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# Advancing drug allergy diagnosis in Tunisia using flow cytometry: a promising first step

**Rania Mzoughi[a,b]*, Baraa Ben Houria[c], Aymen Tazeghdanti[b], Mouna Ben Azaiz[b], Radhia Kochkar[b], Ezzedine Ghazouani[b]**

*[a]Unit of Immunology and Microbiology, Environmental and Carcinogenesis, Faculty of Sciences of Bizerte Jarzouna, Bizerte, Tunisia*
*[b]Department of Immunology, Military Hospital of Tunis, Montfleury, Tunis, Tunisia*
*[c]Department of Pharmacy, Military Hospital of Tunis, Montfleury, Tunis, Tunisia*

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**KEYWORDS**
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**Abstract**
*Background*: Hypersensitivity reactions to drugs represent a significant and growing health concern worldwide, with the potential to cause severe and life-threatening outcomes. Accurate diagnosis of drug allergies is essential for effective patient management, particularly in differentiating true allergic reactions from other adverse drug reactions.
*Objective*: This study aimed to evaluate for the first time the Basophil Activation Test (BAT) using flow cytometry as a reliable diagnostic tool for immediate-type drug allergies in Tunisia.
*Methods*: This study included 35 BATs, conducted between November 2022 and December 2024. Only one patient underwent two separate BATs on different drugs. From each patient, venous blood was drawn and BAT was assessed using flow cytometry. Serial dilutions of suspected drugs, including antibiotics (e.g., amoxicillin and cefixime), non-steroidal anti-inflammatory drugs (e.g., mefenamic acid), and others, were tested. For each sample, basophils were incubated with the drug dilutions stained with antibodies marked with different fluorescents, such as CRTH2-FITC, CD3-PC7, CD203c-PE, and then analyzed on a Navios Ex flow cytometry.
*Results*: Of all the performed BATs, antibiotics represented the majority of tested drugs accounting for 56%. However, only three tests were positive, in favor of a type I hypersensitivity drug reaction.
*Conclusion*: By applying BAT in the Immunology Department of the Military Hospital of Tunisia, this study seeks to enhance diagnostic accuracy and contribute to personalized patient care.
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***Corresponding author:** Mzoughi Rania, University of Carthage, Faculty of Science of BizerteJarzouna, Bizerte 7021, Tunisia. *Email address*: mzoughi.rania@fsb.ucar.tn

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## Introduction

Drug-induced hypersensitivity reactions (DIHRs) are a major public health issue due to their unpredictable nature and the potential severity of their outcomes. These reactions can range from mild cutaneous symptoms to severe systemic reactions, including anaphylaxis. Conventional diagnostic approaches for drug allergies, such as skin tests and drug-specific immunoglobulin E (IgE) assays have certain limitations. Drug-specific IgE assays often lack sensitivity, even for muscle relaxants. In contrast, skin tests are highly specific (100%) and sensitive can reach 85–95% when performed with the optimal concentration of the drug and under standardized conditions, as reported in the literature.

The BAT is a sophisticated flow cytometry-based assay designed to evaluate the expression of specific activation markers on the surface of basophils when these cells are exposed to particular allergens.[1,2]

This assay reproduces a real immunological reaction *in vitro* by using specific monoclonal antibodies coupled to fluorochromes, enabling the precise detection and quantification of cellular responses.

In the context of an immediate hypersensitivity (IH), the cross-linking of IgE bound to the basophil surface triggers a cascade of intracellular events.[3] These events not only lead to the release of mediators but also have phenotypic modifications.[1,4]

Basophil activation is a key process in IH. One of the most important indicators of this process is the upregulation of CD63 on basophil membrane. The expression of CD63 marker proves an active degranulation associated to IgE-mediated mechanisms and is accompanied by phosphorylation of the p38 mitogen-activated protein kinase (p38 MAPK). However, with flow cytometry, nonactivated basophils can be easily discriminated with low expression of CD63 on resting basophils. Added to CD63, another activation marker is CD203c, whose upregulation is used to identify basophil activation, as its expression increases early during activation.

By combining these two markers, BAT can detect both degranulation and early activation and improves the overall sensitivity and specificity of BAT.[5-7]

The primary objective of this study was to establish and validate BAT in the Immunology Department of the Principal Military Hospital of Tunisia (HMPIT), marking the first instance of its application in this setting. This study aimed to explore the utility of BAT in diagnosing drug IH, thereby providing a novel diagnostic tool that could enhance the accuracy of allergy diagnostics in the region.

Through the implementation of BAT, the study sought to advance our understanding of drug-induced allergic reactions and contribute to the broader field of allergy research in Tunisia.

## Materials and Methods

### *Study design and population*

This prospective, descriptive analysis was conducted on patients who exhibited allergic reactions following medication intake and sought medical consultation at various departments of the Military Hospital of Tunisia between November 2022 and June 2024. Participants were asked to complete a comprehensive questionnaire designed to identify potential drug allergies based on clinical symptoms. The questionnaire was designed to record the time between drug intake and onset of clinical symptoms; some patients reported symptoms within minutes to a few hours; others reported onset of symptoms 1 to 2 days and some of them could not remember the exact timing. In this study, no tryptase follow-up was performed.

In this study, clinical symptoms were classified according the Rang and Messmer classification for injected drugs with the following four grades: grade 1 corresponds to "mild" reactions limited to skin manifestations or general malaise without systemic involvement; grade 2 corresponds to moderate reactions (respiratory or digestive symptoms); grade 3 represents to severe reactions; and grade 4 matches to life-threatening anaphylactic reaction.[8] For analysis of the results, only grades 2 and 4 were retained.

### *Sample collection*

For the purpose of our study, blood samples were drawn from each patient using an Ethylenediaminete-traacetic acid (EDTA) tube. In this test, other anticoagulants could be used to prevent basophil degranulation, such as acid citrate dextrose (ACD) or heparin.

