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Analysis of skin testing and serum-specific immunoglobulin E to predict airway reactivity to cat allergens

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Summary

Background When the clinical history is not conclusive, it may be difficult to make an accurate interpretation of the value of skin tests and serum-specific IgE to cat allergens in asthma cases.

Objective To analyse the diagnostic efficiency of skin testing (ST) and serum-specific IgE to cat allergens, based on the results of bronchial-specific challenge with cat epithelium.

Methods Sixty-four asthma patients (49 with cat exposure and 15 without) who did not clearly relate their asthma symptoms to cat exposure and had a positive skin prick testing and/or a positive cat dander-specific IgE determination (CAP-system) underwent intradermal skin tests and specific bronchial challenge with cat epithelium. The results were analysed by receiver operating characteristics curves (ROC curves) and logistic regression. Sensitivity, specificity, positive predictive values and negative predictive values were calculated for different cut-off points.

Results Twenty-seven patients (42.2%) had a positive bronchial-specific challenge. The area under the ROC curve for serum-specific IgE quantification is 0.85, which makes a good diagnostic tool out of this test. Intradermal ST predicts the outcome of the bronchoprovocation test better than skin prick testing (area under the ROC curve of 0.74 vs. area under the ROC curve of 0.54, respectively).

The logistic regression analysis shows that the estimated probability of a positive bronchial challenge is $\geq 93\%$ if CAP values are ≥ 17 kU_A/L, whereas if CAP values are less than 0.35 kU_A/L the estimated probability of a positive bronchial challenge is 16%. When the intradermal skin test is negative, the estimated probability of a positive bronchoprovocation test is 9%, being the test that better identifies patients with a negative bronchoprovocation test.

Conclusions Levels of serum-specific IgE to cat allergens and intradermal ST can be used to diagnose and treat more accurately asthmatic patients sensitized to cat epithelium when there is uncertainty about cat epithelium causality.

Keywords cat allergy, cut-off, logistic regression, ROC curve, serum-specific IgE, skin tests

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Introduction

The first therapeutic recommendation in the face of pet-induced allergies is withdrawal of the animal. This measure can be a disruptive factor in family life, inducing affective conflicts if the animal is separated, or a sense of guilt and health deterioration if it is nonetheless kept at home. Therefore, the diagnosis should be as accurate as possible. Usually, it is made on the basis of the clinical record and the results of skin prick test (SPT) and/or

specific serum IgE. However, the accuracy of clinical history for predicting atopic sensitization to airborne allergens is modest [1]. On the other hand, not always do positive skin tests or the presence of specific serum IgE correlate with clinical allergy [2]. These limitations have been overcome for food allergy by means of the oral double-blind challenge controlled with a placebo procedure, which is the diagnostic gold standard for this type of allergy [3]. Thanks to this method, several cut-off points have been identified in the size of skin tests and values of

serum-specific IgE, greatly increasing the diagnostic usefulness of both techniques [4–8]. In the case of asthma due to sensitization to inhalants, bronchoprovocation testing (BPT), although not the only gold standard, yields useful information on the existence of specific and symptomatic bronchial reactivity, adding to data obtained with skin tests and determination of specific IgE [9]. When there are doubts on whether the cat is really causing clinical allergy because of an inconclusive clinical history, we perform BPT as part of our standard diagnostic protocol. Based on the results obtained with BPT, we have now analysed the diagnostic efficiency of skin tests and specific serum IgE (as determined by CAP) with cat allergens with respect to airway reactivity.

Methods

Subjects

Subjects (64) were asthmatic patients evaluated at the Allergy Department of Hospital 12 de Octubre in Madrid, Spain. Initially, a thorough clinical history was performed in order to confirm the diagnosis and severity of asthma (following the Global Initiative For Asthma Guidelines) [10], and its possible relationship with allergy to inhaled allergens. In this regard, subjects were asked about the presence of house pets or any kind of exposure to domestic animals. Patients were also questioned on the possible relationship between their allergic symptoms and animal exposure. After the clinical history and physical exam, patients underwent skin testing (ST) with a battery of allergens explained later. This study was performed and assessed on the same day of the interview, unless the patient was taking some type of impeding medication.

