

# Comparison of conjunctival and nasal provocation tests in allergic rhinitis children with *Dermatophagoides pteronyssinus* sensitization

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## Summary

**Background:** Nasal provocation tests (NPTs) are indicated in confirming the diagnosis of allergic rhinitis if the clinical history, skin tests or sIgE are inconclusive. NPTs are time-consuming, technically difficult and expensive to perform. Consequently, conjunctival provocation tests (CPTs), which are easier, cheaper and safer should be considered as an alternative method. No recent study has compared CPTs with NPTs in allergic rhinitis children.

**Objective:** To compare CPTs with NPTs in allergic rhinitis children with house dust mite sensitization

**Methods:** Fifty-five children with allergic rhinitis were included. Thirty-six children had positive skin prick tests (SPTs) to *Dermatophagoides pteronyssinus* (Dp). NPTs were performed by spraying 0.1 ml of Dp extract with concentrations of 50, 200 and 500 AU/ml to each nostril at 15 minute interval. The clinical symptom scores, anterior rhinomanometry results and nasal peak flow testing were performed to assess the responses. For CPTs, 0.1 ml of the same concentration of allergen extract was dropped

into one eye and the control solution was dropped into the other. The responses were assessed by clinical symptom scores. The tests were stopped when the subject reported a positive response, or continued to the maximum concentration.

**Results:** The sensitivity, specificity, positive predictive value, negative predictive value and accuracy of CPT compared with NPT are 97.1% (84.7-99.9), 90.5% (69.6-98.8), 94.3% (80.8-99.3), 95% (75.1-99.9) and 94.5 (84.9-98.9), respectively in all patients. Among individual allergic rhinitis subjects the sensitivity, specificity, PPV and NPV are 100%.

**Conclusions:** CPT can be an alternative test for NPT in allergic rhinitis children with house dust mite sensitization, even if they do not have conjunctival symptoms. (*Asian Pac J Allergy Immunol* 2013;31:227-32)

**Key words:** allergic rhinitis, allergic rhinoconjunctivitis, nasal provocation test, conjunctival provocation test, house dust mite sensitization

## Introduction

The diagnosis of allergic rhinitis can be made by clinical history, physical examination and diagnostic tests, for example skin prick tests (SPT) or serum specific IgE measurements. However, the results of these tests can be inconclusive and there is then a need for more specific investigations. Nasal provocation tests (NPT) are a gold standard for the diagnosis of allergic rhinitis, as they act directly on the affected organ. They reproduce the response of the upper airway to natural allergen exposure under controlled conditions. Since identification of exact allergens that cause allergic rhinitis is important for allergen avoidance and allergen immunotherapy, NPTs can be indicated if clinical history, skin test and/or specific IgE testing are inconclusive.

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However, NPTs are time consuming and hard to perform, as they need special equipment, rhinomanometry and skilled technicians. Moreover, only one allergen can be tested at any one time. Systemic reactions, such as bronchospasm, can also occur.<sup>1</sup> Consequently, conjunctival provocation tests (CPTs), which are easier to perform, less expensive, less time consuming and have less systemic side effects should be considered as an alternative method to NPTs in allergic patients. CPTs have been performed to confirm the clinical diagnosis of nasal allergy in clinical studies and as a measure of outcome in various studies.<sup>2-5</sup>

Reports on whether CPTs are appropriate as diagnostic tests for nasal allergies are conflicting. Malmberg et al. found the conjunctiva was less sensitive to allergen challenge than the nasal mucosa.<sup>6</sup> By contrast, Ortega et al. reported CPTs may be most useful in the diagnosis of mono-sensitized patients. They found positive CPTs are frequently elicited in allergic patients who had never complained of clinical symptoms of allergic conjunctivitis.<sup>7</sup> Riechelmann et al. concluded that CPTs are an acceptable alternative to NPTs in allergic rhinitis patients sensitized to HDM, even if they have no conjunctival symptoms.<sup>8</sup> There is no recent study comparing CPTs with NPTs in allergic children. Therefore, the concordances of CPTs and NPTs in allergic children needed to be confirmed.

The most common allergen in Thai children is house dust mite, *Dermatophagoides pteronyssinus*.<sup>9-11</sup> We aimed to compare CPTs with NPTs, which is the gold standard, in allergic rhinitis children with house dust mite sensitization.

