BRIEF REPORT

Real-Life Utility of Basophil Activation Test in the Diagnosis of Immediate Hypersensitivity Drug Reactions

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ABSTRACT

Introduction: The basophil activation test (BAT) is a flow cytometry laboratory technique that assesses the level of activation indicators expressed on the surface of basophils. We conducted a real-life study in a prospective cohort of patients with reported drug hypersensitivity reactions to determine the true relevance of BAT as a diagnostic tool for assessing immediate hypersensitivity reactions to medicines.

Methods: We prospectively assessed individuals with clinical suspicion of immediate hypersensitivity reactions to drugs over a 2-year period. The allergological evaluation was carried out in accordance with European Academy of Allergy and Clinical Immunology (EAACI) guidance. All patients underwent BAT using the activation marker CD63.

Results: In total 13 patients with 54 reported immediate drug hypersensitivity reactions to medications were included in this study. Twelve were female (92.3%) and one was male (7.70%). The mean ± SD age of the patients was 47.31 ± 19.94 years. Antibiotics were tested in 35.2% (19/54) of patients, corticosteroids in 24.1% (13/54), iodinated contrast medium in 14.8% (8/54), and NSAIDs in 5.6% (3/54). There was no correlation between the BAT results and the age of patients, gender, type of medication, or time interval between the allergic reaction and BAT procedure. The sensitivity of BAT 5% $CD63^+$ basophils to drugs was 97.6%, specificity was 96% for drug allergies, positive predictive value (PPV) was 94.3%, and negative predictive value (NPV) was 95.2%.

Conclusions: The sensitivity of BAT for drug allergies is limited, but it can nevertheless be very helpful before contemplating provocation testing in cases of life-threatening drug allergies where patients cannot be rechallenged or in cases of medications for which no other tests are available or their results are ambiguous.

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Keywords: Anaphylaxis; Angioedema; Basophil activation test; Hypersensitivity; Immediate drug reaction

Key Summary Points

Why carry out this study?

This study aimed to evaluate the actual utility of the basophil activation test (BAT) as a diagnostic tool for immediate drug reactions in real-world situations.

What was learned from the study?

Of the 54 drug allergens tested 25.9% (14/54) had a BAT positive result, whilst 74.1% (40/54) had a negative BAT result.

Our study found a 97.6% sensitivity, 96% specificity, 94.3% positive predictive value (PPV), and 95.2% negative predictive value (NPV) of BAT in drug allergy.

INTRODUCTION

The basophil activation test (BAT) is a flow cytometry laboratory technique that assesses the level of activation indicators expressed on the surface of basophils [1]. Since Edward Knol identified CD63 in 1991, BAT has become increasingly significant in the identification and monitoring of allergy disorders [1–4]. BAT uses flow cytometric detection of changes in certain activation markers on the surface (CD63, CD203c) or inside cells (phosphorylated p38 mitogen-activated protein kinase, P-p38MAPK) [3, 4]. Using particular monoclonal antibodies coupled to a fluorochrome, flow cytometry can identify and quantify these changes on a single-cell basis. Obviously, BAT is only appropriate when the final effector function depends on basophil activation (e.g., IgE-mediated immediate-type hypersensitivity) [5–9]. However, one must be mindful that other cell activation mechanisms (such as the complement system or even nonimmunologic, pharmacologic basophil activation) could also take place [4]. We prospectively assessed a cohort of patients with immediate sensitivity drug reactions to evaluate the actual effectiveness of BAT as a diagnostic tool for immediate drug reactions in real-world situations.