In patients with house pets and perennial symptoms, serum-specific IgE for that animal allergens were also determined, independently of the outcome of ST and patient's own assessment of relationship with symptoms. This determination was also performed in patients who did not mention exposure to cat but had a complex clinical history with multiple positive skin tests, including cat, as well as in patients without obvious cat exposure but a positive skin test exclusively for cat allergens. The results were analysed together with the patient approximately 1 month after the first interview. At this time, all patients underwent a BPT with cat allergens to try and clarify the clinical relevance of sensitization because either they were sensitized to cat allergens without apparent cat exposure or because being cat owners sensitized to cat allergens, they did not clearly relate their asthma symptoms with cat exposure. Therefore, we consider that the patients we have analysed here have an unclear clinical history of cat allergy.

Skin prick test

Initially, subjects underwent SPT with a battery of allergens including grass, tree and weed pollens, dust mites (*Dermatophagoides pteronyssinus* and *farinae*), dog and cat epithelium and moulds (*Aspergillus fumigatus*, *Alternaria alternata* and *Cladosporium herbarum*). All the extracts were standardized in biological units (BU; ALK-Abelló, Madrid, Spain). The cat extracts contain 100 BU/mL and 40 mcg/mL of Fel d 1. The SPTs were performed on the forearm with an ALK-Lancet (ALK-Abelló, Horsholm, Denmark). Control tests were performed with 50% glycerin-saline and histamine (10 mg/mL). The orthogonal diameters of the skin prick weal induced by allergens and the negative and positive controls were measured after 20 min. A positive SPT result was defined as a weal at least 3 mm larger than the diameter of the negative control. All subjects avoided short-acting antihistamines for 72 h, long-acting antihistamines for 5 days and tricyclic antidepressants for 2 weeks before ST [2].

Intradermal skin test

Immediately before BPT, subjects underwent intradermal skin testing (IDST). The same extracts as for SPT were used here but with different concentrations (1, 0.1 and 0.01 BU/mL). We injected 0.02 mL into the forearm with a 26-gauge hypodermic needle. Control tests were saline solution and histamine (0.1 mg/mL). A positive IDST result was defined as a weal at least 5 mm larger than the diameter of the negative control [11].

Serum-specific immunoglobulin E

The CAP system (CAP-FEIA, Pharmacia diagnostics, Uppsala, Sweden) was used for the determination of specific serum IgE to cat allergens. A value ≥ 0.35 kU_A/L was considered indicative of the presence of allergen-specific IgE.

Bronchial test

Following the method described by Chai et al. [12], all patients underwent a BPT with cat epithelium. A Devilbiss 646 nebulizer (DeVilbiss Co., Somerset, Pa, USA) with a fitted output of 0.28 mL/min and intermittent aerosol generation was used. The same extract as for ST with concentrations ranging in tenfold increments from 0.001 to 10 BU/mL was used. The allergenic extract (10 BU/mL) was dried and reconstituted with saline solution on the same day as the BPT. Flow-volume curves were carried out with a Jaeger Pneumotach (Viasys Healthcare GmbH, Hoechberg, Germany). Briefly, asymptomatic patients were studied when the baseline forced expiratory volume in 1 s (FEV₁) was 80% or greater with respect to the

standard value. Pulmonary function studies were repeated ten minutes after inhalation of five breaths of saline solution. The specific bronchial test was carried out if a decline in FEV₁ value $\leq 10\%$ was obtained. The challenge begins with five breaths of the 0.001 BU/mL cat epithelium extract. The next dilution was given after 10 min, if less than a 15% reduction from the control FEV₁ had occurred. If the reduction in FEV₁ was between 15% and 19%, an additional 10 min delay was implemented. The test finishes when an FEV₁ decline $\geq 20\%$ occurs (positive) or no significant decline occurs after the inhalation of the most concentrated cat epithelium extract [equivalent to administration of 55.5 breath units (BAU)] (negative). The cumulative dose of cat extract in BAU was plotted on a semilogarithmic paper to determine the cumulative dose, causing a 20% decline in FEV₁.

Before the BPT, all subjects avoided short-acting bronchodilators and inhaled steroids for 12 h, long-acting bronchodilators, theophylline and leucotriene antagonists for 24 h, short-acting antihistamines for 72 h, long-acting antihistamines for 5 days and tricyclic antidepressants for 2 weeks.

In case of a positive test, patients were given a dose of a short-acting bronchodilator and spirometry tests were repeated at 20-min intervals until normalization of pulmonary function. Later, patients performed hourly peak-flow measurements by means of a peak-flow meter (Mini-Wright, Clement Clarke International, Harlow, UK) up to 12 h after the last inhalation.

All patients gave informed consent.

