Molecular Sensitization Profile to Dermatophagoides pteronyssinus Dust Mite in Portugal

Short title: Dust Mite Molecular profile in Portugal

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Abstract

Objective: to analyze component resolved diagnosis to *Dermatophagoides pteronyssinus* (*Der p*) in patients with respiratory allergy and its relationship with clinical severity in different geographical areas.

Methods: 217patients (mean age 25.85±12.7 years; 51.16% females) were included, selected from 13 centers in Portugal (5 from North, n=65). All had allergic rhinitis, with or without asthma, and had positive skin prick tests to at least one dust mite.Specific IgE (sIgE) to *Der p, Dermatophagoides farinae*, *Lepidoglyphus destructor*, *Der p1*, *Der p 2*, *Der p 10* and *Der p 23* were determined by ImmunoCAP. Statistical analysis(Mann Whitney U test) compared patients with rhinitis vs rhinitis and asthma; mild vsmoderate-to-severe rhinitis; North vs South.

Results: Prevalence of sensitization was 98.2% to *Der p*, and 72.4%, 89.4%, 9.7% and 77% to *Der p 1*, *Der p 2*, *Der p 10* and *Der p 23*, respectively. Corresponding median sIgE levelswere 8.56, 17.7, 0.01 and 3.95 kU_A/L. sIgE to all allergens was higher in patients with moderate-to-severe rhinitis and rhinitis with asthma but not statistically significant (NSS). sIgE to *Der p 2* was significantly higher in the South when compared with the North (p=0.0496).

Conclusions: sensitization to *Der p* is the most common in Portugal. *Der p 2* had the highest prevalence and median sIgE levels. All sIgE to molecular components were higher in more symptomatic patients (NSS). sIgE to *Der p 2* was higher in the South, which may be related to the warmer temperature and/or the larger sample size.

Key words: Allergy. Asthma. Component Resolved Diagnosis. *Dermatophagoides pteronyssinus*. Dust Mites. Rhinitis.Specific IgE.

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Resumen:

Objetivo: analizar el diagnóstico por componentes para Dermatophagoides pteronyssinus (Der p) en pacientes con alergia respiratoria y su relación con la gravedad clínica en diferentes áreas geográficas.

Métodos: se incluyeron 217 pacientes (edad media 25.85 ± 12.7 años; 51.16% mujeres), seleccionados de 13 centros en Portugal (5 del Norte, n = 65). Todos tenían rinitis alérgica, con o sin asma, y tenían pruebas positivas en prick a al menos un ácaro del polvo. La IgE específica (sIgE) para Der p, Dermatophagoides farinae, Lepidoglyphus destructor, Der p 1, Der p 2, Der p 10 y Der p 23 se determinaron por ImmunoCAP. El análisis estadístico (prueba U de Mann Whitney) comparó pacientes con rinitis frente a rinitis y asma; rinitis leve frente a moderada-grave; Norte frente a Sur.

Resultados: La prevalencia de sensibilización fue de 98.2% para Der p, y 72.4%, 89.4%, 9.7% y 77% para Der p 1, Der p 2, Der p 10 y Der p 23, respectivamente. Las medianas de sIgE fueron de 8.56, 17.7, 0.01 y 3.95 kUA12 / L. Las medianas de sIgE de todos los alérgenos fue mayor en pacientes con rinitis de moderada a grave y rinitis con asma, pero no estadísticamente significativo (NSS). El valor de Der p 2 fue significativamente mayor en el Sur en comparación con el Norte (p = 0.0496).

Conclusiones: la sensibilización a Der p es la más común en Portugal. Der p 2 tuvo la prevalencia más alta y los niveles medios más altos. Todos los componentes moleculares fueron mayores en pacientes más sintomáticos (NSS). El valor de Der p 2 fue mayor en el Sur, lo que puede estar relacionado con la temperatura más cálida y/o el tamaño de muestra más grande.

Palabras clave: Alergia. Asma. Diagnóstico por componentes. Dermatophagoides pteronyssinus. Ácaros del polvo. Rinitis. IgE específica.

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Introduction

House dust mites (HDM) are a major perennial allergen source and a significant cause of allergic rhinitis and allergic asthma. HDM allergen sensitization varies from 65 to 130 million persons in the general population worldwide. The prevalence and the relative abundance of various species varies from one region to another[1-3]. Dust mite allergens, namely those of *Dermatophagoides pteronyssinus (Der p)*, are the most prevalent ones in Portugal[4, 5].