These samples were used for BAT, a diagnostic procedure aimed at identifying drug-induced allergic responses at cellular level.

### *Application of basophil activation test in drug allergy diagnostics*

The BAT methodology employed in this study relied on injectable forms of medications, standardized at a concentration of 1 mg/mL for the tested drugs (Table 1).

Table 1 lists optimal concentration ranges of various drugs used in BAT. Beta-lactams list concentration ranges for drugs such as benzylpenicillin, amoxicillin, and cefuroxime. Quinolones include concentration ranges for ciprofloxacin and levofloxacin. Neuromuscular blocking agents (NMBAs) provide ranges for atracurium and vecuronium, both used as muscle relaxants. Non-steroidal anti-inflammatory drugs (NSAIDs) list concentration ranges for metamizole.

For each patient, basophil activation was assessed using at least three different concentrations of allergenic extract, spanning multiple logarithmic scales. These concentrations were prepared from a stock solution using phosphate-buffered saline (PBS) with 2% bovine serum albumin (BSA), resulting in final dilution ratios of 1/10, 1/100, 1/1000, and 1/10,000.

This approach was designed to determine optimal concentration capable of inducing significant basophil activation.

**Table 1** Optimal concentration ranges of some drugs used in BAT.

| Group | Drug | Concentration range (mg/mL) |
|---|---|---|
| Beta-lactams | Benzyl penicillin | 3.9-0.4 |
| | Amoxicillin | 4-0.01 |
| | Clavulanic acid | 1.25-0.05 |
| | Ampicillin | 2.5-0.01 |
| | Cefuroxime | 1.25-0.01 |
| | Cefazolin | 10-0.006 |
| Quinolones | Ciprofloxacin | 2-0.1 |
| | Levofloxacin | 4-0.111 |
| NMBA | Atracurium | 5-0.000025 |
| | Vecuronium | 2-0.00008 |
| NAIDs | Metamizole | 5-0.00025 |

Note: Beta-lactams list concentration ranges of drugs such as benzylpenicillin, amoxicillin, and cefuroxime. Quinolones include concentration ranges of ciprofloxacin and levofloxacin. NMBAs provide ranges of atracurium and vecuronium. NSAID lists concentration range of metamizole.

### *Controls and sample handling*

Each patient's sample was subjected to two internal control conditions:

1. A negative control was employed to assess the baseline or spontaneous activation of basophils, thereby establishing a threshold for test positivity.
2. A positive control anti-IgE was used to verify the viability and responsiveness of basophils.

It is critical that BAT is performed on whole blood samples within 4 h of collection to ensure maximal basophil viability. If immediate processing is not possible, samples are stored at +4°C and analyzed within 24 h. Human basophil's lifespan is relatively short (1–2 weeks). The admitted delay of approximately 6 weeks before performing BAT after an IH reaction accounts for the generation of young basophils, re-granulation period, and possible eventual persistence of the offending drug, this period is considered as possible anergic (or post-anaphylactic anergy) period.

Consequently, the protocol is built on an analytical strategy comprising:

**Tube# "Neg" = Negative Control Tube**.
Its purpose is to evaluate the spontaneous activation of basophils within the tested subject, establishing a baseline for comparison.
**Tube # "Pos" = Positive Control Tube**.
In this tube, a monoclonal anti-IgE reagent is introduced, facilitating IgE-mediated activation at a concentration of 5 μg/mL.
**Tube # "Test":** It contains specific molecule or drug under scrutiny.

### *Procedure*

Allergenicity Kit®, Cellular Analysis of Allergy (Beckman Coulter®, CA, USA), was used in this study. This kit is based on a three-color combination of fluorescent monoclonal antibody reagents: CRTH 2 (FITC)/CD3 (PC 7)/CD203 c (PE). This kit can be applied for 100 tests.

For each test, 100-μL venous blood is incubated with the suspected drug, 20 μL of monoclonal antibodies reagents, and 100 μL of activation solution, a calcium-enriched buffer that allows the activation of basophils, in a water bath at 37°C for 15 min. For negative and positive controls, suspected drugs are substituted with 20-μL PBS and 20-μL of monoclonal anti-IgE, a positive control.

Following the first incubation, a 100-μL of stop solution is added to each tube; it is an optimized EDTA-enriched buffer to stop activation, and then the mixture is vortexed gently. After staining, the fix (8% formic acid) and lyse (cyclic amine-based) solutions, which are provided separately in the Allergenicity Kit, are mixed and added to the samples (50-μL fix solution and 2-mL lysing solution for each tube). This step lyses red cells and fixes leukocytes, preserving basophil activation markers. Then the sample undergoes a second incubation at 18–20°C for 10 min. The sample is centrifuged for 5 min and the supernatant is aspirated. A wash step is performed with 3 mL of PBS, followed by another centrifuge. Finally, the cells are resuspended in 0.5 mL of PBS and analyzed by flow cytometry.

### *Flow cytometric analysis and gating strategy*

The application of BAT is based on flow cytometry, an analytical technique based on the principle of immunofluorescence. It allows the identification from cell suspension, the specific characteristics of each cell (shape, size, and granulation), and the expression of markers (extra and/or intracellular), following labeling with fluorochrome-conjugated antibodies.

In this study, flow cytometric data were acquired using the NAVIOS EX flow cytometer, available in the Immunology Department of the Military Hospital of Tunisia. This instrument is equipped with forward scatter (FSC), side scatter (SSC), and fluorescence detection channels suitable for the fluorochromes employed in this study, specifically Fluoresce in Isothiocyanate (FITC), Phycoerythrin (PE), and Phycoerythrin-cyanine 7PC7.

Prior to data acquisition, the flow cytometer was meticulously calibrated to ensure proper alignment and optimal color compensation. A minimum of 400 basophils were analyzed per sample to obtain statistically reliable results.

