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ORIGINAL ARTICLE

The values of nasal provocation test and basophil activation test in the different patterns of ASA/NSAID hypersensitivity

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KEYWORDS

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Summary

Background: The oral provocation test (OPT) is the current gold standard to diagnose aspirin hypersensitivity syndrome although it is time-consuming and contains some systemic risks. Other reliable methods with lower side effects and shorter test duration are being investigated.

Objective: The purpose of this study was to evaluate the efficacy of the nasal provocation test (NPT) and the basophil activation test (BAT) in the diagnosis of different subtypes of aspirin sensitivity.

Methods: Thirty aspirin sensitivity patients with cutaneous and respiratory manifestations underwent NPT and BAT with lysine-ASA. NPT result was interpreted as recommended in EAACI/GA2LEN guidelines and receiver operating characteristic analysis of BAT was performed by using 15 NSAIDs tolerant volunteers as a control group.

Results: NPT was positive in 60% (18/30) of patients and BAT was positive in 76.7% (23/30) of patients. The incubation of basophils with 0.31 mg/ml of lysine-aspirin and using 4.6% activated basophils gives the best predictive values to diagnose aspirin sensitivity. The combination of both tests yielded positive results in 80% and 93.3% of aspirin-induced cutaneous and respiratory patterns. The agreement between NPT and BAT results was 63.3%.

Conclusions: NPT and BAT are beneficial to detect patients with aspirin sensitivity. The combination of both tests have additional diagnostic values; less time-consuming than OPT and their complications are negligible. A reliable alternative method with minimum side effects is needed to diagnose aspirin sensitivity in suspected patients who have contraindications for OPT.

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Introduction

The immediate reaction to aspirin (ASA) and other non-steroidal anti-inflammatory drugs (NSAIDs) is one of the common problems in allergy practice.^{1,2} Clinical manifestations vary from cutaneous symptoms such as urticaria or angio-oedema to respiratory symptoms such as naso-ocular oedema or asthmatic attacks, or in rare cases a mixed type or systemic reaction.³ Although clinical onset reactions generally occur within minutes to a few hours after drug exposure mimicking type I hypersensitivity, specific IgE to the responsible drug is rarely discovered, suggesting that the mechanism is most likely non Ig-E mediated. Current data indicates that the imbalance of arachidonic acid metabolism may play a role in this syndrome leading to erratic mast cell degranulation in patients exposed to the drug.⁴ Several NSAIDs with non-selective cyclooxygenase inhibitor properties exacerbate the derangement of leukotriene pathways and aggravate clinical symptoms in susceptible patients.⁵

The diagnosis of this syndrome largely depends on a patient's clinical history since skin testing and specific IgE measurements are not helpful. Unfortunately, using a patient's clinical history has several limitations and is often unreliable. Currently, the oral provocation test (OPT) with aspirin is the diagnostic gold standard test.⁶ This procedure is very time-consuming as it requires at least two days and places patients at risk of systemic reactions from oral aspirin challenge. As a consequence, OPT is not routinely performed in clinical practice. An alternative method to confirm the diagnosis of ASA/NSAIDs hypersensitivity is required to avoid this time-consuming process and to minimise patient risk while still preserving the accuracy of the test.

The bronchial provocation test (BPT) and the nasal provocation test (NPT) have been introduced in the last decade as alternative procedures to diagnose ASA/NSAID hypersensitivity syndrome.^{7,8} However, the studies of bronchial and nasal challenge tests with lysine-aspirin (L-ASA) were mainly performed on patients with aspirin-induced respiratory reaction, not in patients with aspirin-induced cutaneous reaction.⁹ In contrast to North American studies, reports of aspirin hypersensitivity in southern Europeans and Asians suggest that clinical manifestations of aspirin-induced cutaneous reaction and blended type are not uncommon.^{10–13} The role of bronchial and nasal provocation tests with L-ASA to diagnose aspirin-induced cutaneous reaction is still unclear. To date, only a few papers report the applicability of nasal provocation test in the diagnosis of aspirin-induced urticaria.¹⁴

