Minimal agreement between basophil activation test and immunoassay in diagnosis of penicillin allergy.

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Abstract

Background: Basophil activation test (BAT) and immunoassays are the most widely used in vitro tests to diagnose IgE-mediated allergic reactions to penicillin. However, study to determine if one test is interdependent from another is limited.

Objective: The present study aimed to measure the agreement between BAT and immunoassay in diagnosis of penicillin allergy.

Method: Twenty-five adult patients with clinical history of immediate allergic reactions to penicillin whom referred to the Allergy Clinic, Hospital Kuala Lumpur (HKL) were assessed. BAT was performed using penicillin G (Pen G), penicillin V (Pen V), penicilloyl-polylysine (PPL), minor determinant mix (MDM), amoxicillin (Amx) and ampicillin (Amp) within 12 months from the reaction. Immunoassay of total IgE (tIgE) and specific IgE (sIgE) antibodies to Pen G, Pen V, Amx and Amp were quantified. Skin prick test (SPT) using PPL-MDM, Amx, Amp and Clavulanic acid were also performed.

Results: Minimal agreement was observed between BAT and immunoassay (Cohen's kappa Index, k=0.25). Two patients were BAT-positive, and four patients were sIgE-positive. Of two BAT- positive patients, one patient is positive to Amx (59.27%, SI=59) and Amp (82.32%, SI=82) but sIgE-negative to all drug tested. This patient also SPT-positive to both drugs. Another patient is BAT-positive to Pen G (10.18%, SI=40), Pen V (25.07%, SI=100) and Amp (19.52%, SI=79). The results were in concordance with sIgE testing results (Pen G=10.7 kU/L, Pen V=10.8 kU/L, Amp=8.29 kU/L) except for Amx. In sIgE immunoassay, four patients were sIgE-positive to at least one of the drugs tested. The sIgE level of three patients were between low (0.35-0.69 kU/L) and moderate (0.70-3.49 kU/L) and they were BAT-negative. One BAT- positive patient has high level of sIgE antibodies (3.50-17.5 kU/L) and this patient also has relatively high specific to total IgE ratio $\geq$ 0.002 (0.004-0.007).

Conclusions: The agreement between BAT and immunoassay is minimal. Performing both tests may not increase the sensitivity of allergy diagnosis work-up for immediate reactions to penicillin.

Introduction

Mislabeled of penicillin allergy is an increasing clinical issue leading to increase antibiotic resistance and healthcare costs. Most patients labeled as penicillin allergy are not really allergic to penicillin [1]. It was estimated that only 15.8 – 28.6% of patients labeled as penicillin allergy are true allergy [2,3]. Individuals with history of allergy to penicillin including self-reported patients should be evaluated and subject to routine penicillin allergy testing thus prevent mislabeling [4]. Such individuals can be evaluated by different methods that include clinical history, skin tests, in vitro quantification of specific IgE-antibodies, and drug provocation tests [5].

With the advancement of flow cytometry technique, basophil activation test (BAT) has been proposed as a useful in vitro assay for diagnosis of penicillin allergy. The BAT is a functional assay that measures IgE function which is the ability to induce activation of basophils in the presence of allergen. The activation of basophil is usually assessed by determining CD63 or CD203c expression on the surface of basophil [6].

Determination of total and specific IgE (sIgE) antibodies by immunoassay is another safer method to diagnose allergic reactions to penicillin [7]. This method however, measures both functional IgE (capable of activating mast cells and basophils by binding to Fc region of IgE I (FcϵRI) on the cell surface) and non-functional IgE [8]. Furthermore, the serum level of sIgE antibodies can decline rapidly, where the test often becoming negative within six months to three years after the last exposure [9].

The present study aimed to compare the diagnostic performance of BAT and immunoassay in diagnosis of penicillin allergy by measuring the agreement between the tests.

Materials and Methods

Subjects

Twenty-five adult patients referred to Allergy Clinic, Hospital Kuala Lumpur with suggestive clinical history of immediate allergic reactions to penicillin antibiotics within one to twelve months of reactions (median=3 months) were recruited. Patients were evaluated for penicillin allergy using the European Network for Drug Allergy (ENDA) recommended clinical questionnaire [10]. Patients who received anti-IgE and antihistamines within 24 hours of consultation were not included. Controls were 25 volunteered individuals without any history of drug allergy. This study was approved by Medical Research of Ethics Committee of the Ministry of Health in Malaysia (KKM/NIHSEC/P13-901, NMRR-13-922-17589) and informed consent for all testing were obtained from all patients and controls.

