Basophil activation test using flow cytometry in the diagnostic of antibiotic allergy

Testarea prin flow citometrie a activării bazofilelor în diagnosticul alergiei la antibiotice

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Abstract

Background: *Hypersensitivity to antibiotics is not always easy to assess. The basophil activation test with up-regulation of CD63 quantification is a new technique that might improve diagnostic accuracy. Objectives: the aim of the study was to determine the usefulness of flow cytometry in the diagnosis of β-lactam allergy, to investigate whether flow cytometry might help to identify cross-reactive and safe alternative antibiotics and to determine the adequate time interval between the allergic reaction and testing.* ***Methods:*** *A total of 58 patients with previous history suggestive for an immediate-type hypersensitivity caused by antibiotics were tested. We performed skin tests and the basophil activation test using up-regulation of CD63.* ***Results:*** *There was a fair agreement between the basophil activation test and positive history plus skin tests (0.35 when BAT was performed within two years and 0.25 after two years). There were significantly more frequent positive basophil activation tests within 2 years than after (30.76% vs. 7.69%, Fisher exact test, p=0.032). There were 5 patients with positive history, negative skin tests and a corresponding positive flow cytometry.* ***Conclusion:*** *The agreement between the basophil activation test and skin tests is fair, but their diagnostic values are complementary. Flow cytometry is particularly useful in patients with positive history and negative skin tests as potential dangerous provocation tests might be avoided. The diagnostic value of flowcytometry is increased when recent reactions are investigated.*

Keywords: *drug allergy, flow cytometry, skin test*

Rezumat

Premise: *Diagnosticul de hipersensibilitate la antibiotice nu este întotdeauna direct. Testul de activare a bazofilului cu cuantificarea markerului CD63 poate să îmbunătăţească acurateţea diagnostică.* ***Obiective:*** *Scopul acestui studiu a fost de a stabili utilitatea citometriei de flux în diagnosticul de alergie la β-lactamine, de a determina dacă prin citometrie se poate identifica reactivitatea încrucişată şi antibioticele alternative şi de a determina intervalul de*

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timp adecvat pentru testare. Metodă: Am testat 58 de pacienți cu istoric sugestiv de hipersensibilitate de tip imediat la antibiotice. Am efectuat teste cutanate alergologice şi testul de activare a bazofilului. ***Rezultate:*** *Concordanța între testul de activare a bazofilului versus istoric pozitiv şi teste cutanate pozitive a fost moderată (0.35 când testul de activare a bazofilului a fost efectuat sub doi ani de la reacția alergică şi 0.25 peste doi ani). Au fost semnificativ mai multe teste de activare a bazofilului pozitive atunci când testarea s-a efectuat la mai puțin de doi ani de la reacție (30.76% vs. 7.69%, Fisher exact test, p=0.032). 5 pacienți cu istoric pozitiv şi teste cutanate negative au avut un test de activare a bazofilului pozitiv.* ***Concluzii:*** *Corelația între testul de activare a bazofilului şi testele cutanate este moderată, dar valoarea lor diagnostică este complementară. Citometria este utilă mai ales la pacienții cu istoric pozitiv şi teste cutanate negative pentru că poate evita un test de provocare potențial periculos. Valoarea diagnostică a testului de activare a bazofilului este mai mare când sunt investigate reacții recente.*

Cuvinte cheie: *alergie medicamentoasă, citometrie, teste cutanate*

Introduction

Hypersensitivity to antibiotics is not always easy to assess. Diagnosis is based upon history, in vivo allergy skin tests and in vitro tests (the detection of drug specific IgE antibodies and the basophil activation test using flow cytometry).

Self reported penicillin allergy occurs in up to 10% of the general population, but more than 90% of them are found to have negative test results and they can tolerate the antibiotic safely (1,2). This is a proof that medical history is not always reliable in the diagnosis of drug allergy. Therefore, reliable diagnostic tests are needed. The lack of the gold standard is due to the fact that the performance of provocation tests is limited to few drugs (3), though the importance of drug challenge tests is highlighted (4). In vivo skin tests are usually considered to be the reference standard for the diagnosis of β-lactam (BL) allergy, even if there is a risk of false positive and false negative results (1). The use of drug specific antibodies in the diagnosis of allergy is restricted to few antibiotics and is limited by a low sensitivity (5,6). The basophil activation test (BAT) with up-regulation of CD63 quantification is a new technique that might improve diagnosis, yet there are few published papers to demonstrate its validity (7). Current evidence indicates that flow cytometry-assisted techniques may be a useful tool for both experimental and clinical studies of the functions of basophils in allergic diseases (8). BAT is useful in the diagnosis of both IgE mediated and non-IgE mediated hypersensitivity reactions. In Romania, flow cytometry as a cellular diagnostic tool for perianaesthetic drug allergies was developed within a research program of the University of Medicine and Pharmacy "Iuliu Haţieganu" Cluj-Napoca (National Plan II, Priority Domain Partnership number 41-062/2007).