Basophil activation test (BAT) has been introduced in the evaluation of immediate hypersensitivity reaction to drugs.¹⁵ CD63 and CD203c are markers of activated basophils representing basophil activation and degranulation.¹⁶ Both basophils and mast cells share common characteristics in mediator release by IgE and non-IgE dependent pathways upon allergen exposure. Therefore, BAT is potentially helpful to diagnose this syndrome since drug-specific IgE is not necessary for the test.¹⁷ Basophil activation tests have been studied in patients

with ASA/NSAID hypersensitivity; however, the value of BAT in this syndrome has yet to be concluded as the reported sensitivities varied from 16% to 70%.^{18–21} Some researchers reported that basophil responses to in vitro aspirin challenge had low predictive values to identify aspirin sensitivity.²²

NPT is recommended in aspirin-induced respiratory reactions but only few studies mentioned its role in aspirin-induced cutaneous reactions. The magnitude of basophil activation response and the applicability of BAT to diagnose aspirin sensitivity are currently under hot debate again. The value of each test in different manifestations of aspirin sensitivity has not much been emphasized. The purpose of this study was to evaluate the diagnostic value of NPT and BAT in different manifestations of aspirin sensitivity, the relationship between NPT and BAT results, and the possibility of combining both tests in the diagnosis of ASA/NSAID hypersensitivity in clinical settings where the standard OPT is not available or in patients with suspected aspirin sensitivity who have contraindications for OPT.

Materials and methods

Thirty patients (aged 15–70 years) with a clear cut history of immediate hypersensitivity reactions to ASA/NSAIDs (probability drug allergy category A, $P > 0.9$, according to Nyfeler, B. and Pichler, WJ.) were recruited into this study and underwent nasal provocation testing (NPT) with lysine-aspirin (L-ASA).²³ All patients experienced at least two episodes of immediate hypersensitivity reactions from aspirin or NSAIDs, or had several reactions from different NSAID types, and/or had a positive oral provocation response with aspirin. Fifteen patients developed acute urticaria and/or angio-oedema and 15 patients developed naso-ocular symptoms and/or acute asthmatic attack after ASA or NSAID consumption. Prior to the performance of the nasal provocation test, subjects were asked to stop nasal and oral sympathomimetic drugs for 24 h, short-acting antihistamines for three days, leukotriene modifiers, nasal and systemic corticosteroids for one week. Subjects who had factors interfering with the nasal provocation test such as a massive nasal polyp, nasal septal perforation, or total nasal obstruction of at least one nostril were not included in the study. Patients who were pregnant, had an exacerbation of allergic rhinitis/asthma, had an upper respiratory tract infection within two weeks prior to the test, had nose surgery within eight weeks prior to the test, or had severe systemic disease(s) were also excluded from the study. Fifteen healthy individuals with no history of ASA/NSAID hypersensitivity were enrolled as normal controls.

The single-blind placebo controlled nasal provocation test (NPT) with L-ASA was performed and interpreted according to EAACI/GA2LEN guidelines.²⁴ After non-specific nasal hyper-reactivity had been excluded by nasal instillation of 0.9% NaCl, 30 min later L-ASA (Aspegic, Sanofi-Aventis, France) 80 μ l was instilled into each nostril using an Eppendorf pipette (the total dose equivalent to 16 mg of aspirin). Nasal symptoms were recorded based on thirteen-point symptom score method and the total nasal volume

