

# Is quantitative sIgE serology suitable to distinguish between silent sensitization and allergic rhinitis to *D. pteronyssinus*?

Short running title:  
**IgE serology instead of nasal challenge?**

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**ABSTRACT**

**Background:** Recently, an increasing number of studies focus on the debate whether provocation tests might be replaceable by specific IgE serology in patients sensitized to airborne allergens.

**Objective:** Our study aimed to analyse the concordance between a nasal provocation test with *Dermatophagoides pteronyssinus* and specific IgE measurements in real-life data.

**Patients and Methods:** In 223 patients with proven sensitization to *Dermatophagoides pteronyssinus*, the concordance between the result of the provocation test and the IgE titer against several house dust mite components and extracts was retrospectively analysed.

**Results:** In contrast to other studies, the anti-Der p 1-level alone was not suitable at all to distinguish between silent sensitization and allergy to *Dermatophagoides pteronyssinus*. ROC curve analysis revealed that the sum of sIgE against Der p 1 and Der p 2 is – after adjustment to the total serum IgE – the best parameter to discriminate between clinically silent and relevant sensitization, however far from reaching a sufficient diagnostic validity.

**Conclusions:** Despite the high correlation between sIgE levels and symptoms, no serologic parameter had a sufficiently high accuracy to distinguish between silent sensitization and clinically relevant allergy. Therefore, nasal provocation tests remain the gold-standard to investigate the clinical relevance in *Dermatophagoides pteronyssinus* sensitization.

**KEY WORDS**

Correlation, IgE serology, Nasal provocation test, Allergy, Sensitization, House dust mite

## RESUMEN

**Antecedentes:** Recientemente, un número cada vez mayor de estudios se han centrado en el debate sobre si las pruebas de provocación podrían ser reemplazables por medición de IgE específica en suero en pacientes sensibilizados a aeroalérgenos.

**Objetivo:** Nuestro estudio tuvo como objetivo analizar la concordancia entre la prueba de provocación nasal con *Dermatophagoides pteronyssinus* y la IgE específica con datos de vida real.

**Pacientes y métodos:** En 223 pacientes con sensibilización probada a *Dermatophagoides pteronyssinus*, se analizó retrospectivamente la concordancia entre el resultado de la prueba de provocación y el título de IgE frente a varios componentes y extractos de ácaros del polvo doméstico.

**Resultados:** A diferencia de otros estudios, el nivel de anti-Der p 1 no fue adecuado para distinguir entre una sensibilización silente y la alergia a *Dermatophagoides pteronyssinus*. El análisis de las curvas ROC reveló que la suma de sIgE frente a Der p 1 y Der p 2, después del ajuste a la IgE sérica total, es el mejor parámetro para discriminar entre sensibilización clínicamente silente y relevante, aunque lejos de alcanzar una suficiente validez diagnóstica.

**Conclusiones:** A pesar de la alta correlación entre los niveles de sIgE y los síntomas, ningún parámetro serológico tenía una precisión suficientemente alta para distinguir entre la sensibilización silente y una alergia clínicamente relevante. Por lo tanto, las pruebas de provocación nasal siguen siendo el patrón estándar para investigar la relevancia clínica de la sensibilización a *Dermatophagoides pteronyssinus*.

### Palabras clave:

Correlación, IgE en suero, provocación nasal, alergia, sensibilización, ácaros del polvo.

## INTRODUCTION

House-dust mites (HDM) are the third most relevant allergen in many countries, following grass and birch pollen [1]. The clinical and socioeconomic impact of allergy to HDM is even increased given the fact that non-treated allergies to HDM run the increased risk of developing asthma [2-4]. Consequently, the correct diagnosing of assumed HDM allergy is crucial in order to initiate an appropriate treatment to prevent the development of asthma. However, as the reliability of the anamnesis is not always high regarding HDM allergy [5], HDM allergy might suppose difficulties to be diagnosed, especially due to the lack of seasonal symptoms. Skin prick test and the determination of allergen specific IgE (sIgE) are the predominantly chosen means to test patients suspected to suffer from HDM allergy [6]. However, both tests yield to detect sensitization, which is, though, not always equivalent to a clinically relevant allergy. To determine the clinical relevance of sensitization, allergen provocation is necessary. Especially in allergic rhinitis, provocation tests are usually performed as nasal allergen challenge [7,8], which is time-consuming. Consequently, many doctors avoid performing nasal provocation test (NPT).