Statistical analysis

Quantitative variables were summarized by central tendency measures (mean or median), together with a dispersion measure such as standard deviation or range. Qualitative variables were described by their distribution of frequencies and percentages, both globally and in special interest subgroups. A model of binary logistic regression was used to generate probability curves that depict the likelihood of patients with a particular size of skin test and serum-specific IgE concentration to cat allergens generating a positive outcome in BPT. The odds ratios are shown with Wald's 95% confidence intervals. For this analysis, an SAS 12.1 (SAS Institute Inc., Cary, NC, USA) statistical package was used. The receiver operating characteristic (ROC) analysis was used to quantify the accuracy of the diagnostic tests (skin tests and CAP) to discriminate between a positive or negative outcome in the BPT with cat epithelium. The points along the ROC curve represent the sensitivity-specificity pairs corresponding to all possible decision thresholds for defining a positive test result. On the *y*-axis, sensitivity is plotted and on the *x*-axis 1-specificity is plotted. The area under the ROC curve is an overall index of diagnostic accuracy

that does not depend on a decision threshold. An area of 0.5 indicates that the discriminatory ability of the test is not better than chance. An area of 1.0 indicates perfect discriminatory ability. This statistical analysis was performed using an SPSS (version 11; SPSS Inc., Chicago, IL, USA) statistical package. Assay performance was computed using the following definitions (where FN is a false-negative diagnostic test result, FP is a false-positive diagnostic test result, TP is a true-positive diagnostic test result and TN is a true-negative diagnostic test result). Sensitivity $[TP/(TP+FN) \times 100]$ is the percentage of positive tests in subjects with a positive BPT. Specificity $[TN/(FP+TN) \times 100]$ is the percentage of negative tests in subjects with a negative BPT. the predictive value for a positive test $[TP/(TP+FP) \times 100]$ describes the percentage of subjects with a positive test and a positive BPT. The predictive value for a negative test $[TN/(TN+FN) \times 100]$ is the percentage of subjects with a negative test and a negative BPT. In order to analyse whether simultaneously positive SPT and CAP tests can improve the diagnostic accuracy in comparison with the single positivity of one of the two tests, we created a new composite variable formed by SPT- and CAP-positive tests ($CAP \geq 0.35 \text{ kU}_A/L + SPT \geq 3 \text{ mm}$). Sensitivity, specificity, PPV and PNV were calculated for this new variable.

The association between qualitative variables has been analysed by means of the χ^2 test. The relationship between normally distributed continuous data and a dichotomic variable was assessed by Student's *t*-test, whereas the Mann-Whitney *U*-test was used for non-normally distributed continuous data. Comparisons were considered to be significant at a *P*-value of < 0.05 .

Results

Subject characteristics

The group included 37 females and 27 males with ages ranging between 14 and 55 years (mean 28.7 years). In accord with GINA guidelines, 18 (28.1%) had intermittent asthma, 34 patients (53.1%) had mild persistent asthma and 12 (18.8%) had moderate persistent asthma. Forty-three (67.2%) patients were cat owners, six (9.4%) patients had occasional exposure to cats and 15 (23.4%) patients had no direct cat exposure. In answer to the question 'symptoms when with cat', 47 (73.4%) patients thought their symptoms were not related to cat, nine (14.1%) patients were in doubt about the relationship between cat exposure and their respiratory symptoms and eight (12.5%) patients related rhinitis or urticaria but not asthma. Forty-seven patients (73.4%) had other concomitant allergies, the majority of them being pollen allergy.

Table 1 shows how these data are distributed among patients with BPT-positive and -negative results.

Table 1. Clinical characteristics, ST sizes and CAP values in patients according to BPT outcome

	Positive BPT patients N = 27 (42.2%)	Negative BPT patients N = 37 (57.8%)	Statistical significance P < 0.05
Age, year (mean ± SD)	32.15 ± 12.12	26.19 ± 9.64	0.032
Sex: female/male	17/10	20/17	NS
Asthma severity			NS
Mild intermittent	5	13	
Mild persistent	16	18	
Moderate persistent	6	6	
Cat exposure			NS
Cat owners	20	23	
Occasional	2	4	
Not apparently	5	10	
Symptoms when with cat:			NS
No	21	26	
Uncertain	4	5	
Rhinitis	2	6	
Cat skin tests mm (mean ± SD)			
SPT – 100 BU/mL	5.4 ± 2.6	4.8 ± 2.8	NS*
IDST – 1 BU/mL	11.2 ± 3.2	8.2 ± 5.0	0.013**
IDST – 0.1 BU/mL	9.4 ± 3.9	5.4 ± 4.2	0.000**
IDST – 0.01 BU/mL	6.9 ± 3.5	3.6 ± 4.29	0.003*
CAP kU _A /L (mean ± SD)	21.7 ± 28.9	1.9 ± 2.67	0.000*

*Non-normally distributed data.