METHODS

Patients with clinical suspicion of immediate hypersensitivity reactions to medications and referred to the Dermatology Department of the University Hospital of Heraklion in Crete, Greece between 2020 and 2022 were included. The allergological evaluation was carried out in accordance with European Academy of Allergy and Clinical Immunology (EAACI) guidance. All patients underwent a detailed clinical evaluation. Skin prick tests (SPTs) and intradermal skin testing (IST) of potential culprit drugs were performed with histamine as the positive control and NaCl 0.9% as a negative control. BAT was performed using the activation marker CD63 according to the instructions of BÜHLMANN Flow (Cellular Allergy Stimulation Test) CAST® and CAST® ELISA kits. The BAT BÜHLMANN Elisa kit is a commercially available diagnostic test used to diagnose drug allergies. The kit measures the activation of basophils in response to specific drugs, allowing for the detection of drug-specific IgE antibodies. The test involves multiple steps. Initially, the patient's blood is collected in a heparinized tube and the sample is prepared by diluting the blood with a buffer solution. The sample is then incubated with a mixture of allergen-specific antibodies and CD63 antibodies, which bind to the basophil cell surface upon activation. After incubation, the cells are washed to remove unbound antibodies. The next step involves detecting the bound antibodies using a biotinylated detection antibody and a streptavidin–AP conjugate. The cells are then incubated with a substrate solution that produces a colorimetric reaction upon binding to the AP conjugate. The intensity of the reaction is proportional to the extent of basophil activation. Finally, the cells are analyzed using flow cytometry, and the percentage of activated basophils is calculated. A positive result indicates the presence of drug-specific IgE antibodies and is indicative of a drug allergy [10, 11].

This study was approved by the Ethical Committee of the University Hospital of Heraklion, Heraklion, Greece (Reference number 6552/06-08-2020). The study was performed in accordance with the Helsinki Declaration of 1964, and its later amendments. All subjects provided informed consent to participate in the study. The patients in this manuscript have given written informed consent to the publication of their case details.

Stimulation Index

The ratio of the number of cells expressing CD63 post drug exposure to the number of cells expressing CD63 with wash buffer was used to calculate the stimulation index (SI). A positive result was indicated by an SI value of 2 or higher and a CD63 expression percentage of 5% or higher [12].

Statistical Analysis

To evaluate BAT performance, we calculated sensitivity and specificity using clinical history as the gold standard. The data were compared using various tests such as the unpaired t test, Mann–Whitney test, analysis of variance (ANOVA), or Kruskal–Wallis test. Correlations were assessed using Spearman's or Pearson's correlations. The receiver operating characteristic (ROC) curve was employed to calculate BAT sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV). A result was deemed significant when the p value was less than 0.05. All statistical analyses were conducted using SPSS IBM 25.

RESULTS

Thirteen patients with a history of drug hypersensitivity to 54 drugs who were referred to the Dermatology Department of the University Hospital of Heraklion in Heraklion, Crete, Greece between 2020 and 2022 for examination were included in this study with a complete allergological workup including clinical history, SPTs, ISTs, and BAT. In total, there were 12 (92.3%) female patients and one (7.70%) male patient. The mean ± SD age of the patients was 47.31 ± 19.94 years. The onset of symptoms after drug uptake was less than 15 min for 30.8% (4/13) of patients, between 15 and 30 min in 38.5% (5/13), and more than 30 min in 30.8% (4/13). The mean time interval between an allergic reaction and BAT was 14.12 months (SD ± 7.18). Almost, one-third of patients, 30.8% (4/13), had a history of immediate drug reaction (IDR) of less than a year, and 69.2% (9/13) of more than a year. Nine out of 13 patients (69.2%) experienced anaphylaxis, 1/13 (7.7%) anaphylactic shock, and 23.1% (3/13) experienced urticaria or angioedema. Twelve out 13 patients (92.3%) did not have a past medical history (PMH) of atopy, whilst only 7.7% (1/13) were atopic. These 13 patients were tested for 54 suspected allergens. Antibiotics were tested in 35.2% (19/54) of patients, corticosteroids in 24.1% (13/54), iodinated contrast medium in 14.8% (8/54), and NSAIDs in 5.6% (3/54). SPTs and ISTs were positive in 40.2% of cases and negative in 59.3% of cases: 69.2% (4/13) of the patients yielded a positive BAT, whereas 30.8% (4/13) had a negative BAT result. Of the 54 drug allergens tested 25.9% (14/54) had a positive BAT result, whilst 74.1% (40/54) had a negative BAT result. The clinical and laboratory characteristics of these patients are shown in Table 1.

