

Basophil Activation Test Application in Drug Hypersensitivity Diagnosis: An Empirical Approach

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Keywords

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Abstract

Background: The diagnosis of drug hypersensitivity reactions (DHRs) is based both on clinical history and in vivo tests, such as specific IgE and cutaneous tests, when available. **Objectives:** The aim of this work was to evaluate the basophil activation test (BAT) as a supplementary tool for drug challenges and drug allergy diagnosis. **Method:** We evaluated 204 outpatients reporting DHRs. Available serum-specific IgE drugs were determined and cutaneous tests were performed when appropriate. BAT was performed immediately after blood sampling. The expression of CD63 was evaluated with flow cytometry. The test was considered positive when CD63 expression was >5% and the stimulation index (the ratio of the percentage of CD63-expressing cells with drug exposure/percentage of CD63-expressing cells with wash buffer) was >2. Patients who reported mild to severe reactions and those with a discrepancy between clinical history and BAT underwent a challenge test. **Results:** The drugs that caused adverse reactions were mainly antibiotics

(49%). Non-steroid anti-inflammatory drugs (NSAID) were cited as responsible for DHRs in 37%, with the remaining 14% being due to other drugs. BAT revealed a high specificity (92%) and low sensitivity for antibiotics (40%). For the suspected reactions to penicillin, both the in vitro tests supported 94% of the diagnoses. We also observed a high specificity in the case of challenge with NSAIDs (100% specificity). **Conclusions:** BAT is effective in discriminating adverse drug reactions, whilst only more critical cases require integrated evaluations and more complex clinical examinations. It is relevant that the concordance of anamnesis and in vitro tests reduce the need for challenge testing, limiting them to selected cases.

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Introduction

Adverse drug reactions (ADRs) are noxious, unintended responses to a drug that are triggered at a dose that is usually safe for prophylaxis, diagnosis, or therapy [1]. Drug hypersensitivity reactions (DHR) mediated by im-

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munological mechanisms represent about 15% of ADRs [2]. DHRs commonly involve urticaria and angioedema, but may also include rhinoconjunctivitis, asthma, gastrointestinal symptoms and, in the most serious cases, anaphylaxis and anaphylactic shock [3].

The diagnosis of DHR is based on clinical history, and on tests such as skin prick tests (SPTs), intradermal tests (ITs) and specific immunoglobulin E (sIgE) assays, when available. Because clinical histories are often unreliable, such tests are essential for detecting hypersensitivity to β -lactam antibiotics, including penicillin, although drug challenge testing is considered the gold standard in DHR diagnoses [4, 5]. Therefore, on one hand such a test may be time consuming and needs to be performed by expert personnel, while on the other hand in vitro tests such as specific IgE to β -lactams have good specificity, but low sensitivity [5]. The basophil activation test (BAT) has been introduced as a potential supplementary tool for drug challenge assessment planning and DHR diagnosis [6, 7]. The aim of this “real-life” study was to examine the relationship between symptoms and drug exposure in outpatients who reported previous ADRs and to assess the utility of an in vitro BAT in drug allergy diagnosis. We evaluated BAT and drug challenge results in terms of sensitivity and specificity, and tried to identify the right position of BAT in the diagnostic flow-chart of DHR.

Methods

Subjects

We evaluated outpatients who came to our center for DHR in the 3-year period from 2012 to 2015. A detailed clinical history was collected by allergists and serum IgE testing was performed when appropriate for environmental, food, and indoor allergens.

Clinical examinations were conducted in accordance with the recommendations outlined in the Declaration of Helsinki [8]. Written informed consent was given by the in vivo test participants.

Diagnostic Flow Chart

BAT was performed according to the clinical history in all subjects included in the study. Available serum-specific IgE drugs were evaluated in subjects with attributed reactions to β -lactam. SPTs and ITs were performed when sera were negative (IgE and BAT), and were also conducted for other drugs (e.g., local anesthetics, opioids, heparins, and curares) [5]. Challenge tests were administered to patients with: (a) mild-to-severe reactions, (b) doubtful anamnesis, (c) discrepancy among clinical history/cutaneous sIgE and BAT results, and (d) patients who needed an alternative drug therapy.

