



## Appropriate Antigen Concentrations and Timing of a Nasal Provocation Test

Young Hyo Kim

Department of Otorhinolaryngology, Head and Neck Surgery, Inha University School of Medicine, Incheon, Korea.

**Purpose:** We aimed to determine appropriate antigen concentrations and the right time to evaluate intranasal changes when performing a nasal provocation test (NPT). Also, we sought to analyze the diagnostic usefulness of individual nasal symptom and peak nasal inspiratory flow (PNIF).

**Materials and Methods:** We divided 46 patients into allergic rhinitis (AR) group (n=19) and a non-allergic rhinitis (NAR) group (n=27). We performed intranasal challenge with 100 AU/mL of *Dermatophagoides pteronyssinus* (DP) and measured changes in nasal symptoms [scored using the visual analogue scale (VAS)] and PNIF%. If the patient showed significant changes, VAS and PNIF were assessed again after another 15 minutes. In patients without significant changes, we administered 1000 AU/mL and measured changes in nasal symptoms and PNIF% after 15 and 30 minutes.

**Results:** Fifteen minutes after the 100 AU/mL challenge, the AR group showed more significant VAS changes in all nasal symptoms, total nasal symptom score (TNSS), and PNIF% change than the NAR group. Among the AR group, patients who did not respond to 100 AU/mL exhibited less significant differences relative to the NAR group, even after 1000 AU/mL challenge. Receiver operating characteristic curve analysis for VAS changes 15 minutes after 100 AU/mL challenge revealed that all nasal symptoms had area under the curve (AUC) values of  $\geq 0.84$  ( $p < 0.001$ ). TNSS change had an AUC value of 0.929 ( $p < 0.001$ ), while PNIF% change had an AUC value of 0.834.

**Conclusion:** We could determine the optimal concentration (100 AU/mL), timing (15 minutes after challenge), and parameters (changes in TNSS and PNIF%) when performing NPT.

**Key Words:** Rhinitis, Allergic; nasal provocation tests; ROC curve

### INTRODUCTION

Allergic rhinitis (AR) can be defined as systemic sensitization to allergens, with an allergic response in the nasal cavity upon exposure to causative allergens. AR is a worldwide health problem that adversely affects quality of life and poses enormous medical expenses and significant socio-economic burden.<sup>1</sup> To

directly measure anatomical and physiological changes in the nasal cavity from which to diagnose AR, the nasal provocation test (NPT) is useful. In an NPT, a causative antigen is directly administered, and various nasal cavity changes are evaluated.<sup>2</sup> NPT can also be used to diagnose local allergic rhinitis (LAR),<sup>3,4</sup> assess the effectiveness of allergen immunotherapy,<sup>5</sup> or diagnose occupational rhinitis.<sup>6,7</sup>

Despite its advantages, NPT has not been widely used for the diagnosis of AR, wherein researchers use different concentrations of antigens and test protocols. Furthermore, there has been no work to compare test results among researchers and to standardize them. To overcome these shortcomings and standardize NPT, the European Academy of Allergy and Clinical Immunology (EAACI) recently published a position paper on the implementation of NPT. This position paper systematically suggested laboratory conditions, indications and contraindications, and subjective and objective parameters to be evaluated during an NPT.<sup>8</sup>

**Received:** April 2, 2021 **Revised:** May 21, 2021

**Accepted:** May 28, 2021

**Corresponding author:** Young Hyo Kim, MD, PhD, Department of Otorhinolaryngology, Head and Neck Surgery, Inha University School of Medicine, 27 Inhang-ro, Jung-gu, Incheon 22332, Korea.

Tel: 82-2-890-2437, Fax: 82-32-890-3580, E-mail: inhaorl@inha.ac.kr

•The authors have no potential conflicts of interest to disclose.

© Copyright: Yonsei University College of Medicine 2021

This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<https://creativecommons.org/licenses/by-nc/4.0>) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

Nevertheless, we still do not know the proper antigen concentration that should be administered into the nasal cavity when performing NPT: the EAACI position paper recommends starting with a low concentration and continue with a higher concentration if there is no response.<sup>8</sup> This can be time-consuming poses limitations to practical application. Therefore, there is an urgent need to develop an NPT protocol that can be quickly performed using a single concentration of an antigen.

Therefore, in this study, we aimed to determine the optimal concentration of allergen for diagnosing AR patients, while faithfully following the recommendations of the EAACI position paper; to determine the appropriate timing at which to assess intranasal changes after antigen administration by evaluating changes in nasal symptoms and objective indicators (peak nasal inspiratory flow, PNIF) at 15 and 30 minutes after nasal allergen challenge; and to analyze the diagnostic usefulness of individual nasal symptoms and PNIF before and after allergen challenge through receiver operating characteristic (ROC) curve analysis.

## MATERIALS AND METHODS

### Subjects

We enrolled 46 patients (27 males and 19 females, aged 9 to 81 years, with a mean age of 38.4±19.5 years) who had visited our outpatient clinic complaining of long-lasting symptoms of rhinitis (nasal stuffiness, watery rhinorrhea, and/or sneezing) from June 2020 to October 2020. As a routine diagnostic work-up for systemic allergic sensitization, we performed a skin prick test (SPT) for all of these patients. We conducted SPT using more than 40 allergens, including house dust mite extracts [*Dermatophagoides pteronyssinus* (DP) and *D. farinae* (DF)], pollen, pets dander, fungi, cockroaches, saline (as a negative control), and histamine (as a positive control).