Skin prick test (SPT)

SPT was performed on 24 patients according to conventional techniques using freshly prepared mixture of benzylpenicilloyl octa-L-lysine (PPL) and sodium benzylpenilloate (MDM), sodium amoxicillin (Amx), potassium clavulanate (Clav A) and an in-house preparation of ampicillin (Amp). Histamine hydrochloride (10 mg/ml) was used as positive control and 0.9% saline solution as negative control. All commercial allergens and controls were purchased from Diater LABORATORIOS S.A, Madrid, Spain. SPT is considered positive when the wheal diameter equals to 3 mm or greater than the negative control within 20 minutes. Basophil Activation Test (BAT).

Peripheral blood samples in ethylene-diamine tetra acetic acid (EDTA) tubes were tested for CD63 expression. The flow cytometric analysis of the in vitro activated basophils was carried out using Flow CAST® (Bühlmann Laboratories AG, Schönenbuch, Switzerland) following manufacturer's instruction and cutoff recommendation. Briefly, basophil stimulation in whole blood were performed immediately (within 2 hours) upon blood collection using drug allergens by CAST® (Bühlmann Laboratories AG, Schönenbuch, Switzerland) namely benzylpenicilloyl-polylysine (PPL), minor-determinant mix (MDM), penicillin G (Pen G), penicillin V (Pen V), ampicillin (Amp) and amoxicillin (Amx). Stimulation buffer and mixture of anti-FcεRI and fMLP were used as negative and positive controls respectively. Mixture of anti-CCR3-PE and anti-CD63-FITC were used as staining reagents. The samples were analyzed using CellQuest® software (FACSCalibur BD Analyser) within 2 hours. The number of events acquired was set to contain at least 400 basophils. Percentage of activated basophils (CD63 positive cells) was expressed for each drug. The stimulation index (SI) was defined as the activated basophil percentage after drug stimulation/basally active basophil percentage. A SI ≥ 2 and an absolute activated basophil percentage ≥ 5 were considered positive.

Total and sIgE immunoassay

Quantification of total IgE in 18 patients and specific IgE antibodies to c1 (penicilloyl G), c2 (penicilloyl V), c5 (ampicilloyl) and c6 (amoxilloyl) in all patient's plasma were performed by fluoroenzymeimmunoassay (FEIA) method using UniCAP® Phadia 250 systems (Thermo Fischer Scientific, Uppsala, Sweden) following manufacturer's instructions. Specific IgE results were obtained by direct comparison with standards run in parallel, considering a value of sIgE ≥ 0.35 kU/L were positive. The ratio of specific to total IgE was also determined in patients with serum total IgE >200 kU/l.

Statistical analysis

Agreement between BAT and immunoassay was calculated using Cohen's kappa Index (k) and interpreted using the interpretation described by McHugh (2012) [11]. Cohen's kappa Index was calculated as k=(pa-pe)/(1-pe), where: pa= proportions of observation in agreement; pe=proportions of agreement due to chance.

Results

Patient's clinical manifestations and SPT

Of 25 patients, 14 are females and 11 are males, median age 36 years old (interquartile range (IR): 28-43). Patient's clinical manifestations, SPT, immunoassays and BAT results (Table 1). Clinical manifestations include angioedema in 14 cases (56%), anaphylaxis in 7 (28%), urticaria in 4 (16%), diarrhea in 2 (8%), followed by vomiting, macular exanthema, pruritis, bronchospasm, dyspnea and hypotension with one case (4%) for each. Only one patient (patient 23) was SPT-positive to Amx and Amp.

Basophil CD63 expression

Twenty-five patients and twenty-five controls evaluated for BAT. Basophil gating strategy (Figure 1). Two patients (patient 9 and 23) positively expressed CD63-FITC (Figures 2 and 3), respectively. Patient 9 positively expressed CD63 on its activated basophil when tested with Pen G (10.18%, SI=40), Pen V (25.07%, SI=100) and Amp (19.52%, SI=79). The results were in concordance with immunoassay results (Pen G=10.7 kU/L, Pen V=10.8 kU/L, Amp=8.29 kU/L) except for Amx (6.77 kU/L). Patient 23 positively expressed CD63 on activated basophil tested with Amp (82.32%, SI=82) and Amx (59.27%, SI=59). Interestingly, patient 23 was sIgE-negative

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(sIgE ≤ 0.35 kU/l) to the entire drug tested. Minimal agreement between BAT and immunoassay results was observed (k=0.25).

