the same locations on every occasion that he ingested one of the aforementioned beverages or foods. He noted that the larger the amount he drank the more severe the eruption. The urticaria usually resolved in 1 or 2 hours without treatment. He also referred to a 20-year history of aspirin idiosyncrasy, developing generalized urticaria, predominantly on the upper trunk, after ingestion of aspirin. He had never suffered from dyspnea or any other extracutaneous symptom with these substances. By avoiding the ingestion of aspirin-like drugs and caffeine-containing foods, he remained asymptomatic. The patient's serum IgE was 129 IU/ml. Skin prick and intradermal tests with an aqueous solution of pure caffeine (1:60 gm/ml) were negative as well as a leukocyte histamine release test (fluoromatic automatized method) with several fivefold dilutions of this solution. Patch tests with undiluted and 1:10, 1:100, and 1:1000 dilutions in saline were negative at 20 minutes, 48 hours, and 96 hours. On two separate occasions, double-blind, placebo-controlled, oral challenge with caffeine (25 mg) administered in white, opaque, tartrazine-free capsules produced itching and urticaria on the face, neck, and trunk in 20 minutes. Precipitating antibodies could not be demonstrated by double gel diffusion in Ouchterlony gel.

To evaluate the role of endogenous histamine in this reaction, the patient was administered terfenadine (60 mg) 1 hour before administering the caffeine. This pretreatment

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