In gating strategy, the whole leukocyte population was first selected in FSC/SSC plot (to avoid loss of rare cells), CD3+ were excluded and the fraction FSCC/SSC/CD3− basophils were selected as CRTH2+ (Figure 1).

During this analysis, basophils were classified into two distinct populations:

- Resting basophils: identified by the phenotype $CRTH2^{+}CD3^{-}$ and $CD203C^{LOW}$;
- Activated basophils: identified by the phenotype $CRTH2^{+}CD3^{-}$ and $CD203C^{HIGH}$.

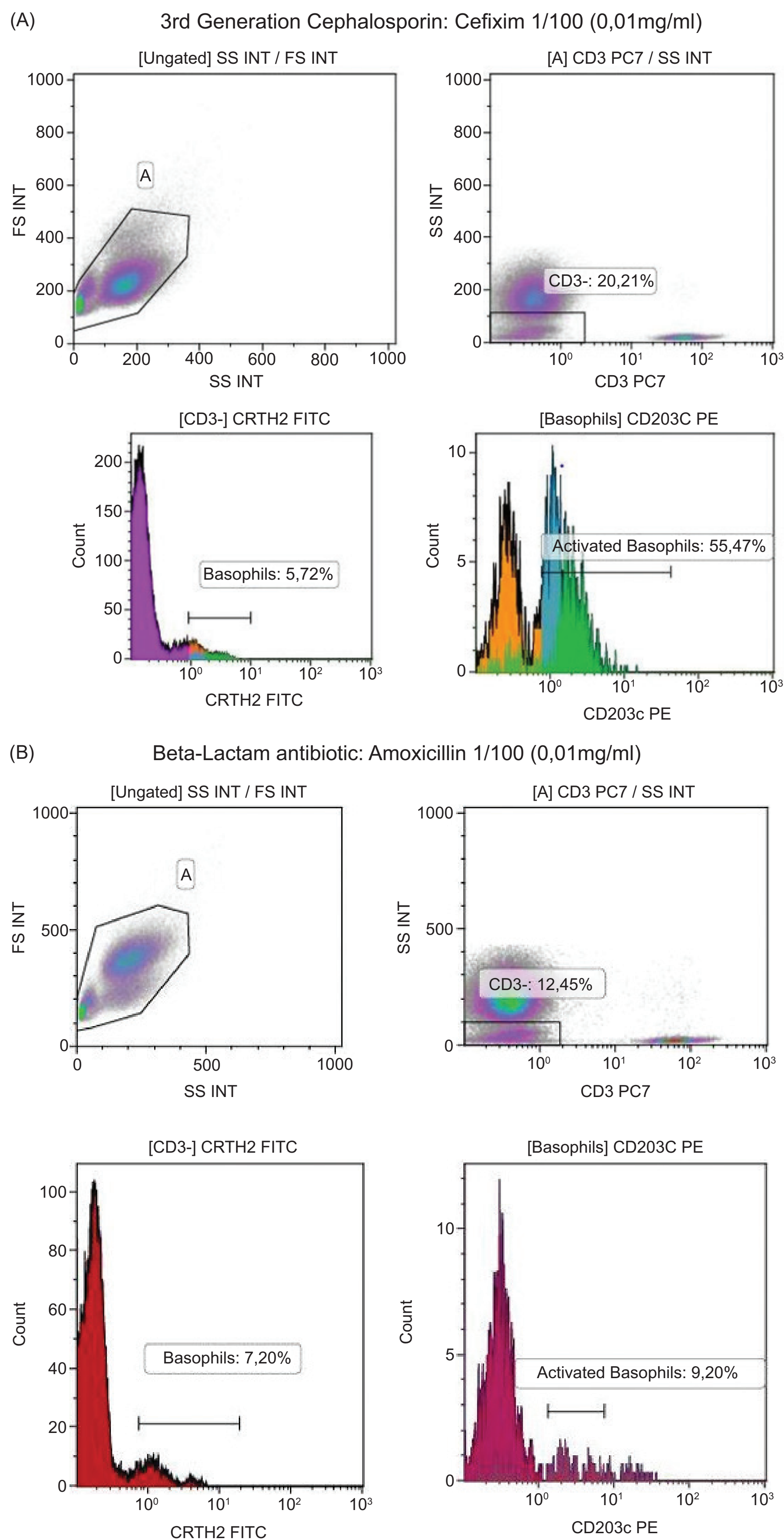


**Figure 1** Example of positive BAT tested with cefixime with a significant basophil activation upon exposure to tested drug at 1:100 dilution, in contrast to a negative BAT tested with amoxicillin treated with Kaluza C analysis software (Beckman Coulter®) evaluating the expression of CD203c.

The value of 55.47% shown in Figure 1 corresponds to the proportion of basophils (CRTH2+CD3-) that upregulated CD203c after stimulation (activated basophils are CRTH2+CD3- and CD203c$^{HIGH}$).

This gating strategy was employed to differentiate between basophils in their resting and activated states, thereby allowing for precise quantification of basophil activation in response to allergenic stimuli.

### *Data collection and analytical methods*

A total of 35 BATs were performed. The cohort comprised 42.8% females and 54.2% males. The mean age of female patients was 37.86±14.29 years, while the mean age of male patients was 56.32±9.65 years.

In terms of clinical manifestations, 74% of the patients reported general discomfort. Urticarial reactions were observed in 68.5% of patients, respiratory issues in 62.8%, and digestive disorders in 17%. Notably, only 7% of patients had a documented history of drug allergic reactions.

The drugs under investigation included antibiotics (56%), analgesics (17%), NSAIDs (9%), corticosteroids (6%), and other medications (12%). Among the drugs tested, BAT results were positive in only three instances: mefenamic acid (an NSAID), cefixime (an antibiotic), and deferasirox (an iron chelator).