The aim of the study was to determine the usefulness of flow cytometry in the diagnosis of BL allergy, to investigate whether BAT might help to identify cross-reactive and safe alternative antibiotics and to determine the adequate time interval between the allergic reaction and testing.

Material and methods

A total of 58 patients with previous history suggestive for an immediate-type hypersensitivity caused by antibiotics were tested for the culprit drugs (65 tests) and for alternative drugs (57 tests). The culprit drug was the antibiotic reported to have caused an allergic reaction and the alternative tested drugs were antibiotics that were not used previously by the patient. From the 58 patients, 47 were females and 11 males; 18 of them presented atopy. None of them was under treatment with steroid medication, antihistamines or antidepressants. After obtaining their informed consent and the approval of the Research Ethics Committee of our hospital, the skin prick test (SPT) and the intradermal test (IDT) were performed using the commercially available antibiotics. Normal saline (NaCl 0.9%) was used to dilute the substances. In our allergology center, we used concentrations recommended by current guidelines (4). We used 1% histamine as positive control and NaCl 0.9% as negative control. The res-

ult of the SPT was considered positive when the wheal diameter was superior to 3mm within 20 minutes. The IDT was considered positive when the reading wheal after 20 minutes was double compared to the injection wheal (4,9). The skin tests (ST) were regarded as positive when either of the SPT or IDT was positive and negative when neither of these was positive.

We performed the flow cytometric analysis of the in vitro activated basophils (BAT) with Flow2Cast technique (Bühlmann Laboratories™, Switzerland). For each test we used six test tubes, each containing 50µl of whole blood collected on EDTA. We performed the cell stimulation immediately after collection of the blood and we did not store the blood samples. The first sample was mixed with 50µl stimulation buffer as negative control, the next two samples were the positive controls and they were mixed with 50µl solution of anti-FcεRI (a highly specific monoclonal antibody for the IgE receptor) and 50µl solution of FMLP (a non-specific cell activator - the chemotactic peptide N-Formyl-Met-Leu). The other three samples were mixed with antibiotic solutions (with different concentrations). Anti-CCR3-PE (human chemokine receptor labeled with phycoerythrin) and anti- CD63-FITC (a glycoprotein expressed on activated basophils) were used as staining reagents and were added in each test tube. After an incubation period of 15 minutes at 37°C in a water bath, 2 ml of pre-warmed lysing solution was added to each tube and incubated 10 minutes at room temperature. After centrifuging and washing, the cells were suspended in 300µl wash buffer. The quantification of the increase of the CD63 marker on basophils was detected using CellQuest software (FACSCalibur BD Analyser). Our flow cytometer is equipped to detect Forward Scatter, Side Scatter and the two fluorochromes FITC and PE. Our laboratory limit of basophilic cell analyzed for allergies is set to 500. We set the gate by including the entire basophil population CCR3 with low Side Scatter (SSC low) and calculated the percentage of CD63 positive cells compared to the total amount of basophilic cell gated. The result was considered positive when the percent of activated basophils was 5% or more over spontaneous activation observed for the negative control and the stimulation index calculated as the ratio between the percentage of activated basophils with the allergens and the negative control was ≥ 2 (10).

The statistical analysis was performed using Excel software (2003, Microsoft Corporation™, Seattle, USA). Cohen's Kappa Index (k) for agreement and the exact test Fisher were used. Cohen Kappa Index was calculated as $k= (a+b) \times (a+c) + (c+d) \times (b+d)/ N \times N$, where: a= number of positive skin tests in patients with positive allergy history (the culprit drugs) ; b= number of positive skin tests in patients with negative allergy history (the alternative drugs); c= number of negative skin tests in patients with positive history; d= number of negative skin tests in patients with negative allergy history; N= total number of tests.

Results

A total of 122 tests were performed for 65 culprit drugs and 57 antibiotic alternatives. We observed 34 positive skin tests and 26 positive BAT. From our results an example of optimal basophil gating in the CD_{63} can be seen in *Figure 1.*

The substances and the correspondence between positive BAT and positive skin tests are presented in *Table 1.*

There was a fair agreement between BAT and history plus skin tests. The agreement (Cohen Kappa Index) was higher when BAT was performed within 2 years after the allergic reaction (0.35) than after (0.25).

