Proceedings of the Tenth International Conference on Urban Pests
Rubén Bueno-Marí, Tomas Montalvo, and Wm. H Robinson (editors) 2022
CDM Creador de Motius S.L., Mare de Deu de Montserrat 53-59,
08930 Sant Adrià de Besòs, Barcelona, Spain

SENSITIZATION TO AVIAN OR FUNGAL PROTEINS IN DIFFERENT WORK ENVIRONMENTS

[1]SILVIA SÁNCHEZ DÍEZ, [1,2]MARÍA JESÚS CRUZ CARMONA, [1,2]IÑIGO OJANGUREN ARRANZ, [1]CHRISTIAN ROMERO MESONES, [5]JUAN CARLOS SENAR JORDA, [3]SANDRA FRANCO GUTIÉRREZ, [3]VICTOR PERACHO TOBEÑA, [1,2]XAVIER MUÑOZ GALL, AND [3,4]TOMÁS MONTALVO PORRO

[1] Servicio de Neumología, Departamento de Medicina, Hospital Universitario Vall d'Hebron, Passeig de la Vall d'Hebron 119, 08035 Barcelona, Spain
[2] CIBER Enfermedades Respiratorias (Ciberes), Av. Monforte de Lemos 3-5, 28029 Madrid, Spain
[3] Servei de Vigilància i Control de Plagues Urbanes Agència de Salud Pública de Barcelona, Pl. Lesseps 1, 08023 Barcelona, Spain
[4] CIBER de Epidemiología y Salud Pública (Ciberesp), Av. Monforte de Lemos 3-5, 28029 Madrid, Spain
[5] Museu de Ciències Naturals de Barcelona, Passeig Picasso s/n, Parc Ciutadella, 08003 Barcelona, Spain

Abstract Hypersensitivity pneumonitis (HP) is usually caused by the inhalation of avian and fungal proteins. The present study assesses a cohort of Urban Pest Surveillance and Control Service (UPSCS) workers with high exposure to avian and fungal antigens, in order to identify their degree of sensitization to these antigens and the potential risk of developing HP. The study population was formed by bird investigators and/or managers at UPSCS of the Public Health Agency, Parks and Gardens staff in Barcelona and employees of private urban pest control firms. Workers were divided according to their work activity in two groups: Nests pruning and Others. All individuals underwent a medical interview regarding exposure, pulmonary function tests and specific IgG antibodies. Antigenic proteins of pigeon sera were investigated using 2-dimensional immunoblotting with sera from patients with HP, asymptomatic exposed controls and healthy volunteers. Proteins of interest were sequenced by liquid-chromatography-mass scpectrometry (LC-MS). One hundred and one workers have been included in the study (76 men, average age: 42 years); 41 in the Nests pruning group and 60 in the others group. In the nests pruning group, specific parakeet IgGs were higher (p=0.03) and FVC% and DLCO/VA% were lower (p=0.04 and 0.01, respectively). Two-dimensional immunoblotting showed protein bands of 20-30 KDa recognized by HP patients but not by workers. LC-MS analysis identified Ig Lambda chain and Apolipoprotein A-I as candidate proteins to distinguish HP patients from exposed workers. We observed a high degree of sensitization to avian and fungal antigens in the study population. In the nests pruning group, alterations in some pulmonary function parameters were found. We identified two pigeon proteins that may play a role in the development of pathological differences between HP patients and exposed workers. This study was funded by ISCIII (PI15 / 01954), FEDER and FUCAP.

Key words hypersensitivity pneumonitis, antigens, exposed workers, biomarkers

INTRODUCTION

Hypersensitivity pneumonitis (HP) is an interstitial lung disease characterized by bronchoalveolar inflammation that occurs, in some genetically predisposed individuals, after the repeated inhalation of certain organic substances (e.g. avian and fungal proteins) (Quirce et al., 2016; Selman et al., 2004). HP has been recently classified into two different forms: acute/inflammatory and chronic/fibrotic. The acute is related to cellular inflammation and appears after discontinuous exposure to high antigenic levels. Nevertheless, it is often resolved with causal antigen avoidance. The chronic form is characterized by fibrotic areas inside lungs that appear due to repetitive exposure to low antigenic doses. It causes respiratory insufficiency, which compromises patient's survival, and in most cases it is irreversible (Lacasse et al., 2009; Vasakova et al., 2017).