There was no correlation between the BAT results and the age of patients, gender, type of medication, or time interval between the allergic reaction and BAT. The sensitivity of BAT to drugs was 97.6%, specificity was 96%, PPV was 94.3%, and NPV was 95.2% (Table 2). The ROC curve was employed to calculate BAT sensitivity, specificity, PPV, and NPV (Fig. 1).

DISCUSSION

In this study, we evaluated BAT in patients reporting IDRs [13] to confirm or rule out a suspected drug allergy. Several studies have investigated the sensitivity and specificity of BAT in drug allergy diagnosis with variable results [13–26]. Determining the sensitivity and specificity of BAT is a crucial step in clinical

Table 1 Details of the 54 basophil activation test (BAT) determinations for 13 patients who experienced immediate drug hypersensitivity: 69.23% (9/13) of patients had a positive BAT, while 30.77% (4/13) had a negative BAT

Patient no.	Gender	Age (years)	Type of reaction	Time since last reaction	Time interval between allergic reaction and BAT	PMH of atopy (Yes/No)	Culprit medication	Negative control (%)	Positive control (%)	Medication tested	Activated basophils (%)[a]	SI[b]	BAT 5% CD63+ basophil result	ST result	IST result
1	F	55	Anaphylaxis	> 1 year	14 months	No	Amoxicillin	1.74	10.62	Penicillin G	3.57	2.05	Positive	Positive	Positive
								1.74	10.62	Penicillin V	3.51	2.02	Positive	Positive	Positive
							Iopromide	1.74	10.62	Iopromide	5.85	3.36	Positive	Positive	Positive
							Iodixanol	1.74	10.62	Iodixanol	2.53	1.45	Negative	Positive	Positive
							Iobitridol	1.74	10.62	Iobitridol	1.80	1.03	Negative	Negative	Negative
							Iomeprol	1.74	10.62	Iomeprol	2.09	1.20	Negative	Negative	Negative
							Iopamidol	1.74	10.62	Iopamidol	2.92	1.68	Negative	Negative	Negative
							Methylprednisolone	1.74	10.62	Methylprednisolone	1.90	1.09	Negative	Negative	Negative
							Lidocaine	1.74	10.62	Lidocaine	1.97	1.13	Negative	Negative	Negative
2	F	57	Anaphylaxis	> 1 year	18 months	No	Clarithromycin	1.63	10.18	Clarithromycin	8.90	5.46	Positive	Positive	Positive
							Clavulanic acid amoxicillin	1.63	10.18	Clavulanic acid amoxicillin	2.43	1.49	Negative	Positive	Positive
							Clindamycin	1.63	10.18	Clindamycin	2.00	1.23	Negative	Positive	Positive
							Methylprednisolone	1.63	10.18	Methylprednisolone	2.43	1.49	Negative	Negative	Negative
							Prednisolone	1.63	10.18	Prednisolone	1.77	1.09	Negative	Negative	Negative
							Hydrocortisone	1.63	10.18	Hydrocortisone	1.89	1.16	Negative	Negative	Negative
							Iomeprol	1.63	10.18	Iomeprol	2.27	1.39	Negative	Negative	Negative
							Iopromide	1.63	10.18	Iopromide	1.92	1.18	Negative	Negative	Negative
							Iodixanol	1.63	10.18	Iodixanol	1.98	1.21	Negative	Negative	Negative
							Levocetirizine dihydrochloride	1.63	10.18	Levocetirizine dihydrochloride	1.70	1.04	Negative	Negative	Negative