Oral food challenges which are the gold standard of diagnosing food hypersensitivities are even more troublesome for patients and their doctors [9]. Therefore, concerning food allergies, there are many attempts to reduce the need of provocation tests. In the recent years, the question whether *in vivo* challenges might be replaced by less risky *in vitro* tests, is increasingly up to debate with regard to airborne allergens [10].

On the one hand, this trend from *in vivo* diagnostics of IgE-mediated allergies to *in vitro* diagnostics might be due to the great progress in component resolved diagnostics. On the other hand, this tendency towards *in vitro* tests has been anticipated in the European Union because of increased regulatory demands [11]: Diagnostic test allergens used in the EU have to be authorized for each test method in each EU member state. This development leads to a decrease of availability of allergen-specific *in vivo* tests although *in vitro* assays cannot completely substitute the diagnostic value of *in vivo* tests [12].

Consequently, there are an increasing number of publications dealing with the question whether sIgE measurements might help to distinguish between clinically silent sensitization and symptomatic allergy. Whereas earlier studies on this topic primarily used allergen extracts [13,14], recent studies are mainly performed with recombinant allergen components [10,15]. Two recent publications even raised hope that measurement of specific IgEs to Der p 1 might consign HDM provocation tests to history [15,16]. Our study aimed to analyse real-life data if NPT with *Dermatophagoides pteronyssinus* (*D. pteronyssinus*) is replaceable by sIgE measurements. In our department, every patient with assumed allergic rhinitis undergoes a wide skin prick test (SPT), amongst others also to *D. pteronyssinus* which is the

most important HDM in Germany. Additionally, every patient with positive SPT result to HDM and/or a clear anamnesis is tested on sIgE reactivity against natural HDM extract, Der p 1 and Der p 2. Further, every patient sensitized to HDM undergoes a NPT without exception. Consequently, we access a rather large number of patients sensitized to *D. pteronyssinus* with well-known SPT results, sIgE measurements and NPT result. Therefore, an analysis of our large and rather representative patient data appeared meaningful in order to investigate whether sIgE measurements are capable to distinguish between clinically silent sensitization and relevant allergy to *D. pteronyssinus*.

## PATIENTS AND METHODS

### Study population

Patient data were retrospectively selected from the allergy database of the Department of Otorhinolaryngology of the Ludwig-Maximilians-University Munich.

The database was scanned for patients with a proven sensitization to HDM according to a positive skin prick test result or a positive sIgE measurement. All patients underwent skin prick test (SPT) and allergen-specific provocation as routine *in vivo* tests and total IgE and allergen-specific IgEs measurements in serum as *in vitro* tests. Further, the database includes data from a standardized clinical history questionnaire enfolding seasonal complaints, demographic data, living environment, oral allergy syndrome, and medical history. Of 241 patients presenting a positive prick tests with HDM, 9 patients were excluded as false positive due to completely negative IgE measurements.

The retrospective study using anonymized data was approved by the local ethics committee. All patients provided written informed consent concerning the use of their data for scientific purpose.

### Fluorescence enzyme immunoassay (FEIA)

IgE reactivity to natural allergen extract (d1) and allergen components Der p 1 and Der p 2 was measured using the FEIA method (UniCAP-FEIA, Thermo Fisher Scientific, Freiburg, Germany) with a commercially available test kit (Phadia Diagnostics, Freiburg, Germany). In case of a positive SPT to further allergens than HDM, specific IgE antibodies to the corresponding native extracts and allergen components were also measured. Results were reported as concentrations (kU/l).

