

REVIEW

Do skin prick and conjunctival provocation tests predict symptom severity in seasonal allergic rhinoconjunctivitis?

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Summary

Background In the investigation of seasonal allergic rhinoconjunctivitis (SAR), quantitative skin and conjunctival allergen challenge tests are used to measure individual allergen sensitivity. These tests are reproducible and relate well to prevalence but their relationship to symptom severity is less well established.

Objective We wished to determine if quantitative skin prick tests (QSPT) and conjunctival provocation tests (CPTs) using a single grass pollen allergen extract are reproducible and predict symptom severity in SAR.

Methods We retrospectively analysed data from 91 participants in a previously published randomized placebo controlled study of low dosage allergen immunotherapy who were randomized to receive placebo treatment. We examined the relationship between pre-seasonal QSPT, CPT and SAR symptoms.

Results We found a high level of reproducibility when repeated measures were compared for both the QSPT ($P < 0.001$) and the CPT ($P < 0.001$) and moderate correlation (0.49) between the standard skin prick test (SPT) and the QSPT ($P < 0.001$). We found weak negative correlation (-0.27) between the QSPT and the CPT ($P < 0.001$). We found no correlation between seasonal symptom, use of rescue medication or quality of life (QOL) scores and pre-seasonal QSPT or CPT.

Conclusion In the assessment of seasonal rhinoconjunctivitis, quantitative skin and conjunctival allergen challenge tests are strongly reproducible, although there is no correlation between these tests and seasonal symptom, use of rescue medication or QOL scores.

Keywords allergic rhinitis, conjunctival provocation test, hay fever, rhinoconjunctivitis, skin prick test

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Introduction

Allergen challenge tests have long been used as a way of measuring individual sensitivity to allergen exposure [1]. In addition to the standard skin prick test (SPT), two incremental tests have been developed that employ serially diluted allergen extracts in order to increase accuracy; the quantitative skin prick test (QSPT) [2] and the conjunctival provocation test (CPT) [3].

It is commonly assumed that there is a relationship between SPT weal size and symptom severity. Data collected from 10854 US citizens showed that the prevalence of seasonal allergic rhinoconjunctivitis (SAR) increases in tandem with increasing airborne allergen skin

test reactivity [4]. House dust mite weal diameters have been shown to correlate significantly with nasal challenge threshold in mite-allergic rhinitis sufferers [5]. However, no association was observed between common airborne allergen skin test diameter and nasal airway hyper-reactivity in SAR sufferers [6].

QSPT was introduced as a quantitative and reproducible assay of allergen potency and stability [1, 7]. It has also been used as a method of assessing the efficacy of desensitization in the treatment of allergic rhinoconjunctivitis [8, 9].

Conjunctival allergen challenge is known to cause symptoms similar to those encountered in allergic rhinoconjunctivitis [10]. The CPT was developed as an accessible and reproducible human model of ocular allergy

[3, 11]. The effectiveness of anti-allergic therapy is commonly evaluated using CPT with different doses, drugs or desensitization treatments [9, 12]. There are significant relationships between conjunctival, skin [13], nasal [13–15] and bronchial [14] challenge thresholds. Studies that have separately examined subjects with positive RAST but negative CPT, and subjects with positive CPT but negative SPT or RAST suggest that SPT and RAST may be misleading because systemic sensitization may not affect the conjunctiva. Conjunctival sensitization may occur in the absence of systemic sensitization [16].

We therefore analysed data from 91 control subjects from our previous clinical trial to examine the relationship between the results of the pre-seasonal CPT, SPT and QSPT and subsequent SAR symptoms.

Methods

Study population

The randomized controlled trial from which we extracted the data for the present study was undertaken by us in Hampshire (UK) and the clinical outcome has been reported previously [17]. We recruited hay fever sufferers aged between 18 and 64, with a history of hay fever for two or more years, poorly responsive to standard pharmacotherapy and who gave a positive SPT to timothy grass pollen extract (*Phleum pratense* pollen; Soluprick ALK, Abelló, Denmark). Participants were excluded if they gave a history of perennial rhinitis or asthma sufficient to warrant regular topical corticosteroids, if their main rhinitis season was not June and July, if they had a history of a significant medical condition, or if they were taking antidepressant or antipsychotic medication.

Assignment and intervention

On two occasions at an interval of 8–11 weeks between January and March 2001, 90 participants received active treatment (an injection of 0.05 mL of enzyme-potentiated desensitization) and 93 participants received placebo treatment (an injection of 0.05 mL of buffered saline).

Measurements and assessments

Both at the time of randomization (October–December 2000) and 6–8 weeks post-treatment (April–May 2001) we performed the following quantitative and incremental tests of grass pollen sensitivity.