Table 1 continued

Patient no.	Gender	Age (years)	Type of reaction	Time since last reaction	Time interval between allergic reaction and BAT	PMH of atopy (Yes/No)	Culprit medication	Negative control (%)	Positive control (%)	Medication tested	Activated basophils (%)[a]	SI[b]	BAT 5% CD63+ basophil result	ST result	IST result
3	F	54	Anaphylaxis	> 1 year	16 months	No	Thiocolchicoside	3.11	68.96	Thiocolchicoside	21.56	6.93	Positive	Positive	Positive
							Acetaminophen/acetylsalicylic acid	3.11	68.96	Acetaminophen/acetylsalicylic acid	3.15	1.01	Negative	Negative	Negative
							Etoricoxib	3.11	68.96	Etoricoxib	3.37	1.08	Negative	Negative	Negative
							Diclofenac	3.11	68.96	Diclofenac	3.13	1.01	Negative	Positive	Positive
4	F	16	Anaphylaxis	< 1 year	4 months	No	Ibuprofen	1.04	57.84	Ibuprofen	2.83	2.72	Positive	Positive	Positive
5	F	85	Anaphylaxis	> 1 year	13 months	No	Moxifloxacin	1.31	91.67	Moxifloxacin	1.48	1.13	Negative	Positive	Positive
							Ciprofloxacin	1.31	91.67	Ciprofloxacin	2.27	1.73	Negative	Positive	Positive
							Sultamicillin tosylate	1.31	91.67	Sultamicillin tosylate	4.10	3.13	Positive	Positive	Positive
6	F	44	Anaphylaxis	> 1 year	24 months	No	Prednisolone	1.54	53.45	Prednisolone	4.44	3.61	Positive	Positive	Positive
							Methylprednisolone	1.54	53.45	Methylprednisolone	1.69	1.37	Negative	Negative	Negative
							Hydrocortisone	1.54	53.45	Hydrocortisone	1.57	1.28	Negative	Negative	Negative
							Iopromide	1.54	53.45	Iopromide	3.11	2.53	Positive	Positive	Positive
							Sulfamethoxazole & trimethoprim	1.54	53.45	Sulfamethoxazole & trimethoprim	2.03	1.65	Negative	Negative	Negative
							Sulfur hexafluoride	1.54	53.45	Sulfur hexafluoride	1.55	1.26	Negative	Negative	Negative
7	F	75	Angioedema	< 1 year	1 month	No	Allopurinol	3.61	66.40	Allopurinol	3.78	1.05	Negative	Positive	Positive
							Ibrutinib	3.61	66.40	Ibrutinib	4.78	1.32	Negative	Negative	Negative
8	F	45	Angioedema	> 1 year	1 month	Yes	Prednisolone	1.05	26.91	Prednisolone	1.33	1.27	Negative	Negative	Negative
							Methylprednisolone	1.05	26.91	Methylprednisolone	1.26	1.20	Negative	Negative	Negative
							Levocetirizine	1.05	26.91	Levocetirizine	3.28	3.12	Positive	Positive	Positive
							Budesonide/formoterol	1.05	26.91	Budesonide/formoterol	2.00	1.90	Negative	Negative	Negative

Table 1 continued

Patient no.	Gender	Age (years)	Type of reaction	Time since last reaction	Time interval between allergic reaction and BAT	PMH of atopy (Yes/No)	Culprit medication	Negative control (%)	Positive control (%)	Medication tested	Activated basophils (%)[a]	SI[b]	BAT 5% CD63+ basophil result	ST result	IST result
9	F	23	Anaphylaxis	> 1 year	17 months	No	Clarithromycin	1.38	76.23	Clarithromycin	3.42	2.48	Positive	Positive	Positive
							Clavulanic acid amoxicillin	1.38	76.23	Clavulanic acid amoxicillin	3.33	2.41	Positive	Positive	Positive
							Amoxicillin	1.38	76.23	Amoxicillin	2.86	2.07	Positive	Positive	Positive
							Clindamycin	1.38	76.23	Clindamycin	1.98	1.43	Negative	Negative	Negative
10	F	25	Anaphylaxis	> 1 year	18 months	No	Ciprofloxacin	1.98	66.42	Ciprofloxacin	2.03	1.03	Negative	Positive	Positive
11	F	20	Anaphylaxis	> 1 year	20 months	No	Acetaminophen	4.58	62.28	Acetaminophen	4.61	1.01	Negative	Negative	Negative
12	M	53	Angioedema	< 1 year	1 month	No	Budesonide	1.34	89.60	Budesonide	2.32	1.34	Negative	Negative	Negative
							Ipratropium bromide/ albuterol sulfate	1.34	89.60	Ipratropium bromide/ albuterol sulfate	1.71	1.28	Negative	Negative	Negative
							Beclomethasone dipropionate	1.34	89.60	Beclomethasone dipropionate	2.34	1.75	Negative	Negative	Negative
							Vilanterol/ umeclidinium bromide	1.34	89.60	Vilanterol/ umeclidinium bromide	1.41	1.05	Negative	Negative	Negative
13	F	43	Anaphylaxis	> 1 year	18 months	No	Levofloxacin	1.17	16.96	Levofloxacin	2.18	1.86	Negative	Positive	Positive
							Clarithromycin	1.17	16.96	Clarithromycin	11.55	9.87	Positive	Positive	Positive
							Amoxicillin	1.17	16.96	Amoxicillin	4.00	3.42	Positive	Positive	Positive
							Methylprednisolone	1.17	16.96	Methylprednisolone	2.18	1.86	Negative	Negative	Negative
							Metronidazole	1.17	16.96	Metronidazole	1.40	1.20	Negative	Negative	Negative