We observed 13 (33.33%) positive skin tests when the tests were performed within 2 years from the allergic reaction, more than those observed for the tests performed after more than 2 years. This difference did not reach the statistical significance (Fisher exact test p=0.15). Also, there were more frequent positive BAT when the test was performed within 2 years from the reaction (30.76% vs. 7.69%), significantly more than those observed for the tests performed after (Fisher exact test p=0.032) (*Table 2).*

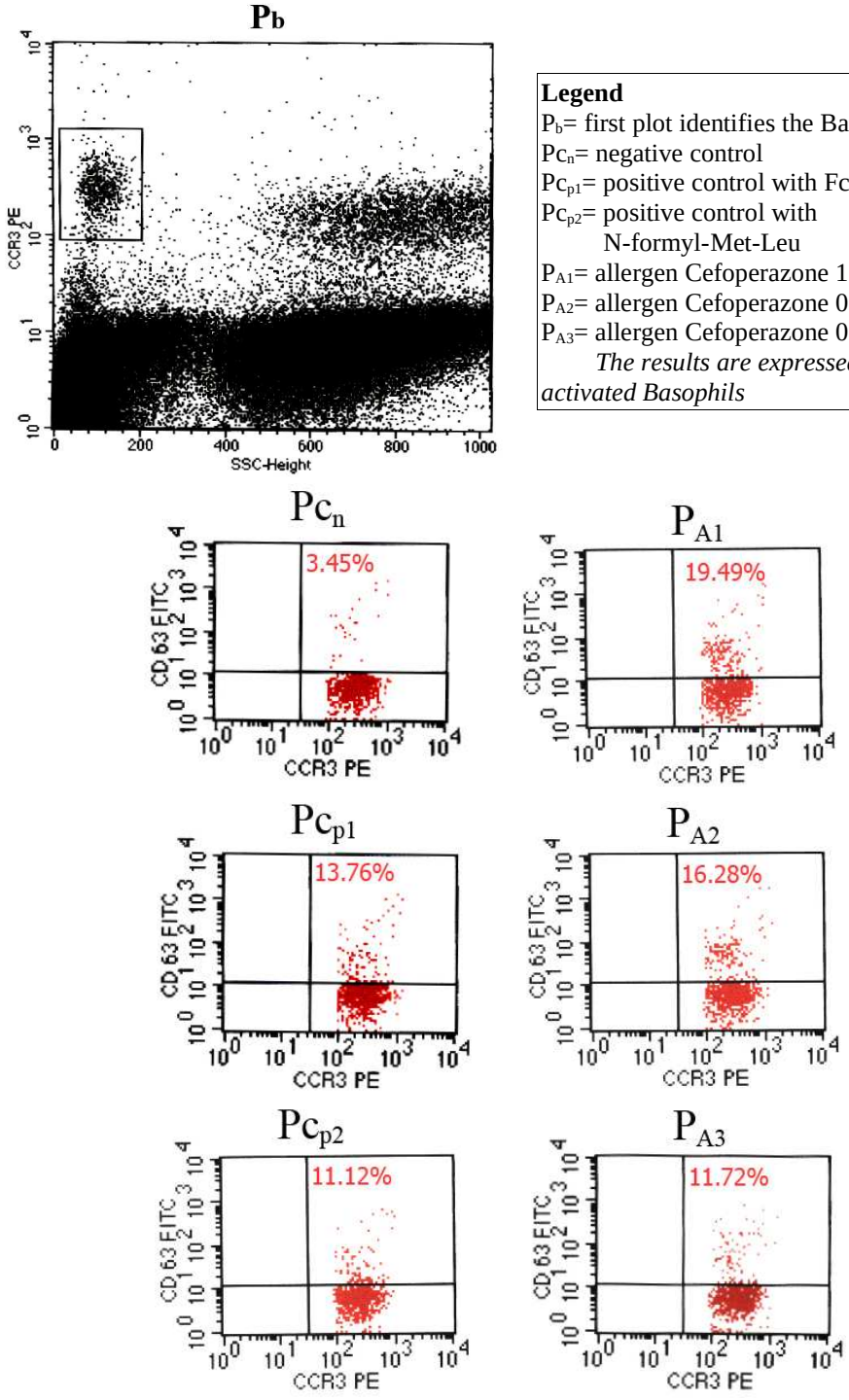

Figure 1. Positive flow cytometry results from a patient with Cefoperazone anaphylactic shock

Table 1. Positive skin tests and their corresponding positive flow cytometry

Substance	BAT+/ST+	n
penicillin	2/9	32
amoxicillin	3/5	17
ampicillin	1/8	29
oxacillin	2/2	9
cefoperazone	0/1	2
ceftazidime	0/1	1
ceftriaxone	0/1	4
cefuroxime	3/3	18
ciprofloxacin	1/2	3
clarithromycin	-	2
clindamycin	-	1
gentamicin	0/1	2
moxifloxacin	-	1
trimetroprim - sulfamethoxazole	0/1	1

ST=skin test; BAT= basophil activation test.