Serum IgE Evaluation

As the first step, allergists required serum-specific IgE for penicilloyl G, penicilloyl V, amoxicillin, ampicillin, and cefaclor to

evaluate subjects with DHR to β -lactam drugs. sIgEs were considered positive when greater than 0.10 kU/L (ImmunoCAP System, ThermoFisher, Sweden).

Basophil Activation Test

Patients with DHR underwent BATs for suspected, same class, and alternative drugs (range 5–12 drugs per patient). BAT was performed according to the manufacturer's instructions with a few changes as previously reported [9]. Briefly, immediately after blood sampling, 50 μ L of heparinized peripheral blood were preincubated with 20 ng/mL of interleukin-3 at 37 °C for 10 min and then with the diluted drug for 20 min at 37 °C. Drugs were freshly prepared from commercially available injectable solutions. Optimal experimental conditions and drug concentrations were determined in previous experiments (data not shown). Wash buffer (50 μ L, Biotest, Glycotope, Heidelberg, Germany) and monoclonal anti-IgE (50 μ L, 200 ng/mL, clone G7-18, BD Biosciences, San Jose, CA, USA) were used as negative and positive controls of IgE-induced basophil activation, respectively. Formyl-methionyl-leucyl-phenylalanine was not used as a control for in vitro cell reactivity because the testing was performed immediately after blood sampling. The reaction was blocked for 8 min on ice. Then, basophils were stained with 20 μ L of a mixture of purified anti-IgE PE and anti-CD63-FITC (Glycotope) for 30 min on ice. Erythrocytes were lysed with NH_4Cl solution (DAKO, Glostrup, Denmark). Cells were evaluated with a flow cytometer (FACSCalibur, BD Biosciences, San Jose, CA, USA) and analyzed with CellQuest software. A range of 400–1,000 basophils was considered suitable for the analysis.

Stimulation Index

The stimulation index (SI) was calculated as the ratio of CD63-expressing cells following drug exposure to the percentage of CD63-expressing cells with wash buffer. The SI and CD63 percentage cut-off values for a positive result were ≥ 2 and $\geq 5\%$, respectively [10].

Cutaneous Tests

SPTs/ITs were performed in subjects with negative sIgE for β -lactam compounds as previously reported [4]. Cutaneous tests were also carried out for other drugs (e.g., local anesthetics, opioids, heparins, and curares). No cutaneous tests were carried out for non-steroid anti-inflammatory drugs (NSAIDs). SPTs/ITs were considered positive when the mean weal diameter was greater than 3 mm.

Challenge Test

Challenge tests are generally administered when the drug triggering hypersensitivity cannot be identified reliably on the basis of history, cutaneous tests, and blood tests, and when the benefits of the information obtained from it is thought to outweigh the risks [11]. In our study, a challenge test was administered to patients with mild-to-severe reactions, according to Ring and Messmer's scale [12]. A total of 120 challenges were administered, including 84 for antibiotics, 21 for NSAIDs, and 15 for other drugs. Most (80/120) were carried out with the suspected drug. In the remaining 40 challenges, we administered an alternative drug selected on clinical history and BAT results, and according to the possibility of cross-reactivity based on chemical similarity. Under close monitoring, the patients were submitted to progressive administration of the target drug [13].

