

Specific skin tests in subjects with chronic bronchitis exposed to pigeons

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We have studied the diagnostic reliability of the specific skin tests done in 30 subjects who presented with chronic bronchitis (CB) as the only clinical manifestation related to exposure to pigeons and in 21 subjects with CB and known risk factors. Additionally, two control groups were included (24 asymptomatic subjects exposed and sensitized to pigeons and 10 subjects not exposed and not sensitized to pigeons). The skin prick tests with pigeon serum were negative in all the subjects tested. The intradermal skin tests showed an immediate positive reaction in 16 of the 30 CB-affected patients and in six of the control group of exposed asymptomatic subjects (chi square: 3.376) ($P < 0.1$; nearly significant); after 6 h, a positive reaction was recorded in 14 of the CB-affected patients and in three subjects of the control group (chi square: 5.187) ($P < 0.005$). A delayed reaction was seen in 10/30 CB patients and in only three of the 24 subjects of the control group (chi square: 2.218) (nonsignificant). In the group of the 21 CB patients with known risk factors and not sensitized to pigeons, only two patients showed immediate skin reactivity; the remaining readings were negative. Lastly, in the control group of 10 unexposed, nonsensitized subjects, the intradermal skin tests in the different readings were negative. Our results show that although the skin tests with pigeon serum have low sensitivity, they can be a useful supplement in distinguishing cases of CB attributable to chronic pigeon exposure from those cases attributable to another cause, especially in the consideration of immediate and late readings.

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Exposure to pigeon-derived antigens can induce sensitization in susceptible persons. A few subjects exposed to pigeons may develop respiratory diseases such as hypersensitivity pneumonitis (8) and, more rarely, rhinitis or asthma (17). Recently, it has been suggested that chronic bronchitis (CB) may be a manifestation of morbidity associated with pigeon exposure (2–4) and exposure to other antigens (12). The diagnosis of pigeon breeder's lung (PBL) may be very easy in those cases in which the course of the disease is acute, but it is more difficult when the course of the disease is chronic or when it manifests itself exclusively as simple CB. One of the commonest diagnostic procedures is serologic detection of specific antibodies against avian derivatives. However, these antibodies are present not only in the patients but also in a considerable number of exposed asymptomatic subjects (9, 15). On the other

hand, it is possible to find patients without sensitization, even though they suffer or have suffered from the disease.

In the Canary Islands, Spain, there is great interest in the breeding and care of birds, especially pigeons. Usually the pigeon lofts are built on the flat roofs of the houses; therefore, the number of subjects exposed to pigeon-derived antigens is very high. Subjects exposed to pigeons frequently show symptoms of rhinitis, asthma, hypersensitivity pneumonitis, and CB associated with this exposure (20). It has been previously shown that intradermal skin tests with avian antigens may be a useful aid in the diagnosis of PBL, especially in the immediate reading (16).

The goal of this study was to evaluate the diagnostic value of the specific skin tests in patients with CB as the only clinical manifestation related to ex-

posure to pigeons, in order to distinguish them from those with nonspecific CB.

Material and methods

Patients

A total of 343 pigeon breeders belonging to five pigeon breeder's societies from Gran Canaria (Canary Islands, Spain) took part in this study. A team of three nurses and a physician visited the local pigeon-raising clubs at weekly meetings during the 1989–90 raising season. At the time of the visit, all members in the club were encouraged to complete a standard questionnaire (administered by a physician), which included the following areas: 1) personal data: age, sex, profession, and smoking habits; 2) circumstances and degree of exposure to pigeons: number of pigeons kept, duration of exposure in years, daily contact in hours, and location of pigeon coop. An exposure score was defined on the basis of the hours spent in the loft in a week. Also included in the questionnaire was current or previous history of the following: rhinitis: nasal itching, sneezing, nasal blockage, and watery discharge immediately after contact with pigeons; asthma-like symptoms: cough, wheezing, breathlessness, and thoracic oppression when entering or cleaning the loft; CB: chronic cough with expectoration for at least 3 months per year for at least 2 years (19); hypersensitivity pneumonitis (for verification of this disorder, it was required that one or more delayed respiratory symptoms such as cough or breathlessness and at least one systemic symptom such as fever, chills, and arthromyalgias had occurred on at least three different occasions, 6–8 h after exposure) (7). A venous blood sample was also taken from each fancier who participated in the study, for further serologic analysis.