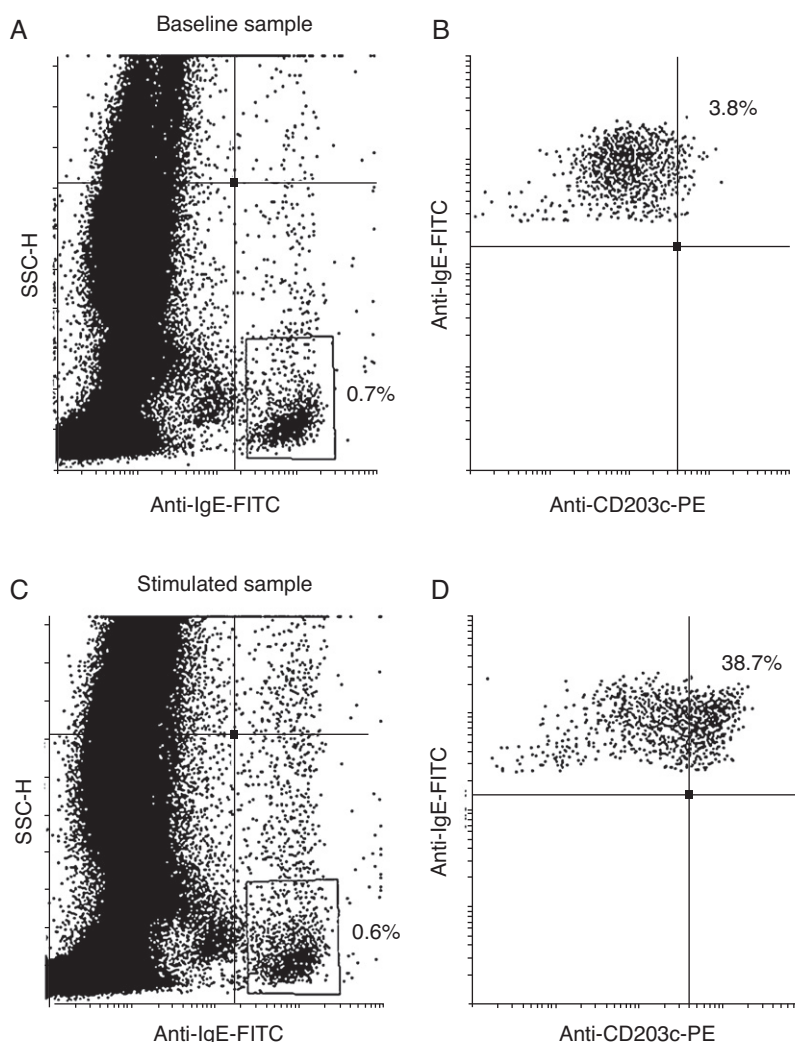


Figure 1 Examples of flow cytometry results demonstrating percentage of activated (IgE+/CD203c+) basophils at baseline and after the incubation with lysine-aspirin. Activation of basophils by lysine-aspirin was demonstrated in aspirin sensitivity patients. (A) Negative control: basophils were detected in 0.7% of all leukocytes. (B) There were 3.8% of spontaneous activated basophils (anti-CD203c +ve/anti-IgE highly +ve cells) at baseline. (C) Basophil population in sample incubated with lysine-aspirin. (D) 38.7% of basophils were activated after stimulated with lysine-aspirin in this patient with aspirin sensitivity.

values were measured during the following 2 h at 10-min intervals by acoustic rhinometry (RhinSCAN, Rhinometrics A/S, Denmark) as recommended.^{25,26} The appearance of nasal symptoms following the challenge and a 25% decrease of total nasal volume at 12 cm from baseline was defined as a positive NPT as previously validated.²⁷

Basophil activation test was performed as previously described with some modifications.²⁸ One hundred millilitres of each patient's whole blood was incubated with L-ASA at final concentrations of 0.31, 1.25, and 5 mg/ml at 37°C for 40 min. The reaction was stopped by putting on ice, and centrifuged for 5 min at 4°C, 1000 × g. The basophils from the pellet were then double-labelled by adding anti-CD203c-PE (Immunotech, France) and anti-IgE fluorescein isothiocyanate-FITC (Caltag, USA). After incubation for 30 min at 4°C, erythrocytes were lysed and then a flowcytometric analysis was performed at 488 nm on a FACScan flowcytometer (Becton Dickinson) and

analysed by CellQuest software. Basophils were gated around the lymphocyte area and the second gate was defined around anti-IgE highly positive cells. Double-positive IgE⁺ and CD203c⁺ cells were defined as activated basophils as shown in Fig. 1. At least 400 basophils were assessed from each assay. Receiver operating characteristic (ROC) analysis was performed to determine the accuracy of BAT to diagnose ASA/NSAID hypersensitivity by using different doses of lysine-aspirin.

The study was approved by the Faculty of Medicine's ethics committee and all subjects gave informed consent. Student's *t* test was used to compare the difference of NPT and BAT between patients and normal controls. The sample size was calculated at 15 patients per group to detect a 20% difference of sensitivity between each patient group with a power of 80% and Alpha₂ = 0.05. Data were analysed through the statistical program SPSS 15.0. Values of *p* < 0.05 were considered statistically significant.