Table 1. *Patient's clinical manifestations with SPT, immunoassay and BAT results.*

Patient	Age	Sex	SPT					Immunoassay (kU/L)					BAT (SI)					
			Clinical manilectas	PPL-MDM	Amp	Amox	Clav A	T-IgE	Pen G	Pen V	Amp	Amox	Pen Cr	Pen V	PPL	VIDM	Amp	Amox
1	75	F	Diarrhea & vomiting					NA	0	0	0.03	0.03	1	1	1	1	1	1
2	36	M	Pruritis					NA	0	0.02	0.04	0.07	1	1	1	1	1	1
3	30	F'	Urticaria & macular					578	0.36	0.31	0.39	0.56	1	1	1	1	1	1
4	25	F	Diarrhea					34.1	0	0	0.04	0.04	1	1	1	1	1	1
5	23	F	Angioedema					348	0.02	0.05	0.06	0.15	1	1	1	1	1	1
6	25	F	Urticaria					164	0.01	0.03	0.04	0.11	1	1	1	1	1	1
7	28	F	Angioedema					202	0	0.04	0.03	0.06	1	1	1	1	1	1
8	30	M	Angioedema & anaphylaxis					106	0.01	0	0.03	0.05	1	1	1	1	1	1
			Anaphylactic shock	ND	ND	ND	ND	1398			8.29	6.77	40	100	1	1	79	1
10	33	F	Anaphylaxis					57.4	0	0	0.04	0.05	1	1	1	1	1	1
11	40	F	Angioedema					NA	0	0.01	0.08	0.10	1	1	1	1	1	1
12	65	M	Angioedema					310	0.03	0.04	0.10	0.11	1	1	1	1	1	1
13	60	M	Anaphylaxis					NA	0.49	0.31	0.52	0.74	1	1	1	1	1	1
14	52	F	Angioedema					238	0	0.06	0.08	0.13	1	1	1	1	1	1
15	58	F	Angioedema					314	0	0.04	0.08	0.11	1	1	1	1	1	1
16	16	M	Anaphylaxis					871	0.05	0.06	0.17	0.15	1	1	1	1	1	1
17	26	M	Anaphylaxis, angioedema, urticaria & bronchospasm					102	0.03	0.04	0.08	0.08	1	1	1	1	1	1
18	35	M	Dyspnea					111	0.01	0.02	0.08	0.06	1	1	1	1	1	1
19	43	F	Urticaria & angioedema					NA	0	0	0.03	0.08	1	1	1	1	1	1
20	37	M	Angioedema					214	0.11	0.02	0.09	0.03	1	1	1	1	1	1
21	39	M	Hypotension					1410	0.17	0.25	0.36	0.56	1	1	1	1	1	11
22	59	F	Angioedema					1113	0.06	0.13	0.13	0.27	1	1	1	1	1	1
23	38	M	Anaphylaxis & angioedema					6.41	0	0	0.03	0.02	1	1	1	1	82	59
24	32	M	Angioedema					NA	0.01	0.10	0	0.05	1	1	1	1	1	1
25	22	F	Angioedema					NA	0.04	0.01	0.06	0.08	1	1	1	1	1	1

Immunoassay for total and specific IgE quantification

Total IgE were elevated (≥ 100 kU/L) in 15 (83%) of 18 patients. Four patients (patient 3, 9, 13 and 21) were sIgE positive (≥ 0.35 kU/L) to at least one of the drugs tested. Of the four patients, three patients (patient 3, 13 and 21) had sIgE level between low (sIgE level: 0.35-0.69 kU/L) and moderate (sIgE level: 0.70-3.49 kU/L). Only one patient (patient 9) with high sIgE antibodies level (sIgE level: 3.50-17.5 kU/L) shows

positive BAT. This patient also had relatively high specific to total IgE ratio ≥ 0.002 (0.004-0.007).
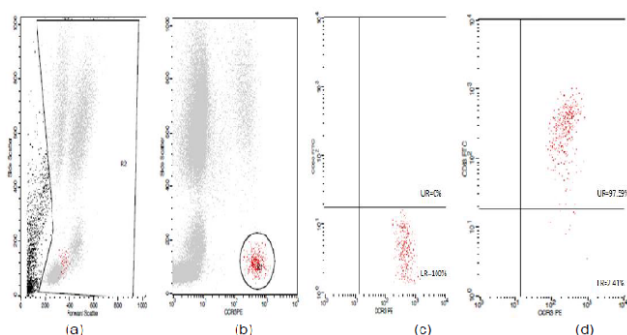

