



Research paper

A comparison of specific IgE and skin prick test results to common environmental allergens using the HYTEC™ 288

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ABSTRACT

Although skin-prick testing (SPT) is commonly used by allergists in the evaluation of allergy, in-vitro testing for specific IgE (sIgE) is an attractive alternate because it can be performed remotely and is of utility when SPT is contraindicated, as in patients on anti-histamines, or with dermatitis or severe eczema. It is, however, necessary to determine the extent of correlation between the in-vitro and in-vivo methods. In this study, we examined the qualitative concordance between SPT and sIgE as measured on the HYTEC™288 platform for 10 commonly encountered inhalant allergens in 232 subjects, and analysed the performance characteristics for the HYTEC™288. Overall concordance between SPT and sIgE was > 70% for all allergens tested. Sensitivity ranged from 25% to 95%, depending on the allergen, while specificity was significantly higher for all allergens (78–97%). NPV was > 85% for all allergens tested, while PPV was more variable, ranging from 22% to 88%. These results are similar to findings in other studies comparing SPT with sIgE. Lack of concordance in a percentage of samples might be partly attributed to differences in allergen preparations for SPT and HYTEC™ 288. Follow-up studies utilizing identical allergen preparations for both in-vivo and in-vitro testing may address these discrepancies.

1. Introduction

Skin prick testing (SPT) and the analysis of IgE levels to specific allergens (sIgE) are two diagnostic tools frequently used in the evaluation of allergy. SPT and sIgE results are reviewed in conjunction with the patient's history, clinical evaluation, and allergen challenge results where applicable (Sicherer, and M., Robert A., 2012). Many allergists consider sIgE levels as a complement to the more commonly utilized SPT results; however, sIgE testing is of additional value when SPT is contraindicated. This may be the case in patients with dermatological conditions that preclude skin testing, such as dermatographism, extensive eczema or urticaria (Stromberg, 2002; Dreborg, 1989), or for those who are on medications that may interfere with accurate interpretation of the test (anti-histamines or glucocorticoids) (Gradman and Wolthers,

2008; Hammarlund et al., 1990; Pipkorn et al., 1989). In such situations sIgE levels can provide valuable information to support clinical diagnosis and treatment or lifestyle decisions.

There are currently three FDA approved platforms for analysis of specific IgE levels; however, results from one platform may not always agree with those obtained from another (Hamilton, 2010). It is therefore important to compare sIgE results to the accepted SPT diagnostic technique when assessing platform performance. Knowledge of the extent of correlation becomes particularly important when sIgE is the diagnostic test of choice due to SPT contraindication. Several studies have been performed to compare sIgE measured on a variety of platforms with SPT results (Ewan and Coote, 1990; Ciprandi et al., 2010; Borchini et al., 2007). This study was undertaken to evaluate the performance of the HYTEC™ 288 platform in patients that had undergone a

Abbreviations: SPT, Skin prick test; sIgE, Specific immunoglobulin E; PPV, Positive predictive value; NPV, Negative predictive value

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SPT as part of their clinical evaluation. sIgE levels to a panel of 10 allergens were compared with SPT results to evaluate concordance as well as the performance characteristics of the platform i.e., sensitivity, specificity, positive and negative predictive values.

2. Materials and methods

2.1. Samples

Human sera were obtained from the National Jewish Health (NJH) Bio bank (institutional bio repository for biological samples and associated clinical and laboratory data) after approval from the NJH Institutional Review Board, the NJH Honest Broker Services for allocation of de-identified biological samples, and the Bio bank Sample Board. Data for these samples were downloaded from the National Jewish Health Research Database (Data Set Identifiers: 140-2871-11-13-2012, 140-2884-12-07-2012, 140-2912-01-11-2013) in a de-identified manner using the NJH Honest Broker Services. Samples included in the analysis were associated with a request for SPT. The data associated with the samples included SPT results to a variety of common environmental allergens. The analyst running the sIgE assay was blinded to these results until all analyses were complete.

2.2. Skin prick testing (SPT)

SPT was performed using the Duotip-test device (Lincoln Diagnostics, Decatur, IL) according to the practice at National Jewish Health (Harold and Nelson, n.d.). SPT controls included histamine as a positive control and saline as a negative control. A reaction was termed positive if histamine and the allergen elicited a wheal ≥ 3 mm and erythema ≥ 10 mm after 15 min and the negative control was < 3 mm wheal and < 10 mm erythema. Reactions that were positive for one parameter but not the other were termed equivocal.

2.3. Specific IgE analysis

The HYTEC™ 288 (Hycor Biomedical, Garden Grove, CA) was used for the measurement of specific IgE against 13 common environmental allergens (*D. pteronyssinus*, *D. farinae*, cat hair, dog dander, Timothy grass, Johnson grass, common ragweed, silver birch, white oak, cockroach, box-elder tree, *Aspergillus fumigatus* and *Alternaria tenuis*) according to the manufacturer's instructions.