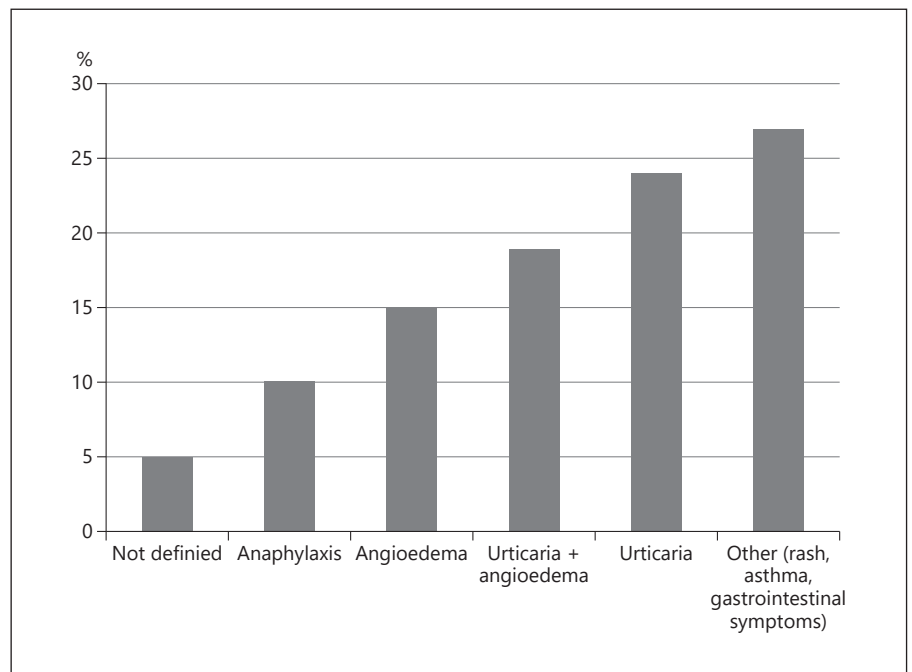


Fig. 1. Percentage of ADR presentations in the examined patients.

Statistical Analysis

We calculated prevalence ratios of food allergy in patients exhibiting atopic reactions versus those without atopic reactions. To evaluate BAT performance, we calculated sensitivity, specificity, and 95% CIs using either clinical history or challenge as gold standards. Concordance between serum IgE tests and BAT results were evaluated for β -lactams with Cohen's kappa (κ) statistics. Statistical analyses were performed in Stata 14 (StataCorp, 2015).

Results

Patient Characteristics

We enrolled 204 outpatients (48 males, 23.5%, and 156 females, 76.5%, with a mean age 50.1 ± 16.6 years). Twenty-two (10.8%, 18 females and 4 males) had allergic respiratory reactions (rhinitis and/or asthma). In total, 32 out of 204 subjects (15.7%, 25 females and 7 males) reported a previous diagnosis of food allergy. Food allergy was found in 6 out of 22 (27.2%) atopic patients, compared with 26 out of 182 (14.3%) nonatopic patients (prevalence ratio 1.91; 95% CI 0.88–4.13).

Patient Reactions

DHR was associated mainly with antibiotics (127/258 DHRs, 49.2%, in 204 patients), followed by NSAIDs (95/258 DHRs, 36.8%). For the remaining 36 DHRs (13.9%), the suspected drugs included local anesthetics,

opioids, heparins, curares, and corticosteroids. Eleven patients (4.3%) presented multiple DHRs (≥ 3 reactions), 7 (2.7%) had reactions with 2 different classes of antibiotics, and 2 (0.8%) attributed their reactions to a drug-food interaction. Moreover, 22 (8.4%) subjects showed reactions to more than one drug.

Most of the subjects (194/204, 95.1%) supplied clinical documentation (visits to emergency departments, treatment and therapies in hospitalized patients) related to previous reactions to drugs. The documentation showed a high prevalence of angioedema and urticaria (58%), with the occurrence of anaphylaxis being relatively rare (10%). Only 10 subjects (5%) reported prior ADRs with no clinical documentation or event details (Fig. 1). Regarding reaction timing, 74 subjects (36%) had an early adverse reaction (<1 h after consumption), 78 subjects (38%) had late reactions (>1 h), and the remaining 52 (26%) patients were unable to remember the time that had elapsed between drug consumption and adverse event onset. Overall, 61.5% of DHRs occurred within the first year of our study, with the remaining 28.5% of cases recorded in the second and third years; about 10% occurred after 2 years and were due to NSAIDs and not β -lactams. Considering antibiotics and NSAIDs, BAT sensitivity and specificity did not vary in relation to the time of execution (within 6 months, <1 year, or >1 year after the reaction; unpubl. data).