QSPTs were undertaken using serially diluted allergen extracts on the flexor surface of the forearm [2]. We used Aquagen *P. pratense* pollen extract, 100 000 SQ-U/mL – ALK Abelló, as the strongest testing solution and serial ($5 \times$) dilutions were made using the Aquagen Diluent to

produce five testing solutions at the following strengths 160, 800, 4000, 20 000 and 100 000 SQ-U/mL.

Each dilution was tested in duplicate using a parallel line assay method [2]. To obtain a single measure of sensitivity, the mean weal diameter for each pair of tests was plotted against the log concentration of the test solution. The area under the semi-log skin test titration curve (AUC) was calculated as the arithmetic mean of the weal diameters at each dilution of allergen extract.

We performed CPTs following Möller's method [3]. We used the same Aquagen *P. pratense* pollen extract as above for the strongest testing solution and serial ($5 \times$) dilutions with the Aquagen diluent to produce six testing solutions numbered from 0 to 5 at the concentrations of 32 (0), 160 (1), 800 (2), 4000 (3), 20 000 (4) and 100 000 SQ-U/mL (5) using Aquagen diluent as the negative control. A single drop of the diluent was placed in the right conjunctival sac. Successively stronger solutions were then placed into the left conjunctival sac at 10-min intervals, each step in the test sequence being contingent on a negative response in the immediately preceding test. Ten minutes after each drop was inserted we inspected for redness and enquired about eye weeping, eye itch or burning, nose dripping or nose blockage, and scored each reaction absent = 0, mild = 1, moderate = 2, and severe = 3. The test was concluded when a reaction score of five was reached, and a CPT score between 0 and 5 assigned according to the test solution.

During calendar weeks 20 to 31 inclusive of 2001 (May 14 to August 5 inclusive) each participant was asked to record a single daily symptom score ('How bad were your nose and eye symptoms during the last 24 h') using a seven-point scale from 'no symptoms' to 'very severe symptoms'. In addition, at the end of each week each participant was asked to complete the Mini Rhinitis Quality of Life Questionnaire (Mini-RQLQ, McMaster University) [18]. This questionnaire comprises 14 questions within 5 domains: (a) activities, (b) practical problems, (c) nose symptoms, (d) eye symptoms and (e) other symptoms; each is assessed on a seven-point severity scale. It is validated and derived from a previous longer version, the Rhinitis Quality of Life Questionnaire (RQLQ) [19].

During the same period participants were also asked to make a daily record (yes/no) of the primary rescue medications acrivastine (or own preferred antihistamine), cromoglycate nasal spray and cromoglycate eye drops and the secondary rescue medications, beclomethasone nasal spray and prednisolone. We derived a daily rescue medication score by ascribing a score of 1 to use of one or more weak treatments (topical cromoglycate or topical antihistamine), a score of 2 to use of one or more moderate treatments (oral antihistamine, topical steroid) and a score of 3 to use of strong treatment (oral steroid), giving a total weekly score of 42.

Table 1. Baseline data for control and total groups

	Control group	Total group
Total	91	176
Female	46	87
Average age (SD)	36.5 (8.6)	38.0 (9.9)
Male	45	89
Average age (SD)	37.1 (10.5)	37.8 (10.9)
Mean rhinitis duration (years) (SD)	19.94 (10.31)	21.28 (10.94)
Mean asthma duration (years) (SD)	2.96 (7.62)	3.82 (8.44)
SPT positive to house dust mite (%)	23 (25.3)	46 (26.1)
SPT positive to pollens other than grass (%)	29 (31.9)	50 (28.44)

SPT, skin prick test.

Statistical methods

Correlation and regression analyses were used to assess the strength of the relationships between the QSPT results, the conjunctival challenge test scores (CPT), the quality of life (QOL) total scores and the symptom scores over the 12-week study period. A Bland–Altman plot and weighted κ statistics were used to examine the agreement between the two quantitative SPT scores at the initial and second assessment. A sample size of 91 (control subjects) was calculated as sufficient to have an 80% power to detect correlations of about 0.3 using a 5% significance level.

Results

Withdrawals subsequent to randomization (5/90 active and 2/93 control) left 176 participants (85 active group and 91 control) for analysis. We used the data from the 91 control subjects for all analyses but reverted to using data from all 176 when examining agreement between baseline tests. A few tests were not completed due to non-attendance and so some data sets are a little smaller. Baseline data for the control subjects are given (Table 1). We

obtained pollen count data for Winchester, the nearest pollen counting station. We found that both grass pollination and its associated symptom peak were well covered by the 12 study weeks (Fig. 1).