Results

Baseline patient characteristics

Thirty patients with ASA/NSAID hypersensitivity (3 men and 27 women) were recruited to complete this study (Table 1). Fifteen patients had predominantly cutaneous symptoms (eight urticaria, four angio-oedema, and three urticaria with angio-oedema) and the other 15 patients had predominantly respiratory symptoms (six naso-ocular symptoms, two asthma, and seven naso-ocular symptoms with asthma) after ASA/NSAID intake. Both groups shared a similar age, however, patients with ASA/NSAID-induced respiratory symptoms had more common underlying airway diseases (chronic rhinosinusitis and asthma) and less common underlying chronic urticaria than patients with ASA/NSAID-induced cutaneous symptoms. Patients with predominant respiratory pattern had shorter symptom onset than ones with predominant cutaneous pattern (37 min versus 92 min, respectively, p value $<0.05^*$) after exposure to culprit drugs.

Results of nasal provocation test with L-ASA

ASA/NSAID-sensitive patients with cutaneous manifestation had significantly lower symptom scores compared to the ones with respiratory manifestation after L-ASA nasal provocation (2.4 ± 0.6 versus 4.7 ± 0.6 , $*p$ value <0.05), particularly ASA/NSAID-sensitive patients with pure urticarial symptoms (mean nasal symptom scores = 1.4, data not shown). However, when nasal volume was measured by acoustic rhinometry, the decreases of nasal volume were not significantly different between the two groups ($30.4 \pm 3.3\%$ versus $32.7 \pm 4.6\%$ decrease in cutaneous pattern and respiratory pattern, respectively, p value >0.05). ASA/NSAID-tolerant volunteers were asymptomatic and nasal volume remained unchanged (Fig. 2). No patient experienced serious side effects except one patient who developed a stuffy nose up to 6 h after the positive NPT. None of them developed urticaria, angio-oedema, or bronchospasm.

Results of basophil activation test with L-ASA

The results of BAT demonstrated dose-response basophil activation with varying concentrations of L-ASA. Activated basophil percentages in normal controls ($N = 15$) were significantly lower than those in both cutaneous and respiratory types when stimulated with L-ASA at 0.31 and 1.25 mg/ml ($*p$ values <0.05), but not with L-ASA 5 mg/ml (p values = 0.09) (Fig. 3a). Therefore, data from lysine-ASA 5 mg/ml stimulation were not used for further analysis. Data from ROC analysis showed that 0.31 mg/ml of L-ASA had 76.7% sensitivity and 80.0% specificity by using 4.58% activated basophils as a threshold (point A, p value <0.01). The concentration of 1.25 mg/ml L-ASA had 60% sensitivity and 80% specificity by using 14.2% activated basophils as a threshold as well (point B, p value = 0.01) (Fig. 3b).

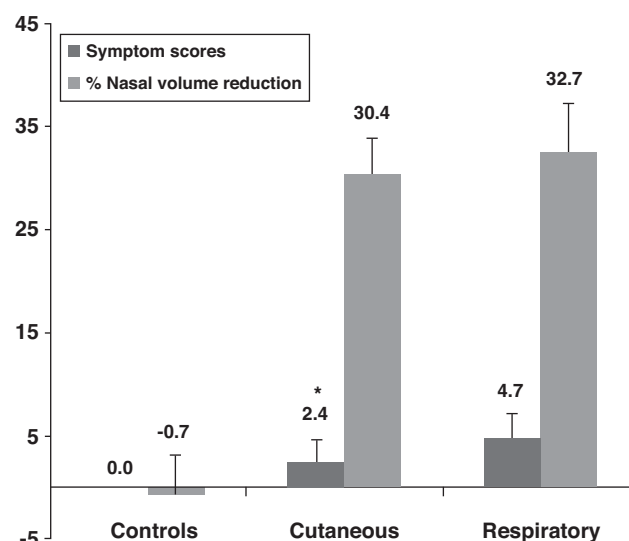


Figure 2 Results of nasal provocation test with lysine-aspirin. Nasal provocation with lysine-aspirin in aspirin sensitivity patients demonstrated that nasal symptom scores were prominent in patients with respiratory pattern but the decreases of nasal volume were not significantly different between the two groups. Symptom scores after L-ASA nasal provocation in patients with cutaneous manifestation were lower than those in patients with respiratory manifestation; *, $p < 0.05$. Data were presented as mean $\pm$ SEM ($n = 15$ each).