## Methods

### Study Population

Subjects were recruited from the pediatric outpatient unit at Ramathibodi hospital during April 2009 to January 2010. The inclusion criteria were: children with allergic rhinitis aged between 6 - 18 years who were skin prick test positive to the *Dermatophagoides pteronyssinus* (Dp) allergen. Allergic rhinitis children who skin prick test were negative for Dp were recruited as controls. Allergic rhinitis was defined as allergy related symptoms with positive skin prick test to at least one aeroallergen. In the case of negative skin tests, the diagnosis was based on allergic symptoms or positive skin tests to other allergens. There were thirty five children with allergic rhinoconjunctivitis and twenty five with allergic rhinitis. The exclusion

criteria included manifestations of allergic symptom exacerbation, other co-morbid allergic diseases, including acute or chronic sinusitis, asthma, acute upper or lower respiratory tract infection, septal deformities, any diseases of the eye and orbit except allergic conjunctivitis, wearing of contact lenses, prior immunotherapy and any current anti-allergic therapy.

The SPTs and NCTs were mainly conducted and interpreted by physicians assisted by a nurse who is skilled at using the relevant techniques. For CPT, the nurse would drop the allergen solution and the control solution to the subject's eyes so that the physician who interpreted the reaction was blinded as to which eye had received which.

The patients were asked to withhold the following medicine before performance of the tests; oral or topical decongestants for one day, antihistamines, anti-leukotrienes and NSAIDs for one week, intranasal steroids and ketotifen for two weeks, antidepressants for three weeks, oral steroids for one month.<sup>12</sup>

The study was approved by the Research Ethical Committee of Ramathibodi Hospital, Faculty of Medicine, Mahidol University. All subjects or parents provided written informed consent.

### Skin prick test (SPT)

Skin prick tests were performed with Dp extract (10,000 AU/ml, glycerin 50% vol/vol and 0.4% phenol as a preservative, ALK Abelló, INC.). Skin prick tests were considered positive if the wheal reaction was at least 3 mm. in diameter greater than that for buffered saline solution.

### Nasal provocation test (NPT)

Subjects waited 10 minutes before the test to allow the nasal mucosa to become acclimatized to the environment. Before the start the test, active anterior rhinomanometry was performed. Baseline symptom scores, nasal peak flow and nasal airway resistant were also recorded.

After that, the control solution (0.9% sodium chloride with 0.4% phenol) was sprayed into each nostril with a metered dose pump delivering a fixed volume of 0.1 ml aqueous solution per puff. After provocation with the control solution, 0.1 ml of increasing concentrations of Dp allergen extract (50, 200, 500 AU/ml) were applied to both nostrils at 15 minute intervals. After each provocation, total nasal symptom scores which were composed of sneezing, pruritus, rhinorrhea, nasal blockage and ocular symptoms were recorded. Additionally, nasal airway

resistance was measured in each nostril separately using active anterior rhinomanometry. (Multifunctional spirometer HI-801, Chest M.I, INC.). Nasal peak flow was also measured using a nasal peak flow meter (In-check inspiratory flow meter, Clement Clarke, Inc.). NPTs were considered positive if the difference in the total nasal symptom scores.<sup>12</sup> (Table 1) before and after allergen provocation was at least 5 or nasal airway resistance by anterior rhinomanometry was increased by at least 100% or nasal peak flow was reduced by at least 50%. Side effects were recorded during the performance of the test. After finishing the test, the patients were asked to stay in the clinic for observation for at least 30 minutes. Oral antihistamines or oral decongestants were given to patient with troublesome nasal symptoms.