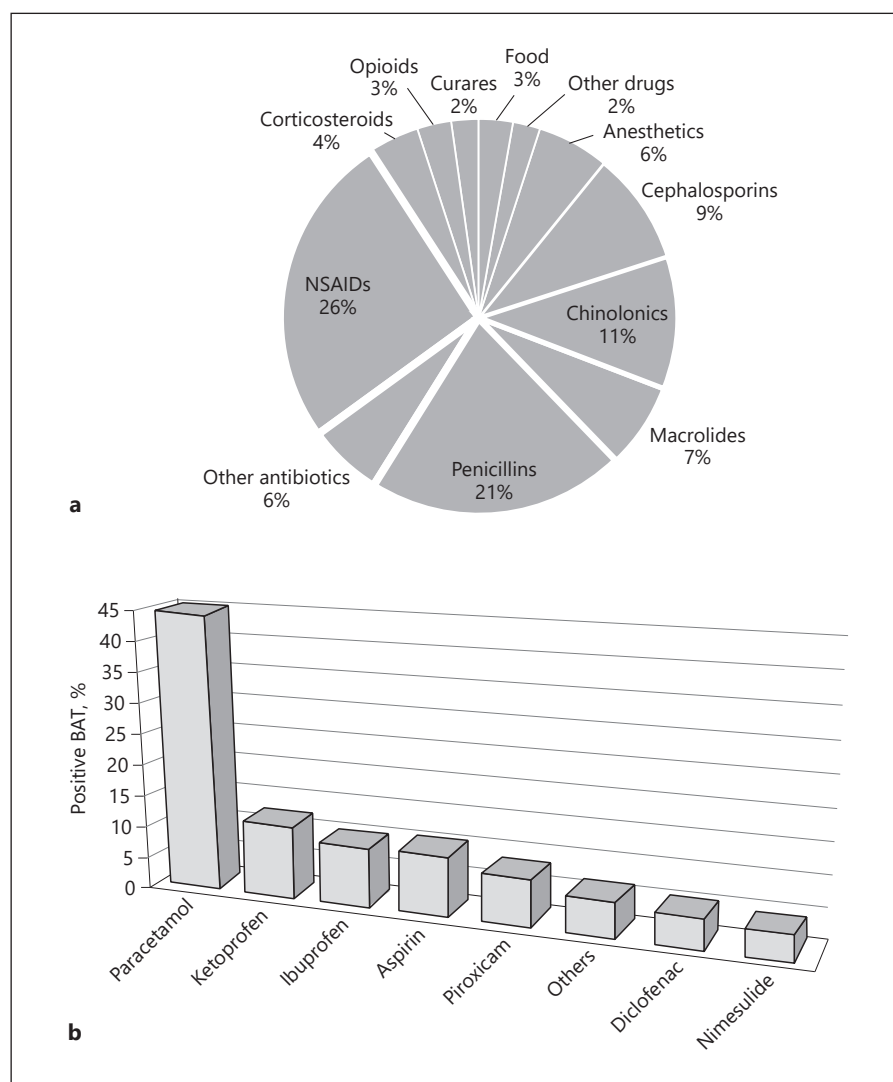


Fig. 2. **a** Distribution of positive BATs in 204 patients: 53% were positive for antibiotics, while NSAIDs represented a quarter of the positive results. **b** A high percentage of paracetamol was observed in positive BAT to NSAIDs.

In vitro Tests

A total of 1,652 BATs were performed in the 204 enrolled patients. Overall, 5.8% of patients had no response to the IgE-positive control, but some of them (4.2%) had a positive response for one of the tested drugs. Positive BAT results are shown in Figure 2. NSAIDs, penicillins, and cephalosporins were the most frequently positive drugs. Paracetamol was the most frequently positive drug amongst NSAIDs (Fig. 2b)

Antibiotics and NSAIDs showed a high BAT specificity: 90.0% (95% CI 85.8–93.3) and 95.1% (95% CI 92.6–96.9), respectively. The sensitivity was 33.6% (95% CI 25.3–42.7) for antibiotics and 22.4% (95% CI 15.2–31.1) for NSAIDs.

We observed a moderate concordance between specific IgE and BAT results for penicillin ($n = 37$, 81.1% agreement; $\kappa = 0.4$, 95% CI 0.1–0.7). Four patients had both positive BAT and sIgE, 2 patients had a positive BAT but negative specific IgE, whilst 5 had a negative BAT but a positive sIgE.