Basophil activation test is an *in vitro* diagnostic assay designed to evaluate hypersensitivity reactions by measuring the percentage of activated basophils in response to suspected allergens or drugs.

To ensure the reliability of BAT results, it is recommended to analyze a minimum of 400 basophils.

The results of BAT are expressed as the percentage of activated basophils. The accurate interpretation of these results depends on comparisons with both negative and positive controls.

The interpretation of BAT results involves evaluating several key parameters; CDmax provides information on the maximum percentage of basophils activated in response to the tested substance, offering insights into the extent of basophil activation. Another crucial parameter is the stimulation index (SI), which is calculated as the ratio of the percentage of activated basophils following exposure to the suspected drug to the percentage of activated basophils in the negative control.

A BAT result is considered positive if SI ≥ 2.

### *Ethical considerations*

The Ethics Committee of the Military Hospital of Tunisia approved the protocol of this study and all research procedures complied with the ethical guidelines.

### *Statistical Analysis*

The statistical analysis of the data was conducted using the SPSS software version 26 (IBM SPSS Statistics, Somers, NY). Clinical and demographic parameters were summarized using descriptive statistics. For categorical variables, frequencies and proportions were calculated. Numerical data were summarized as means and standard deviation (SD) if normally distributed, or medians with interquartile ranges (IQR) if not normally distributed.

To facilitate the visualization of the data, boxplots were generated using Excel and GraphPad Prism version 5. Statistical significance was set at $P < 0.05$.

## Results

A total of 34 patients were included in this cohort, with 35 BATs applied, since one patient underwent two BATs for two different suspected drugs. In this study, we classified patients into two groups:

N1 group: It had symptoms with IH, such as urticarial reaction with general malaise, respiratory symptoms, and digestive symptoms.

N2 group: It exhibited reaction atypical of IH; in this case, IH was isolated general malaise.

Based on distribution, clinical symptoms are summarized in Table 2. In N1 group, 84.4% of patients were characterized with general malaise and urticarial reactions, 62.5% with respiratory symptoms, and 28.1% with digestive symptoms. Only 25% of patients in N1 group had a family history. In contrast, patients in N2 group suffered from general malaise only, without any other IH symptom.

Among the 34 patients, 32 patients were classified in N1 group, and N2 group had only three patients. The sensitivity of our study is around 9.4% (3/32) and the specificity reached 100% (3/3).

For this study, it is clear that antibiotics represented the most frequently drugs tested with BAT.

BAT is an *in vitro* diagnostic assay designed to evaluate hypersensitivity reactions by measuring the proportion of activated basophils in response to suspected allergens or drugs.

The results of BAT are expressed as the proportion of activated basophils. The accurate interpretation of these results depends on comparisons with both negative and positive controls.

**Table 2** Clinical symptoms of the studied population using N1 and N2 classification.

| Symptoms | N1 = 32 | N2 = 3 |
|---|---|---|
| General malaise | 27 (84.4%) | 0 (100%) |
| Urticarial reaction | 27 (84.4%) | 0 |
| Respiratory symptoms | 20 (62.5%) | 0 |
| Digestive symptoms | 9 (28.1%) | 0 |
| Family history | 8 (25%) | 0 |
| Positive BAT | 3 (9.4%) | 0 |

Note: Based on the distribution of clinical symptoms summarized, 84.4% of patients in N1 group were characterized with general malaise and urticarial reactions, 62.5% with respiratory symptoms, and 28.1% with digestive symptoms. Only 25% of the patients in N1 group had a family history. In contrast, patients in N2 group suffered from general malaise only without any other IH symptom.

BAT replicates an immunological reaction *in vitro*, and its results must be interpreted on an individual basis to ensure accurate diagnosis. This individualized approach accounts for variations in immune responses and the specific characteristics of the tested drug. The use of both negative and positive controls is essential for determining positivity threshold and ensuring the reliability of test results.

As demonstrated in Table 3, negative patients exhibited no significant activation across different dilutions, while for positive patients, the results demonstrated a biphasic dose-response curve as illustrated in Figure 2.

Based on this curve, the maximum of the activation CDmax was reached at 1:100 dilution for three tested drugs: mefenamic acid 1%, cefixime and deferasirox.

An example of positive BAT obtained with cefixime with significant basophil activation upon exposure to tested drug at 1:100 dilution, in contrast to negative BAT with amoxicillin, is shown in Figure 1. The tests were treated with Kaluza C analysis software (Beckman Coulter®) evaluating the expression of CD203c.

**Table 3** BAT results with mean ± SD for negative and positive patients across different dilutions.

| | Negative patients Mean ± SD | Positive patients Mean ± SD |
|---|---|---|
| Negative control | 9.24 ± 6.32 | 9.16 ± 1.43 |
| Positive control | 34.78 ± 2.14 | 26 ± 8.94 |
| Dilution 1/10 | 7.84 ± 5.69 | 35.73 ± 15.73 |
| Dilution 1/100 | 7.65 ± 5.57 | 60.86 ± 6.43 |
| Dilution 1/1000 | 7.32 ± 9.87 | 18.36 ± 15.27 |
| Dilution 1/10,000 | 6.68 ± 11.32 | 5.56 ± 0.49 |

Note: Negative patients exhibited no significant activation across different dilutions.

## Discussion

Recent advancements in technology have highlighted the BAT performed by flow cytometry as an effective and reliable tool for investigating various types of allergies, including food,[9] inhalant,[10-12] and drug allergies.[13,14]

The BAT is distinguished by its capacity to replicate an immunological reaction in *in vitro* setting.[1,2]

Over the years, several commercial kits have been used to perform BAT, including the Allergenicity Kit (Beckman Coulter®) used in this assay.

Performing BAT is based on several combinations for the diagnosis of allergy; these combinations include "CCR3/SCC, CRTH2/CD203c/CD3, CD203c/CD63, or even IgE/CD63.