The exclusion criteria were as follows: those who had used anti-allergic medications, such as antihistamines or vasoconstrictors, within the previous 7 days, intranasal steroids within a month, and systemic corticosteroids within the last 3 months. We also excluded those with unstable/severe systemic disease, those who had contraindications to the use of epinephrine in case of an emergency, those who had undergone any nasal surgery within the last 3 months, pregnant or lactating female, those who had chronic rhinosinusitis confirmed by nasal endoscopy and/or imaging study (paranasal X-ray or computed tomography), and those with a history of repeated exposure to chemical irritants or cigarette smoking.

Before performing NPT, we received informed consent after providing patients with sufficient information about this study's purpose. This study was approved by the Inha University Hospital Institutional Review Board Committee on Studies Involving Human Beings (IRB number: 2019-07-026).

According to the SPT results, we divided the patients into

two groups: AR group (n=19, those with strongly positive results for DP/DF) and non-allergic rhinitis (NAR) group (n=27, negative results for all antigens tested, including DP and DF). We defined a “strongly positive” result as that “when the size of a wheal caused by an allergen was the same or larger than that caused by histamine” and a “negative” result as that “when the allergen made absolutely no wheal or the size of the wheal was the same as that caused by saline.” We compared the demographic characteristics of patients according to grouping as summarized in Table 1.

### Protocol for implementing NPT

#### *Laboratory setup and acclimatization before testing*

We thoroughly followed the recently published EAACI position paper guidelines in implementing the NPT.<sup>8</sup> We maintained constant temperature and humidity in the laboratory (temperature 20±1.5°C, relative humidity 40–60%). Patients adapted to temperature and humidity while waiting in the laboratory for at least 15 minutes before the NPT.

#### *DP antigen and sprayer for provocation*

We purchased a 10000 AU/mL stock DP solution (#6692, HollisterStier Allergy, Spokane, WA, USA) that we diluted 1:100 to make a 100 AU/mL solution and 1:10 to obtain a 1000 AU/mL solution. As a control challenge with which to evaluate and rule out nonspecific hyper-reactivity, we used saline. Using a metered-dose pump sprayer, we sprayed 100 µL of saline or DP solution (100 AU/mL or 1000 AU/mL) onto both nostrils of the patient.

#### *Subjective and objective measurements*

We assessed the severity of subjective symptoms (nasal obstruction, rhinorrhea, sneezing, nasal itching, and ocular symptoms) using the visual analogue scale (VAS), as recommended by the EAACI position paper.<sup>8</sup> We used a standardized 100-mm VAS ruler, and the patient was asked to indicate the severity of symptoms from 0 mm (no symptoms) to 100 mm (worst troublesome). We defined the sum of all subjective symptoms as the total nasal symptom score (TNSS).

For objective evaluation, we measured PNIF using a porta-

**Table 1.** Demographic Characteristics of the Study Patients

	AR group (n=19)	NAR group (n=27)	p value
Sex (male:female)	10:9	17:10	NS
Age, mean (SD)	25.4 (16.7)	47.5 (16.0)	<0.001
ARIA classification			
Intermittent:persistent	7:14	12:13	NS
Mild:moderate-severe	4:16	15:11	0.016

AR, allergic rhinitis; ARIA, Allergic Rhinitis Impact on Asthma; NAR, non-allergic rhinitis; NS, not significant; SD, standard deviation. Independent t-test and Fisher's exact test.

ble inspiratory flow meter (Clement Clarke International, Harlow, UK). With a mask connected to the flow meter, we covered the patient's nose and mouth completely. We then asked the patient to inhale as much as possible through their nose with their mouth closed.

*Actual NPT protocol*

We first measured VAS and PNIF at baseline before any challenge was administered to the nose. Afterwards, we applied a control solution (100 µL of saline) into both nostrils of the patient. After 10 minutes of saline challenge, we again measured VAS and PNIF values.

We calculated VAS change as [(Post-challenge VAS)-(Baseline VAS)]. Also, we defined PNIF change as [(Baseline PNIF)-(Post-challenge PNIF)]/(Baseline PNIF)×100 (%). If a patient had a VAS change of ≥27.5 mm and/or a PNIF change ≥20% after the saline challenge, we determined that the patient had nonspecific hyper-reactivity and discontinued the test.<sup>8</sup> In our study, none of the 46 patients had nonspecific hyper-reactivity.

Next, we sprayed 100 µL of DP solution (100 AU/mL) into both nasal cavities. After 15 minutes, we measured VAS and PNIF and calculated changes therein relative to baseline. If the VAS change was ≥55 mm and/or the PNIF change was ≥40% at 15 minutes after 100 AU/mL DP challenge, we determined that they patient had a "positive response" to that concentration. Among 19 patients in the AR group, 14 were positive. For these patients, we waited another 15 minutes (until 30 minutes after the challenge), measured VAS and PNIF again, and calculated changes therein relative to baseline.