Of the 343 pigeon fanciers, 75 were recruited for the skin tests protocol. These fanciers were grouped in three categories according to the symptoms (CB), exposure, smoking habits, and serologic results: 1) 30 subjects with "specific" CB (without any risk factor other than avian exposure and with positive serology); 2) 21 subjects with nonspecific CB (with negative serologic results who were either smokers or occupationally exposed to large amounts of dust); and 3) 24 asymptomatic sensitized pigeon breeders. Additionally, 10 subjects with no history of regular exposure to pigeons were also included in the study as a control group. None of these latter subjects showed specific IgG against pigeon serum as measured by ELISA (6).

Specific IgG against pigeon serum

Specific IgG against serum was measured in all the samples gathered by an indirect ELISA, as previ-

ously reported (6). Values that were 0.2 optical densities (OD) higher than the blanks were considered to be positive.

Antigen

Pigeon serum was used as antigenic material in the skin tests, as well as in specific IgG determinations. Pigeon serum was obtained by cardiac puncture after anesthetizing with ether in the pigeons given by the fanciers. The blood was allowed to clot, the serum was aspirated and centrifuged, and the supernatant was gathered. Finally, the serum obtained was passed through a Millipore filter (pore size 0.24 μ m) (Bedford, MA, USA) and frozen at -4°C until its later use.

Skin tests

For the skin tests, the pigeon serum was diluted to a concentration of 1:100. Histamine phosphate was used as a positive control at 1/1000 (intradermal tests) and at 1/100 (prick tests); physiologic serum was used as a negative control in all cases.

Prick and intradermal skin tests were done on the volar surface of the forearm, following general recommendations (13). In the case of the prick tests, a drop of pigeon serum was instilled on the skin surface and then an intraepidermic puncture was done; bleeding was avoided. Reading was done after 15 min, and the test was considered to be positive if the wheal diameter was equal to or greater than the histamine control. For the intradermal tests, each patient was injected with a volume of approximately 0.03–0.05 ml to obtain a wheal smaller than 5 mm in diameter. In this type of test, the reading was done three times: at 15–20 min (immediate reaction), at 6 h (late reaction), and at 24 h (delayed reaction); every test with a wheal diameter of more than 10 mm was considered to be positive.

Statistical study

Statistical analysis was done with the RSIGMA HORUS SA (Madrid, Spain) package. Data were expressed as the mean $\pm$ standard deviation. Comparison between groups was done by chi-square test and Fisher's exact test for categoric variables. Student's *t*-test was used to compare means.

Results

We finally studied 75 pigeon fanciers (60 males and 15 females) with a mean age of 36.6 ± 16.4 years (range: 7–64). Basic exposure data were as follows: number of years of exposure to pigeons, 18.6 ± 14.3 years (range: 1–60); daily contact, 2.5 ± 1.8 hours/

day (range: 1–10); number of pigeons, 116 ± 79.9 (range: 2–450). Of the 75 subjects included in the protocol of the skin tests, 51 fulfilled CB criteria, and only 21 showed risk factors for the development of such symptoms (19 current smokers and two exposed to industrial dust), whereas in the remaining 30 fanciers, the only potential factor for the development of CB was their exposure to pigeons. Specific IgG against pigeon serum in CB subjects with no risk factors other than pigeon exposure reached a mean value of 1.255 OD (0.700–2.000 OD), whereas in the 21 patients with CB and known risk factors, the average value was 0.576 OD (0.400–0.680 OD) ($P < 0.05$). In the control group of asymptomatic, sensitized subjects, the average specific IgG level was 1.788 OD (0.700–2.000 OD).

The skin prick tests with pigeon serum were negative in all 85 subjects studied. The analysis of the intradermal skin tests with pigeon serum in the 30 fanciers with CB and no apparent risk factor other than pigeon exposure revealed the following results: an immediate reaction was observed in 16 patients (53%), a late reaction in 14 patients (46%), and a delayed reaction in 10 patients (33%). In the group of 21 CB patients with known risk factors, an immediate positive response was obtained in only two patients; neither late nor delayed response was obtained in this group (Table 1).

In the first control group, made up of 24 sensitized and asymptomatic fanciers, a positive immediate response was obtained in six subjects (25%), at 6 h in three subjects (12.5%), and at 24 h in three subjects (12.5%). In the second control group, made up of 10 asymptomatic, unexposed, nonsensitized subjects, the skin tests were negative (Table 1).