Currently, 30 allergens of *Der p* have been identified and sequenced [6]. The "major" allergenic molecules are *Der p 1*, *Der p 2* and *Der p 23* and they are responsible for IgE responses in more than 50% of HDM-allergic patients[7-10].

The term 'respiratory allergic disease' recognizes a unifying allergic mechanism for the pathogenesis of allergic subtypes within asthma and rhinitis[11]. Identification and treatment of HDM allergy is a worthwhile investment for future patient outcomesand componentresolveddiagnosis (CRD) offers the possibility of a higher diagnostic precision and better management of each patient[1, 12, 13].

The aim of this study was to analyze CRD to *Der p* in patients with respiratory allergy to dust mites, and its possible relationships with clinical severity and geographical areas.

Methods

Study design and population

A multicentric study was conducted with a total of 217 HDM-allergic patients followed in 13Allergy and Clinical Immunology Departments/Units in Portugal from different geographical areas, 5 from the North (n=65) and8 from the South (n=152). The patients were randomly selected from January to December 2018. All the patients hada medically confirmed diagnosis of allergic rhinitis, classified as mild or moderate-to-severe, with or without asthma, according to ARIA (Allergic Rhinitis and its Impact on Asthma) and GINA (Global Initiative for Asthma)guidelines[11, 14]. Patient selection was based on the following criteria: 1- allergic respiratory symptoms (rhinitis with or without asthma) after exposure to HDM; 2- positive skin prick test (wheal ≥ histamine 10mg/mL) to *Der p* and/or *Dermatophagoides farinae (Der f)* and/or *Lepidoglyphus destructor (Lep d)* (Diater, Madrid, Spain)*;*

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3-age between 3 and 60 years. None of these patients had been previously treated with immunotherapy with dust mite allergens.

The study was approved by all thehospital ethics committees and signed informed consent was obtained from all patients and parents or legal guardians for those under 18 years old.

Laboratory analysis

Serum specific IgE (sIgE)to *Der p*, *Der f* and *Lep d*, as well as the molecular components of*Der p*, specifically, *Der p 1*,*Der p 2, Der p 10* and*Der p 23*, were determined in all patients (n=217) by the ImmunoCAP® system (ThermoFisher Scientific, Uppsala, Sweden), according to the manufacturer's instructions.Results ≥0.35 kU_A/L were considered positive. Prevalence was defined as percentage of patients with positive serum sIgE.

Western Blot with the*Der p* extract was pwrformed and it presented all the allergens according to the Liquid Chromatography Mass Spectrometry analysis described by the WHO/IUIS [6] ensuring that any patient sensitized to this source was correctly diagnosed. Therefore, proteins from *Der p* extract were separated by sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) according to Laemmli [15] in 15 % polyacrylamide gels under reducing conditions and transferred onto polyvinylidene difluoride (PVDF, Trans-Blot Turbo™. BIO-RAD, Hercules, CA, USA). IgE antibody binding to allergens was analyzed by Western blot using all the patients´ sera from each center and anti-human IgE peroxidase conjugate (SouthernBiotech, Birmingham, USA). *Der p* sIgE were distributed according to 3 level: 0.35-3.5 kU_A/L; 3.5-50 kU_A/L; 50->100 kU_A/L.Chemiluminescence detection reagents (Western Lightning™ Plus-ECL, PerkinElmer. Waltham, MA, USA) were added according to the manufacturer's instructions.

Statistical analysis

Statistical analysis was performed using the IBM SPSS statistical software package (v.22). Descriptive parameters such as means and standard deviations for normally distributed continuous data, frequencies, and percentages for categorical data, were calculated. Parametric quantitative data

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werepresented as the mean and standard deviation. Non-parametric quantitative data were presented as amedian (25% to 75% interquartile range). Categorical data were reported as a percentage showing the proportion of positive results. The Mann Whitney U test was used to compare serum IgE between groups (patients with rhinitis vs rhinitis with asthma, mild vs moderate to severe rhinitis, patients from Northern centers vs Southern centers). Differences were considered statistically significant if $p<0.05$.

Results

Characteristics of study patients

Two hundred and seventeen patients with respiratory allergy and sensitization to at least one dust mitewere included in the study. Seventy-six were children (35%).The mean age was 25.8±12.7 years (minimum 3; maximum 58; median 24 years) and51% werefemale. According to the ARIA guidelines,all the patients had allergic rhinitis, which was mild in 43.8% (n=95) and moderate-to-severe in 56.2% (n=122).Furthermore, 52% (n=113) of patients had concomitant asthma.