Results of combined nasal provocation test and basophil activation test

The sensitivities of NPT, BAT, and the combination NPT/BAT were categorised based on clinical manifestations (Fig. 4). Percentages of subjects with positive nasal provocation test determined by acoustic rhinometry criterion (25% nasal volume reduction) were 60.0% (9/15) in both patient groups. According to the aforementioned criteria (activated basophils $\geq 4.58\%$ by stimulating with 0.31 mg/ml L-ASA), BAT was positive in 73.3% (11/15) and 80.0% (12/15) of patients with cutaneous and respiratory manifestations, respectively. The combination of NPT and BAT could detect 80.0% of ASA/NSAID-sensitive patients who presented with cutaneous manifestations and in 93.3% of patients with respiratory manifestations. The agreement of NPT and BAT results was demonstrated in 63.3% (19/30) of patients. Altogether, ASA/NSAID hypersensitivity can be diagnosed with NPT alone, BAT alone, and NPT plus BAT up to 60.0%, 76.7%, and 86.7% of patients, respectively.

Discussion

ASA/NSAID hypersensitivity or "ASA sensitivity" is a common adverse drug reaction. The diagnosis of ASA sensitivity remains problematic. Unfortunately, the skin test has no diagnostic role since most of the reactions are not Ig-E mediated. Up to recently, oral provocation testing has been mandatory to confirm the diagnosis. While nasal provocation and bronchial provocation tests are being proposed as alternative methods, the sensitivities of these tests are

Table 1 Characteristics of patients with a ASA/NSAID sensitivity ($n = 30$)

	Cutaneous predominant ($n = 15$)	Respiratory predominant ($n = 15$)
Age (years)/range	44.3 (31–66)	42.1 (16–67)
Gender (M/F)	1/14	2/13
Underlying diseases		
Chronic rhinosinusitis	1 (6.7%)	4 (26.7%)
Nasal polyps	0 (0%)	5 (33.3%)
Asthma	2 (13.3%)	5 (33.3%)
Chronic urticaria	6 (40%)*	1 (6.7%)
SPT +ve to aeroallergens	8 (53.3%)	13 (86.7%)
Symptom onset after drug exposure (minutes)	92 (5–360)	37 (10–60)*
Symptom episodes	3.4 (2–10)	5 (2–25)
Multiple NSAID hypersensitivity	5 (33%)	9 (60%)

* = P value < 0.05.

still insufficient and the clinical implications of these two procedures mainly emphasize on patients with respiratory reactions.

The oral provocation test with aspirin is time-consuming and potentially causes systemic side effects. Therefore, this study was performed to investigate the role of less invasive methods in patients with immediate cutaneous or respiratory reactions from taking aspirin/NSAIDs. Although most of the patients recruited in this study were diagnosed ASA/NSAID-hypersensitivity based on clinical history alone and only some of them were confirmed with oral provocation test (OPT), all of those who did not undergo OPT experienced at least two episodes of immediate hypersensitivity reactions from aspirin or NSAIDs, or had several reactions from different NSAID types. It was already demonstrated that patients with two or more prior aspirin- and NSAID-associated respiratory reactions had an 89% chance of having a positive oral aspirin challenge.²⁹ For ethical reasons, these patients should not undergo OPT with aspirin again. Instead, patients who presented with a convincing history of multiple-NSAID intolerance, with or without underlying chronic urticaria, should directly undergo oral provocation test with alternative drugs for further use.³⁰

Our study showed an interesting result that, not only ASA-induced respiratory reaction, but also ASA-induced cutaneous reaction can give a positive response by NPT with lysine-aspirin. Although most of the patients with cutaneous manifestation remained asymptomatic with NPT and the nasal symptom scores alone were not sensitive enough in the cutaneous group, our findings pointed out that nasal volume reduction could be demonstrated in a significant number of these patients. Since patients in this subtype have milder nasal symptoms in comparison to patients with respiratory type, the assessment of nasal patency with acoustic rhinometry is recommended to increase the sensitivity of the test. Patients with ASA-induced urticarial/angio-oedema possibly develop nasal obstruction at a subclinical level and this asymptomatic nasal mucosal oedema could be detectable with a more sensitive technique. Our study supported previous data from Portugal that NPT with L-ASA was positive in most patients with ASA-induced urticaria if the clinical response and nasal expiratory peak flow were both evaluated.¹⁴ NPT with L-ASA had 100% specificity in our study

since no healthy controls had positive reactions. Our data support good specificity of NPT to diagnose aspirin sensitivity, which was around 92.5–95.7% in previous studies.^{8,31} NPT was very specific for ASA sensitivity patients except in few cases who may develop non-specific nasal response to NSS installation so that nasal provocation with L-ASA is unable to proceed.

