Chapter 4

The Performance of Flowcytometry-Assisted Basophil Activation Tests in the Diagnosis of Drug-Induced Immediate-Type Hypersensitivity Reactions

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Abstract

Background: Flowcytometry-assisted basophil activation tests (BAT) are useful in vitro diagnostic tools for drug-induced immediate-type hypersensitivity.

Objective: The study aimed to perform a medical literature review on the performance of CD63 BAT.

Results: Seven studies for antibiotics, eleven for neuromuscular blocking agents (NMBAs), and 10 for nonsteroidal anti-inflammatory drugs (NSAIDs) were included. The reference standards such as history, skin tests, challenge tests, and/or IgE, along with the concentrations, varied among studies. The thresholds for BAT positivity varied from basophils in the range greater than 4–15%, a net percentage of activated basophils greater than 5% or a stimulation index ranging 1.76–1.85, to the use of more complex composite indexes for NMBAs. For antibiotics and NSAIDs, a stimulation index > 2 was generally chosen. BAT sensitivity for β-lactams was 33–55%, while specificity was 79–93.3%. For NMBAs, BAT sensitivity was 36–92%, while specificity was 93–100%. For NSAIDs, BAT sensitivity was 11.7–61% and specificity was 74–100%.

Discussion: BAT for the diagnosis of drug hypersensitivity has good specificity, but only moderate sensitivity. Despite the shortcomings and the methodological differences in BAT, the potential benefits to avoid challenge tests and increase the allergologic survey sensitivity have led to their widespread use in the clinical practice and warrant their future standardization.

Keywords: Basophils, Drug, Hypersensitivity, Sensitivity, Specificity

1. Introduction

Flowcytometry-assisted basophil activation tests (BAT) are useful functional in vitro diagnostic tools available to confirm drug-induced immediate-type hypersensitivity reactions. In order to confirm previous hypersensitivity reactions caused by drugs and to identify safe alternatives, rapid and reliable diagnostic tests are needed.

Drug-induced immediate-type hypersensitivity reactions include localized skin rashes, pruritus, and urticaria as minor manifestations, while angioedema, bronchospasm, hypotension, and cardiac arrest are the possible severe life-threatening clinical manifestations [1]. Thus, the main characteristics of the retrospective diagnostic tests should be a high sensitivity to identify and confirm the previous clinical reaction in order to avoid further re-exposure and a high specificity, drug hypersensitivity representing a low-prevalence disease.

The retrospective diagnosis is established by performing a comprehensive allergological survey that includes the history, the allergological work-up, and laboratory diagnostic tests. In vivo comprising the skin prick tests, the intradermal tests, and the challenge tests carry the risk of exposing the patient to the potential culprit drug, cannot be performed in patients who reject their performance, and in pregnant women. They remain, however, the main diagnostic tests in the investigation guidelines and the reference tests for the detection of the in vitro tests' performance.

The current guidelines recommend drug-specific IgE antibody dosing as the main in vitro diagnosis [2–4]. However, the performance of such tests varied in previous reports and the accuracy is not 100%. Moreover, serum total IgE influences the results of the assays for β-lactam-specific antibody dosing [5]. For the neuromuscular blocking agents (NMBAs), previous studies reported that healthy controls might have uneventful exposures despite positive-specific IgE for muscle relaxants [6, 7].

In the last two decades, studies have focused on the cellular tests: the histamine release tests and the basophil activation tests, which can confirm IgE- and non-IgE-mediated reactions. Flowcytometry-assisted basophil activation tests are useful in vitro diagnostic tools available to confirm drug-induced immediate-type hypersensitivity reactions. The principle of BAT is to quantify, after the basophils come in contact with the allergen, the upregulation of certain activation markers on the cellular surface by staining with specific fluorescent monoclonal antibodies. The use of BAT allows the rapid confirmation of the previous reaction to the culprit drug (the results can be obtained in less than 3 h), together with the avoidance of another exposure during testing. The performance of BAT for drug hypersensitivity has moderate to low sensitivity, ranging from 33% to 75%, while the reported specificity displayed values >80% [1, 3, 8–11]. However, the studies are not comparable as the inclusion criteria for the study populations, as well as the reference tests varied. Few single-drug studies have been published to investigate the most discriminative threshold to accurately identify patients. The testing methodology and drug concentrations varied from one study to another. No standardized technique currently exists for BAT, but recent advances have improved the performance of this assay [3].

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In drug hypersensitivity reactions, extensive research needs have been identified. Among them, one can find the standardization of the test procedures, the development of new diagnostic tools, and the improvement of the already available ones, together with the development of multicenter studies on drug hypersensitivity diagnosis [12].