### **Skin prick test (SPT)**

The SPT solution for HDM by ALK-Abelló, Wedel, Germany was used. The procedure was in line with published guidelines [17]

### **Nasal provocation test (NPT)**

Every patient sensitized to HDM underwent a nasal provocation test (NPT) in accordance with current guidelines [18]. The protocol provided several measurements of active anterior rhinomanometry (RhinoSys, Happersberger otopront GmbH, Hohenstein, Germany), as baseline measurement, after the administration of allergen-free solution (LETI Pharma GmbH, Ismaning, Germany) and finally after the application of allergen-containing solution (*D. pteronyssinus*, 100 HEP/ml; LETI Pharma GmbH) each by a nasal spray pump. Additionally, patients reported on their symptoms leading to a calculated symptom score.

The intranasal challenge was rated positive for patients who showed either a decrease in rhinomanometry of >40% at 150 Pa on the allergen-challenged side, a symptom score of >3, or a combination of a symptom score of >2 and a reduction in airflow of >20%. For the symptom score, several symptoms consisting of secretion, irritation and remote symptoms were semiquantitatively assessed. In case of a negative NPT despite high anamnestic evidence for a clinical relevant HDM allergy, NPT was repeated with a different provocation test solution (Allergopharma GmbH, Reinbek, Germany). In cases of remaining doubts, conjunctival provocation test was carried out in addition: 25 µl of allergen-free test solution was instilled in the inferior-external quadrant of the bulbar conjunctiva of one eye, whereas the same volume of *D. pteronyssinus* containing test solution was instilled to the bulbar conjunctiva of the other eye. After 10 minutes, the response was evaluated by a clinical assessment of itching, redness, tearing and chemosis.

### **Statistical analysis**

Statistical analysis was performed with SPSS 18.0 (IBM, Somers, New York, USA). All of the data failed normality testing. For descriptive statistics we used median values and the range. The Mann-Whitney Rank Sum Test was performed for the comparison of the different groups. A value of  $p \leq 0.05$  was judged significant.

Further, we analysed the receiver operating characteristics (ROC) curve and calculated the area under the curve (AUC), representing the summary of statistic accuracy for sIgE as diagnostic method for distinguishing between clinically silent sensitization and symptomatic allergy.

To compare proportions, the chi-square test was used.

## RESULTS

The database query with the above-mentioned inclusion criteria resulted in 232 patients with a proven sensitization to *D. pteronyssinus*. A sensitization was judged as proven in case of positive sIgE reactivity against the natural HDM extract (d1). Table 1 summarizes the demographics and clinical data of the study population which was divided into two groups: 160 patients with a positive nasal provocation test (NPT) to *D. pteronyssinus* and 72 patients with a negative NPT to *D. pteronyssinus*. Gender distribution showed a slight male predominance in both groups (in average 60% male). The median age was comparable in both groups. Further, the distribution of monosensitization (IgE to one single allergen source), oligosensitization (IgE to 2-4 allergen sources) and polysensitization (IgE to > 4 allergen sources) was similar in both groups, even with similar co-sensitization rates (see Table 1).

34% of all *D. pteronyssinus* sensitized patients suffered from asthma confirmed by lung functioning tests including metacholine bronchial challenge; however, the prevalence for asthma was higher in patients with silent sensitization compared to patients with *D. pteronyssinus* related allergic rhinitis (43% vs. 30%). Among all asthmatic patients, the grade of sensitization was high with 47% being polysensitized. In contrast, *D. pteronyssinus* sensitized patients without asthmatic complaints were only polysensitized in 28% of the cases.

Concerning the laboratory characteristics, which are given in Table 2, more discrepancies between allergic patients and patients with a clinically silent sensitization were found: Despite a higher total IgE serum level, patients without allergic symptoms showed lower sIgE levels against the natural HDM extract (d1) as well as against the tested components Der p 1 and Der p 2. In order to take various total IgE levels into account, the quotient of sIgE levels and the total IgE level in the serum was calculated for each allergen component.