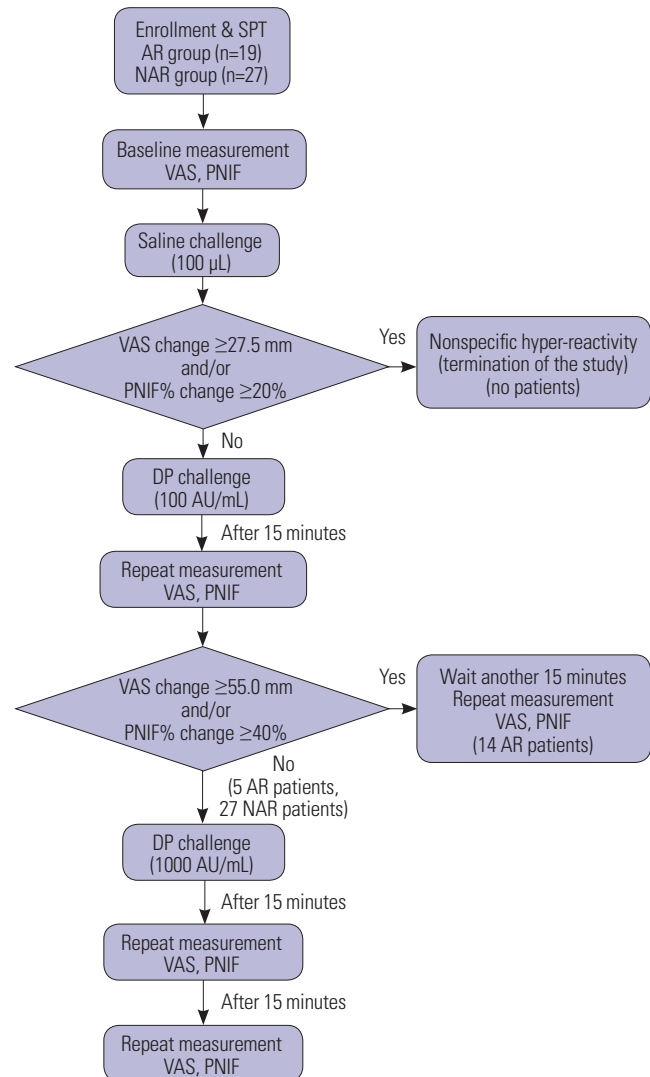
Five patients in the AR group and all 27 patients in the NAR group did not respond to 100 AU/mL antigen. After a 15-minute wash-out period, we sprayed 1000 AU/mL DP solution into both nasal cavities in these patients. Fifteen and 30 minutes after the 1000 AU/mL DP challenge, we measured VAS and PNIF and calculated the amount of change in these indicators according to the formula mentioned above. Fig. 1 summarizes the process of the NPT protocol.

**Statistical analysis**

We adopted the F test to compare variances and the unpaired t-test (with Welch's correction) to compare changes in VAS and PNIF% between the AR and NAR groups. To compare the diagnostic usefulness of various indicators and determine sensitivity and specificity according to cut-off values, we used ROC curve analysis. We used SPSS 22.0 (IBM Corp., Armonk, NY, USA) and Prism 5.0 software (GraphPad Software, San Diego, CA, USA) to conduct all statistical analyses and set statistical significance at a *p* value<0.05.