CD63 and CD203c are considered appropriate markers for the analysis of basophils.[15,16] However, their activation pathways and kinetics are different; CD203c requires 5 min to reach its maximum activation, while CD63 upregulation peaks after 15 min. This difference suggests that these markers follow different signaling pathways.[17,18]

Using flow cytometry, CD63 and CD203c are the main activation markers used in BAT. CD63 is stored in intra-cytoplasmic granules, and through an energy-dependent IgE degranulation process, it appears on basophil membranes.[19] However, CD203c is expressed through non-IgE-dependent pathways. Based on comparative studies, CD63 seems to be more sensitive than CD203c in detecting basophil activation for IH to NMBAs.[20]

In the absence of a standardized protocol, BAT emerges as a dynamic and adaptable tool capable of capturing the intricacies of individual immune responses. As researchers and clinicians navigate the challenges posed by assays, the imperative to balance standardization and personalization remains at the forefront.

This study delves into the complexities of BAT, highlighting its mechanisms, analytical components, and the critical considerations involved in the quest for accurate and clinically relevant results.

The application of BAT has now extended to various types of type I allergies, and it can be used to diagnose food allergies and can be performed using extracts of food or even single allergens.[21] BAT has also proven to be an effective tool for diagnosing patients with respiratory allergies in several contexts, including patients with cystic fibrosis who develop allergic bronchopulmonary aspergillosis (ABPA).[22]

As a key biomarker, BAT is considered as a reliable tool for monitoring the effectiveness of allergen-specific immunotherapy across various allergy types, such as dust mites and peanuts.[23] Moreover, this test is used in venom immunotherapy, particularly for hymenoptera stings.[24]

This study represents the first application of BAT to drug allergies in Tunisia. The study included 34 patients, with a noted under-representation of the pediatric population and a predominance of male over female patients. Our findings indicated that 62.8% of the cases presented with skin reactions characterized by urticaria.

This condition was observed exclusively in female patients, suggesting a predominance of NMBA inducing hypersensitivity in females.[25] Antibiotics were the most commonly tested drugs (56%), particularly beta-lactams. This finding is consistent with the global literature and specifically with the studies conducted in Tunisia.[26,27] High prevalence of beta-lactams among tested drugs is attributed to their frequent prescriptions.

BAT presents unique challenges in the context of drug allergies, compared to other allergy types. Drugs can induce a wide range of immune reactions. Low molecular weight drugs are generally incapable of triggering an immunological response independently. However, they may become immunogenic when coupled with carrier proteins, forming a bivalent molecule that binds to adjacent IgE antibodies.

In this study, BSA was used as a carrier protein. However, as BAT is performed on whole blood, other plasma proteins may act as carrier proteins.

For instance, amoxicillin alone does not induce immunogenic reaction unless it is bound to a protein, a process involving the opening of a beta-lactam ring and creation of an antigenic determinant.[28,29]

It is important to note that this approach is based on injectable forms of medications. Therefore, a prior pharmacologic preparation with a defined drug concentration is recommended before performing BAT.[30]

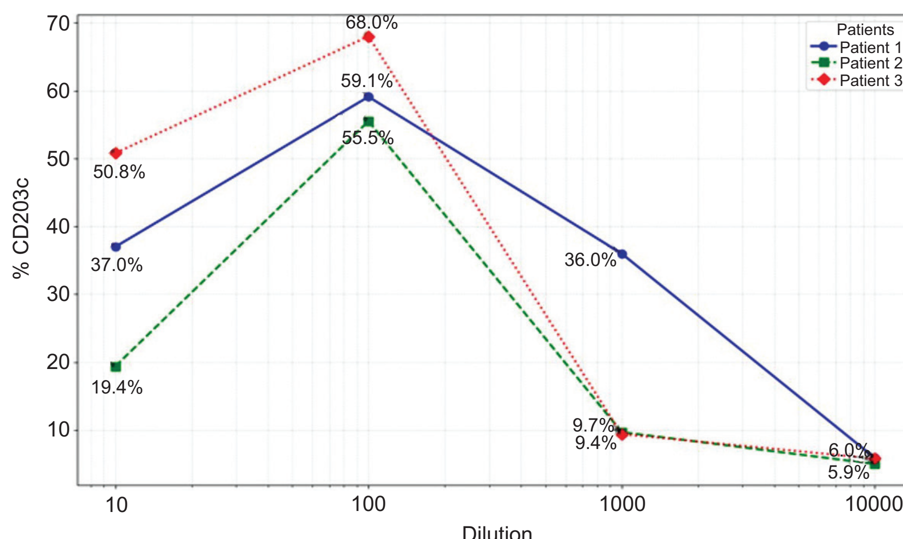


**Figure 2** Response of basophils to different drug dilutions in positive BAT cases. It includes a graph showing how basophil activation varies with dilution levels for each drug tested.

Compared to other types of allergies, such as food allergies, BAT exhibits lower sensitivity for drug allergies. Nevertheless, BAT remains a useful diagnostic tool. Sensitivity varies among different drugs and molecules.[31,32] For example, penicillin shows a high rate of positive BAT results,[33,34] whereas NSAIDs exhibit a lower positive rate, even in confirmed cases of allergy.[35] Additionally, sensitivity is influenced by the concentration of the drug used in BAT.[36]

Our study used wider ranges, and this point should be considered when interpreting our results. These factors must be considered to effectively integrate BAT in the diagnosis of drug allergies. The specificity of our study reached 100%; however, the sensitivity was considerably lower than 50%, the BAT's sensitivity for drugs generally mentioned in the literature. This lower value could be explained by the use of board range of dilutions in this work that may induce basophil toxicity at higher concentrations.