**Normally distributed data.

BPT, bronchoprovocation testing; SPT, skin prick test; ST, skin testing; IDST, intradermal skin testing.

Cat skin test and specific immunoglobulin E results

SPTs with cat epithelium were positive in 54 of the 64 patients (mean weal diameter 6.1 mm, range: 3–13 mm), and the determination of serum-specific IgE was positive in 50 of the 64 patients (the median 4 kU_A/L ranged from 0.35 to ≥100 kU_A/L). Ten patients were SPT negative and CAP positive, 14 patients were SPT positive and CAP negative and 40 patients had both tests, SPT and CAP, positive. Intradermal skin tests were performed in 60 patients, resulting in being positive with cat extract containing 1 BU/mL in 51 patients (mean weal diameter 10.7 mm, ranged from 5 to 19 mm), 0.1 BU/mL in 47 patients (mean weal diameter 8.6 mm, ranging from 5 to 18.5 mm) and 0.01 BU/mL in 36 patients (mean weal diameter 8.1 mm, ranging from 5 to 13.5 mm).

Except for SPT-100 BU, papule size and specific IgE quantification were significantly higher in BPT-positive patients compared with BPT-negative ones (Table 1). There were also statistically significant differences in IgE quantification levels between patients with cats living in their homes (mean 13.4 kU_A/L) and those with only occasional or no apparent exposure to cat (mean 4.49 kU_A/L; $P < 0.015$).

Bronchial provocation test results

The BPT was positive in 27 patients (42.2%). The FEV₁ decline was between 20% and 61% (median 28.3%), and the PD20 FEV₁ range was 0.02–55.5 BAU (median 19 BAU). The majority of patients recovered between 30 and 60 min after bronchodilator administration. Sixty per cent were early asthmatic responses and 40% also showed a decline ≥20% in peak-flow measurements (dual response). The BPT was positive in three SPT-negative subjects and in one patient with negative CAP. The BPT was positive in 21 of 47 (44.6%) subjects who had answered that their cat did not worsen or cause their asthma, four of nine (44%) doubtful ones and two of eight (25%) with other symptoms related to cat exposure. The BPT was positive in 20 of 43 (46.5%) cat owner subjects, two of six (33%) subjects with occasional cat exposure and five of 15 (33%) patients without cat exposure (Table 2).

Five (26%) patients with intermittent asthma, 16 (47%) with mild persistent asthma and six (50%) with moderate persistent asthma were BPT positive (Table 1).

Statistical analysis

Skin test. The estimated area under the ROC curve for SPT-100 BU was 0.54 [95% confidence interval (CI), 0.39–0.69] (Fig. 1), which means that this diagnostic technique has an almost null diagnostic ability for the BPT result at the cut-off point of 3 mm. When predictive values for different cut-off points are analysed, the maximal NPV is 70% for a 3 mm cut-off point and the maximal PPV is 71% for an 8 mm cut-off point (Table 3). The same results are obtained by logistic regression analysis, with a 34% estimated probability of a positive BPT for a negative test and a maximum of 50% for a 10 mm papule test.

The estimated areas under the ROC curve for IDST – 1, 0.1 and 0.01 BU/mL were 0.67 (95% CI, 0.54–0.81), 0.74 (95% CI, 0.61–0.86) and 0.70 (95% CI, 0.57–0.83), respectively (Fig. 1). Overall, the best predictor for airway reactivity to cat is IDST with extract at 0.1 BU/mL.

At the 5 mm cut-off point, the two higher concentrations yield a high sensitivity (96%), and an NPV of 92% with IDST – 0.1 BU/mL (Table 3). Therefore, this test is useful to predict if a patient is going to have a negative bronchoprovocation test. As we increase the cut-off point, we improve the specificity and the PPV up to a 100% (cut-off points of 19 mm and 14 mm, for IDST – 1 BU/mL and 0.1 BU/mL, respectively). However, these cut-off points have a low sensitivity and only a few patients could be diagnosed using these weal sizes (Table 3). Logistic regression analysis of IDST – 0.1 BU/mL shows that a negative test is associated with a 9% estimated probability of a positive BPT. For increasing papule size, the probability