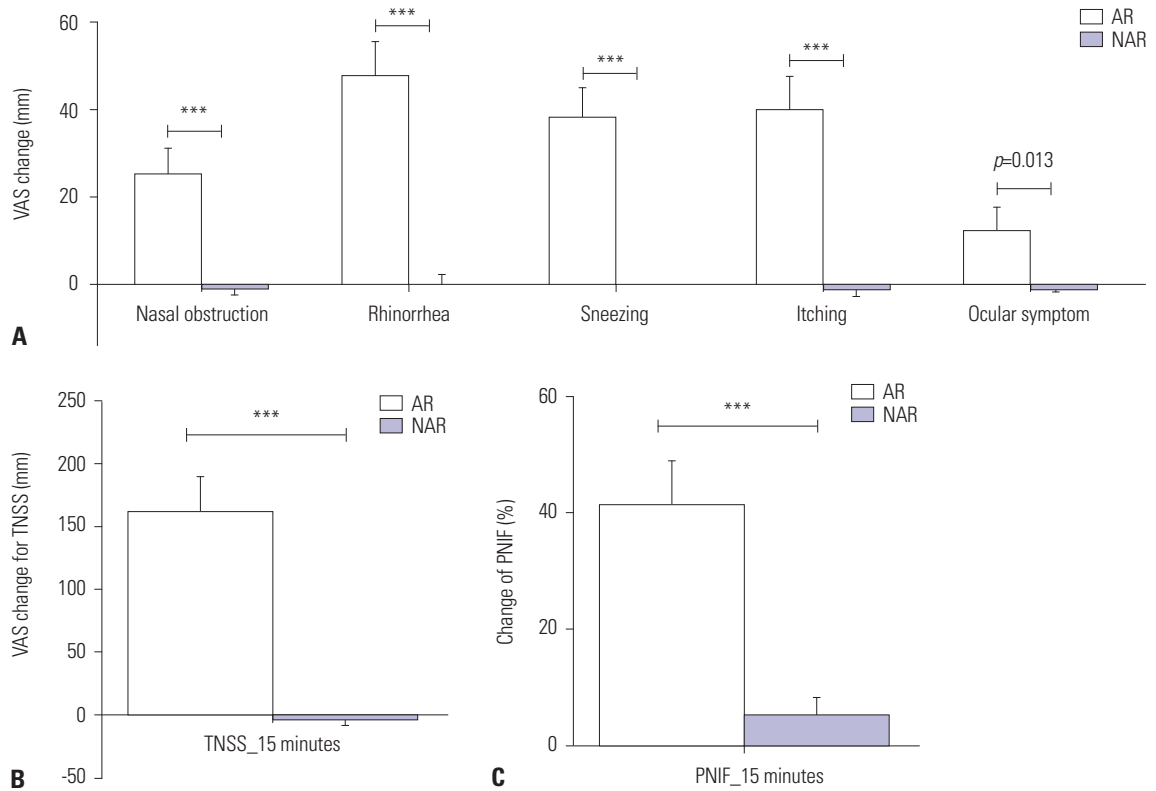
**RESULTS**

After 100 AU/mL DP challenge, the AR group showed more

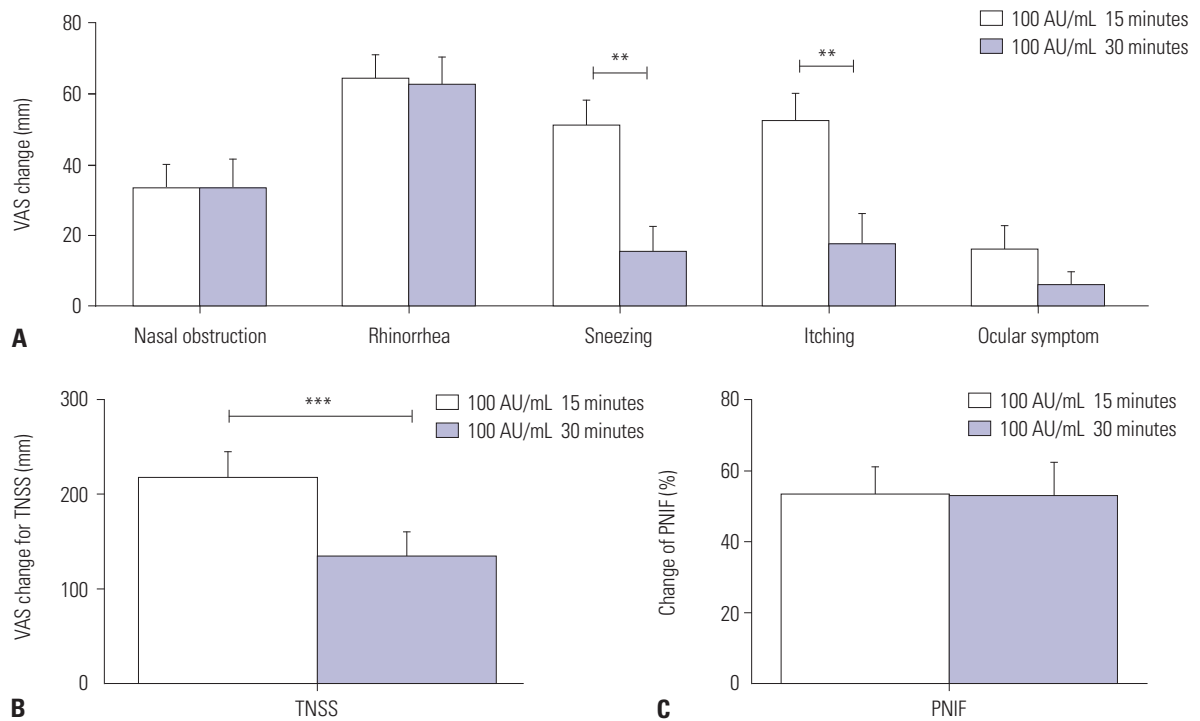


**Fig. 1.** Summary of the nasal provocation test. SPT, skin prick test; AR, allergic rhinitis; NAR, non-allergic rhinitis; VAS, visual analogue scale; PNIF, peak nasal inspiratory flow; DP, dermatophagoides pteronyssinus.

significant VAS changes in all subjective symptoms, including nasal obstruction (AR group 24.7±6.2 mm vs. NAR group -0.7±1.2 mm, *p*<0.001), rhinorrhea (AR group 47.1±8.5 mm vs. NAR group 0.0±1.5 mm, *p*<0.001), sneezing (AR group 37.4±7.5 mm vs. NAR group 0.0±0.0 mm, *p*<0.001), itching (AR group 39.5±7.9 mm vs. NAR group -0.7±1.7 mm, *p*<0.001), and ocular symptoms (AR group 11.6±5.6 mm vs. NAR group -0.7±0.7 mm, *p*=0.013), than the NAR group, which was statistically significant (Fig. 2A). The amount of change in TNSS, the sum of all subjective symptoms, was also statistically significant in the AR group (160.3±30.6 mm), compared to the NAR group (-2.2±3.4 mm, *p*<0.001) (Fig. 2B). After 100 AU/mL DP challenge, PNIF% changes were also statistically more significant in the AR group (40.9±7.8%) than in the NAR group (4.9±3.3%, *p*<0.001) (Fig. 2C). Considering these results, we deemed that a DP solution of 100 AU/mL was quite useful for distinguishing between AR



**Fig. 2.** Change in (A) each nasal symptom, (B) TNSS, and (C) PNIF at 15 minutes after DP 100 AU/mL administration. \*\*\* $p < 0.001$ . Independent t-test. AR, allergic rhinitis; NAR, non-allergic rhinitis; VAS, visual analogue scale; TNSS, total nasal symptom score; PNIF: peak nasal inspiratory flow; DP, dermatophagoides pteronyssinus.



**Fig. 3.** Comparison of changes in (A) each nasal symptom, (B) TNSS, and (C) PNIF at 15 and 30 minutes after DP 100 AU/mL administration. \*\* $p < 0.01$ , \*\*\* $p < 0.001$ . Independent t-test. VAS, visual analogue scale; TNSS, total nasal symptom score; PNIF: peak nasal inspiratory flow; DP, dermatophagoides pteronyssinus.