Sensitization profile

With respect to dust mites, the prevalence of positive serum sIgE was higherfor*Der p*, followed by *Der f* and *Lep d*. *Der p* was by far the allergen with the highest median sIgE level, followed by*Der f* and *Lep d* (Table 1). Regarding CRD, *Der p 2* was the most prevalent, followed by *Der p 23*, *Der p 1* and *Der p 10*. The *Der p 2* molecular component had the highest sIgE level, followed by *Der p 1*, *Der p 23* and *Der p 10* (Table 1). The sensitization profile was similar in children and adults (Table 1). Among the 213 patients sensitized to *Der p*, 7.5% (n=16) were monosensitized to *Der p 2*, 2.8% (n=6) to *Der p 23* and 0.9% (n=2) to *Der p 1*.

Component resolved diagnosis and clinical severity and geographical areas

With respect to clinical severity, median sIgE levels (kU_A/L) to *Der p 1*, *Der p 2*, *Der p 10* and *Der p 23* tended to be higher in patients with moderate-to-severe rhinitis than those with mild rhinitis (18.15vs 14.20, 22.10 vs 17.65, 5.76 vs 1.36, 6.87 vs 6.14, respectively) but this difference was not

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statistically significant. In this context, we also observed that*Der p 2* has the highest IgE value.In addition, sIgE (kU_A/L) levels to *Der p 1*,*Der p 2*, *Der p 10* and *Der p 23* were higher in patients with concurrent rhinitis and asthma when compared with those with rhinitis alone (20.35 vs 17.20, 23.50 vs 20.00, 7.29 vs 5.69, 7.32 vs 6.65, respectively), although this was not statistically significant. Once again, IgE levels were higher for *Der p 2* (Table 2).

According to geographical areas, patients from the Southern centers displayed significantly higher concentrations of *Der p 2*sIgE (kU_A/L)than those from the Northern centers (19.10 vs 11.30; p=0.0496; Table 3 and Figure 1).

TheIgE Western blot to *Der p*that was carried out with all the blood sera from each center according to IgE levels, revealed that each group of patients presented a characteristic pattern of recognition, either by center or by the titration of sIgE to the source studied. Furthermore, all the groups presented a more intense IgE binding for the *Der p 2* allergen which proved to be the most prevalent and the one with the highest levels of sIgE(Figure 2).

Discussion

In this multicenter nationwide study, we not only confirmed that *Der p* sensitization is the most common sensitization to house dust mites in Portugal, as previously described in the literature [4,5], but we also characterized, for the first time in our country, details of molecular sensitization and its relationship to clinical and geographical factors.

This multicentric study in Portugal highlighted the molecular sensitization profile of the most prevalent HDM [16, 17].The *Der p 2*molecular component was the most prevalent, followed by *Der p 23*, *Der p 1* and *Der p 10*, supporting the worldwide recognition of the former three as major allergens [7-9, 18]. Similar studies were performed in other countries around the world with the prevalence of sensitization to*Der p 1* varying between 44.4% and 93%[19-28], *Der p 2* between 53.5% and 96%[19-28], and*Der p 23* between 45% and 71%[22, 24, 25]. Several of these studies, especially those carried out in Europe, reported that the frequency of patients sensitized to *Der p 2* was higher than those sensitized to*Der p 1*, as we observed in this study [19, 20, 22, 23, 24, 25, 28].It should be highlighted

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that, in comparison to similar studies, we obtainedone of the highest levels of prevalence of sensitization to *Der p 2*. Higher values were only reported by Barber et al with 96% in a Vizcaya center in Northern Spain [20].

Although sIgE levels to *Der p*molecular components were higher in more symptomatic patients, that is, in patients with moderate-to-severe rhinitis and in patients with concomitant asthma, this trend was not statistically significant.According to studies of the prevalence of IgE recognition and allergen-specific IgE levels, *Der p 1*, *Der p 2* and *Der p 23* appear to be clinically relevant andthere appears to be a correlation between higher levels of sIgE of molecular components and the severity of allergic respiratory disease[19-28].Some studies have shown that IgE levels to *Der p 1*, *Der p 2* and *Der p 23* were significantly higher in patients with asthma (with or without concomitant rhinitis) compared to patients with rhinitis alone [20, 22, 23, 26, 28]. On the other hand, Bonnert et al found no association between the prevalence of IgE reactivity to HDM components and the diagnosis of asthma or rhinitis [21].