Despite the shortcomings of the basophil activation tests, their benefit in patients with severe reactions who might avoid challenge tests or the potential to increase the allergologic survey sensitivity have led to their widespread use in the clinical practice and warrant their future standardization.

2. Objectives

In the context of large variability in previous studies on BAT using CD63 as activation marker on the surface of the basophils to quantify the intensity of the immune response that characterizes drug-induced anaphylaxis, the objectives of this review are to underline the methodological differences in previous studies, to identify the optimal drug concentrations to be used in BAT, the optimal incubation conditions, the optimal time frame for testing, and to approximate BAT's performance in terms of sensitivity and specificity.

3. Methods

We performed a scientific literature review on BAT for drug-induced immediate-type hypersensitivity reactions by searching several medical electronic libraries (PubMed, Web of Knowledge, EBSCO, and EMBASE) using keywords such as basophil activation test, drug allergy, drug hypersensitivity, sensitivity, and specificity, in order to identify the published studies on CD63 BAT. In addition, other papers were selected from the references of the previously identified studies. The key elements we searched for included the methodology of BAT, the drug concentrations that were used in BAT, the incubation conditions, the time frame for testing, and BAT's performance in terms of sensitivity and specificity. All studies upon drug-induced immediate-type hypersensitivity reactions were included: antibiotics, nonsteroidal anti-inflammatory drugs (NSAIDs), and NMBAs, as well as reports on miscellaneous rare drug allergies. We excluded review papers, letters to the editors, and editorials.

4. Results

Using the keywords such as basophil activation test, drug allergy, drug hypersensitivity, sensitivity and specificity, we identified over 20 review papers, editorials, and invited commentaries, which we excluded, together with all fundamental in vitro studies on BAT for drugs, in which sensitivity and specificity were not assessed. We included seven papers for antibiotics, eleven studies for NMBAs, and ten studies for NSAID's immediate-type hypersensitivity reactions, all of which evaluated the performance of BAT.

4.1. Antibiotic agents

For antibiotics, we identified seven studies in which BAT sensitivity and specificity were assessed for β-lactams. The reference tests that were considered for comparison varied from history only to the performance of the skin tests and the challenge tests and even to the inclusion of drug-specific IgE dosing (**Table 1**). The optimal threshold for positivity was generally a stimulation index (SI) >2 and a percentage of activated basophils >5%, as identified by the receiver operating characteristic (ROC) curve analysis in several studies [13–15]. It was demonstrated by performing the ROC curve analysis that the SI varies from 1.2 to 3, depending on the tested drug and its concentrations [14]. There was a wide variability concerning the tested drug dilutions. For example, for penicillin G, the tested dilutions were 2 and 0.4 [14–16], 1, 0.1, and 0.01 [17], 3.9 and 0.975 [13], and 2, 0.2, and 0.02 mg/mL [18]. The sensitivity of BAT for β-lactam antibiotics was found to be 33–55%, while the specificity was higher 79–93.3%.

Reference	Threshold for positivity	Se%	Spe%	N	Reference
H, ST	SI > 2, Ba% > 5%, **ROC**	50	93	88	[13]
H, ST, IgEs	SI > 2, Ba% > 5%	49	91	110	[16]
H, ST	CN+6%	33	79	41	[17] **amoxicillin**
H, ST, IgEs	SI > 2, Ba% > 5%, **ROC**	48.3	88.9	262	[14]
H, (ST-)	SI > 2, Ba% > 5%	39.1	93.3	53	[19]
H, ST	SI > 2, Ba% > 5%, **ROC**	55	80	39	[15] **cefuroxime**
H, ST	SI > 1.97, Ba% > 5% - **ROC**	51.4	90.3	68	[18]

H, history; ST, skin tests; IgEs, drug-specific antibodies; SI, stimulation index; Ba%, percentage of activated basophils; ROC, receiver operating characteristic curve analysis used in the study methodology; Se%, sensitivity; Spe%, specificity; N, number of patients and healthy controls included.

Table 1. The performance of BAT for antibiotics.

4.2. NMBAs

For NMBAs, our search retrieved 11 studies reporting the sensitivity and specificity of BAT (**Table 2**). BAT methodology varied between the studies, with one using both CD63 and CD203c as activation markers [20]. BAT sensitivity was 36–92%, depending on the drug to be tested (the highest value was obtained in the study by Ebo et al. for rocuronium [21]), on the severity of the reactions (patients with severe anaphylaxis displaying more often positive results compared to those with cutaneous reactions), as well as on the time frame between the hypersensitivity reaction and testing (higher values were obtained with a time lapse below 3.5 years in the study by Kvedariene et al. [22]). BAT specificity was 93–100%. Studies on BAT for NMBAs initially included patients with immediate-type hypersensitivity to all NMBAs [20, 22–25]. We found that three drug-specific studies (including ours) used ROC curve analysis [21, 26, 27]. The thresholds used for the positivity of BAT varied from activated basophils

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ranging >4–15%, a net percentage of activated basophils >5% or an SI >1.76–1.85, to the use of more complex composite indexes [28]. There were also differences in the concentrations of NMBAs to be tested, with all research teams using several dilutions starting from the NMBAs available on the market. Optimal concentrations of 500 μg/mL rocuronium and atracurium were identified in drug-specific studies [21, 27].