The diagnosis of HP remains challenging because of the absence of a gold standard technique. In the clinical practice HP patients are diagnosed depending on a combination of clinical, imaging and laboratory findings such as the presence of specific IgGs against a causative antigen in serum (Schuyler et al., 1997; Ohtani et al., 2000; Lacasse et al.,

2003; Millerick-May et al., 2016). However, IgG determination is just an evidence of antigenic sensitization. So, exposed but asymptomatic individuals can also present high IgG levels against a specific antigen without having the disease. In fact, there are studies demonstrating that up to 50% of healthy individuals exposed to birds can be sensitized to avian antigens (Rodrigo et al., 2000). Regarding fungal proteins, between 30-60% of the exposed subjects can develop specific antibodies (Erkinjuntti-Pekkanen et al., 1999).

In large cities, a possible risk group for the development of HP are individuals who work in pest control, being the avian population control one of its most important functions. Among their work activities we can found the capture of different birds (pigeons, parrots, big parrots...), the destruction of nests and tree pruning. In this sense, they are really exposed to avian and fungal proteins during their workday. The prevalence of sensitization in these workers to avian or fungal proteins and the specific antigenic proteins causing HP pathology are unknown. Several groups have described some antigenic substances that are found in bloom, serum, droppings (Koschel et al., 2010) and/or intestinal mucin of different birds (Nademi et al., 2013). IGLL-1 and ProE are examples of proteins recently identified as causative antigens of bird-related HP (BRHP) (Rouzet et al., 2017; Shirai et al., 2017) but many others have to be discovered.

The present study assesses a cohort of Urban Pest Surveillance and Control Service (UPSCS) workers with high exposure to avian and fungal antigens, in order to determine their degree of sensitization to these antigens and the potential risk of developing HP. The comparison of this cohort with patients diagnosed with HP due to bird exposure could supposed the identification of antigenic proteins with diagnostic value.

MATERIALS AND METHODS

Patients with BRHP due to pigeon (n=5) diagnosed between 2009-2016 in Vall d'Hebron Hospital (Barcelona, Spain), according to the criteria proposed by Schuyler and Cornier (Schuyler et al., 1997), were included in the study to be compared with the exposed group (n=101). This last group was formed by bird investigators and/or managers at UPSCS of the Public Health Agency, Parks and Gardens staff in Barcelona and employees of private urban pest control firms. These individuals underwent a medical interview regarding exposure and were divided according to their work activity and avian exposure degree in two groups: Nests pruning (n=41, basically parks and gardens staff) and Others (n=60, e.g. biologists, administrative and pest control staff). Pulmonary function tests were performed in all individuals of the exposed group to discard respiratory symptoms.

Specific IgG antibodies against avian (pigeon, parakeet, small parrot and parrot) and fungal (*Aspergillus fumigatus and Penicillium frequentans*) proteins were determined in serum samples of all individuals of the study using a direct ELISA method. The positivity of the results depended on previously established cut-offs for each antigen using a cohort of healthy subjects. One dimensional and 2D electrophoresis were performed in order to study antigenic proteins in pigeon and small parrot serum. For 1 D electrophoresis, avian serums were loaded onto a preparative Tris-Glycine gel according to the manufacturer's manual (Bio-Rad, Madrid, Spain). For 2D electrophoresis, avian serums were loaded onto immobilized pH 3-10 gradient strips (General Electric Healthcare, Boston, USA) to carry out the isoelectric focusing. Then, the strips were loaded onto a preparative Tris-Glycine gel (Bio-Rad, Madrid, Spain) to perform the 2D electrophoresis.

After electrophoresis, proteins were blotted to a membrane using a transfer system. Blocked membranes were incubated with diluted human serum samples and were then revealed with a secondary antibody and substrate according to the supplier's recommendation (Bio-Rad, Madrid, Spain). Spots of interest were cut from gels and sent it to an external sequencing service (Institut Oncològic Vall d'Hebron, Barcelona, Spain).

All data were analyzed using Fisher's exact test or chi-square for categorical variables and Mann-Whitney U-test or Unpaired T-test for quantitative variables (GraphPad Prism 6.01, Graphpad Software Inc, San Diego, USA). P-value <0.05 (two-tailed) was considered to be significant.