Basophil activation test has been introduced for the diagnosis of aspirin sensitivity for a while. Since the likely mechanism is not truly allergic, but the imbalance of arachidonic acid pathway, the predictive value of BAT in aspirin sensitivity is still under hot debate.^{22,32} Therefore, we decided to perform ROC analysis to analyse BAT results instead of using the arbitrary cut off criteria and found that with only low concentrations of L-ASA it yielded a significantly higher percentage of activated basophils in aspirin-sensitive patients than in normal controls. Although percentages of CD203C+ve basophils from 5 mg/ml of L-ASA were the highest, the numbers were not different from the healthy subjects; indicating that it was a non-specific response. The lowest concentration of L-ASA (0.31 mg/ml) gives the highest diagnostic accuracy, probably due to its least interference to cyclooxygenase pathway in normal controls while the sensitivity to detect patients with ASA/NSAID hypersensitivity remains preserved.

BAT sensitivity varies greatly depending on testing protocols and diagnostic criteria.^{32,33} The sensitivity might be increased by adding more NSAIDs to the testing panel and by using the leukocyte isolation technique; however, it was our purpose to seek the practical approach to screen ASA sensitivity in clinical service. The whole blood method was selected and single NSAID (ASA) was used for BAT since it is more suitable for routine use than multiple NSAIDs combination. Considering this syndrome is the result of the imbalance of cyclooxygenase and lipoxygenase pathways, the inhibition of cox-1 enzyme by a single NSAID should be sufficient to verify this syndrome except in the rare cases that are Ig-E mediated. Specificity of BAT in our study was 80.0%, which is in the same range as previous studies.¹⁸

By including BAT in addition to NPT, sensitivity of the test was 20% increased in the cutaneous group and 33.3% increased in the respiratory group compared to using NPT alone. When administering NPT and BAT together, 80% of