The results of BAT are expressed as proportion of activated basophils. Crucially, the interpretation of BAT results is based on a meticulous comparison with both negative and positive controls. This dual-control framework ensures a comprehensive understanding of basophil's response, considering both spontaneous and induced facets of activation. The inclusion of two negative controls, rather than one, could reduce variability and increase reproducibility of the activation index. Although only one negative control was applied in our study, this methodological refinement should be considered in future research. Moreover, the determination of positivity thresholds is a nuanced process, intricately linked to the specific drug being tested and the idiosyncrasies of each patient.

However, the identification of basophils that do not respond to a positive control introduces a critical consideration in the interpretation of BAT results, categorizing them as false negatives.[37] The potential explanations for this phenomenon are diverse, ranging from a recent exposure to an allergen to technical errors, such as incorrect manipulation.[38]

Moreover, the intricate dynamics of basophil behavior includes the presence of "non-releasing basophils," highlighting a subset of basophils that does not undergo the expected activation process. BAT is a pivotal assay in the field of immunologic diagnostics, notable for its complexity and the several challenges associated with its interpretation, primarily because of the current lack of standardization. This complexity stems from the need for a personalized approach in diagnosing IH to drugs, underscoring the imperative to tailor assessments to each unique patient profile.

This study was based on Allergenicity Kit, which provided CRTH2, CD3, and CD203c markers. Consequently, our strategy of gating relied on these markers. After stimulation, activated basophils identified were CD3-, CRTH2+, and CD203c$^{HIGH}$. However, in order to improve this gating strategy, it was necessary to minimize the percentage of eosinophils by using CRTH2+ with SCC-low (since eosinophils are SCC-high), or we gate from FSC/SCC graph only basophils present in the lymphocytes population, rather than gating the entire leukocyte population. Other method that could improve the refining of basophil identification and minimize eosinophil events is the use of other markers, such as CCR3 and CD123/HLADR.

Our study identified three patients with confirmed drug allergies to mefenamic acid, cefixime, and deferasirox based on clinical symptoms and BAT results. Other settings can be used to interpret BAT, such as "CDmax"; it indicates the concentration that induces a maximum rate of basophil activation. Other parameter that must be mentioned is stimulation index (BAT is considered positive if SI ≥ 2).

Testing across various dilutions of the suspected drug helps to establish a dose–response curve (Figure 2). In this study, for positive tests, the dose–response curve is a biphasic presentation; the first phase is with ascending form, at a very high concentration (0.1 mg/mL), the receptors of basophils are saturated, leading to an inhibitory effect. The second phase, where the maximum of activation is reached with 0.01 mg/mL, is a peak phase, and the

third phase is the descending phase, where the lower concentrations are insufficient to induce a significant response of basophils.[39,40]

It is crucial to acknowledge that medications contain both active substances and excipients. Consequently, a negative BAT result does not exclude the possibility of a drug allergy. Each molecule within a suspected drug must be considered potentially allergenic. To understand the main reason of drug allergy, it is important to know that the basic composition of drug includes two types of substances—the active ingredient, a chemical compound responsible for therapeutic effect, and the excipients.[41]

However, some excipient can cause allergic reactions or intolerances, such as mannitol, aspartame, sorbic acid and others.[42,43]

A limitation of our study is its relatively small sample size. Hence, to validate the effectiveness of BAT in diagnosing drug allergies, it is essential to compare BAT results with other diagnostic tests, such as skin tests, to evaluate the sensitivity and specificity of BAT and calculate test concordance.

Another important limitation of this study is the choice of the diluted range of tested drugs. While allergens, such as pollens, induce a broad activation profile, drug-induced basophil activation is generally limited to a narrow concentration range. However, using large dilutions may miss correct concentration, where activation is maximum and causes false negative results. In clinical practice, it is recommended to test drugs at three concentrations, each diluted by factor of five, in order to identify correct reaction range. Particularly, this aspect is relevant for atracurium, for which the recommended maximal concentration for both skin testing and BAT is approximately 0.05 mg/L.

In order to accurately validate the diagnostic performance of BAT, comparative analyses are needed using established diagnostic tools, including skin prick tests (SPT), intradermal tests (IDT), serum specific IgE tests, and drug provocation tests. These comparisons are crucial to assess the sensitivity and specificity of BAT in different clinical scenarios. Previous studies have shown varying degrees of correlation between BAT and other diagnostic methods; for example, BAT has shown high agreement with DPT and SPT in IgE-mediated reactions to beta-lactam antibiotics and NMBAs; however, its sensitivity may be lower in non-immediate reactions.[44]

## Conclusion

This study represents the first application of BAT as a drug allergy diagnostic in Tunisia. Our findings demonstrated that BAT effectively confirmed drug IH in suspected patients. However, increasing the number of participants and including other tests, such as tryptase measurements and SPT, could strengthen the reliability of our results.

Using CD203c as an activation marker proved its utility in BAT performance. Future studies, should combine CD203c with CD63 and include IL-3 as an activation stimulant to enhance basophil activation. It is also recommended to optimize gating strategy.

Using double negative controls for each test while avoiding high drug concentrations by applying three 5-fold dilutions could reduce false negatives and limit basophil toxicity.

Overall, our study emphasized the importance of incorporating BAT into diagnostic tool kit for drug allergies and encouraged further research to validate and refine its application in clinical settings.

## Mandatory Disclosure on Use of Artificial Intelligence

The authors declare that AI-assisted tools were used as follows: ChatGPT (OpenAI) was used exclusively for minor language correction and wording refinement. All references have been manually verified for accuracy and relevance.

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## Author Contributions

All authors contributed equally to this article.

## Conflict of Interest

The authors declared no potential conflict of interest with respect to research, authorship, and/or publication of this article.