**Conjunctival provocation test (CPT)**

CPTs were performed using allergen solutions which were identical to the solutions employed for NPTs except for the diluents. A previous study<sup>8</sup> found that a burning sensation could occur, which was suspected to be due to the preservative used, was the major complaint. Therefore we used normal saline without 0.4% phenol for the diluents in CPTs solutions. The solutions for CPTs were prepared day by day. Eye examinations were employed in all subjects before the tests to confirm that they did not have any eye symptoms. 0.9% sodium chloride with 0.4% phenol diluted with normal saline was used as a negative control. A control solution, which was identical to the allergen solution except for the allergen content, was administered to the lower conjunctival sac of one eye (control eye). Immediately after application of the control solution, 0.1 ml of the low-concentrated allergen solution (50 AU/ml) was administered to the lower

conjunctival sac of the opposite eye (provocation eye). Every 15 minutes 0.1 ml of control solution was administered to the conjunctival sac of control eye and 0.1 ml of increasing concentrations of prepared allergen was placed into the provocation eye. The CPT was considered positive when the response was stage two or higher (Table 2). The subjects were informed not to rub their eyes during the tests. Topical antihistamine was administered to the affected eye immediately after the test was positive. Any side effects were recorded during the tests. After the test, the patients were asked to stay in the clinic for observation for at least 30 minutes. The physicians who evaluated the response after administration of the solutions were blinded as to which eye had received which solution.

CPTs and NPTs were performed at least two weeks apart to ensure that the allergen from first the test did not affect the result of the second test.

**Statistical analysis**

Sensitivity, specificity, PPV, NPV, accuracy, likelihood ratio and area under the curve (AUC) were performed to compare the result of CPTs with NPTs. All statistics were analyzed using SPSS version 17 software.

**Results**

There were 55 subjects consisting of 30 boys and 25 girls. Their ages ranged between 6 and 17 years. The mean age was 11.5 (± 3) years. Twenty-five subjects had allergic rhinitis without conjunctival symptoms and thirty subjects had allergic rhinoconjunctivitis. SPTs were positive to *dermatophagoides pteronyssinus* (Dp) in 36 subjects and negative in 19 subjects. In 36 subjects with positive SPTs, NPTs were positive in 34 subjects and CPTs were positive in 35 subjects. In the negative SPTs group, NPTs and CPTs are all negative. The test results are outlined in Figure 1.

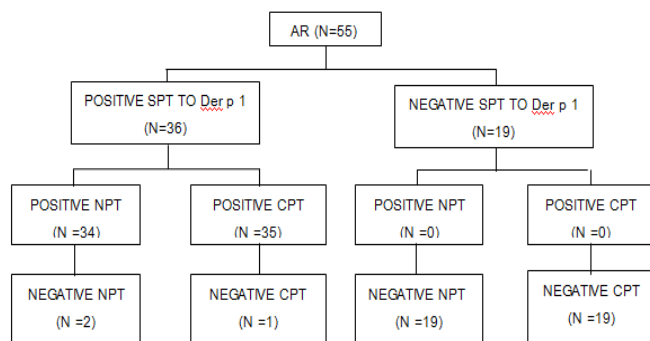
**Table 1.** Nasal symptom scores<sup>12</sup>

Nasal symptoms	Point score	Method
Sneeze		Frequency of sneeze
1-2	1	
3-4	2	
5 or more	3	
Pruritus		Ask the subjects
Nose	1	
Palate	1	
Ear	1	
Rhinorrhea	0-3	Weigh the nasal secretion
Nasal blockage	0-3	Rhinoscope
Ocular symptom	1	Ask the subjects

**Table 2.** Categorization of the response to allergens in the CPT<sup>8</sup>

Stage	Criteria
0	No subjective or visible reaction
1	Itching, foreign body sensation
2	Stage 1 + tearing, vasodilation of bulbar conjunctiva
3	Stage 2 + vasodilation and erythema of tarsal conjunctiva, blepharospasm
4	Stage 3 + chemosis, lid swelling

**Figure 1.** The results of SPTs, NCTs and CPTs in 55 children with allergic rhinitis



AR, Allergic rhinitis  
 SPT, Skin prick test  
 NPT, Nasal provocation test  
 CPT, Conjunctival provocation test

Evaluating the whole study group, the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of CPT are 97.1% (84.7-99.9), 90.5% (69.6-98.8), 94.3% (80.8-99.3), and 95% (75.1-99.9), respectively (Table 3). The accuracy is 94.5% (84.9-98.9). The positive likelihood ratio is 10.2 (2.7-38.1) and the AUC is 0.94 (0.87-1). The kappa value is 0.84. The sensitivity, specificity, PPV and NPV in patients with isolated allergic rhinitis are 100% (Table 4).