A result is considered positive when activated basophils (%) is ≥ 5 or stimulation index is ≥ 2

BAT basophil activation test, PMH past medical history, M male, F female, ST skin test, SI stimulation index, IST intradermal skin test

[a] Percentage of basophils expressing an activation marker minus the value of the non-stimulated control tube

[b] Allergen stimulation divided by negative control

Table 2 Optimal cutoffs for the parameters of BAT to drugs with the largest area under the ROC curve ($n = 54$)

BAT parameters	Cutoff	AUC	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Diagnostic accuracy
%CD63$^+$	8.14 (2.94–16.56)	0.98 (0.92–1.0)	97.6 (87.4–99.9)	96.0 (86.3–99.5)	94.3 (84.2–99.4)	97.0 (89.1–99.9)	94.7 (93.2–100)
Mean %CD63$^+$	4.69 (4.45–11.76)	0.98 (0.94–1.0)	97.6 (87.4–99.9)	95.0 (84.3–99.5)	95.2 (84.2–99.4)	98.0 (89.1–99.9)	95.7 (92.1–100)

Values in parentheses represent 95% CI

CI confidence interval, *AUC* area under the ROC curve, *%CD63$^+$* percentage of CD63 positive basophils, *PPV* positive predictive value, *NPV* negative predictive value

validation. Although demonstrating variations between foods, BAT has a high sensitivity and specificity for food allergies [27–30]. The sensitivity of BAT for drug allergies is lower, but it can still be very helpful before considering provocation tests in cases of life-threatening drug allergies where patients cannot be rechallenged or in cases of drugs for which there are no other tests available or their results are ambiguous [1].

The sensitivity of BAT in drug allergy diagnosis has been extensively studied and several factors can influence its accuracy. One important factor affecting the sensitivity of BAT is the type of drug being tested [31–39]. Some drugs such as penicillin have a high rate of positive BAT results in patients with a confirmed allergy [8, 9]. However, other drugs, such as non-steroidal anti-inflammatory drugs (NSAIDs), have a lower rate of positive BAT results, even in patients with a confirmed allergy [10]. Therefore, the sensitivity of BAT in drug allergy diagnosis can vary, depending on the drug being tested. However, the specificity of BAT may also be influenced by the drug being tested. For example, some drugs may induce non-specific basophil activation, leading to false-
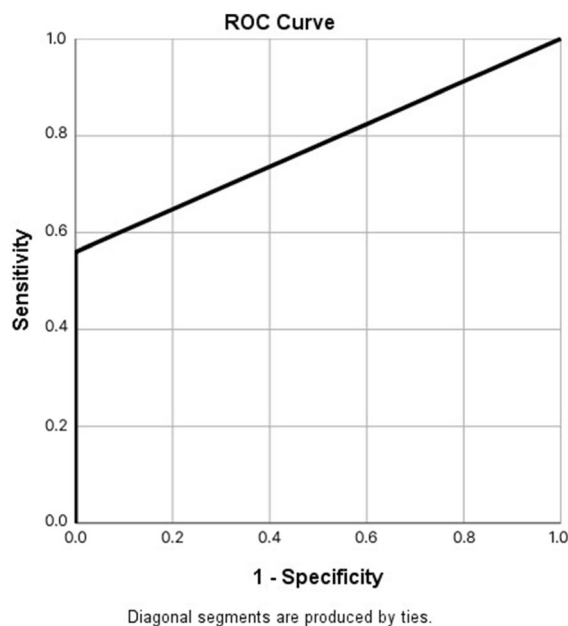
Fig. 1 Evaluation of the receiver operating characteristic (ROC) curve for basophil activation test (BAT)