RESULTS AND DISCUSSION

No significant differences were observed regarding to sex or smoking habits between workers of Nests pruning and Others groups (Table 1). Workers of the Nests pruning group were older and had lower FVC% (forced vital capacity) and DLCO/VA% (diffusion capacity for carbon monoxide). These findings could suggest a restrictive respiratory pattern due to an interstitial disease among others (Enright, 2016). Regarding specific IgGs antibodies, workers in the Nests pruning group had higher levels of IgGs against parakeet. Nevertheless, this levels only demonstrate that workers of that group have been exposed to parakeets and not necessary that they suffer from the disease (Rodrigo et al., 2000).

Table 1. Demographic data, pulmonary function and IgG antibodies in study population.

			Nests Pruning (n=41)	Others (n=60)	p
Age, median (range)			48 (27 - 64)	32 (20 - 62)	<0,0001
Sex, M n(%)			33 (80)	43 (72)	0,3557
Smoking					0,6046
Smoker, n (%)			8 (23)	13 (23)	
Exsmoker, n (%)			12 (34)	14 (25)	
Non smoker, n (%)			15 (43)	29 (52)	
Pulmonary function	FVC%, median (range)		96,1 (70,2 -121,6)	100,3 (76,1 - 126,8)	0,0386
	FVC <80%, n (%)		6 (16)	3 (5)	0,1478
	FEV1%, median (range)		96,75 (70 - 137,4)	100,6 (74 - 143,6)	0,4616
	FEV1 <80%, n (%)		3 (8)	2 (4)	0,3770
	TLC%, median (range)		101,3 (73,2 - 139,6)	95,35 (73,6 - 158,1)	0,1288
	TLC <90%, n (%)		9 (24)	22 (38)	0,1862
	DLCO/SB%, median (range)		82,45 (60 - 110,3)	87,4 (62,4 - 118,8)	0,4533
	DLCO/SB <80%, n (%)		14 (39)	14 (24)	0,1637
	DLCO/VA%, median (range)		79,65 (62,5 - 107,3)	87,9 (63,1 - 113,1)	0,0104
	DLCO/VA <80%, n (%)		19 (53)	14 (24)	0,0072
Specific IgG antibodies	Pigeon (0,284)	median (range)	0,236 (0,031 - 0,843)	0,156 (0,044 - 1,802)	0,1574
		% positives	41,5	31,7	
	Parrot (0,294)	median (range)	0,332 (0,046 - 1,161)	0,238 (0,049 - 1,900)	0,1534
		% positives	58,5	36,7	
	Small Parrot (0,193)	median (range)	0,196 (0,057 - 1,048)	0,167 (0,052 - 1,434)	0,390
		% positives	51,2	41,7	
	Parakeet (0,348)	median (range)	0,258 (0,060 - 0,757)	0,212 (0,036 - 1,715)	0,0306
		% positives	24,4	21,7	
	Penicillium (0,687)	median (range)	0,697 (0,263 - 2,347	0,931 (0,180 - 2,387)	0,8919
		% positives	85,4	81,7	
	Aspergillus (0,417)	median (range)	1,278 (0,346 - 2,623)	1,412 (0,168 - 2,545)	0,3035
		% positives	73,2	77,3	

Table 2. Identification of pigeon serum proteins by liquid chromatography-mass spectrometry.

Spot ID*	MW (kDa)ˡ	Protein identified (theoretical MW in kDa)	Accession Number‡	Score	Peptides	Sequence coverage (%)¶
1	25	Ig Lambda chain (22,9)	A0A2I0LZC1	136,7	3	20,6
1	25	Apolipoprotein A-I (30,6)	A0A2I0LQE2	169,2	8	27,3
2	25	Apolipoprotein A-I (30,6)	A0A2I0LQE2	524,9	18	52,3

MW: molecular weight; *Each spot is described by its number on the 2D gel and WB (Figure 2); ˡ Apparent MW extrapoled from the standard; ‡ Accession number in UniProt database; ¶ Percentage of the protein covered by matched peptides.