Regarding geographical distribution of sensitization patterns, we found that concentrations of *Der p 2* sIgE in the Southern centers were significantly higher when compared with the Northern centers. This may be related to the warmer southern temperature and/or the larger sample size. *Der p 1*is a cysteine protease located in the mite intestine and it is more thermolabile while*Der p 2* is an intracellular lipid-carrier protein that is more thermostable and thismay, therefore, explain the higher intensity of sensitization in the warmer Southern regions of Portugal [7, 29, 30, 31]. However, other reasons may underlie these differences since, in Spain,our neighboring country, Barber et al studied a total of 477 patients from 10 different clinical groups throughout the Mediterranean and Atlantic regions, where mites arerelevant allergenic sources, and they did not observe any differences between the thermal pattern of each region and the prevalence or sIgE levels to *Der p 2* [20].

Our study has several limitations. First, there were more centers from the South than the North of the country and this may have influenced our results. Second, we selected patients on the basis of a high level of sensitization to HDM, based on SPT results (wheal ≥ histamine 10mg/mL) and not on serum levels of *Der p*, *Der f* or *Lep d*-specific IgE. This may have biased our results since correlations

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between CRD and SPT may not have been optimal [28]. Third, we did not determine asthma severity or control or fully characterize how long the patients had had asthma or rhinitis for, or the medication they were on, and this may have hampered our capacity to adequately determine disease severity. Nevertheless, our study is novel, multicentric, and thorough in terms of HDM-related CRD analysis, and itendeavoured to characterize allergic disease severity not only in terms of isolated rhinitis versus concurrent rhinitis and asthma but also in terms of ARIA-based severity and persistence of rhinitis.

In conclusion, our data supports the relevant role of *Der p 2* in mite allergy which presented the greatest intensity of sensitization, especially in the more severe allergic respiratory disease and in the warmer Southernregions of the country. This study confirms the importance of molecular components in improving diagnosis in mite allergic patients. We provide evidence for the importance of major allergens in patients with respiratory allergy to HDM and the clinical implications of CRD. Finally, since mite immunotherapy represents approximately 50% of the total volume of the vaccine market, our study may contribute to the development of HDM immunotherapy treatments with a more precise allergen content and potentially greater efficacy and safety [32, 33, 34].

Presented at conferences:

This study has been presented as an oral communication atthe *European Academy of Allergy and Clinical Immunology* Congress in June/2019 and at the 40th annual meeting of the Sociedade Portuguesa de Alergologia e Imunologia Clínica in October/2019.

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Conflicts of interest

Pineda F was at the time of the study and is currently workinginDiaterLaboratorio de Diagnostico y AplicacionesTerapeuticas SA. However, there are no direct conflicts with the data presented in this study. The remaining authors declare that they have no conflicts of interest, financial or otherwise.

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Table 1. Dust mite sensitization and *Dermatophagoides pteronyssinus*molecular profile by *in vitro* tests.

	ImmunoCAP (sIgE)	Prevalence, N (%)	sIgE, Mean± SD kU_A/L	sIgE, Maximum/ Minimum kU_A/L	sIgE, Median kU_A/L
Global	*Der p*	213 (98.2)	42.5 ± 37.9	100 / 0.03	31.9
	Der f	211 (97.2)	30.6 ± 32.3	100 / 0.02	17.5
	Lep d	184 (84.8)	22.6 ± 30.3	100 / 0.02	8.12
	Der p 1	157 (72.4)	21.7 ± 29.7	100 / 0	8.56
	Der p 2	194 (89.4)	30.6 ± 34.0	100 / 0	17.7
	Der p 10	21 (9.7)	1.6 ± 9.2	100 / 0	0.01
	Der p 23	167 (77)	12.1 ± 19.9	100 / 0	3.95
Children (<18 years)	*Der p*	76 (100)	61.72 ± 37.72	100 / 0.54	66.5
	Der f	76 (100)	44.2 ± 35.63	100/0.32	31.2
	Lep d	63 (83)	30.16 ± 31.2	100/0.02	12.1
	Der p 1	67 (88)	35.16± 35.83	100 / 0	20.4
	Der p 2	74 (97)	47.36 ± 36.85	100 / 0	35.5
	Der p 10	8 (10.5)	2.20± 12.5	100 / 0	0.01
	Der p 23	60 (78.9)	22.61 ±26.40	100 / 0	10.5
Adults (≥18 years)	*Der p*	136 (97.1)	31.78 ± 33.77	100 / 0.03	17.3
	Der f	135 (96.4)	23.33 ± 28.17	100/0.02	11.3
	Lep d	121 (86.4)	18.74 ± 26.73	100/0.02	6.89
	Der p 1	95 (67.8)	13.92 ± 26.73	100/0	5.01
	Der p 2	122 (87.1)	21.07 ±28.40	100/0	9.09
	Der p 10	13 (4.2)	1.25 ± 7.27	100/0	0
	Der p 23	109 (77.8)	6.51 ± 12.28	100/0	2.33

sIgE: specific IgE.