2.4. Data analysis

Data generated by the HYTEC™ 288 platform designates a class to ranges of sIgE levels as shown in Table 1. To enable qualitative comparisons, negative sIgE results were designated '0' and positive results (classes I/0 through VI) were designated '1'. SPT results were designated, qualitatively, as '1', '0', and '-1' for positive, negative and equivocal results respectively. Qualitative concordance, sensitivity, specificity, positive predictive value (PPV) and negative predictive value

Table 1
Modified Classification System (MCS) scoring used by HYCOR HYTEC™ 288 Platform.

Class	conc. IU/ml	Interpretation
Negative	< 0.05	Negative
0/I	0.05–0.08	Positive with increasing antibody Concentration
I	0.08–0.15	
II	0.15–0.50	
III	0.50–2.50	
IV	2.50–12.50	
V	12.50–62.50	
VI	> 62.50	

(NPV) were calculated for the HYTEC™ 288 compared with SPT for 10 allergens that were common to both platforms. Concordance between SPT and sIgE measurements on the HYTEC™ 288 was calculated using a modified kappa statistical method that can be used with nominal and ordinal variables (Quatto and Ripamonti, 2014). Sensitivity was calculated as the number of HYTEC™ 288 positive results among patients with a positive SPT, specificity was calculated as the number of HYTEC™ 288 negative results among patients with a negative SPT. PPV was calculated as the number of positive HYTEC™ 288 results among patients with a positive SPT as a proportion of the total number of positive HYTEC™ 288 results, while NPV was calculated as the number of negative HYTEC™ 288 results among SPT negative patients as a proportion of the total number of HYTEC™ 288 negative patients. The proportions of positive results between HYTEC™ 288 and SPT were compared using Fisher's exact test.

3. Results

A total of 232 serum samples from patients with a request for SPT were tested for serum levels of sIgE to 13 common inhalant allergens. Since this was a blinded study, the sIgE results were correlated with retrospective SPT results for allergens that were common to the SPT panel and the sIgE panel after all testing was completed. Of the 13 allergens tested on the HYTEC™ 288 platform, 10 were also utilized in the SPT allergen panel and had corresponding data. These 10 allergens were examined for concordance, sensitivity, specificity, PPV and NPV as compared with the SPT.

3.1. Concordance between SPT and sIgE measured using the HYTEC288 platform

SPT and sIgE measured by the HYTEC288 were compared taking all positive and negative results by either method into consideration. Equivocal SPT results were $< 10\%$ for all allergens tests and were not included in the calculation of concordance. Since equivocal results are generally interpreted by the allergist in conjunction with history and clinical findings. When both negative and positive results were compared for concordance between the two methodologies, SPT and sIgE showed $> 70\%$ concordance for all allergens tested (concordance ranged from 74% for White oak to 88% for Timothy grass, Table 2).

3.2. Evaluation of performance characteristics

Next, the sensitivity and specificity of the HYTEC™ 288 were determined based on the results of the SPT. Data for individual allergens is presented in Table 3, using 2×2 tables for calculation of sensitivity,

Table 2
Overall concordance between SPT and sIgE results.

Allergen	Concordance (%) ^a
<i>D. pteronyssinus</i>	74 [64–84]
<i>D. farinae</i>	78 [69–87]
Cat Hair	77 [68–86]
Dog Dander	69 [58–79]
Timothy Grass	88 [81–94]
Cockroach	71 [60–81]
<i>Aspergillus fumigatus</i>	76 [66–84]
<i>Alternaria tenuis</i>	79 [69–87]
Box-elder Tree	67 [56–78]
White Oak Tree	53 [29–76]

^a Numbers in brackets represent 95% bootstrap (10,000 replications) percentile confidence intervals (Quatto reference). The concordance statistic is a modified kappa statistic that can be used with nominal and ordinal variables, and it avoids some of the issues with kappa itself when there is strong agreement between raters (or methods).

Table 3

Sensitivity, Specificity, Positive Predictive Value and Negative Predictive Value for Individual Allergens.

<i>D. pteronyssinus</i>			
SPT			
sIgE	Positive	Negative	Totals
Positive	44	4	48
Negative	21	150	171
Totals	65	154	219
Sensitivity	68		
Specificity	97		
PPV	92		
NPV	88		
<i>D. farinae</i>			
SPT			
sIgE	Positive	Negative	Totals
Positive	41	7	48
Negative	16	148	164
Totals	57	155	212
Sensitivity	72		
Specificity	95		
PPV	85		
NPV	90		
Cat Hair			
SPT			
sIgE	Positive	Negative	Totals
Positive	78	12	90
Negative	12	124	136
Totals	90	136	226
Sensitivity	87		
Specificity	91		
PPV	87		
NPV	91		
Dog Dander			
SPT			
sIgE	Positive	Negative	Totals
Positive	53	23	76
Negative	9	136	145
Totals	62	159	221
Sensitivity	85		
Specificity	86		
PPV	70		
NPV	94		
Timothy Grass			
SPT			
sIgE	Positive	Negative	Totals
Positive	84	11	95
Negative	4	118	122
Totals	88	129	217
Sensitivity	95		
Specificity	91		
PPV	88		
NPV	97		

Table 3 (continued)