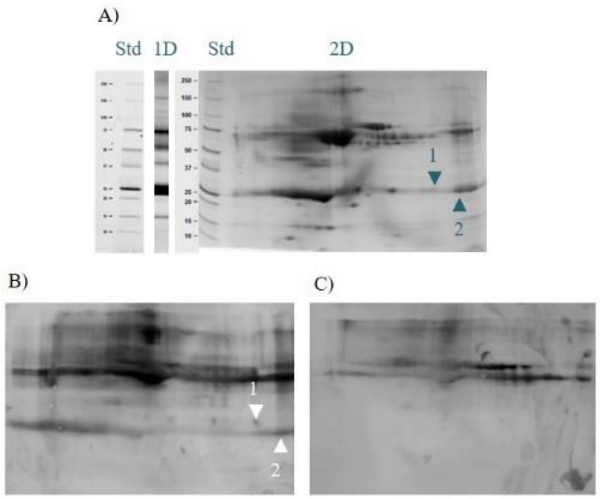

Figure 1. A) 1D and 2D gel of pigeon serum. B) Western blot against pigeon serum of a patient with HP due to pigeon. C) Western blot against pigeon serum of a worker of a private urban pest control firm.

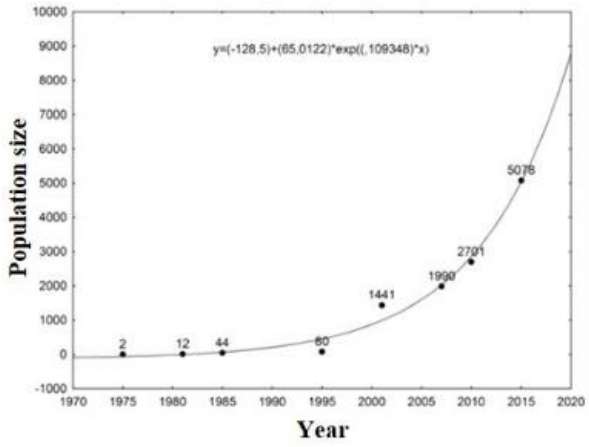

Figure 2. Small parrot population size in Barcelona (1975-2015).

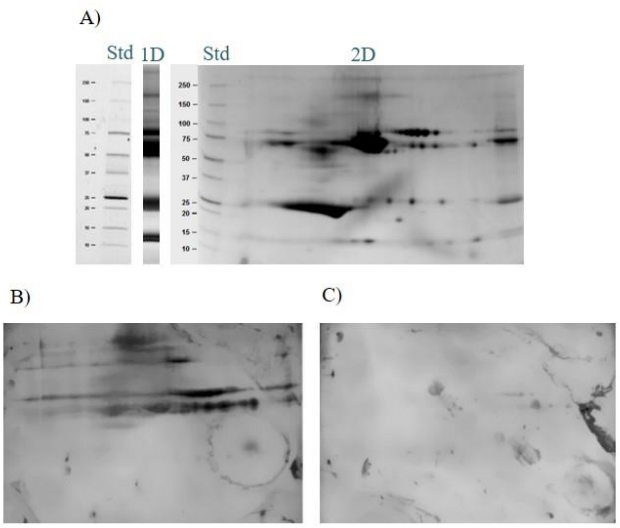

Figure 3. A) 1D and 2D gel of small parrot serum. B) and C) Western blots against small parrot serum proteins separated in 2D of different workers of a private urban pest control firm.

One dimensional and two dimensional electrophoresis showed different spots of interest in the low molecular weight range, between 20-25 KDa, of pigeon serum that could act as causative antigens in HP (Figure 1A). By performing western blots with serum of patients suffering from HP due to pigeon exposure (Figure 1B) and workers of the study exposed to these type of birds (Figure 1C), we identify two proteins of interest that were recognized by all patients with BRHP but not by workers. These proteins were Ig Lambda chain and Apolipoprotein A-I (Table 2). These results are consistent with other studies that have described Immunoglobulin lambda-like polypeptide-1 as a disease-specific protein in serum and pigeon droppings (Rouzet et al., 2017; Shirai et al., 2017).

The analysis of the population size of small parrots in the city of Barcelona from 1975 to 2015 (Figure 2) showed that this type of specie has increased a lot in the last decades. In fact, nowadays it has become a problem of pest control in some locations. That's why we want to know the sensitization and the degree of exposure to these types of birds of the workers of these study. One dimensional and two dimensional electrophoresis of small parrot serum showed different proteins of high and low molecular weight (Figure 3A). Western blots against small parrot sera of different workers exposed to these type of birds had different recognition profiles. One worker recognized a lot of proteins, mostly of high molecular weight (Figure 3B), while the other only recognized a few proteins (Figure 3C). The first worker had a decreased level of DLCO% (<80%, which is considered under the normal stablished cut-off) was diagnosed with sarcoidosis 15 years ago and had crackles during the clinical exploration.