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## References

1. Hemmings O, Kwok M, McKendry R, Santos AF. Basophil activation test: Old and new applications in allergy. Curr Allergy Asthma Rep. 2018;18:77. https://doi.org/10.1007/s11882-018-0831-5
2. Lei Y, Gregory JA, Nilsson GP, Adner M. Insights into mast cell functions in asthma using mouse models. Pulm Pharmacol Ther. 2013;26:532–9. https://doi.org/10.1016/j.pupt.2013.03.019
3. Johansson SGO, Hourihane JO'B, Bousquet J, Bruijnzeel-Koomen C, Dreborg S, Haahtela T, et al. A revised nomenclature of allergy: An EAACI position statement from the EAACI nomenclature task force. Allergy. 2001;56(9):813–824. https://doi.org/10.1034/j.1398-9995.2001.t01-1-00001.x
4. Holgate ST. The role of mast cells and basophils in inflammation. Clin Exp Allergy. 2000;30:28–32. https://doi.org/10.1046/j.1365-2222.2000.00093.x

5. Buhring HJ, Simmons PJ, Pudney M, Foster SJ, van der Walt J, Morrison C, et al. The monoclonal antibody 97A6 defines a novel surface antigen expressed on human basophils and their multipotent and unipotent progenitors. Blood. 1999;94(7):2343-56.
6. Ebo DG, Hagendorens MM, Bridts CH, De Knop KJ, Stevens WJ. The basophil activation test in immediate drug allergy. Acta Clin Belg. 2009;64(2):129-35. https://doi.org/10.1179/acb.2009.022
7. Sainte-Laudy J, Boumediene A, Touraine F, Orsel I, Cogné M. Analysis of IgE downregulation induced by basophil activation. Application to the diagnosis of muscle relaxant allergic hypersensitivity by flow cytometry. Inflamm Res. 2006;55(Supp I): S21-2. https://doi.org/10.1007/s00011-005-0023-3
8. Garvey LH, Ebo D, Mertes PM, et al. An EAACI position paper on the investigation of perioperative immediate hypersensitivity reactions. Allergy. 2019;74:1872-84. https://doi.org/10.1111/all.13820
9. Moneret-Vautrin DA, Sainte-Laudy J, Kanny G, Fremont S. Human basophil activation measured by CD63 expression and LTC4 release in IgE-mediated food allergy. Ann Allergy Asthma Immunol. 1999;82:33-40. https://doi.org/10.1016/S1081-1206(10)62657-9
10. Cozon G, Ferrandiz J, Peyramond D, Brunet J. Detection of activated basophils using flow cytometry for diagnosis in atopic patients. Allergol Immunopathol (Madr). 1999;27:182-7.
11. Hauswirth AW, Natter S, Ghannadan M, Wolf M, Krauth MT, Valenta R, Sperr WR. Recombinant allergens promote expression of CD203c on basophils in sensitized individuals. J Allergy Clin Immunol. 2002;110:102-9. https://doi.org/10.1067/mai.2002.125257
12. Paris-Kohler A, Demoly P, Persi L, Lebel B, Bousquet J, Arnoux B. *In vitro* diagnosis of cypress pollen allergy by using cytofluorimetric analysis of basophils (Basotest). J Allergy Clin Immunol. 2000;105:339-45. https://doi.org/10.1016/S0091-6749(00)90085-X
13. Abuaf N, Rajoely B, Ghazouani E, Bricard H, Nicolas JF, Nguyen QT. Validation of a flow cytometric assay detecting *in vitro* basophil activation for the diagnosis of muscle relaxant allergy. J Allergy Clin Immunol. 1999;104:411-8. https://doi.org/10.1016/S0091-6749(99)70386-6
14. Sanz ML, Gamboa PM, Antepara I, Fernández J, Gastaminza G, Sanz ML. Flow cytometric basophil activation test by detection of CD63 expression in patients with immediate-type reactions to betalactam antibiotics. Clin Exp Allergy. 2002;32:277-86. https://doi.org/10.1046/j.1365-2222.2002.01305.x
15. Kahlert H, Cromwell O, Fiebig H. Measurement of basophil-activating capacity of grass pollen allergens, allergoids and hypoallergenic recombinant derivatives by flow cytometry using anti-CD203c. Clin Exp Allergy. 2003;33:1266-72. https://doi.org/10.1046/j.1365-2222.2003.01756.x
16. Pâris-Köhler A, Demoly P, Persi L, Lebel B, Bousquet J, Arnoux B. *In vitro* diagnosis of cypress pollen allergy by using cytofluorimetric analysis of basophils (Basotest). J Allergy Clin Immunol. 2000;105:339-45. https://doi.org/10.1016/S0091-6749(00)90085-X
17. Bühring H-J, Simmons PJ, Pudney M, Müller R, Jarrossay D, van Agthoven A, et al. The monoclonal antibody 97A6 defines a novel surface antigen expressed on human basophils and their multipotent and unipotent progenitors. Blood. 1999;94:2343-56.
18. Buhring HJ., Streble A., Valent P. The basophil-specific ectoenzyme E-NNP3 (CD203c) as a marker for cell activation and allergy diagnosis. Int Arch Allergy Immunol. 2004;133:317-29. https://doi.org/10.1159/000077351
19. Mac Glashan Jr D. Expression of CD203c and CD63 in human basophils: Relationship to differential regulation of piecemeal and anaphylactic degranulation processes. Clin Exp Allergy. 2010 Sep;40(9):1365-77. https://doi.org/10.1111/j.1365-2222.2010.03572.x
20. Ene-Cocis M, Petrisor C, Sfichi M, et al. CD63 vs. CD203c for the diagnosis of neuromuscular blocking agents immediate-type hypersensitivity. Human Vet Med. 2020;12:65-9.
21. Santos AF, Douiri A, Bécares N, Wu SY, Stephens A, Radulovic S, et al. Basophil activation test discriminates between allergy and tolerance in peanut-sensitized children. J Allergy Clin Immunol. 2014;134:645-52. https://doi.org/10.1016/j.jaci.2014.04.039
22. Gernez Y, Waters J, Mirković B, Lavelle GM, Dunn CE, Davies ZA, et al. Blood basophil activation is a reliable biomarker of allergic bronchopulmonary aspergillosis in cystic fibrosis. Eur Res J. 2016;47:177-85. https://doi.org/10.1183/13993003.01068-2015
23. Varshney P, Jones SM, Scurlock AM, Perry TT, Kemper A, Steele P, et al. A randomized controlled study of peanut oral immunotherapy: Clinical desensitization and modulation of the allergic response. J Allergy Clin Immunol. 2011;127(3):654-60. https://doi.org/10.1016/j.jaci.2010.12.1111
24. Eržen R, Košnik M, Silar M, Korošec P. Basophil response and the induction of a tolerance in venom immunotherapy: A long-term sting challenge study. Allergy. 2012;67(6):822-30. https://doi.org/10.1111/j.1398-9995.2012.02817.x
25. Peck G, Hashim MJ, Shaughnessy C, Muddasani S, Elsayed NA, Fleischer AB. Global epidemiology of urticaria: Increasing burden among children, females and low-income regions. Acta Derm Venereol. 2021;101:adv00433.
26. Darragi MK, Daghfous H, Ben Mansour A, Slim A, Ben Saad S, Tritar F. Investigation of drug hypersensitivity in Tunisia. Rev Fr Allergol. 2023;63(3):103539. https://doi.org/10.1016/j.reval.2023.103539
27. Ghozzi H, Sahnoun Z, Hammami S, Chaabane N, Ben Hassine L. Adverse effects due to antibiotics: a retrospective study over three years at the Regional Pharmacovigilance Center of Sfax. Rev Tun Infectiologie. 2011;5(1):16-21.
28. Amsler E, Soria A. *Hypersensitivity reactions to beta-lactam antibiotics*. Rev Med Interne. 2017;38(11):737-748. https://doi.org/10.1016/j.revmed.2017.06.020
29. Ponvert C, LeBourgeois M, Scheinmann P, Paupe J. Allergic reactions to commonly used anti-infective drugs. Rev Fr Allergol Immunol Clin. 1999;39(5):455-467.
30. Mayorga C, Çelik GE, Pascal M, Campana R, de Chaisemartin L, Netchiporouk E, Klossek JM, et al. Flow-based basophil activation test in immediate drug hypersensitivity. Allergy. 2024;79(3):580-600.
31. Knol EF, Mul FP, Jansen H, Calafat J, Roos D. Monitoring human basophil activation via CD63 mono-clonal antibody 435. J Allergy Clin Immunol. 1991;88:328-38. https://doi.org/10.1016/0091-6749(91)90094-5
32. Hoffmann HJ, Santos AF, Mayorga C, Eberlein B, Heeringa M, de Weck AL, et al. The clinical utility of basophil activation testing in diagnosis and monitoring of allergic disease. Allergy 2015;70:1393-405. https://doi.org/10.1111/all.12698
33. Fernandez TD, Mayorga C, Salas M, Doña I, Torres MJ, Blanca M. Evolution of diagnostic approaches in betalactam hypersensitivity. Expert Rev Clin Pharmacol. 2017;10:671-83. https://doi.org/10.1080/17512433.2017.1313110
34. McGowan EC, Saini S. Update on the performance and application of basophil activation tests. Curr Allergy Asthma Rep. 2013;13:101-9. https://doi.org/10.1007/s11882-012-0324-x
35. Sturm GJ, Kranzelbinder B, Sturm EM, Heinemann A, Groselj-Strele A, Aberer W. The basophil activation test in the diagnosis of allergy: Technical issues and critical factors. Allergy. 2009;64:1319-26. https://doi.org/10.1111/j.1398-9995.2009.02004.x