In vivo Tests

A total of 80 challenges were performed with the suspected drugs (26 for β -lactam/penicillin antibiotics, 43 for quinolone/macrolide antibiotics, and 11 for NSAIDs). Twenty-six subjects negative to both the *in vitro* tests underwent cutaneous testing and challenges: 24 out of 26 were negative to the challenge test, 1 had a positive cutaneous test, and 1 was negative to the *in vitro* test and cu-

Fig. 3. a Summary of findings in subjects with a clinical history of penicillin-associated ADRs. Only patients with negative BAT, serum IgE, and SPT/IT results were submitted to a challenge. Of the 25 patients with negative SPT/IT results, only 1 showed a positive reaction to the challenge. The positive serum-specific IgE test and/or BAT obviated the need for SPT/IT and challenge tests in 11 out of 37 patients. **b** Cases of ADR with macrolides and quinolones submitted to challenge.

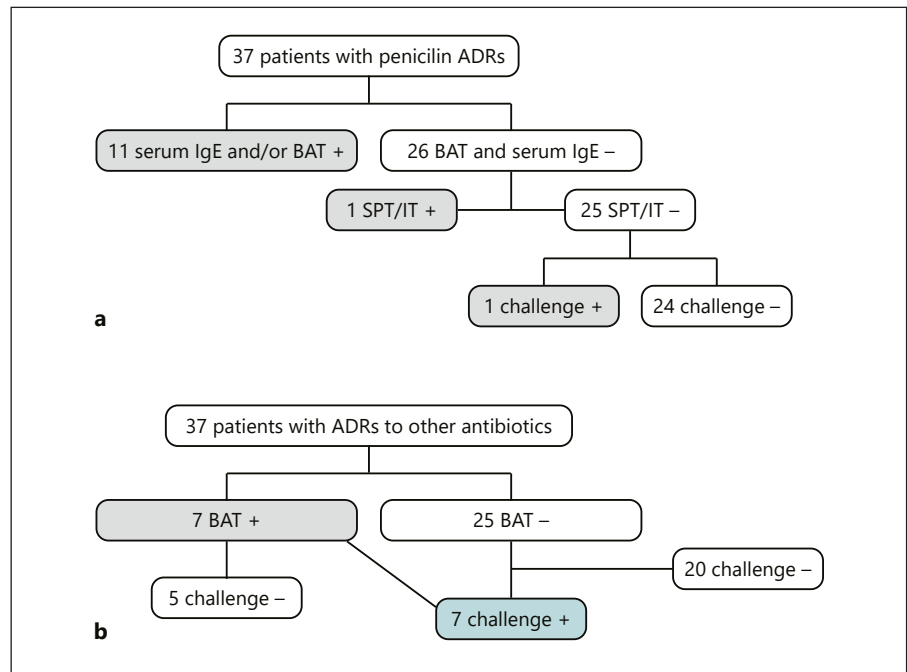


Table 1. Results of 80 drug challenges for NSAIDs and antibiotics with the suspected drug

Parameter	Antibiotics		NSAIDs	
	positive challenge	negative challenge	positive challenge	negative challenge
BAT positive, <i>n</i>	2	5	0	0
BAT negative, <i>n</i>	3	59	0	11
Sensitivity, % (95% CI)	40 (5–85)		not determined	
Specificity, % (95% CI)		92 (83–97)		100 (72–100)

taneous test but had a positive challenge test (Fig. 3). The challenge with the suspected drug and BAT for antibiotics and NSAIDs showed high specificity but low sensitivity (Table 1).

The remaining 42 of the 120 challenges carried out in order to test an alternative drug (16 quinolones/macrolides, 10 NSAIDs, and 14 other drugs) showed that no subject with a negative BAT had a positive challenge.

Discussion

Consistent with the literature, the cohort of DHR patients we examined were frequently over 50 years old [6] and predominantly female [6, 14]. Compared to prior re-

ports [14, 15], atopy was relatively infrequent in this study. From our data no relationship was observed between respiratory allergy and drug allergy, although there was a relatively high prevalence of food allergy. The prevalence rates for antibiotic (53%) and NSAIDs (26%) in our study were similar to previously published rates in patients treated in emergency department [16, 17]. In contrast with other data in the literature [18], paracetamol, propionic acid derivatives (ketoprofen, ibuprofen), and aspirin were the most frequent NSAID drugs in our study, probably related to the high frequency of national consumption of these compounds [19]. With respect to symptoms, urticaria and angioedema were frequently observed, with documented anaphylaxis occurring in 10% of the examined population.