Comparison between the allergic rhinoconjunctivitis and rhinitis groups indicated that the results of SPTs, NCTs and CPTs in rhinitis children are concordant but discordant in some rhinocconjunctivitis children. Among allergic rhinoconjunctivitis children (N=30), 19 (63.3%) children had positive SPTs, NCTs and CPTs, 8 (26.7%) children had negative SPTs, NCTs and CPTs, 2 (6.7%) children had positive SPTs and CPTs but negative NCTs, 1 (3.3%) child had positive SPTs and NCTs but negative CPTs. By contrast, among children with isolated rhinitis (25), 14 (56%) children had positive SPTs, NCTs and CPTs, 11 (44%) children had negative SPTs, NCTs and CPTs.

Among the patients with positive NPTs, most patients had positive results at the concentration of 200 AU/ml (20 patient, 57%). Eight (23%) and seven (20%) patients had positive results at 500 and 50 AU/ml, respectively. Similarly, most patients with positive CPTs were positive at 200 AU/ml (16 patients, 46%). Fifteen (43%) and four (11%) patients had positive results at 500 and 50 AU/ml, respectively.

**Table 3.** The results of CPTs and NPTs in all subjects, with NPT serving as the reference method. (N = 55)

Test	Positive CPT	Negative CPT	Total
Positive NPT	33	1	34
Negative NPT	2	19	21
Total	35	20	55

Sensitivity = 97.1% (84.7-99.9)  
 Specificity = 90.5% (69.6-98.8)  
 Positive predictive value = 94.3% (80.8-99.3)  
 Negative predictive value = 95% (75.1-99.9)  
 NPT, Nasal provocation test  
 CPT, Conjunctival provocation test

### Discussion

Routine diagnosis of clinical allergy is based on clinical history and skin-prick tests. SPTs cannot be performed in some situations, for example skin rash or severe dermatitis. Moreover, SPTs and serum specific IgE cannot distinguish true allergy from asymptomatic sensitization.<sup>13-14</sup> Therefore, provocative tests are necessary.

The gold standard for the diagnosis of allergic rhinitis is the nasal provocation test, which evokes a response in the affected organ. Nevertheless, NPTs are time consuming, technically difficult, expensive and can cause more adverse effects. Conjunctival provocation tests could be an attractive alternative method. In several studies, CPTs have been used as a surrogate for NPTs in patients with allergic rhinitis<sup>2-5</sup> and it was found that CPTs are safe.<sup>8,15,16</sup>

The concordance of CPTs and NPTs in children with allergic rhinitis was investigated in this study. The children with allergic rhinitis, regardless of whether they had allergic conjunctivitis as well, were chosen because we wanted to determine whether CPTs could be successfully performed in allergic rhinitis patients, even if they do not have conjunctival symptoms. Normal children, are not included in the negative control group for ethical reasons.

The sensitivity, specificity, positive predictive value, negative predictive value and accuracy of CPTs, with NPT serving as a reference method, are all more than 90%. Furthermore, the positive likelihood ratio reached 10 and the area under the curve (AUC) almost reached one. These results indicate that the CPT is accurate and the results are comparable to NCT, which is the gold standard. In contrast to studies in Western countries,<sup>6,17</sup> we used allergen concentration for NPTs and CPTs equal to 50, 200 and 500 AU/ml which is lower and

**Table 4.** The results of CPTs and NPTs in isolated allergic rhinitis subjects, with NPT serving as the reference method (N=25)

Test	Positive CPT	Negative CPT	Total
Positive NPT	14	0	14
Negative NPT	0	11	11
Total	14	11	25

Sensitivity, specificity, positive predictive value and negative predictive value = 100%

NPT, Nasal provocation test

CPT, Conjunctival provocation test

reproducible. Kanthawatana et al used a highest concentration for NPTs in adult Thai subjects equal to 1,000 AU/ml and found that it was reproducible.<sup>18</sup> Moreover, Roongapinun et al found the concentration that produced a significant discrepancy from the baseline was 50 AU/ml for congestion and pruritus and 100 AU/ml for rhinorrhea and sneezing. Nasal airway resistance was different from controls at a concentration of 1000 AU/ml.<sup>19</sup> Both these studies were performed in adults and the youngest subject was 20 years old. In this study, performed in children age between 6-18 years, we decided to reduce the highest concentration of allergen to 500 AU/ml because of the risk of adverse reactions. There was no published data on CPTs in Thai children, so we used the same concentrations as for NPTs. We have found that with these concentrations, NPTs and CPTs are reproducible and no severe adverse reactions were observed.