Table 2. Characteristics of patients with positive BPT

	Sex	Age (years)	Asthma severity	SPT (mm)	CAP (kU _A /L)	PD20-FEV ₁ (BAU)	% fall on FEV ₁	Cat exposure	Symptoms when cat
1	F	44	MP	4	5.56	18	31	No	Uncertain
2	F	34	MP	6	0	24	32	No	No
3	F	55	MI	5.5	19	20	25	Yes	No
4	F	39	MP	0	9.4	15	61	Yes	No
5	F	19	MP	4.5	1.1	20.55	20	Yes	No
6	F	52	MD	6	8.53	18	33	Yes	No
7	M	42	MD	6.5	>100	19	28	Yes	No
8	F	22	MD	3	39	35.5	36	Yes	No
9	F	22	MP	0	>100	13	28.6	Yes	Uncertain
10	F	34	MP	4	1.6	11	44	No	No
11	F	36	MP	4	1	55.5	21	Yes	No
12	F	16	MP	0	3.86	16	30	Yes	No
13	M	20	MP	6	38.6	20.5	32	Yes	Uncertain
14	M	29	MP	6	1.12	15.55	28	Yes	No
15	F	35	MP	7.5	1.1	26	37	Yes	No
16	M	21	MP	8.5	1.33	18	32	Yes	Uncertain
17	M	33	MI	10	39.8	55.5	21	Occasional	No
18	M	16	MI	5	18.8	6	53	No	No
19	F	16	MP	7.5	5.7	12.5	28	Occasional	No
20	F	45	MD	4	3.26	20.5	26	Yes	No
21	F	31	MP	10	83.7	40	22	Yes	No
22	F	25	MI	5	9.4	17	27	Yes	No
23	F	46	MP	9	21.7	20	22.8	Yes	Rhinitis
24	M	25	MI	5	22.1	42	22	Yes	Rhinitis
25	M	27	MD	6	13	0.02	36	No	No
26	M	55	MD	5.5	16.9	20	23	Yes	No
27	M	33	MP	8	22.5	5.5	21	Yes	No

Cat exposure: no, no apparent exposure; yes, cat owners; occasional, sporadic cat exposure.

Cat-related symptoms: no, subjects who do not relate their asthma symptoms with cat; uncertain, subjects who do not know if cat is the cause their allergic symptoms, including bronchial hyperreactivity; rhinitis, subjects who relate cat exposure with upper airway symptoms but not with their bronchial hyper-reactivity.

BPT, bronchoprovocation testing; SPT, skin prick test; FEV₁, forced expiratory volume in 1 s; BAU, breath units; MI, mild intermittent; MP, mild persistent; MD, moderate persistent.

also increases: 27% for a 5 mm papule, 51% for 9 mm, 70% for 12 mm and a maximum of 92% for an 18 mm size (Fig. 2).

Serum-specific immunoglobulin E. The estimated area under the ROC curve for CAP values was 0.85 (95% CI, 0.75–0.94), (Fig. 1), which makes a good diagnostic tool out of this test. A cut-off point of 0.35 kU_A/L yields a sensitivity of 96.3% and an NPV of 92.31%. If the cut-off point is increased to 0.9 kU_A/L, the negative predictive value increases up to 94.4%, becoming useful in identifying those patients with a negative BPT. The value of 13 kU_A/L yields a specificity of 100% (PPV 100% and NPV 72%) (Table 3). Figure 3 depicts the predicted relationship between CAP results and BPT outcome. As can be seen, the curve shows an ascending slope such that an increasing value corresponds to a higher probability of a positive BPT. For a CAP < 0.35 kU_A/L, the estimated probability of having a positive BPT is 16%, 20% for 1.5 kU_A/L, 30% for 3.05 kU_A/L, 50% for 6.28 kU_A/L, 70%

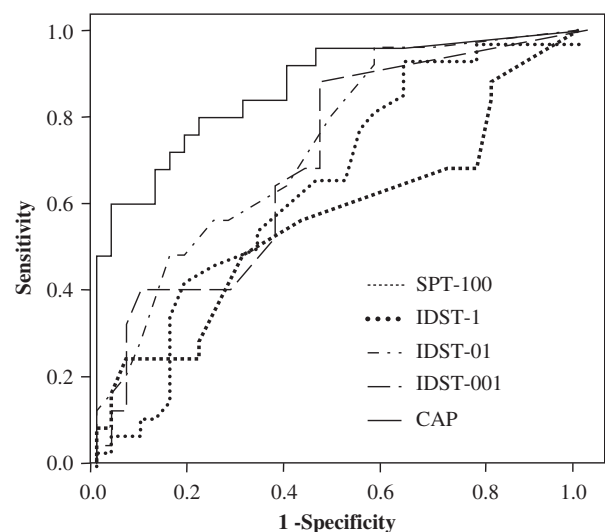


Fig. 1. Receiver operating characteristics (ROC) curves: skin prick test (SPT), intradermal skin testings (IDSTs) and serum-specific IgE vs. bronchoprovocation testing (BPT) with cat allergens.