The relatively small number of subjects with reactions to more than one drug is fairly consistent with estimated multidrug hypersensitivity prevalence rates of approximately 10% in the literature [20–22]. The diagnosis is always empirical because the pathogenic mechanisms are complex and have not yet been resolved [20].

Half of the enrolled subjects reported DHR onset within 1 h of drug intake. Although we used a 1-h delineation, it should be noted that reactions occurring as much as 6 h after drug exposure have been described as immediate reactions [5]. It is well known that it is not always possible to determine the onset interval accurately, and patient recall regarding onset in anamnesis can be unreliable. Therefore, it is often prudent to employ more than one diagnostic tool (e.g., SPTs/ITs and BAT). With appropriate cut-offs, BAT in particular has been reported to be helpful for antibiotic and NSAID DHR diagnosis in cases of negative/doubtful anamnesis [10, 23, 24].

The cutaneous tests can support a diagnosis when applicable, avoiding challenge tests. With respect to anamnesis, we obtained quite good specificity with low false positive rates (11–31%), but relatively low sensitivity with substantial false negative rates, for both antibiotics and NSAIDs. Low sensitivity for challenges was also observed in previous studies [25–27].

Surprisingly, for NSAIDs, we observed a high specificity in DHR with BAT. It is worth noting that the mechanism of NSAID hypersensitivity is thought to be mainly nonimmunological, and rather to involve the inhibition of cyclooxygenase, which metabolizes arachidonic acid into prostaglandins (i.e., thromboxane and prostacyclins) [28]. Our data suggest that BATs for NSAIDs can be used to distinguish metabolic from immunological reactions, the latter of which may be underestimated.

Although challenge tests are the “gold standard,” in our “real-life study,” they are performed only when prior diagnostic efforts (i.e., anamnesis, specific IgE tests, BATs, SPTs, ITs) have been negative or ambiguous and are designed based on the findings of the prior diagnostic tests to minimize safety risks [5, 29, 30]. We also administered challenge tests for potential alternative classes of drugs with the aim of finding safe alternative drugs in patients with ADRs. When we performed challenges in selected subjects, we observed good specificity for antibiotics and a modest concordance between BAT and specific IgE for β -lactam antibiotics. BAT proved to be useful, particularly in cases of non- β -lactam reactions in which it is the only possible test alternative to the challenge. Moreover, we only needed to administer β -lactam antibiotic challenges to 2 out of 3 patients who also underwent

cutaneous tests, showing that in vitro test procedures such as sIgE and BAT can, in many cases, obviate the need for more risky, complex, expensive, and time consuming in vivo tests. In this regard, it is important to note that challenges confirmed negative NSAID BAT results in our patients, demonstrating good specificity for BAT, particularly in cases where there was a suspicious anamnesis in conflict with a negative BAT result. In contrast with other previous published studies, in which patients were accurately selected [4, 10], the present work was a “real-life” study based strictly on clinical experience and outpatient monitoring.

In conclusion, the BAT procedure is useful for discriminating DHR sources. The introduction of this test in the routine diagnostic flow-chart of DHR is useful and saves time and resources [31, 32]. In fact, subjects with a concordant (positive or negative) clinical history and BAT results do not need to undergo more complex in vivo evaluations. Moreover, BAT with an alternative drug has a high negative predictive value and avoids further challenges. We found that BAT was a reasonably complementary prechallenge tool for antibiotics, especially β -lactam antibiotics, and that BAT may also be helpful in understanding NSAID DHRs. We suggest that BAT is a tool that should not be used alone in ADR diagnosis, but must be applied in a multifaceted ADR evaluation. Therefore, in such cases it may be the first line of flexible in vitro tests necessary in order to assess the patient and to better define the diagnostic/therapeutic approach.

Disclosure Statement

The authors have no conflicts of interest to declare.

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