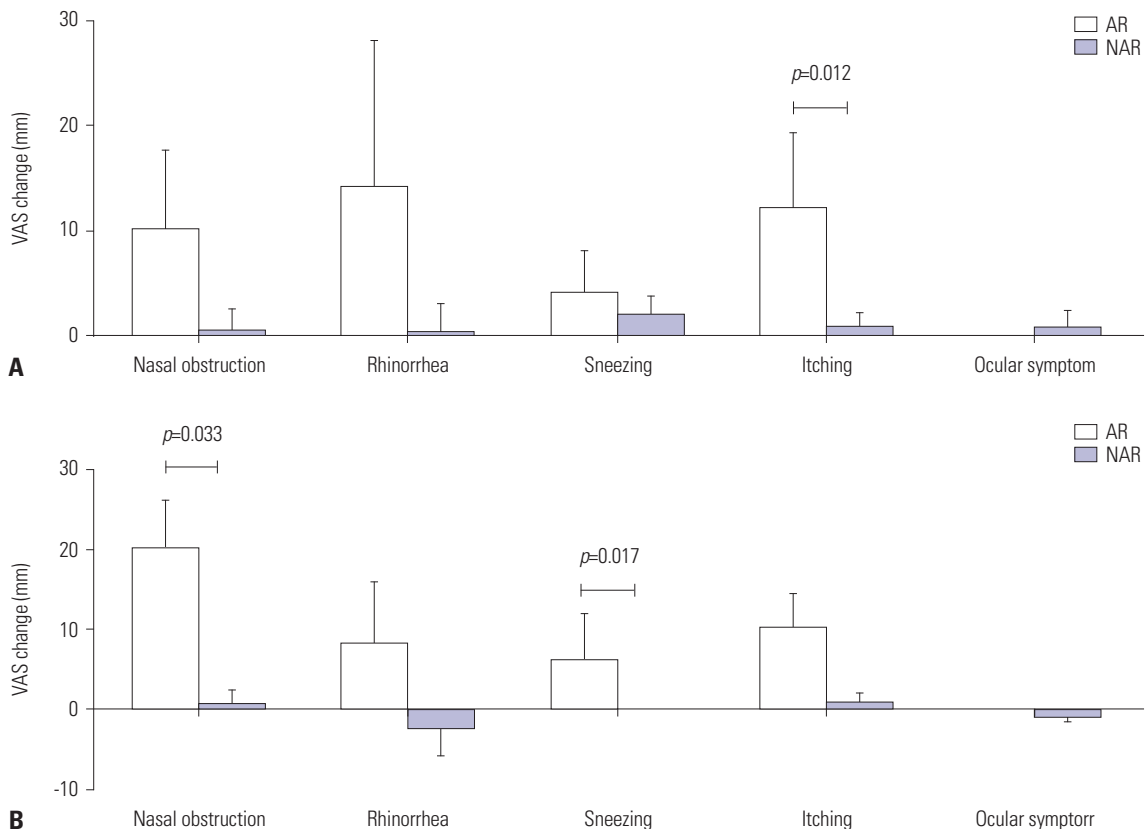
and NAR individuals using an NPT.

For 14 patients in the AR group who had a positive response after 100 AU/mL DP challenge, we compared changes in VAS and PNIF% after 15 and 30 minutes. In result, we found no significant difference in VAS changes for symptoms of nasal obstruction, rhinorrhea, and ocular symptoms, at 15 and 30 minutes after the challenge ( $p>0.05$ ). For sneezing and itching, the VAS change was significantly smaller after 30 minutes ( $p<0.01$ ) (Fig. 3A). After 30 minutes of DP administration, TNSS changes decreased considerably, compared to that after 15 minutes ( $p<0.001$ ) (Fig. 3B). PNIF% changes at 15 and 30 minutes after 100 AU/mL DP challenge did not show a significant difference ( $p>0.05$ ) (Fig. 3C). In summary, changes in VAS scores and PNIF% at measured 15 and 30 minutes after the DP challenge showed no significant difference or significantly decreased after 30 minutes.

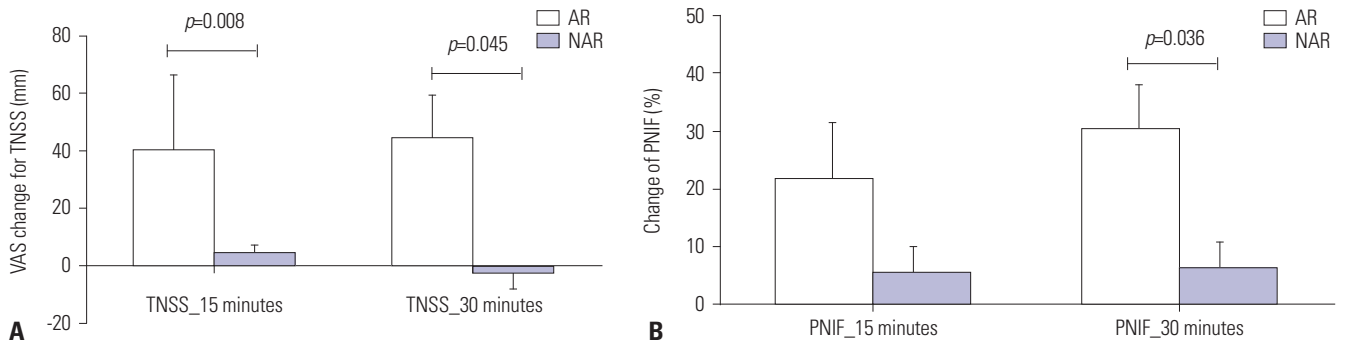
Among patients in the AR and NAR groups who did not respond to the 100 AU/mL DP challenge, we performed a 1000 AU/mL DP challenge and compared VAS and PNIF%. As there was no significant difference in VAS changes between the groups in most symptoms. VAS changes in itching at 15 minutes after challenge (AR group  $12.0\pm 7.3$  mm vs. NAR group  $0.7\pm 1.3$  mm,  $p=0.012$ ) and those for nasal obstruction (AR group  $20.0\pm 6.3$  mm vs. NAR group  $0.4\pm 2.0$  mm,  $p=0.033$ ) and sneezing (AR group  $6.0\pm 6.0$  mm vs. NAR group  $0.0\pm 0.0$  mm,  $p=$