Table 3. Predictive values for BPT of different skin tests and CAP cut-off points

TEST	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	PPV (%) (95% CI)	NPV (%) (95% CI)
SPT – 100 BU (mm)				
3	88.89 (77.00–100)	18.92 (6.30–31.54)	44.40 (31.19–57.74)	70.00 (41.60–98.46)
8	18.52 (3.87–33.17)	94.59 (87.31–100)	71.43 (37.96–100)	61.40 (48.77–74.04)
IDST – 1BU (mm)				
5	96.00 (88.32–100)	20.59 (7.00–34.18)	47.06 (33.36–60.76)	87.50 (64.58–100)
19	4 (– 3.68–11.68)	100 (100–100)	100 (100–100)	58.00 (45.95–71.3)
IDST – 0.1BU (mm)				
5	96.00 (88.32–100)	34.29 (18.56–50.00)	51.06 (36.77–65.36)	92.31 (77.82–100)
14	12 (– 0.74–24.74)	100 (100–100)	100 (100–100)	61.40 (48.77–74.04)
IDST – 0.01BU (mm)				
5	80.00 (64.32–95.68)	54.29 (37.78–70.79)	55.56 (39.32–71.79)	79.17 (62.92–95.41)
13	4.00 (– 3.68–11.68)	97.14 (91.62–100)	50.00 (– 19.3–100)	58.62 (45.95–71.30)
CAP (kU _A /L)				
0.35	96.30 (89.17–100)	33.33 (17.93–48.73)	52.00 (38.15–65.85)	92.31 (77.82–100)
0.9	96.30 (89.17–100)	47.22 (30.91–63.53)	57.78 (43.35–72.71)	94.44 (83.86–100)
13	48.15 (29.30–67.00)	100 (100–100)	100 (100–100)	72.00 (59.55–84.45)
SPT ≥ 3 mm + CAP ≥ 0.35	85.19 (71.79–98.59)	54.05 (38.10–70.00)	57.56 (42.18–72.82)	83.3 (68.42–98.24)

BPT, bronchoprovocation testing; SPT, skin prick test; CI, confidence interval, IDST, intradermal skin testing.

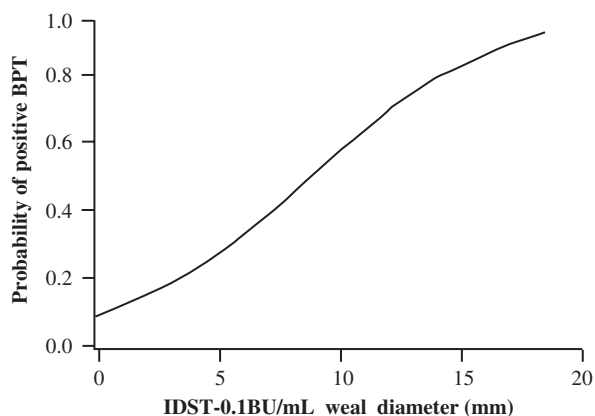


Fig. 2. Predicted relationship between intradermal skin testings (IDST) – 0.1 BU/mL and bronchoprovocation testing (BPT) with cat allergens. The odds ratio estimate was 1.293 (95% Wald confidence limits 1.098–1.522).

for 9.4 kU_A/L, 84% for 13 kU_A/L, 93% for 17 kU_A/L and 99.99% for 30 kU_A/L.

The combined variable 'SPT positive' and 'CAP positive' improves on the results obtained with SPT alone at the cut-off level of 3 mm, especially by increasing specificity, PPV and NPV; however, the results are not much better than those obtained with the cut-off level of 0.35 kU_A/L in CAP (Table 3).