As shown in Table 2 and Figure 1, the levels of sIgE against natural HDM extract d1, r Der p 1 and r Der p 2 were significantly higher in allergic patients than in cases of clinically silent sensitization. As many patients displayed sIgE either to Der p 1 or Der p 2, we summated the sIgE level against Der p 1 and Der p 2 in each group (see Table 2). Comparing the sum of sIgE to Der p 1 and to Der p 2 between the groups improved the level of statistical significance again.

As shown in Figure 2, the proportion of patients with HDM-induced rhinitis was higher among individuals who showed sIgE reactivity to both Der p 1 and Der p 2 (98/120, 82%) than

among those patients who reacted to only one allergen (Der p 1 or Der p 2, 35/63, 56%) or none of them (26/49, 53%).

ROC analysis pointed out a low accuracy for sIgE against the HDM extract (AUC = 0.641; 95% confidence interval = 0.557-0.725), against Der p 1 (AUC = 0.652; 95% confidence interval = 0.573-0.73) and against Der p 2 (AUC = 0.654; 95% confidence interval = 0.578-0.73). Again, taking the sum level of sIgE to Der p 1 and to Der p 2 improves the result slightly by enhancing the AUC to 0.659 (95% confidence interval = 0.583-0.736).

As patients with clinically silent sensitization had a higher total IgE level in serum than allergic patients, we calculated a quotient of the sIgE levels and the total IgE levels before re-analysing the ROC curves for each parameter. For total IgE-adjusted sIgE against the HDM extract, AUC changed to 0.661 (95% confidence interval = 0.582-0.74), against Der p 1 to 0.678 (95% confidence interval = 0.599-0.756) and against Der p 2 to 0.671 (95% confidence interval = 0.596-0.745). Again, the sum of both sIgE to Der p 1 and sIgE to Der p 2, adjusted to the total IgE level, showed the best correlation to the NPT result (AUC = 0.680; 95% confidence interval = 0.603-0.757; see Figure 3).

Due to earlier studies, the age might influence the results of *in vivo* and *in vitro* tests [19-21]. Therefore, we analyzed the ROC curves after excluding data from patients younger than 12 years and older than 60 years. However, the age-adjustment did not lead to a relevant change of the above-mentioned results.

## DISCUSSION

The present study on real-life data clearly demonstrates a significant correlation between allergen specific IgE serum levels and nasal provocation testing in HDM sensitized patients. However, in contrast to other studies [15,16], the concordance between *in vitro* and *in vivo* measurements is not strong enough to make a method exchangeable one by another. A recent study by Comite P *et al.* suggested a Der p 1 threshold value of 5.5 kU/l for distinguishing between silent sensitization and symptomatic allergy in patients positive to Der p 1 - with a corresponding sensitivity of 94% and a specificity of 84% [15]. Applying this cutoff value to our Der p 1 positive patients led to a sensitivity of 78% and a specificity of 55%. In order to analyse and understand this discrepancy between our data and other studies, a precise comparison of the methods used and populations studied among different publications is crucial.

To our knowledge, the present study based on 232 patients deals with the largest study population which was ever analyzed in the context of correlation between *in vivo* and *in vitro* tests of HDM allergy in times of component resolved diagnostics. Most published studies using recombinant allergen components enrolled a number of patients lower than 100 [15,16,19]. However, according to Metz *et al.*, a valid qualitative conclusion can be drawn from ROC curves including approximately 100 clinical cases [22].

Despite the large number of patients, our study results have to be interpreted with caution as data were obtained during clinical routine and, therefore, do not reach the quality standards of prospective trials. For example, neither the doctors nor the patients were blinded to the SPT and serology results when performing the NPT. However, although the included patients do not represent the general population, the study population appears relatively representative for the population with allergic rhinitis: Every patient with assumed allergic rhinitis was tested on HDM sensitization and, in a second step, every patient with any hint on HDM sensitization underwent sIgE measurements and NPT. Due to this approach, which did not accept any exception, a bias regarding the selection of study patients is less probably. Further, every patient underwent NPT to distinguish between sensitization and allergy even if anamnesis might be suggestive for either the existence or the absence of allergic symptoms under HDM exposure. Many other studies have the limitation that the clinical relevance of sensitization is not always proven by provocation tests, but partially only assumed due to anamnesis [10,15,23]. The condition of rhinitis can be caused by a huge variety of different factors; concerning nasal obstruction, as one major symptom of rhinitis, a recent review gives a detailed overview [24]. Especially in patients sensitized to HDM, quality and severity of symptoms are rather comparable in patients with negative and positive NPT [25]. As the anamnesis is, therefore, not suitable to evaluate the clinical relevance of HDM sensitization,