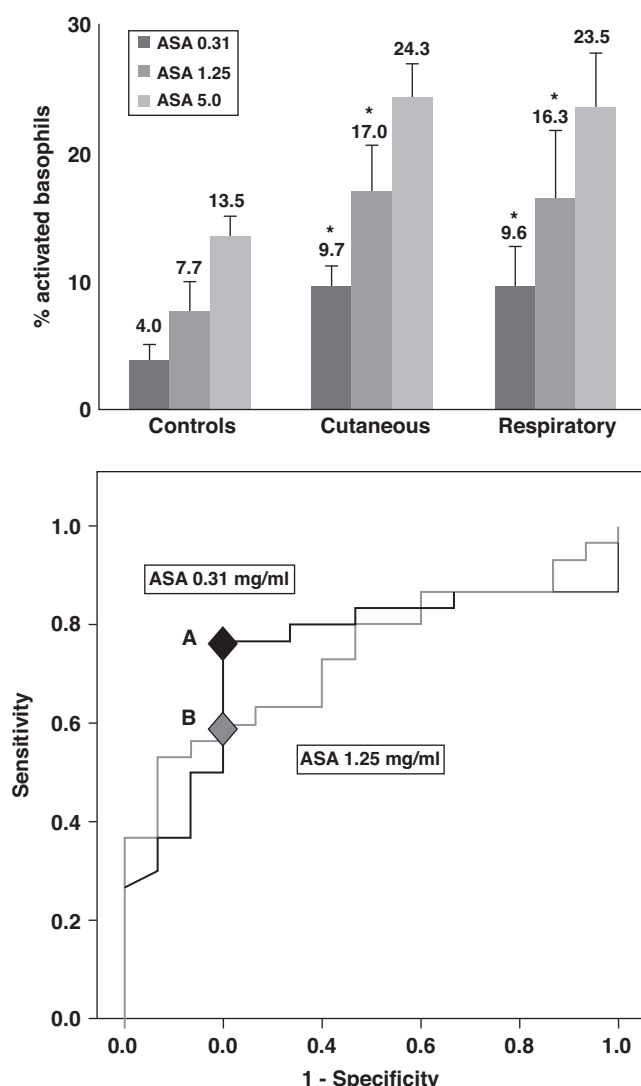


Figure 3 Results of basophil activation test with lysine-aspirin ($n = 30$). Basophil activation test (BAT) with lysine-aspirin in aspirin sensitivity patients demonstrated that percentages of activated basophils were dose-dependent and significantly higher than those of normal controls when incubating with 0.31 and 1.25 mg/ml of lysine-aspirin. Data were presented as mean $\pm$ SEM ($n = 15$ each); *, $p < 0.05$. The sensitivity of 76.7% and specificity of 80% were achieved by stimulation with 0.31 mg/ml L-ASA and using 4.6% activated basophils as a cut-off point (area under curve = 0.741, p value < 0.01).

patients with cutaneous type and almost all cases of respiratory type could be diagnosed, which is more sensitive than performing BAT with multiple NSAIDs combination. The trade-off of this approach is that the specificity of the test would be decreased from 100% by NPT alone to 80% with BAT combination. Specificity of the test remains an issue and a better technique to increase the positive predictive value of the test should be explored.

Up to date, no reliable test other than OPT is available to diagnose ASA/NSAID hypersensitivity. Several alternatives have been proposed but the sensitivity of each individual test is still unsatisfactory. Other methods such as leukotriene

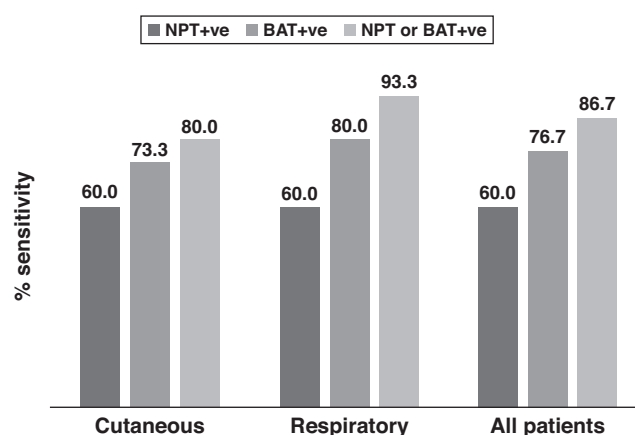


Figure 4 The sensitivities of nasal provocation test and basophil activation test in ASA/NSAID sensitivity syndrome. NPT alone was positive in 60.0% of patients with aspirin sensitivity. NPT and BAT combinations could detect up to 80.0% and 93.3% of patients with cutaneous pattern and respiratory pattern, respectively.

measurement by ELISA have some prognostic values in the diagnosis of ASA/NSAID hypersensitivity,^{32,34,35} however, the ELISA assays still need to be tested in pooled samples, as such they are not suitable for practical use. Our study demonstrated that a NPT-BAT combination can screen up to 86.7% of patients with a history of immediate reactions from ASA/NSAIDs and leave only 13.3% of patients for further testing with OPT. Complications are rare and mild, and the test is less time-consuming than standard OPT. This approach could be considered as an alternative option to detect patients with suspected ASA sensitivity when the standard OPT is not feasible, with patients not wanting to take risks, or those who demonstrate contraindications for OPT. Patients whose tests are negative can be directed for further OPT before the status of ASA/NSAID tolerance can be confirmed. In conclusion, our study demonstrated that the nasal provocation test was able to detect 60% of ASA sensitivity patients in both cutaneous and respiratory manifestations if the changes of nasal volume were taken into account. The combination of administering NPT and BAT with L-ASA further enhances the sensitivity of the test. Its good sensitivity, negligible side effects, and the fact that it is a less time-consuming method make this approach a good candidate to diagnose ASA/NSAID hypersensitivity syndrome. Additional methods to improve diagnostic sensitivity while maintaining the method's specificity should be explored.

Conflict of interest

The authors have no conflict of interest to declare.

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