Cockroach			
SPT			
sIgE	Positive	Negative	Totals
Positive	28	18	46
Negative	18	168	186
Totals	46	186	232
Sensitivity	61		
Specificity	90		
PPV	61		
NPV	90		
Aspergillus fumigatus			
SPT			
sIgE	Positive	Negative	Totals
Positive	16	14	30
Negative	12	178	190
Totals	28	192	220
Sensitivity	57		
Specificity	93		
PPV	53		
NPV	94		
Alternaria tenuis			
SPT			
sIgE	Positive	Negative	Totals
Positive	40	18	58
Negative	8	156	164
Totals	48	174	222
Sensitivity	83		
Specificity	90		
PPV	69		
NPV	95		
Box-elder Tree			
SPT			
sIgE	Positive	Negative	Totals
Positive	34	27	61
Negative	7	136	143
Totals	41	163	204
Sensitivity	83		
Specificity	83		
PPV	56		
NPV	95		
White Oak Tree			
SPT			
sIgE	Positive	Negative	Totals
Positive	3	11	14
Negative	1	49	50
Totals	4	60	64
Sensitivity	75		
Specificity	82		
PPV	21		
NPV	98		

* Skin Prick Test Positives were determined by Wheal ≥ 3 mm $\times$ 3 mm and Erythema ≥ 10 mm $\times$ 10 mm.

specificity, PPV and NPV. Samples that had a corresponding equivocal SPT result were not included in the analysis of these parameters. Sensitivity on the HYTEC™ 288 ranged from 57% (*Aspergillus fumigatus*) to 95% (Timothy grass) and specificity ranged from 82% (White Oak) to 97% (dust mite). Sensitivity was in general lower than specificity and is in agreement with similar, previously published studies (Calabria et al., 2009). PPV ranged from 21% (White oak) to 92% (dust mite). NPV was in general higher and close to 90% or greater for all allergens, again in agreement with similar studies (Calabria et al., 2009; Asha'ari et al., 2011).

4. Discussion

We compared the SPT results, obtained retrospectively, for patients attending the National Jewish Health allergy practice with sIgE levels measured on the HYTEC™ 288 platform for 10 commonly encountered environmental allergens. In general, we found good concordance between both diagnostic methods, with an overall > 70% agreement between methods.

In this particular study we found, qualitatively, a marginally higher percentage of positive sIgE results (class 1/0 and greater) than positive SPT results in 8/10 allergens. This difference, however, was not significant except in the case of Box Elder tree and White oak (data not shown). Similar comparisons between SPT and sIgE measured on other platforms for a variety of inhalant allergens have also shown variable results. For instance, the percentage of positive sIgE results tested on the Phadia ImmunoCAP platform were found to be higher than SPT results in a study of polysensitized patients with allergic rhinitis (Ciprandi et al., 2010), whereas Calabria, CW et al, in a study examining 53 different allergens in patients with chronic rhinitis found that SPT tended to have a higher number of positive results (Calabria et al., 2009). Discordance between SPT and sIgE results in these studies, as with our study, may be attributed to differences among allergen preparations utilized for SPT and in the individual sIgE test platforms. Similar studies have noted inconsistencies between SPT and sIgE results for several mold species studied in a cohort of school children, noting that the agreement between sIgE and SPT results was around 30% (Taina Taskinen et al., 2001). Mari et al noted discrepancies between SPT and sIgE results for reactivity to *Alternaria* that were highly dependent on the source of the mold preparation (Mari et al., 2003). In our study, the agreement between the two molds (*Alternaria* and *Aspergillus*) studied in this particular group of patients showed a significantly higher correlation at about 80%.

SPT and sIgE results to White oak showed the greatest discordance as well as the lowest PPV. The numbers of subjects with both SPT and sIgE results for White oak were much lower (64 compared with > 180 for all other allergens). This might account for the greater variation in correlation as well as the lower PPV. In addition, the SPT utilizes scrub oak allergen while HYTEC™ 288 utilizes White oak allergen. While both of these allergens belong to the oak family, it is possible that antigenic differences between the two preparations may exist and account for the discrepancy in test results.

Because allergen preparations are in general non-standard extracts, differences may exist among the proteins present in SPT reagents and those used in in-vitro testing platforms and therefore, these systems may detect different populations of IgE resulting in discrepant results. Despite these differences, we found a reasonably acceptable concordance between SPT and sIgE for the 10 allergens tested.

A small percentage of SPT results (< 10%) were considered equivocal by the criteria that either the wheal was < 3 mm or the flare was < 10 mm. In these patients, the sIgE levels tended to be negative or low positive (class 1/0 to 3) and were never greater than class 3, suggesting that low levels of IgE tended to correlate much more variably with the SPT result.

In summary, we tested the performance characteristics of the HYTEC™ 288 platform in comparison with SPT for 10 commonly encountered environmental allergens. The overall agreement between the two platforms was > 70%. However, as with similar published studies, sIgE and SPT agreement can vary widely depending on the allergen. In order to address the role of allergen preparations in contributing toward discrepancies that exist between sIgE and SPT, future studies should focus on using identically prepared extracts for both SPT and sIgE.

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