Discussion

The diagnosis of clinical allergy depends initially on a correlation between clinical history and the results of ST and/or serum-specific IgE. Nonetheless, even when using the question that Murray and Milner [1] described as most

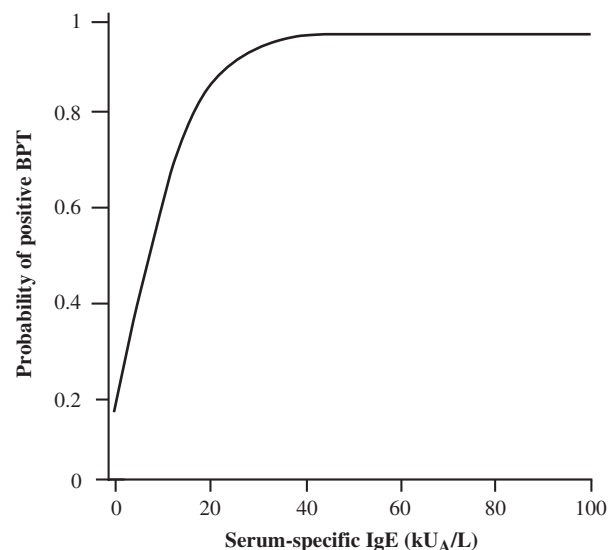


Fig. 3. Predicted relationship between CAP results and bronchoprovocation testing (BPT) with cat allergens. The odds ratio estimate was 1.287 (95% Wald confidence limits 1.096–1.512).

efficient to detect patients with cat allergy ('symptoms when with cat?'), the predictive value of the clinical history to detect allergy to inhalants is low. Possible reasons for the modest degree of accuracy of this question include multiple sensitization, occurrence of several different allergens together and probably reluctance of the patients to acknowledge involvement of their pets in their symptoms. On the other hand, it is known that the presence of positive skin tests and/or specific serum IgE are not always followed by clinical symptoms. In the study of asthma, there is another diagnostic tool that can

be used: the specific BPT. This test is not considered a gold standard diagnosis tool, because false positives can occur due to the fact that allergic patients may have bronchor-eactivity to allergens that are clinically unrelated to their asthma, and false negatives inherent to the technique and material used for the test [9]. However, there is a general agreement that bronchial challenges are useful when the history and skin tests and/or serum-specific IgE determinations are not conclusive [9]. We have carried out a standard bronchoprovocation test following the guidelines described for the method of intermittent delivery of the allergens [13]. The cat allergens have been administered by means of a nebulizer that generates optimal size particles to reach the lower respiratory tract, i.e. a size similar to the actual particles that transport Fel d 1 [14, 15]. We have carefully tested the extract and doses administered with respect to the reproducibility of results of BPT [16].

Other authors use the method of live-cat-room challenge, which has the potential advantage of exposing both the upper and lower respiratory tracts to cat allergens. An excellent correlation in bronchial response has been observed between both methods, with subjects responding almost identically [17]. However, these tests are expensive, time consuming and cannot easily be performed in every hospital. An ideal solution would be to establish a correlation among ST sizes, serum-specific IgE levels and BPT results so that we can have an additional objective assessment to diagnose and treat patients sensitized to cat epithelium. For example, based on our analysis a particular patient can be told that not only has he or she a positive ST or detectable serum-specific IgE levels but also that the skin test size or specific IgE level predicts that BPT would also be positive if it were to be performed, even if he/she is not aware of any association between symptoms and his/her cat. Therefore, it would help reinforce the message that adequate environment control measures and treatment compliance are necessary.

This approach has been used in the setting of food allergy based on the results of a double-blind oral provocation controlled with placebo. Data have been published regarding the size of prick testing and values of specific IgE for several foods [4–7, 18], which facilitates the diagnosis of actual allergy to those foods, thereby avoiding the need for challenges in many cases. In some instances, the levels have been used to follow-up the state of sensitization and define the point in which allergy has been overcome and a provocation test would be negative [19].

In the allergy to inhalants, some attempts have also been made despite the lack of a unique diagnostic gold standard. Pastorello et al. [20] observed that patients with a clinical history compatible with allergy to seasonal or perennial allergens showed amounts of specific IgE significantly higher than patients without symptoms and

positive skin tests. Schafer et al. [21] have analysed the value of skin tests and CAP in hayfever cases. Choi et al. [22] have found that both the specific IgE levels and the SPT reactivity are useful parameters in the prediction of positive airway responses to house dust mites in asthma.

We have analysed the capacity of skin tests and serum-specific IgE to predict the airway response to cat allergens in a population of asthma subjects sensitized to cat allergens with an unclear clinical history of allergy to that animal.