every patient sensitized to HDM underwent a nasal challenge in our study. However, one methodological limitation of our study might be the various age among our patients ranging from 6 to 73 years. According to earlier studies [19-21], the age affects the skin test and sIgE reactivity. Therefore, we performed age-adjusted subgroup-analysis, which, however, did not yield any change in our results.

Further, the reliability of NPT is still on debate. However, we performed NPT according to current German guidelines [18] which are similar to the Spanish guidelines [26]. In a very recently published position paper on the standardization of nasal allergen challenges, the European Academy for Allergy and Clinical Immunology recommends a protocol which highly concurs with the method used in this present study [27].

The patients' demographic and clinical features seem to confirm the representative character of our study population: The rate of clinical relevance of HDM-sensitization was 69% in our population which is, according to Burbach *et al.*, typical for Germany [28].

The overall prevalence for self-reported asthma was 34%. A meta-analysis on 13.558 subjects with HDM sensitization from 16 countries reported an overall prevalence for asthma of 21%, however with interpopulation heterogeneity [29]. In a recent observational survey on 1589 patients with proven HDM allergy in France, approximately 42% of all patients with HDM related allergic rhinitis had allergic asthma [30]. In our study population, only 30% of patients with allergic rhinitis due to *D. pteronyssinus* also had asthma. This discrepancy might be due to the fact that the interaction between exposure to HDM allergens and symptoms is very complex in asthmatic patients and influenced by environmental and genetic factors [2].

Among patients without HDM-caused allergic rhinitis, the prevalence for asthma was unexpectedly even higher (43%). Though, a negative NPT to HDM does not imply the absence of any other allergy as potential eliciting factor for asthma: 47% of our asthmatic patients were polysensitized which makes it difficult to determine the contribution of a specific allergen to airway inflammation [31].

In sum, we can conclude from the demographic, clinical and laboratory characteristics of our study population that our collective appears representative. Given the representative character of our study population, the immense discrepancy between the results of the present study and earlier publications cannot be explained only by methodological limitations of our study. Comite *et al.* who presented a remarkable AUC of 0,95 for Der p 1 to distinguish between sensitization and allergy [15], had based their study on a rather artificial study population of 73 patients sensitized to Der p 1. The hypothesis that this study population is not representative is emphasized by the low rate of clinical relevance among the study group: Only a half of the study patients had allergic symptoms. However, according to Burbach *et al.* [28], the rate of clinical relevance among HDM sensitized patients in Italy is

about 90%. Another methodical reason for our discrepant results compared to literature might be the fact that the total IgE level in serum has not been taken into account in others studies. For example, in the study by Minami *et al.* who showed a clear concordance between clinical relevance and sIgE measurements, the total IgE values in sera of allergic patients were more than three-fold higher compared to patients with clinical silent sensitization [16]. One could debate that the correlation between sIgE measurements and clinical relevance would have been weaker than published, if this relevant difference in total IgE level had been taken into account. Another methodological difficulty which every study on the concordance of *in vivo* and *in vitro* test of HDM-allergy is confronted with, is the definition and following exclusion of false-positive patients. We excluded nine patients as false-positive after their positive SPT was followed by negative sIgE measurements against the natural HDM extract. In some studies, there is a rather large number of patients with positive SPT to HDM, but missing IgE reactivity against allergen components: In the study by Haxel *et al.*, for example, 27 patients out of 50 cases with silent sensitization (54%) displayed this constellation with positive SPT, but negative sIgE measurements [6]. As it remains unclear whether sIgE against the natural HDM extract were determined, false-positive SPT results cannot be excluded.