$0.017$ ) at 30 minutes after challenge were significantly greater in AR group. However, when compared with 100 AU/mL challenge, the amount of VAS change was smaller between groups (Fig. 4). Meanwhile, although TNSS changes were statistically significant in the AR group, compared to the NAR group ( $p<0.05$ ) (Fig. 5A), those after 100 AU/mL challenge ( $160.0\pm 30.6$  mm) were greater than those after 1000 AU/mL challenge ( $40.0\pm 26.7$  mm). After 30 minutes of 1000 AU/mL DP challenge, PNIF% changes in the AR group were significantly greater than those in the NAR group (AR group  $30.2\pm 8.0\%$  vs. NAR group  $6.0\pm 5.1\%$ ,  $p=0.036$ ) (Fig. 5B), albeit at lesser degrees than those achieved with 100 AU/mL challenge. Overall, these results suggested that AR patients who did not respond to 100 AU/mL did not react to a higher concentration of 1000 AU/mL or had smaller changes even if they were positive.

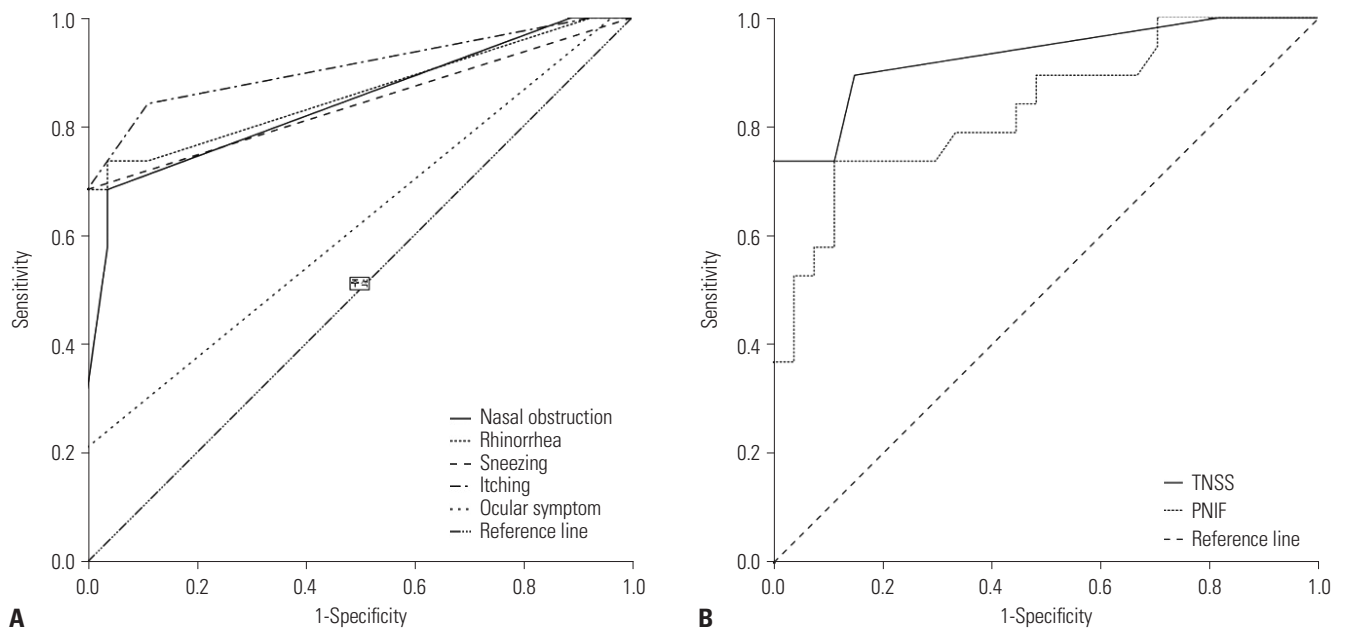
ROC curve analysis for VAS changes at 15 minutes after 100 AU/mL DP challenge revealed that all symptoms but ocular symptoms had area under the curve (AUC) values of 0.84 or more (all  $p<0.001$ ). Ocular symptoms had an AUC of 0.620 ( $p=0.170$ ), which had less diagnostic value than the other symptoms (Fig. 6A). TNSS changes at 15 minutes after 100 AU/mL DP challenge had an AUC of 0.929 ( $p<0.001$ ), while PNIF% change had an AUC of 0.834 ( $p<0.001$ ) (Fig. 6B). When we set the cut-off value for TNSS change to  $\geq 50.0$  mm, the sensitivity was 73.7%, and the specificity was 100.0%. With a cut-off val-



**Fig. 4.** Changes in each nasal symptom at (A) 15 minutes and (B) 30 minutes after DP 1000 AU/mL administration. Independent t-test. AR, allergic rhinitis; NAR, non-allergic rhinitis, VAS, visual analogue scale; DP, dermatophagoides pteronyssinus.



**Fig. 5.** Changes in (A) TNSS and (B) PNIF at 15 and 30 minutes after DP 1000 AU/mL administration. Independent t-test. AR, allergic rhinitis; NAR, non-allergic rhinitis; VAS, visual analogue scale; TNSS, total nasal symptom score; PNIF: peak nasal inspiratory flow; DP, dermatophagoides pteronyssinus.



**Fig. 6.** Receiver operating characteristic curve analysis for (A) changes in each nasal symptom, (B) TNSS, and PNIF at 15 minutes after DP 100 AU/mL administration. TNSS, total nasal symptom score; PNIF: peak nasal inspiratory flow; DP, dermatophagoides pteronyssinus.