positive results [8–10]. Therefore, the specificity of BAT can be affected by the drug class and concentration used in the test [1]. In our study, we found a 97.6% sensitivity in drugs. BAT has previously been examined as a potential in vitro diagnostic tool for patients with drug allergies in antibiotics, such as amoxicillin (AX) and clavulanic acid (CLV); and the sensitivity and specificity values were 48–55% and 89–93%, respectively [19, 36, 40]. All of these studies, however, were conducted on retrospectively chosen patients with well-characterized phenotypes and therefore the true clinical utility of BAT to identify allergic patients was unknown. Owing to the invention of flow cytometry, the identification of activation markers like CD63, and the development of specific markers recognizing basophil granulocytes, BAT has become a widely used diagnostic for allergic reactions [14]. The clinical significance of BAT is due to the unique capacity of basophil granulocytes to degranulate upon cross-linking of the specific IgE (sIgE) bound on membrane-bound high-affinity IgE receptor (FceRI) by allergen exposure [41, 42].

BAT eliminates the need for in vivo procedures which can cause unpredictable and severe reactions. BAT closely reflects the patients' phenotype in most cases, making it useful in diagnosing drug and food allergies and predicting and monitoring the clinical response to immunomodulatory treatments [43–45].

Sensitivity and specificity values reveal the accuracy of diagnosing allergic and nonallergic individuals but cannot determine the likelihood of an individual patient being allergic. To assess this probability, analyzing the PPV and NPV of the test is essential. These values offer insight into the probability that a subject with a positive or negative test result has been accurately diagnosed [2–6].

BAT, despite its high diagnostic potential, is not widely employed in diagnosing drug allergies because of a lack of validation and standardization, hindering comparisons of results across different laboratories. Universal protocols need to be developed, encompassing an appropriate reference test for validation, considering the potential limitations of such references. Additionally, the selection and significance of allergens for specific patient groups and the choice of suitable activation markers must be defined. These factors are essential prerequisites for the universal adoption of BAT in clinical diagnosis and research.

Currently, there is a lack of standardization in detecting CD63 or CD203c including setting a positive threshold value. Additionally, in various studies, differences in CD63 and CD203c expression have been observed among allergic and sensitized patients at different allergen concentrations [7–10].

The continued use of BAT in clinical practice and allergy research depends on standardization, ongoing quality assurance, and training of healthcare workers in the interpretation of BAT results. Ongoing efforts are underway, to establish a platform for quality assurance and certification that could be used in laboratories in Europe and the USA [1].

The main limitation of our study is the small number of participants. Another limitation of our study is that it was conducted in patients recruited in a specialized clinical setting and therefore may not reflect the results of BAT with drugs in the general population.

CONCLUSION

This study provides real-world data on sensitivity and specificity in BAT for drug allergies in the clinical setting. The sensitivity of BAT (5% $CD63^+$ basophils) to drugs was 97.6%, with a specificity of 96% for drug allergies. The PPV was 94.3%, and the NPV was 95.2%. In our study, BAT outperformed both skin and intradermal testing. The actual rate of false positive skin test results in our patients remains unknown because, for ethical considerations, we refrain from conducting oral provocation tests on patients who test positive in the skin test. In our case, the clinical history was considered the gold standard for allergy assessment true positive when it accurately aligned with the patient's symptoms and was supported by reliable evidence, confirming the presence of an allergy. Implementation of BAT in clinical practice might be valuable in the workup of drug allergies.

ACKNOWLEDGEMENTS

Declarations

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