There was a strong relationship between the two QSPT measures of sensitivity for the same subject at the two visits. A Bland–Altman plot investigating the differences between the two QSPT assessments for the same subjects demonstrated the mean of these differences was 3.96 (Fig. 2). There was fair agreement between the two CPT assessments ($\kappa = 0.34$, $n = 78$, $P < 0.0001$; Fig. 3), with 73 (93.6%) of the 78 subjects who had both assessments having the second assessment within one level of the first.

Moderate correlation was noted between the standard grass pollen SPT (mean weal diameter) and the QSPT (AUC; correlation = 0.493, $n = 176$, $P < 0.001$) (Fig. 4a). A weak negative relationship between the CPT score and the QSPT (AUC) was found, (correlation = -0.27 , $n = 170$, $P < 0.001$; Fig. 4b).

We found no correlation between pre-seasonal SPT and either symptom scores (correlation = 0.025, $n = 84$, $P = 0.825$) or total miniRQLQ score (correlation = -0.036 , $n = 83$, $P = 0.749$; Fig. 5a) for the peak symptom week. We found no correlation between pre-seasonal QSPT (AUC) and either symptom scores (correlation = -0.043 , $n = 84$, $P = 0.695$) or miniRQLQ scores (correlation = -0.078 , $n = 86$, $P = 0.482$; Fig. 5b) for the peak symptom week. We found no correlation between pre-seasonal CPT and eye domain miniRQLQ scores for the peak symptom week (correlation = -0.002 , $n = 83$, $P = 0.988$; Fig. 5c).

We found no relationship between symptoms and either pre-seasonal QSPT or CPT for any individual week of the pollen season (Table 2).

We found no significance when we compared the rescue medication score with either pre-seasonal QSPT (AUC;

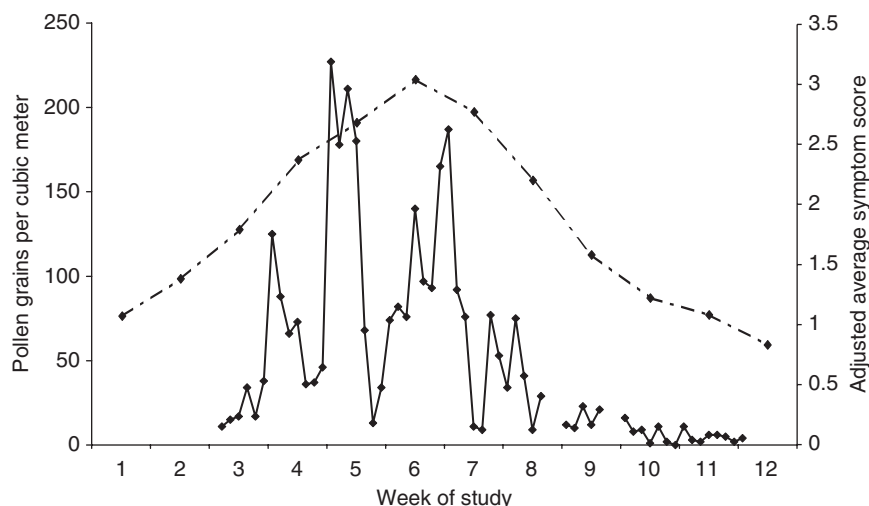


Fig. 1. Adjusted average weekly symptom scores and daily grass pollen counts as measured at Winchester for the 12 study weeks.

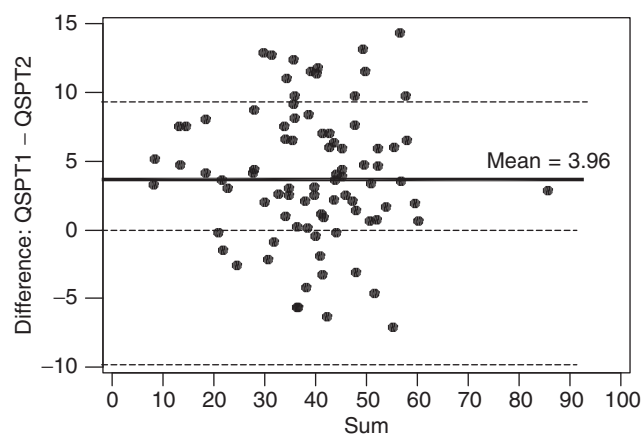


Fig. 2. Reproducibility of quantitative skin prick tests (QSPTs). Comparison of first and second QSPTs (area under curve) for control group (Bland-Altman plot).