Reference test	Se%	Spe%	N	Threshold for positivity	Reference
H	64	93	62	↑Ba% > 15%	[23]
H, ST	54	100	60	↑Ba% > 10%	[24]
H	44.44	100	31	↑Ba% > 10%	[20]
H, ST	36-86	93	92	↑Ba% > 15%	[22]
H, ST	92	100	22	Ba% > 4%- **ROC**	[21] **rocuronium**
H, ST, IgEs	68	100	49	Composite index	[28]
H, ST	80	96	104	Net percentage > 4%	[8]
H, ST	68.18	100	56	SI > 1.76, Ba% > 5%- ROC	[25]
H	42.9	90	35	SI > 2	[29]
H, ST	63	100	15	Net percentage > 5%	[13]
H, ST	66.66	100	27	SI > 1.85, Ba% > 5%-**ROC**	[26] **atracurium**

H, history; ST, skin tests; IgEs, drug-specific antibodies; SI, stimulation index; Ba%, percentage of activated basophils; ROC, receiver operating characteristics curve analysis used in the study methodology; Se%, sensitivity; Spe%, specificity; N, total number of patients and healthy controls included.

Table 2. The performance of BAT for NMBAs.

4.3. Nonsteroidal anti-inflammatory drugs (NSAIDs)

We identified 10 studies on aspirin and multiple NSAID-induced hypersensitivity syndrome and three studies on metamizole hypersensitivity (**Table 3**).

History alone or history and the challenge tests represented the reference tests for multiple drug-induced hypersensitivity, starting from the identification of the sensitivity and specificity of BAT [30–34]. In the four studies including ROC curve analysis, an optimal SI >2 and a percentage of activated basophils >5% were found to be most discriminative [30, 32–34]. Using these criteria, the sensitivity of BAT varied widely from 11.7 to 61%, depending on the drug that was tested and the concentrations that were used in the test, while the specificity was 74–100% (**Table 3**).

For metamizole, there were three studies to assess BAT sensitivity and specificity [35–37] (**Table 3**). In the three studies that included ROC curve analysis to identify the optimal cutoff for positivity, SI varied from an SI >5 [35] to an SI >2 [37]. We identified an optimal SI >1.71 from the ROC analysis. Using this lower cutoff, we obtained a sensitivity of 70%, the highest recorded (**Table 3**) [36]. The methodology of BAT differed greatly concerning the drug

dilutions that were tested. Gamboa et al. used 5 and 1 mg/mL and Gomez et al. 2.5 and 0.25 mg/mL, while we used lower concentrations such as 25, 2.5, and 0.25 μg/mL [36]. The specificity of BAT for metamizole was 85.71–100%.

Reference test	Se%	Spe%	N	Threshold for positivity	Reference
H, ST, PT	42	100	56	SI > 5 - **ROC**	[35] **metamizole**
H,ST	54.9	85.71	107	SI > 2, Ba% > 5% - **ROC**	[37] **metamizole**
H, ST	70	100	30	SI > 1.71 - **ROC**	[13] **metamizole**
H, PT	15-55	74-100	90	SI > 2, Ba% > 5% - ROC	[30]
H	43	100	72	SI > 5	[31]
H, PT	11.7-41.7	93.3-100	90	SI > 2, Ba% > 5%	[32]
H,PT	76.7	80	45	Ba > 4.58 - ROC	[38]
H	61	91	29	SI > 2, Ba% > 5% - **ROC**	[13]
H	37	90	80	SI > 2, Ba% > 5% - **ROC**	[34]
H,PT	21.7-55.5	51.1-91.1	91	SI > 2, Ba% > 5%	[39]

H, history; ST, skin tests; IgEs, drug-specific antibodies; SI, stimulation index; Ba%, percentage of activated basophils; ROC, receiver operating characteristic curve analysis used in the study methodology; Se%, sensitivity; Spe%, specificity; N, number of patients and healthy controls included.

Table 3. The sensitivity and specificity of BAT for NSAIDs.

5. Discussion

5.1. Epidemiologic characteristics of drug-induced hypersensitivity and allergologic diagnosis

Drug allergy is a significant health problem with serious consequences of diagnostic errors [8, 40]. Most frequently, immediate-type hypersensitivity reactions triggered by drug agents are caused by β-lactam antibiotics and NSAIDs in the general population [41].