From the 65 culprit drugs, 17 had a corresponding positive skin test and 14 had a corresponding BAT. There were 5 patients with positive history, negative skin tests, but positive BAT. 7 patients presented positive tests for tested alternatives (skin tests or BAT).

Discussion

To date, there is no reliable diagnostic test that confirms allergy accurately (11). Even though skin tests are usually performed, their diagnostic value is limited. New reliable diagnostic tools are needed and increasing the number of tests might improve efficiency (2).

BAT is a new instrument in the diagnostic management of immediate type allergy (12). Different markers and techniques have been used after stimulation with various allergens (5). At present, there is no consensus on the use of the basophil activation test in the diagnosis of antibiotic allergy (1).

BAT based on the expression of CD63 in the presence of specific allergens was suggested to be important for the diagnosis of IgE mediated hypersensitivity, including BL antibiotics (7,13). Flow2CAST technique is more sensitive than Flow-CAST technique for the diagnosis of antibiotic allergy (5), thus we used double labeling with monoclonal antibodies to determine the up-regulation of CD63 on the stimulated basophils using Flow2CAST technique.

It has been suggested that the optimal time for allergy tests is between 1 month and 6 months after the reaction (2). Skin sensitivity declines with time if antibiotics are avoided in allergic patients (1,14). The sensitivity of flow cytometry might be improved if patients are tested soon after the allergic reaction (1). We found that the usefulness of BAT clearly increases when recent reactions are investigated within 2 years.

A significant correlation between ST results and positive CD63 was not found when antibiotics were tested (1). The previously cited concordance among in vivo and in vitro tests was low-0.22 (10). In our study, the agreement

Table 2. Positive diagnostic tests for the culprit and alternative drugs observed when they were performed within or after 2 years

	Positive allergy test	Δt < 2years	Δt >2 years
Culprit drug N=65	positive ST	13 (33.33%)	4 (15.38%)
	positive BAT	12 (30.76%)	2 (7.69%)
Alternative drug N=57	positive ST	11 (17.54%)	6 (10.52%)
	positive BAT	9 (15.78%)	3 (5.26%)

Δt = time lapse between the allergic reaction and the test; ST = skin test; BAT = basophil activation test; N = total number of tests

between BAT and history plus skin tests was 0.35 when BAT was performed within 2 years after the allergic reaction and 0.25 when BAT was performed after 2 years, thus the concordance between flow cytometry and history plus skin tests seems to be higher when the time interval is short. False positive and false negative results might explain the moderately good correlation between tests, as both skin tests and flow cytometry have been shown to have false results (1).

A positive BAT result in ST negative patients might be explained by the occurrence of a false negative skin test. Skin tests are negative in 10-36% of patients allergic to BL (10). In previous studies it has been shown that patients with history of BL allergy, a negative skin test and negative IgEs, might have a positive provocation test (15). Thus, BAT is useful in the diagnosis of patients with IgE mediated allergy to BL antibiotics and negative skin tests as potential life threatening provocation tests might be avoided (10). In our study, 5 negative skin tests for the culprit drug had a positive corresponding BAT. 7 patients presented positive tests for alternatives (skin tests or BAT), ascertaining cross-reactivity among BL antibiotics.

The joint use of in vivo and in vitro tests might improve the efficiency of the antibiotic allergy diagnosis, but flow cytomety is a new diagnostic tool used mainly for research and additional studies are necessary to allow its entrance in clinical practice.

In conclusion, the agreement between the basophil activation test and skin tests is fair, but their diagnostic values are complementary. Flow cytometry is particularly useful in patients with negative skin tests as potential dangerous provocation tests might be avoided. The diagnostic value of flow cytometry is increased when recent reactions are investigated.

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Abbreviations

SPT = skin prick test
IDT = intradermal test
ST = skin test
BAT = basophil activation test
N = total number of tests
k = Cohen Kappa Index
EDTA = ethylenediaminetetraacetic acid
Δt = time lapse between the allergic reaction and the test
CAST = cellular allergen stimulation test

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