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Table 2. Association between Component Resolved Diagnosis to *Dermatophagoides pteronyssinus* and severity of respiratory allergic disease.

	sIgE, median (IQ) kU_A/L			**sIgE, median (IQ) kU_A/L**		
	Mild Rhinitis	**Moderate-to-severe Rhinitis**	***P* value**	**Rhinitis**	**Rhinitis with asthma**	***P* value (Mann Whitney U test)**
Der p	28.30 (5.54-74.80)	36.00 (8.20-99.78)	0.354	32.80 (6.80-83.40)	38.30 (9.00-100.0)	0.259
Der p 1	14.20 (5.43-42.20)	18.15 (6.55-49.78)	0.382	17.20 (5.96-46.15)	20.35 (6.90-49.33)	0.501
Der p 2	17.65 (5.46-42.60)	22.70 (6.41-66.90)	0.192	20.00 (6.14-56.78)	23.50 (9.29-67.20)	0.232
Der p 10	1.36 (1.13-23.50)	5.76 (3.66-22.40)	0.4	5.69 (1.38-22.60)	7.29 (3.16-25.05)	0.654
Der p 23	6.14 (2.31-13.40)	6.87 (2.26-23.60)	0.416	6.65 (2.26-18.40)	7.32 (2.56-27.40)	0.489

sIgE: specific IgE. IQ: 25% to 75% interquartile.

Table 3. Association between Component Resolved Diagnosis to *Dermatophagoides pteronyssinus* and geographical areas.

	sIgE, median (IQ) kU_A/L		
	Northern centers	**Southern centers**	***P* value (Mann Whitney U test)**
Der p	20.80 (5.35-73.95)	35.35 (7.17-84.18)	0.192
Der p 1	7.06 (0.04-23.30)	8.84 (0.14-39.28)	0.342
Der p 2	11.30 (1.96-32.25)	19.10 (4.71-56.08)	0.0496*
Der p 10	0.00 (0.00-0.03)	0.01 (0.00-0.03)	-
Der p 23	2.53 (0.28-9.16)	4.99 (0.81-13.33)	0.083

sIgE: specific IgE. IQ: 25% to 75% interquartile.

*Statistically significant

Figure legend

Figure 1. Specific IgE levels of Component Resolved Diagnosis to *Dermatophagoides pteronyssinus* according to geographical area.

*Statistically significant (*P*=0.0496)