CONCLUSIONS

A high degree of sensitization to avian (especially parrot and small parrot) and fungal antigens was observed in the study population. In addition, we found workers of the Nests pruning group with alterations in the FVC% and DLCO/VA%, pulmonary parameters related to lung function. These could be related to the exposure of these workers

to antigenic proteins during their workday. However, further studies are needed to stablish that correlation. Moreover, we identified two pigeon proteins (Ig Lambda chain and Apolipoprotein A-I) that may play a role in the development of pathological differences between HP patients and exposed workers. Nevertheless, more analyses have to be carried on before considering these proteins as possible biomarkers of the disease.

REFERENCES CITED

Enright P. 2016. Office-based DLCO tests help pulmonologists to make important clinical decisions. Respir Investig. 54: 305-311.

Erkinjuntti-Pekkanen R, Reiman M, Kokkarinen JI, Tukiainen HO and Terho EO. 1999. IgG antibodies, chronic bronchitis, and pulmonary function values in farmer's lung patients and matched controls. Allergy. 54: 1181-1187.

Koschel D, Lützkendorf L, Wiedemann B and Höffken G. 2010. Antigen-specific IgG antibodies in feather duvet lung. Eur J Clin Invest. 40: 797-802.

Lacasse Y, Selman M, Costabel U, Dalphin JC, Ando M, Morell F and Erkinjuntti-Pekkanen R. 2009. Classification of Hypersensitivity Pneumonitis. Int Arch Allergy Immunol. 149: 161-166.

Lacasse Y, Selman M, Costabel U, Dalphin JC, Ando M, Morell F, Erkinjuntti-Pekkanen R and Mueller NL. 2003. Clinical Diagnosis of Hypersensitivity Pneumonitis. Am J Respir Crit Care Med. 168: 952-958.

Millerick-May ML, Mulks MH, Gerlach J, Flaherty KR, Schmidt SL, Martinez FJ, Leveque RM and Rosenman KD. 2016. Hypersensitivity pneumonitis and antigen identification – An alternate approach. Respir Med. 112: 97-105.

Nademi Z, Todryk S and Baldwin C. 2013. Characteristics of antibody responses in Pigeon Fanciers' Lung. Mol Immunol. 54: 227-232.

Ohtani Y, Kojima K, Sumi Y, Sawada M, Inase N, Miyake S and Yoshizawa Y. 2000. Inhalation provocation tests in chronic bird fancier's lung. Chest. 118: 1382-1389.

Quirce S, Vandenplas O, Campo P, Cruz MJ, de Blay F, Koschel D and Moscato G. 2016. Occupational hypersensitivity pneumonitis: an EAACI position paper. Allergy. 71: 765-779.

Rodrigo MJ, Benavent MI, Cruz MJ, Rosell M, Murio C, Pascual C and Morell F. 2000. Detection of specific antibodies to pigeon serum and bloom antigens by enzyme linked immunosorbent assay in pigeon breeder's disease. Occup Environ Med. 57: 159-164.

Rouzet A, Reboux G, Dalphin JC, Gondouin A, de Vuyst P, Balliau T, Millon L, Valot B and Roussel S. 2017. An immunoproteomic approach revealed antigenic proteins enhancing serodiagnosis performance of bird fancier's lung. J Immunol Methods. 450: 58-65

Schuyler M, Albuquerque N and Cormier Y. 1997. The Diagnosis of Hypersensitivity Pneumonitis. Chest. 111: 534-536.

Selman M. 2004. Hypersensitivity pneumonitis: a multifaceted deceiving disorder. Clin Chest Med. 25: 531-547.

Shirai T, Furusawa H, Furukawa A, Ishige Y, Uchida K, Miyazaki Y, Eishi Y and Inase N. 2017. Protein antigen of bird-related hypersensitivity pneumonitis in pigeon serum and dropping. Respir Res. 18: 65.

Vasakova M, Morell F, Walsh S, Leslie K and Raghu G. 2017. Hypersensitivity Pneumonitis: Perspectives in Diagnosis and Management. Am J Respir Crit Care Med. 196: 680-689.