Irrespective of the methodological differences and discrepancies regarding results among the different publications concerning HDM allergy, all studies - including the present analysis - clearly demonstrate that the risk of allergic symptoms increases with the level of sIgE against the natural HDM extract, Der p 1 and Der p 2. This finding is in line with a study by Olivieri *et al.*, who proved sIgE level to be the most important predictor for allergen-related symptoms [32]. The highest risk of having a clinical relevant HDM sensitization was found in patients who reacted to both Der p 1 and Der p 2. Vidal *et al.* had found a similar association between simultaneous reactivity to Der p 1 and Der p 2 and asthma [33]. When both allergens Der p 1 and Der p 2 were analyzed individually, both titers performed equally well for the prevalence of clinical relevant sensitization. This result is in contrast to the study by Sylvestre *et al.* who had found higher titers of Der p 2 in patients with severe atopic asthma [34]. However, this subgroup of patients was not present among our population.

However, despite this reproducible observation that sIgE levels correlate with the prevalence of allergic symptoms, our data demonstrate that the statistical accuracy of sIgE against any tested allergen component as well as natural HDM extract is too low to distinguish between sensitization and allergy. This finding is in line with earlier investigations which, however, usually determined sIgE only against the natural HDM extract [25,35]. There are several potential explanations why quantitative sIgE serology is not suitable to predict the clinical relevance of HDM sensitization: First of all, the allergic tissue reaction induced by an allergen does not only depend on the level of sIgE, but also on the number and sensitivity of effector

cells in the skin or the nasal mucosa. Further, the skin or nasal sensitivity can be influenced by allergen-specific neutralizing IgG which might inactivate allergic effector cells by competing with IgE. Additionally, the structure of IgE epitopes on an allergen can vary, as well as the affinity of sIgE.

## CONCLUSION

In summary, our real-life data on 232 patients sensitized to HDM show - in contrast to recently published studies - that measuring sIgE against Der p 1 or Der p 2 or natural HDM extract is not suitable to predict the clinical relevance of HDM sensitization. According to our data, quantitative sIgE serology cannot replace the NPT which remains the gold-standard test to evaluate the clinical relevance of HDM sensitization. We consider this an important message in times of growing interest in molecular-based allergy diagnostics: The current attempt of the industry to reduce the testing portfolio endangers the reliable diagnosing of allergic rhinitis, for which availability of NPT solutions is crucial.

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## TABLES

**Table 1. Demographics and clinical data of patients allergic to *D. pteronyssinus* compared to patients with a clinically silent sensitization to *D. pteronyssinus*:** Except for the rate of self-reported asthma which was higher in patients with negative nasal provocation test, the clinical and demographic data were comparable in both groups.

Characteristics	Positive NPT (n=160)	Negative NPT (n=72)	Total (n=232)
Gender			
Male	98 (61%)	42 (58%)	140 (60%)
Female	62 (39%)	30 (42%)	92 (40%)
Age (yrs)	29 (range: 18-39)	30 (range: 18-48)	29 (range: 17-41)
Monosensitized to HDM	35 (22%)	13 (18%)	48 (21%)
Oligosensitized (to 2-4 allergens)	65 (40%)	37 (51%)	102 (44%)
Polysensitized (to $\geq 5$ allergens)	60 (38%)	22 (31%)	82 (35%)
Co-sensitization against			
Grass	101 (63%)	46 (64%)	147 (63%)
Birch tree	77 (48%)	37 (51%)	114 (49%)
Ash tree	89 (56%)	30 (42%)	119 (51%)
Mugwort	33 (21%)	11 (15%)	44 (19%)
Cat	60 (38%)	31 (43%)	91 (39%)
Asthmatic complaints	48 (30%)	31 (43%)	79 (34%)
Severity of rhinitis (according to ARIA criteria)			
Not known	50 (31%)	5 (7%)	55 (24%)
Known	110 (69%)	67 (93%)	177 (76%)
Intermittent mild	12 (11%)	10 (15%)	22 (12%)
Intermittent moderate-severe	23 (21%)	18 (27%)	41 (23%)
Persistent mild	11 (10%)	10 (15%)	21 (12%)
Persistent moderate-severe	64 (58%)	29 (43%)	93 (53%)
<i>Except for the age which is given as median and range, values are number of patients total and percent of each evaluated subgroup.</i>			