		CPT threshold (2nd test)						Total
		0	1	2	3	4	5	
CPT threshold (1st test)	1	1		4				5
	2		4	13	11	1		29
	3			9	15	6	1	31
	4			2	3	5	2	12
	5				1			1
Total		1	4	28	30	12	3	78

Fig. 3. Reproducibility of conjunctival provocation tests (CPTs). Comparison of first and second CPT scores (provocation thresholds) for control group.

correlation 0.051, $n = 84$, $P = 0.64$) or pre-seasonal CPT (correlation -0.10 , $n = 83$, $P = 0.36$).

For each unadjusted analysis we made proportional adjustments to the observed symptom scores using the rescue medication score. These adjustments made no difference to our conclusion that neither the pre-seasonal QSPT nor CPT predicts symptom scores during the peak hay-fever season.

Discussion

We took 91 SAR sufferers and compared pre-seasonal skin and conjunctival tests of *P. pratense* pollen sensitivity with seasonal symptom and QOL scores. Neither SPT, QSPT nor CPT showed any correlation when compared with symptom scores, rescue medication score, rhinitis

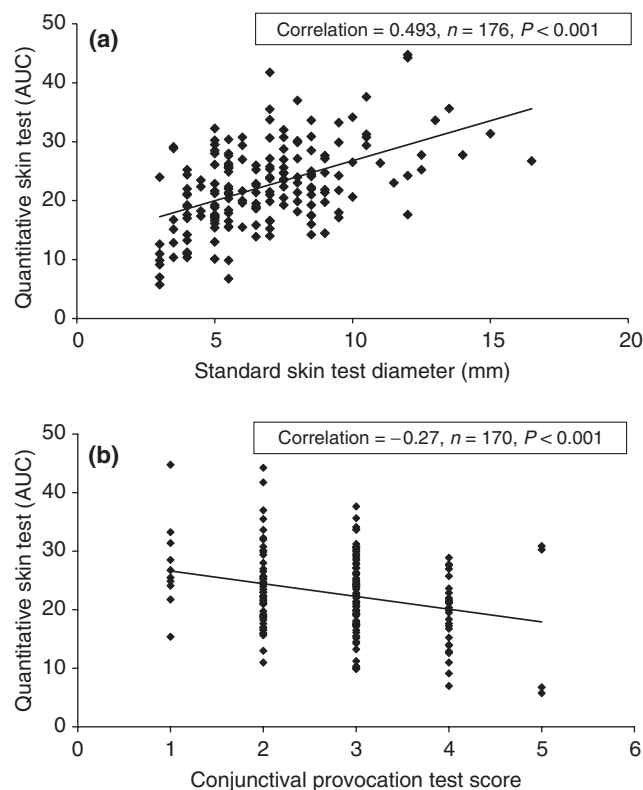


Fig. 4. Comparison of quantitative skin prick tests with standard skin prick tests (SPT) and conjunctival provocation test (CPT) scores (provocation thresholds). Quantitative SPTs (area under curve) for all subjects at visit 1 compared with (a) standard SPTs and (b) CPT scores (regression plots).

QOL, or eye domain QOL scores. Pre-seasonal QSPT with grass pollen was strongly reproducible.

In SAR, impairments outside the respiratory tract affect the patient's QOL. Daytime sleepiness is a feature of seasonal SAR that relates to disease severity [20]. Other non-nasal symptoms such as thirst, poor concentration, headaches and irritability also affect QOL [19]. Rhinitis-specific QOL instruments, such as the miniRQLQ, were developed to complement conventional markers of nasal inflammation (such as symptom scores, rhinomanometry, nasal cytology) by encompassing the breadth of symptoms as perceived by the patient. When studies have compared rhinitis-specific QOL and nasal symptom scores, only weak to moderate correlations have been found [19, 21]. This may explain why tests conducted on a single target organ such as the skin (not an organ primarily involved in aeroallergen disease) correlate poorly with the patients' perception of disease severity.

Seasonal rhinitis symptoms are associated with allergy to a range of grass pollens [22]. Studies using both CPT and QSPT commonly use a single pollen when assessing seasonal rhinitis [9]. However, the assumption that a single pollen is a satisfactory surrogate for the effects of mixed pollens may not be correct. Had we used a mixed pollen extract it would have introduced confusion due to