When the intradermal skin tests were separately analyzed, it was found that the immediate reading was positive in 16/30 sensitized patients without risk factors for CB (53%), and in six exposed, asymptomatic subjects of the control group (25%) (chi square: 3.376) ($P < 0.1$). At 6 h, a positive reaction was shown in 14/30 CB-affected patients and in 3/24 subjects of the control group (chi square: 5.187)

($P < 0.05$). Finally, a positive delayed result was obtained in 10/30 CB patients and in only three subjects of the control group (chi square: 2.128) (non-significant).

The sensitivity of these skin tests, considered as a whole, among CB patients was 53% in the first reading, 46% in the second reading, and 33% in the third reading. As for the specificity of this type of test, calculated by comparing CB-affected patients in relation to the group of asymptomatic, sensitized, exposed subjects, it was 75% at 20 min, 88% at 6 h, and 88% at 24 h.

Discussion

A significant number of persons exposed to pigeons suffered from CB without any other apparent risk factor than exposure to avian antigens (6). Different authors (1, 5) have also proved that fanciers with CB have a significantly high level of IgG against pigeon serum. Therefore, CB should be considered to be part of the clinical spectrum of PBL, although the relationship between this clinical manifestation and exposure to pigeons is sometimes difficult to determine.

In 1968, Fink et al. (10) first studied the role of specific skin tests in the diagnosis of PBL. Since then, numerous studies on the reliability of this type of specific skin test have shown controversial results (8, 10, 18, 21). Attention has been drawn, in some of these studies, to the greater specificity of the late reactions (10), whereas, in others, better results have been obtained with the immediate reading (14, 16). In most of these studies, not only pigeon serum but also pigeon dropping was used. The possible contamination of this latter material by microorganisms and its powerful irritating effect (caused by its high content of teichoic acids) led us to use only pigeon serum (easily obtained and sterilized) as antigenic material. However, the great disparity of the results obtained with this type of test in different studies (10, 11, 14, 18, 21) must be noted. That is possibly due to the great differences existing in the type of patients included in some of these series.

Fifty-one subjects with CB were included in our study. Thirty of them had no apparent risk factor for the development of such symptoms other than their exposure to pigeon material. No positive pigeon serum prick test was obtained in any of our patients or in the two control groups. With regard to this, McSharry et al. (14) obtained positive prick test with pigeon serum in 21% of their patients. Perhaps these differences could be explained by the different inclusion criteria in our study. In the analysis of the intradermal skin tests, although the immediate reaction showed better sensitivity, the test was also positive in 6/24 exposed, sensitized, and asymptom-

Table 1. Results of intradermal skin tests with pigeon serum in patients with chronic bronchitis and in control groups

	CB		Control groups	
	Sensitized CB (n=30)	Nonsensitized CB (n=21)	Sensitized exposed (n=24)	Nonsensitized unexposed (n=10)
Reading time	Pos/Neg	Pos/Neg	Pos/Neg	Pos/Neg
20 min	16/14	2/19	6/18	0/10
6 h	14/16*	0/21	3/21	0/10
24 h	10/20	0/21	3/21	0/10

* Chi square: 5.187; $P < 0.05$.

atic subjects. The maximum specificity of the intradermal tests was obtained in the late and the delayed reaction (88%). All of the 21 CB patients with known risk factors showed positive intradermal tests at the second and third readings. Moreover, there was no irritating effect, because none of the 10 subjects included in the control group of unexposed subjects gave a positive result in any of the three readings. When the results of the intradermal skin tests in the different groups studied (chi square) were compared, the best results were obtained in the late reaction ($P < 0.05$); this finding agreed with those reported by others (10).

Our results suggest that specific skin tests with pigeon serum allow one to distinguish between subjects with CB developed from exposure to pigeons and CB from another cause. However, for the group of asymptomatic but sensitized subjects, the discriminatory power of the skin tests is very inferior, because 34% of these fanciers presented positive skin tests. It should be noted that, in this latter group, specific IgG levels against pigeon serum reached maximum values (greater than 2000 OD) in the three fanciers (of the six with immediate positive skin test) who in the second and third readings also showed significant skin reactivity (Table 1). However, we have not found any correlation between serologic and skin test results, even when all groups were taken together. The significance of these tests, as well as of specific IgG against pigeon serum, is unknown. In our experience, however, specific skin tests did not allow us to distinguish between asymptomatic, sensitized, exposed subjects and patients with CB.

Intradermal specific skin tests with pigeon serum are a useful, quick, easy, and cheap means to study CB subjects regularly exposed to pigeons.

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