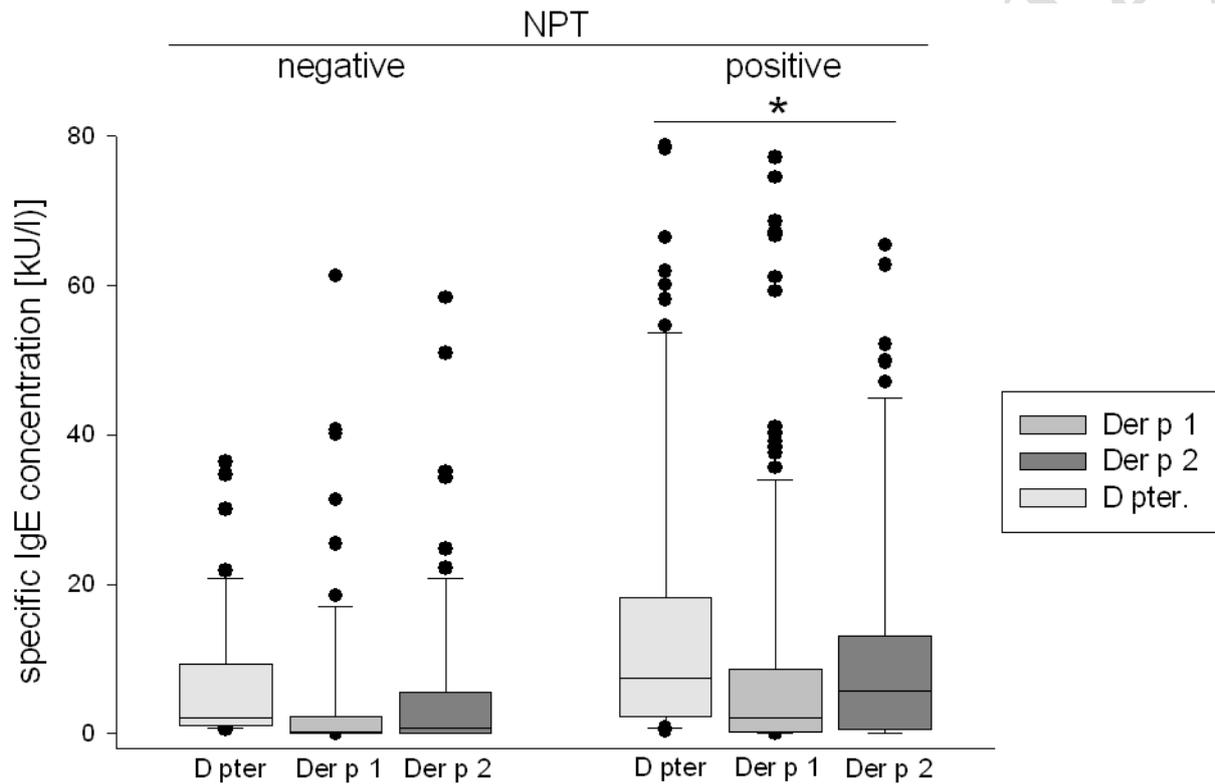
**Table 2. Laboratory characteristics of patients allergic to *D. pteronyssinus* compared to patients with a clinically silent sensitization to *D. pteronyssinus*.** Despite a higher total IgE level, patients with negative nasal challenge had lower titers of sIgE against Der p 1 and Der p 2. As shown in the lowest section of the table, 21% of all cases did not show any sIgE against either Der p 1 or Der p 2. The rate of cases missing sIgE against both major allergens was higher among patients with negative nasal challenge compared to patients with positive nasal provocation test (38% vs. 16%).