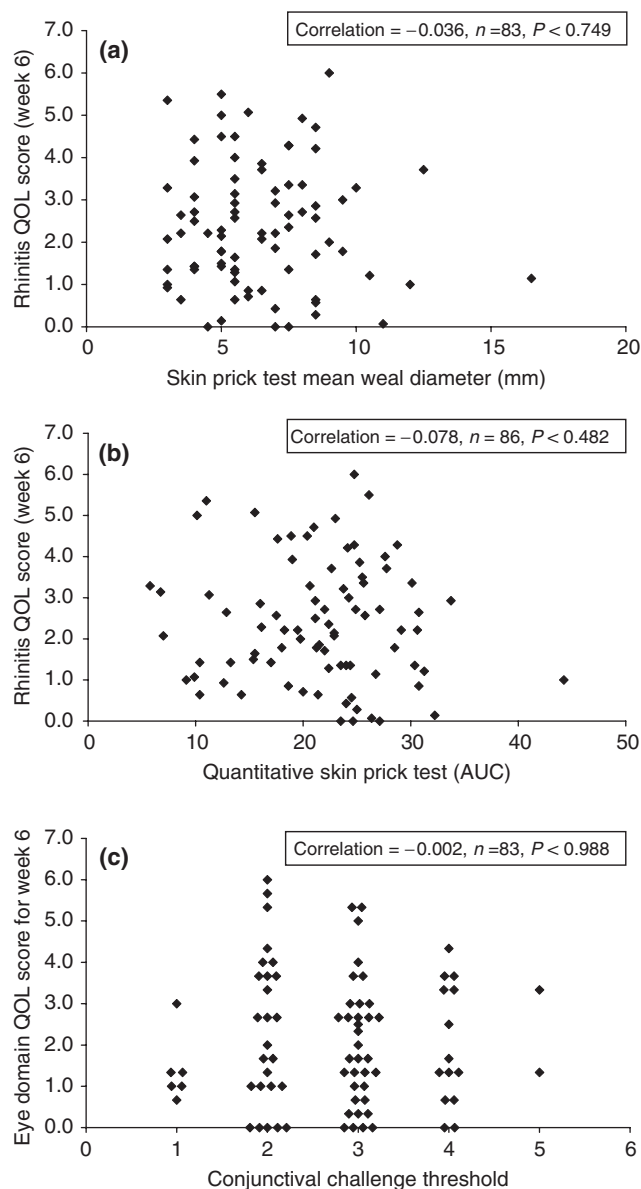


Fig. 5. Predictability of (a) standard skin prick tests (SPTs) (b) quantitative skin prick tests (c) conjunctival provocation test (CPT) scores (provocation thresholds). The relationship between pre-seasonal SPTs (standard and quantitative) and CPTs with *Phleum pratense* pollen and the quality of life scores for the peak symptom week (week 6).

variation in response between pollens. *P. pratense* was chosen as it exhibits strong cross-reactivity with other allergenic grass pollens and is effective in desensitizing to multiple grass pollens [9].

The study population had received placebo treatment in a trial of EPD [17]. We have no evidence or mechanism to suggest that placebo can selectively interfere with the relationship between allergy testing and clinical response. Bousquet et al. came to similar conclusions about the placebo in their study demonstrating stability of SPT in the placebo group [23]. However, they were able to demonstrate correlation between QSPT and SAR, while

Table 2. Predictability of CPT and QSPT: Correlation between daily symptom scores (weekly means) and the pre-seasonal QSPTs and conjunctival tests taken at visit 1

Week	QSPT	CPT
1	-0.012	0.137
2	-0.046	0.047
3	-0.021	0.032
4	-0.002	-0.027
5	-0.024	-0.032
6	-0.043	-0.142
7	-0.007	-0.184
8	-0.057	-0.332
9	-0.075	-0.222
10	-0.027	-0.097
11	-0.206	0.079
12	-0.127	-0.072

CPT, conjunctival provocation test; QSPT, quantitative skin prick test.

recognizing that three out of four previous studies had failed to find correlations [24–26].

Release of pre-formed mediators from mast cells has been suggested as the basis for the clinical response that is measured by the CPT with an early phase response (EPR) resulting in increases in tear histamine and tryptase maximally at 20 min and a subsequent late phase response (LPR) [27]. The LPR can therefore be viewed as the most relevant model for clinical disease [27] but CPT and SPT are measures of EPR. Consequently, the assumption that the CPT predicts clinical outcome may be inaccurate. The lack of a correlation between symptoms and SPT or CPT could also be due to the presence of differing tissue specific factors. In the context of allergen challenge in allergic asthma, SPT more closely correlates with the early allergic response if bronchial hyper-reactivity is taken into account [28] suggesting that both mast cell mediator release and non-specific bronchial reactivity are separate factors influencing mucosal response to an airborne allergen.

We found no correlation between pre-seasonal CPT, SPT and QSPT and subsequent SAR symptoms, QOL measures or use of rescue medication in this study. We therefore cannot support the assumption that CPT and QSPT predict seasonal symptoms.

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