There are no standard guidelines for either NPTs or CPTs. We used the nasal symptom scores as escribed in Middleton's Allergy 6<sup>th</sup> edition<sup>12)</sup> which we usually use in our institute. We used conjunctival symptom scores as described in the study of Riechelmann et al.<sup>8</sup> They categorized the conjunctival symptom scores into five stages which are not difficult to determine and did not need ophthalmologist or special equipment. The ophthalmologist involved in this study determined whether the subjects had allergic conjunctivitis by using the slit lamp.

A burning sensation was not the major problem in our patients. No serious side effects were observed in this study.

Three patients had discondordance between SPTs, CPTs and NPTs. Two patients had positive SPTs and CPTs but negative NCTs. OIn one of them the clinical history was reviewed and it was found that he probably had pure allergic conjunctivitis. His conjunctival symptoms predominated

and the diagnosis of allergic conjunctivitis was confirmed by an ophthalmologist. In contrast his nasal symptoms are mild. He often has periods free of nasal symptoms. Moreover, his nasal examination is not typical of allergic rhinitis. In the other subject, who had positive SPTs and CPTs but negative NCTs, *Dermatophagoides Farinae* (Df) is probably the main cause of nasal symptoms, but not Dp. His SPTs were positive to both Dp and Df.

In the third subject who had positive SPTs and NPTs but negative CPTs, it was concluded that this might be a false negative test result. He has both nasal and conjunctival symptoms. His SPTs are positive only to Dp. He probably needed more concentration of allergen to reveal the positive result.

The results of this study are consistent with the results reported by Riechelmann et al. They found that NPTs and CPTs yield concordant results in 90% of the subjects successfully tested. They also reported the diagnostic efficacy of CPTs, with NPTs as the reference method was 89%, whether or not conjunctival symptoms had been reported in addition to rhinitis symptoms. The sensitivity, specificity, PPV and NPV of CPTs were 91%, 87%, 89% and 90%, respectively. Finally, they concluded that CPTs are an acceptable alternative to NPTs in patients with allergic rhinitis to house dust mite, even if they have no conjunctival symptoms.<sup>8</sup> The subjects in Riechelmann et al study were all adults. They included healthy people as negative control subjects. To the best of our knowledge, this is the first study in allergic rhinitis children and we found that the results were consistent with the reports in adults. However, we used a concentration of allergen lower than they used in adults; this probably suggests that children have more sensitivity of their mucosa than adult. Furthermore, the results of this study are partially consistent with the reports from Mosbech et al. They performed bronchial provocation tests (BPTs), NPTs and CPTs in 50 asthmatic patients with house dust mite sensitization and found concordant results for the three diagnostic techniques in 40/50 patients.<sup>18</sup> In patients with seasonal allergic rhinitis, 100% concordance between NPTs and CPTs was also found by Petersson et al.<sup>20</sup> Ortega et al. found that conjunctivae react in a similar way to skin in patients with and without clinical conjunctivitis.<sup>7</sup>

According to the accuracy of the CPTs shown in this study, they should be considered as an alternative method to NPT. CPT, which is simple,



technically easy, fast and low-cost can replace NPT to confirm the diagnosis of allergic rhinitis, if data obtained by clinical history, skin tests or specific IgE are not conclusive. Moreover, it can be used to identify the exact allergen that causes the symptoms which will be useful for allergen avoidance and specific immunotherapy. It should probably be considered as an objective tool to evaluate the efficacy of allergen specific immunotherapy. The differences between the level of allergen concentration required to produce positive test results before and after immunotherapy may reflect the efficacy of the therapy. Further study is needed to prove this advantage.

In conclusion, the conjunctival provocation test appears to be a valuable tool in the diagnosis of allergic rhinitis in children with house dust mite sensitization. It may serve as an alternative method to the nasal provocation test in the diagnosis of allergic rhinitis, even in patients without conjunctival symptoms.

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