Laboratory characteristics	Positive NPT (n=160)	Negative NPT (n=72)
Total IgE (kU/l)	148 (range: 60-294)	187 (range: 88-408)
Natural extract d1	160 (100%)	72 (100%)
CAP class	3 (range: 2-3.25)	2 (range: 2-3)
Serum IgE level (kU/l)	7.33 (range: 2.33-17.6)	2.07 (range: 1.05-9.35)
Quotient sIgE/total IgE	0.056 (range: 0.021-0.13)	0.0157 (range: 0.008-0.051)
Der p 1+	112 (70%)	31 (43%)
CAP class	3 (range: 2-3)	3 (range: 2-3)
Serum IgE level (kU/l)	4.91 (range: 1.65-15.1)	3.86 (range: 1.43-13.8)
Quotient sIgE/total IgE	0.016 (range: 0.0019-0.06)	0.001 (range: 0.0002-0.02)
Der p 2+	133 (83%)	40 (56%)
CAP class	3 (range: 2.75-4)	2.5 (range: 2-3)
Serum IgE level (kU/l)	8.55 (range: 3.56-18.9)	4 (range: 1.41-12.5)
Quotient sIgE/total IgE	0.04 (range: 0.003-0.1)	0.0047 (range: 0-0.031)
(Der p 1+2) > 0	134 (84%)	49 (68%)
sum serum IgE level (kU/l)	12.23 (range: 4.73-32.95)	4.65 (range: 1.64-17.15)
Quotient sIgE/total IgE	0.103 (range: 0.038-0.205)	0.039 (range: 0.011-0.137)

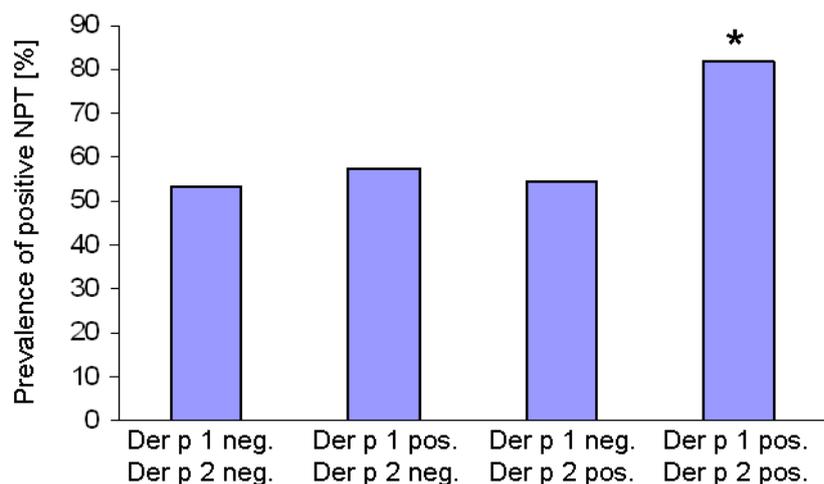
*Concentrations and CAP classes are given as median and range, further values are number of patients total and percent of each group.*

## FIGURE AND TABLE LEGENDS

**Figure 1. sIgE titer against HDM extract (D pter), Der p 1 and Der p 2 in patients with positive NPT to *D. pteronyssinus* compared to patients with negative NPT: All measured sIgE titers were significantly higher in patients with positive nasal challenge compared to patients with clinically silent sensitization (\*  $p < 0.05$ ).**



**Figure 2. Prevalence of clinical relevant allergy to HDM in relation to positivity or negativity of sIgE to Der p 1 and Der p 2:** The proportion of HDM allergic individuals was higher among patients with sIgE reactivity against both HDM components compared to patients reacting to only one component or none (\* $p < 0.001$ ).



**Figure 3. Receiver operating characteristic (ROC) curve of sIgE testing (anti-Der p 1/total IgE + anti-Der p 2/total IgE) and results of the nasal provocation test:** The ROC curve analysis reveals a positive correlation between sIgE titer and the result of nasal challenge – with low accuracy, however.