**Table 2.** Results of ROC Curve Analysis of Symptom Changes after NPT: Determination of Cut-Off Value, Sensitivity and Specificity

VAS changes for	Cut-off value (mm)	Sensitivity (%)	Specificity (%)
Nasal obstruction	5.0	68.4	96.3
Rhinorrhea	12.5	73.7	96.3
Sneezing	15.0	68.4	100.0
Itching	5.0	84.2	88.9
Ocular symptom	20.0	21.1	100.0

ROC, receiver operating characteristic; NPT, nasal provocation test; VAS, visual analogue scale.

ue of  $\geq 23.0\%$  for PNIF% change, the sensitivity was 73.7%, and the specificity was 88.9%. A summary of the cut-off values, sensitivity, and specificity for VAS changes in individual symptoms is provided in Table 2.

## DISCUSSION

In addition to evaluating the clinical characteristics of AR patients, NPT has utility in various aspects. LAR can be defined as a condition in which rhinitis symptoms are manifested by Th2 type inflammation localized in the nasal cavity without systemic allergy.<sup>3,9,10</sup> Therefore, to diagnose LAR, it is essential to diagnose hyper-reactivity to antigens in the nasal cavity by performing NPT.<sup>11</sup> Also, in patients undergoing allergen immunotherapy, NPT can also be useful as an in vivo biomarker to evaluate its effectiveness. Schiavi and colleagues administered immunotherapy for 2 years in pediatric AR patients with nasal hyper-reactivity against grass pollen and found that in the group receiving immunotherapy, only about 21% were positive for NPT after 2 years, whereas in the control group, about 90% were positive.<sup>12</sup> Ramírez-Jiménez, et al.<sup>13</sup> found that patients with aspirin-exacerbated respiratory disease who received montelu-

kast had fewer positive responses (13 of 82 patients) from NPT using lysine-acetylsalicylate than those who did not receive medication (35 of 37 patients).

In previous studies, we used a DP solution of 1000 AU/mL for NPT.<sup>14</sup> While we have not experienced any anaphylactic reactions after nasal allergen challenge while conducting NPT research,<sup>3,15-18</sup> in order to determine a safe and useful concentration, we investigated NPT with a concentration of 100 AU/mL in this study. In result, we noted that, even when using a low concentration, AR patients had more significant changes in VAS and PNIF, compared to NAR patients. Accordingly, we deemed that NPT can be performed successfully even with an antigen concentration 10 times more diluted than that used in previous studies.

Repeatedly measuring VAS, PNIF, and possibly other parameters at 15 and 30 minutes after nasal allergen challenge can be laborious. Therefore, we compared VAS and PNIF changes at 15 and 30 minutes after NPT implementation to determine which may be more useful. Interestingly, VAS and PNIF changes at 30 minutes after the DP challenge did not differ from those at 15 minutes or significantly decreased. Therefore, the changes after 30 minutes had less diagnostic usefulness in distinguishing between AR and NAR than those after 15 minutes. Based on these findings, we were able to determine an appropriate antigen concentration (DP 100 AU/mL) for performing an NPT and an appropriate timing (15 minutes after the challenge) at which to assess NPT results.

To evaluate the diagnostic usefulness of NPT and to determine cut-off values of use as diagnostic criteria, we performed a ROC curve analysis. In doing so, we found that TNSS changes at 15 minutes after 100 AU/mL DP challenge had an AUC of 0.929 and that PNIF% changes had an AUC of 0.834. In general, if an AUC value is 0.8 or higher, the diagnostic criterion is deemed to have high usefulness. Therefore, we could identify that TNSS change and PNIF% change had significantly high diagnostic usefulness when performing NPT using an appropriate antigen concentration (DP 100 AU/mL) and timing (15 minutes after nasal challenge).

As this study was conducted in AR patients mono-sensitized to house dust mite antigen, we performed NPT using only DP antigen. Therefore, would be challenging to apply this study's results to patients sensitized to antigens other than DP (e.g., grass, cockroach, cats, and dogs). Therefore, we should carry out similar studies using other allergen extracts. In addition, as this study enrolled a relatively small number of patients, it is necessary to confirm our results with a larger number of patients.

In previous studies, we measured the total nasal volume and minimal cross-sectional area using an acoustic rhinometer and evaluated changes in these parameters.<sup>18,19</sup> However, repetitive acoustic rhinometry for NPT requires a lot of effort and time. Besides, unless experienced personnel performs it, the results of acoustic rhinometry can be incorrect. One of the

objectives of this study was to establish a quick and reproducible NPT protocol. Therefore, we adopted only PNIF, which can be measured with little inter-tester error and high reproducibility.

For comparison of challenge concentrations, we performed a 1000 AU/mL DP challenge in patients who did not respond to 100 AU/mL among the AR and NAR groups. As can be seen in Fig. 4, there was a difference in the amount of VAS change between the groups, and some of the VAS scores (nasal obstruction and sneezing after 30 minutes of DP challenge) showed statistically significant differences. Therefore, based on these results, one could argue that testing at 1000 AU/mL is better than 100 AU/mL. However, looking closely at the results, we can see that although there was statistical significance, the difference between groups after the 1000 AU/mL challenge was significantly less than that after the 100 AU/mL challenge: For example, at 15 minutes after 100 AU/mL challenge, the VAS change for rhinorrhea in the AR group was  $47.1 \pm 8.5$  mm. On the other hand, the rhinorrhea VAS change after the 1000 AU/mL challenge was  $14.0 \pm 14.0$  mm. Therefore, when ROC analysis was performed, it did not have diagnostic usefulness. Of course, if all patients are challenged at 1000 AU/mL, it could have diagnostic usefulness; however, we aimed to determine the lowest concentration at which diagnostic utility was comparable to that of higher concentrations. Additionally, we initially planned to study four concentrations (100/200/500/1000 AU/mL) when planning the study to find the optimal concentration of DP solution. In the initially enrolled patients, 100 AU/mL and 1000 AU/mL were tried first. However, since excellent results were obtained at 100 AU/mL, additional studies were not conducted on 200 and 500 AU/mL. Since the concentration could be lowered to 1/10 compared to the previous routine test, no further study was conducted for the lower concentration.