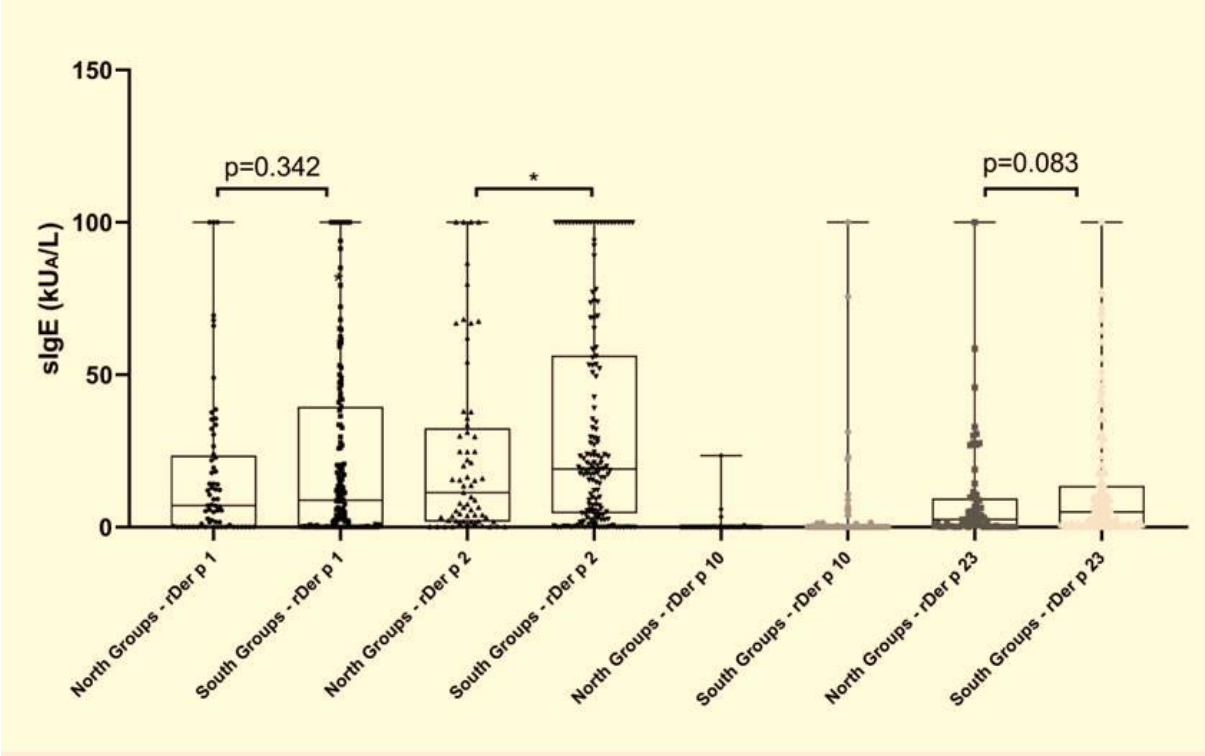

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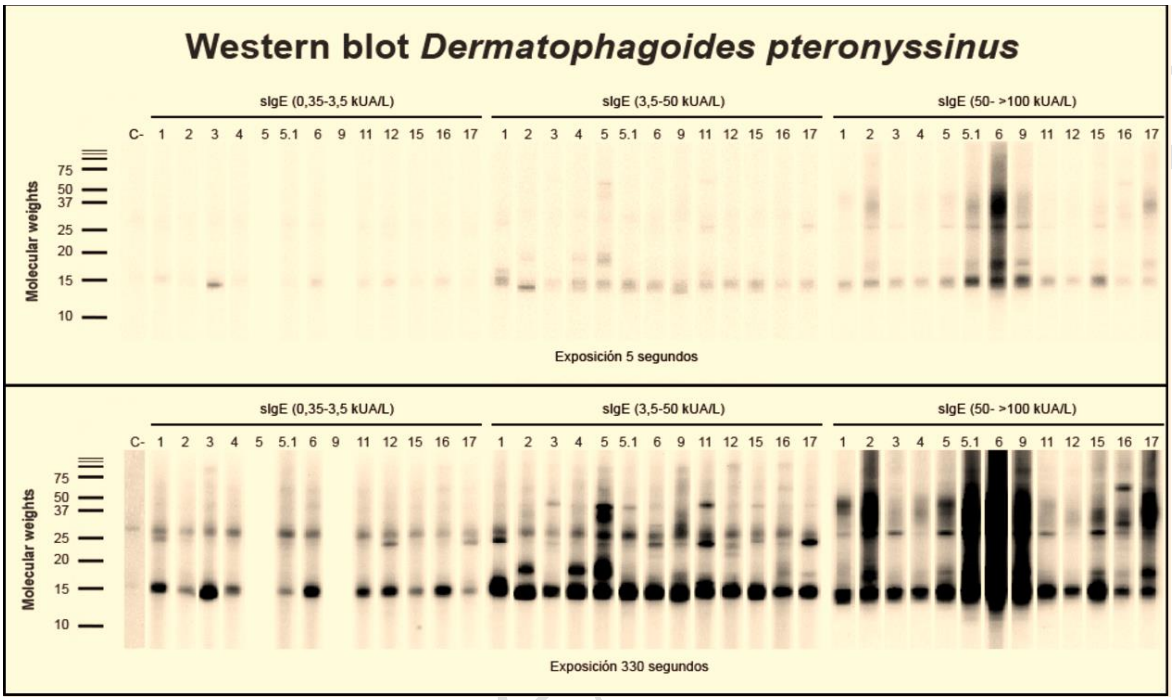
Figure 2. Distribution of the pool of sera from each center according to specific IgE levels.

Center 1 (S), center 2 (S), center3 (S), center 4 (S), center5 (S),center 5.1 (S), Lane 6 (S),center 9 (N), center 11 (N), center 12 (N), center15 (S), center16 Lane 16 (N), center 17 (N).

N: North. S: South.



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