According to our results, determination of serum-specific IgE is an optimal diagnostic test. With this test, a very good area under the ROC curve is obtained, which points out that this determination is able to distinguish between BPT-positive and -negative patients (Fig. 1). Cut-off points with good predictive values can be established (Table 3) and by logistic regression an ascending probability curve is obtained that includes probabilities for positive BPT between 16% and 99.9%, depending on CAP results (Fig. 3). For example, in the present series, there are 14 patients with $CAP < 0.35 \text{ kU}_A/\text{L}$ and 20 with a CAP between 0.37 and $2.29 \text{ kU}_A/\text{L}$. In all these cases, the probability of a positive BPT is 16–26%. For 10 patients with a CAP level between 3.05 and $6.28 \text{ kU}_A/\text{L}$, the estimated probability of a positive BPT is 30% to 50%. The other eight patients have a CAP level between 7.7 and $17 \text{ kU}_A/\text{L}$ and their probability of a positive BPT is 60–93%. Finally, 12 patients show CAP levels between 17 and $100 \text{ kU}_A/\text{L}$, and the probability of a positive BPT is 93–99.99%.

The main limitation of this technique resides in the low levels of IgE. For example, for the cut-off point of $0.35 \text{ kU}_A/\text{L}$ the estimated probability of a positive BPT is not zero but 16%, which is similar to the one shown by serum-specific IgE values of up to $1.5 \text{ kU}_A/\text{L}$ (20%). Levels of serum-specific IgE with a high probability of a positive BPT are more frequently found in cat-owner patients. However, they are also found in some subjects without apparent exposure. This is not surprising because Bollinger et al. [23] have described that in many homes without a cat, there are levels of Fel d 1 sufficient to cause symptoms.

With respect to skin testing, the screening test used by us – SPT-100 BU/mL – shows an area under the curve of low significance, which indicates a very high percentage of false positives and also a significant number of false negatives in relation to BPT outcomes (Fig. 1). Cut-off points with balanced predictive values were not found (Table 3) and the probability curve reaches a maximum of 50% probability (data not shown). In our series, it is more useful to use IDST with the 0.1 BU/mL extract than SPT to diagnose cat allergy, given the fact that the values of the area under the ROC curve obtained make it an acceptable diagnostic test [24] (Fig. 1). Also, in the logistic regression model we obtain low (9%) probabilities of a positive BPT

when it is negative and high (92%) ones for larger size papules (Fig. 2). However, as only a few patients have large size papules (only seven patients have size papules >12 mm), this test is mainly useful to identify patients who will have a negative BPT. Therefore it can be used as a first diagnostic step.

As we usually perform both SPT and CAP in patients owning pets, we have also analysed whether having both tests positive is better than only one of them. Specificity is the only parameter that is slightly improved when both tests are positive, with respect to the 3 mm SPT cut-off point or 0.35 kU_A/L in CAP, allowing for an improved probability of correctly classifying subjects with negative BPT (Table 3).

Other authors [25, 26] have investigated the efficiency of skin tests and determination of specific IgE in diagnosing allergy to cat epithelium with different results. Sicherer et al. [17] did not find a statistically significant association between manifestations of bronchial symptoms by cat exposure and the value of specific IgE determined by RAST or ID titration. In this study, the cumulative dose of allergen causing a 20% decline in FEV₁ was highly correlated with methacholine challenges. It had been established that the allergic reaction and the non-specific bronchial reactivity are interrelated in the production of allergen-induced asthma [27]. We do not have data on non-specific bronchial hyper-reactivity. However, ROC curve analyses of skin test size and specific IgE values allow us to make an individual assessment on the ability of these tests to predict the result (positive or negative) of BPT.

Wood et al. [28] compared SPT, IDST and RAST for the diagnosis of clinical reactivity to cat allergen and found that the diagnostic efficiency of SPT and RAST was good, while IDST performed in patients with negative SPT did not add to the other two techniques.

Our analysis is different from the one by Wood et al. [28] First, almost all our patients have undergone IDST, and not just those with negative SPT. Then, we have described ROC curves and logistic regression to analyse globally all the results, and not just predictive values of different cut-off points. Also, their patients had symptoms related to cat exposure, whereas our patients had an unclear history of cat allergy and this could explain the low specificity and PPV obtained in the assessment of SPT at the cut-off point of 3 mm.

In summary, from our results it can be deduced that quantification of specific serum IgE can be used to improve the diagnostic accuracy of asthmatic patients sensitized to cat allergens and an unclear clinical history. The higher the level of IgE antibodies, the more likely the existence of cat-related bronchial hyper-reactivity. To identify patients with a negative BPT, it is more useful to perform IDST (with a 0.1 BU/mL extract in our series) because a negative IDST is associated with a lower-

estimated probability of a positive BPT (9% vs. 16% with CAP). In the rest of the cases, a specific probability of a positive BPT could be estimated according to each CAP value (Fig. 3).

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