36. Cañas JA, Pérez-Sánchez N, Lopera-Doblas L, Moreno Aguilar C, Torres MJ, Blanca M, et al. Basophil activation test utility as a diagnostic tool in LTP allergy. Int J Mol Sci. 2022;23:4979. https://doi.org/10.3390/ijms23094979
37. Ebo DG, Bridts CH, Hagendorens MM, De Clerck LS, Stevens WJ. Basophil activation test by flow cytometry: Present and future applications in allergology. Cytomet B Clin Cytomet. 2008;74B:201–10. https://doi.org/10.1002/cyto.b.20419
38. Ebo DG, Sainte-Laudy J, Bridts CH, Mertens CH, Hagendorens MM, De Clerck LS, et al. Flow-assisted allergy diagnosis: Current applications and future perspectives. Allergy. 2006;61:1028–39. https://doi.org/10.1111/j.1398-9995.2006.01039.x
39. MacGlashan DW Jr. Basophil activation testing. J Allergy Clin Immunol. 2013 Oct;132(4):777–87. https://doi.org/10.1016/j.jaci.2013.06.038
40. Santos AF, Lack G. Basophil activation test: Food challenge in a test tube or specialist research tool? Clin Transl Allergy. 2016;6(1):10. https://doi.org/10.1186/s13601-016-0098-7
41. Dureuil A. *Pharmaceutical industry: a difficult market to regulate. Industrie.com* [online]. 2010 Dec 1. Available at: http://www.industrie.com
42. Rowe RC, Sheskey PJ, Quinn ME, editors. Handbook of Pharmaceutical Excipients, 6th ed. London, UK & Washington, DC: Pharmaceutical Press & American Pharmacists Association; 2009.
43. European Medicines Agency (EMA). Human regulatory – Marketing authorisation – Excipients labelling - EMA. [online]. Available at: http://www.ema.europa.eu
44. Sudheer PS, Hall JE, Read GF, Rowbottom AW, Williams PE. Flow cytometric investigation of perianaesthetic anaphylaxis using CD63 and CD203c. Anaesthesia. 2005;60:251–6. https://doi.org/10.1111/j.1365-2044.2004.04086.x