Figure 1. *Example of optimal basophil gating with (a) gated area shows white blood cells; (b) circle area shows CCR3-PE positive cells; (c) negative control (stimulation buffer); (d) positive control (anti FcεRI + fMLP).*
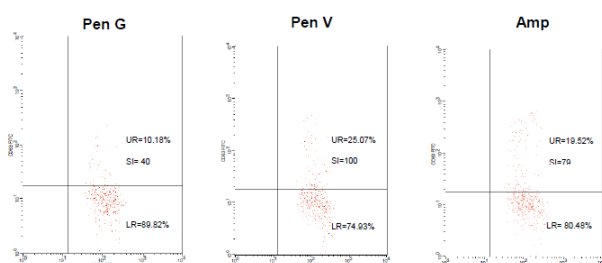

Figure 2. *CD63-FITC expression on activated basophil in patient 9 tested with Pen G (10.18%, SI=40), Pen V (25.07%, SI=100) and AMP (19.52%, SI=79).*
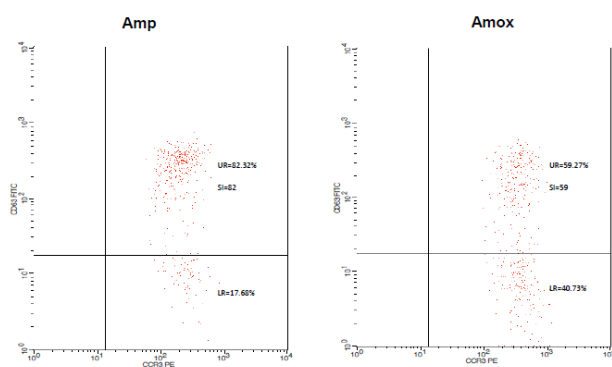

Figure 3. *CD63-FITC expression on activated basophil in patients 23 tested with AMP (82.32%, SI=82 and AMX (59.27%, SI=59).*

Discussion

A reliable *in vitro* test is very important to diagnose true penicillin allergy to prevent mislabeling and subsequently reduce the need to perform risky drug provocation test. Immunoassays and BAT are the most widely used in vitro tests to diagnose IgE-mediated allergic reactions. However, study to determine if one test is complementary or independent from another is limited.

In the present study, the agreement between the tests is relatively low (k=0.25). One of two BAT-positive patients has BAT results correlated with immunoassay but not in another.

False negative and false positive results might explain the discrepancies between results as both tests have been shown to have false results due to relatively low sensitivity. The average sensitivity and specificity of BAT is 51.7% and 89.2% respectively while immunoassay at 50.1% and 81.01% respectively [12].

Furthermore, earlier negativization of IgE bound to basophils as compared to serum IgE antibodies was demonstrated in patient allergic to amoxicillin. Therefore, BAT become negative before immunoassay [13]. This early negativization might explain symptomatic patients with negative BAT results.

Our analysis involving patients with total IgE >200 kU/l (n=11) revealed one BAT- positive patient with relatively high specific to total IgE ratio ≥ 0.002 (0.004-0.007). It has been reported that the ratio of penicillin specific to total IgE is able to improve the diagnostic performance of immunoassay in identifying allergic patients particularly patients with serum total IgE >200 kU/l [14]. An elevated specific to total IgE ratio was associated with a high level of allergen-sIgE on basophils or mast cells, whereas this is rare when the ratio is low. Thus, application of specific to total IgE ratio may improve overall diagnostic performance of IgE- mediated drug allergy.

It is noteworthy, in the present study, only one SPT-positive patient reactive to Amx and Amp but shows good tolerance to Pen G and V. This patient may have selective reactions to R-group side chain of the Amx and Amp which play an important role as antigenic determinant in some allergic reactions related to penicillin antibiotic [15]. Nevertheless, this was not detected in immunoassay. Such has indicated the limitation of the test to identify patient allergic to side chain of the drugs.

In summary, the agreement between BAT and immunoassay is minimal and performing both tests has very little increase on the sensitivity of allergy diagnosis work-up for immediate reactions to penicillin. This study also suggests that SPT and specific to total IgE ratio is useful to improve diagnosis of patients allergic to penicillin antibiotics therefore we recommend BAT and determination of specific to total IgE ratio in cases where the diagnosis of drug allergy is highly suspected thus avoiding life-threatening provocations tests. In this study, BAT was performed along with sIgE determination based on clinical history alone and future studies investigating the accuracy of both tests should include subjects that positive to at least one causative drug in intradermal test or drug provocation test.

Acknowledgement

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