We used a solution from a company developed for subcutaneous immunotherapy in this study. Each company uses different biological units (e.g., BU/mL, SBU/mL, or  $\mu\text{g/mL}$ ) while preparing the DP solution. Therefore, the fact that the results of different companies' products cannot be applied uniformly is a significant limitation in the standardization of NPT research. Therefore, we selected a company's product that uses AU/mL, which is the most common, readily available, and relatively well-known unit for conducting research. If other researchers utilize the same product in the future, comparable results should be obtained.

In conclusion, we determined the optimal concentration of allergen (DP 100 AU/mL), appropriate timing (15 minutes after nasal challenge), and feasible parameters (TNSS VAS change and PNIF% change) of use in performing NPT while still following the recommendations of the EAACI position paper on performing NPT.

## ACKNOWLEDGEMENTS

This study was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF-2020R1F1A1064194).

## ORCID iD

Young Hyo Kim <https://orcid.org/0000-0002-3623-1770>

## REFERENCES

- Han DH, Shin JM, An S, Kim JS, Kim DY, Moon S, et al. Long-term breastfeeding in the prevention of allergic rhinitis: Allergic Rhinitis Cohort Study for Kids (ARCO-Kids Study). *Clin Exp Otorhinolaryngol* 2019;12:301-7.
- Tantilipikorn P, Vichyanond P, Lacroix JS. Nasal provocation test: how to maximize its clinical use? *Asian Pac J Allergy Immunol* 2010; 28:225-31.
- Jang TY, Kim YH. Nasal provocation test is useful for discriminating allergic, nonallergic, and local allergic rhinitis. *Am J Rhinol Allergy* 2015;29:e100-4.
- Rondón C, Campo P, Herrera R, Blanca-Lopez N, Melendez L, Canto G, et al. Nasal allergen provocation test with multiple aeroallergens detects polysensitization in local allergic rhinitis. *J Allergy Clin Immunol* 2011;128:1192-7.
- Leśniak M, Dyga W, Rusinek B, Mazur M, Czarnobilska E. Comparison of the basophil activation test versus the nasal provocation test in establishing eligibility for specific immunotherapy. *Pol Arch Med Wewn* 2016;126:521-9.
- Airaksinen L, Tuomi T, Vanhanen M, Voutilainen R, Toskala E. Use of nasal provocation test in the diagnostics of occupational rhinitis. *Rhinology* 2007;45:40-6.
- Hytönen M, Sala E. Nasal provocation test in the diagnostics of occupational allergic rhinitis. *Rhinology* 1996;34:86-90.
- Augé J, Vent J, Agache I, Airaksinen L, Campo Mozo P, Chaker A, et al. EAACI position paper on the standardization of nasal allergen challenges. *Allergy* 2018;73:1597-608.
- Kim YH, Park CS, Jang TY. Immunologic properties and clinical features of local allergic rhinitis. *J Otolaryngol Head Neck Surg* 2012;41:51-7.
- Kim YH, Jang TY. Clinical characteristics and therapeutic outcomes of patients with localized mucosal allergy. *Am J Rhinol Allergy* 2010;24:e89-92.
- Vardouniotis A, Doulaptsi M, Aoi N, Karatzanis A, Kawauchi H, Prokopakis E. Local allergic rhinitis revisited. *Curr Allergy Asthma Rep* 2020;20:22.
- Schiavi L, Brindisi G, De Castro G, De Vittori V, Loffredo L, Spalice A, et al. Nasal reactivity evaluation in children with allergic rhinitis receiving grass pollen sublingual immunotherapy. *Allergy Asthma Proc* 2020;41:357-62.
- Amírez-Jiménez F, Vázquez-Corona A, Sánchez-de la Vega Reynoso P, Pavón-Romero GF, Jiménez-Chobillon MA, Castorena-Maldonado AR, et al. Effect of LTRA in L-ASA challenge for aspirin-exacerbated respiratory disease diagnosis. *J Allergy Clin Immunol Pract* 2021;9:1554-61.
- Joo SH, Hyun KJ, Kim YH. Korean modification of nasal provocation test with house dust mites antigen following EAACI guidelines. *Clin Exp Otorhinolaryngol*. 2020 Jul 7 [Epub]. Available at: <https://doi.org/10.21053/ceo.2020.00563>.
- Park KI, Jang TY, Yang SC, Hong HS, Kim YH. Correlation of nasal eosinophilia and response after nasal provocation test in patients with nonallergic rhinitis. *Otolaryngol Head Neck Surg* 2018;159: 231-7.
- Kim KS, Jang TY, Kim YH. Usefulness of Allerkin house dust mite extract for nasal provocation testing. *Clin Exp Otorhinolaryngol* 2017;10:254-8.
- Chang GU, Jang TY, Kim KS, Choi H, Kim YH. Nonspecific hyper-reactivity and localized allergy: cause of discrepancy between skin prick and nasal provocation test. *Otolaryngol Head Neck Surg* 2014;150:194-200.
- Kim YH, Jang TY. Proposed diagnostic standard using visual analogue scale and acoustic rhinometry in nasal provocation test in allergic patients. *Auris Nasus Larynx* 2011;38:340-6.
- Kim YH, Yang TY, Lee DY, Ko KJ, Shin SH, Jang TY. Evaluation of acoustic rhinometry in a nasal provocation test with allergic rhinitis. *Otolaryngol Head Neck Surg* 2008;139:120-3.