Anaphylaxis in the setting of anesthesia is a rare, but potentially lethal, event [42]. NMBAs account for a significant number of intra-anesthetic drug hypersensitivity reactions, followed by antibiotics, hypnotics, analgesics, latex, and chlorhexidine, the latter being frequently overlooked [43–46]. The investigation of adverse reactions to drugs administered in anesthetic conditions and in the perioperative period should be performed in specialized centers and in close collaboration with anesthetists [2, 47].

The diagnosis of an immediate-type hypersensitivity begins with the clinical history and physical examination, confirmed through skin tests and allergen specific IgE measurement [48, 49]. After more than a century of relying on skin testing and 40 years of relying on quantification of specific IgE antibodies in drug allergy diagnosis, clinicians now have access to flow-

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assisted quantification of activated basophils [50]. To date, BAT is used in more than 50% of the drug allergy centers [51].

5.2. The principle for the basophil activation tests for drugs

Basophils are the rarest granulocytes and represent one of the most enigmatic cells, whose precise biological roles in allergic responses and immune regulation are still unclear [52–55].

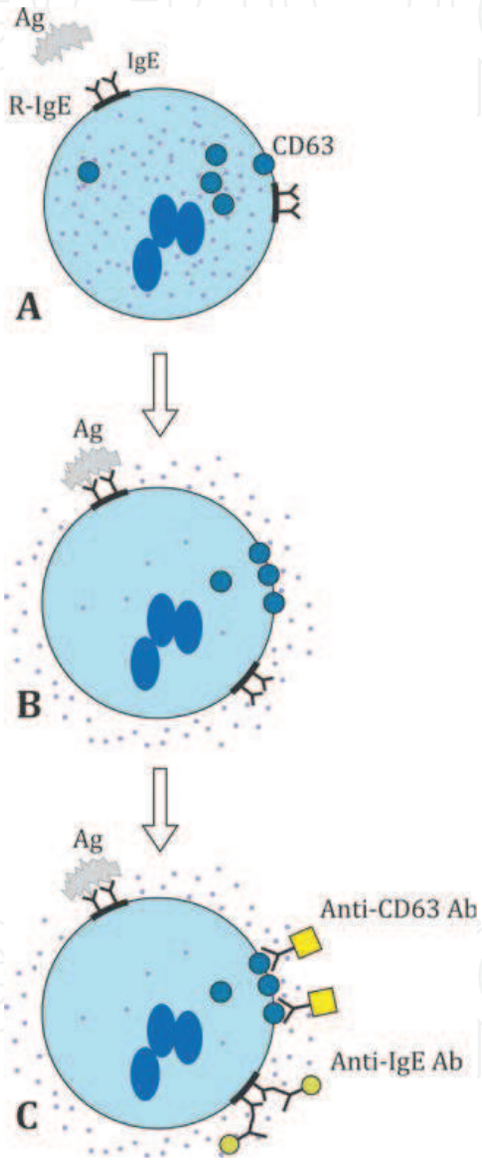

Figure 1. The basophil activation test steps: (A) basophils in contact with the culprit drug in vitro; (B) basophil degranulation after the contact with the allergen, the expression of CD63 activation markers on the cellular membrane; (C) identification of the reactive cells after double staining with monoclonal antibodies. Ag, antigen; IgE-R, the surface receptor for IgE; Ab, antibody. Adapted from [59].

The intensity of the immune response that characterizes anaphylaxis can be detected in vitro when the blood basophils (the key cells in peripheral blood involved in the development of

anaphylaxis) are exposed to the culprit drug, the antigen. Thus, BAT represents a functional diagnostic tool [56]. The activation of the basophils involves complex immunological mechanisms, with intracellular changes followed by the release of the mediators in the systemic circulation. CD63 is a transmembrane protein which is anchored in the basophil membrane and is exposed on the cellular surface upon activation [20, 57, 58]. The principle of BAT is to quantify, after the basophils come in contact with the allergen, the upregulation of certain activation markers such as CD63 on the cellular surface by staining with fluorescent, specific monoclonal antibodies (**Figure 1**, adapted from [59]).

CD63 is expressed on the surface of the basophils upon contact with the allergen. CD203c is constitutively expressed on basophils. Both markers have been studied, but most published data have included CD63 flow-assisted BAT [40, 56, 60].

5.3. Flow-assisted basophil activation test: technique

Different protocols and commercial kits are available for BAT [40]. We described the basophil activation test with the quantification of the CD63 activation marker on the surface of the basophils using monoclonal antibodies [61], as follows:

1. Fresh whole blood is needed for the assay and collected into K-EDTA tubes, from a peripheral vein, without using a tourniquet.
2. The assay is performed immediately, without storing blood. We used stimulation buffer as a negative control and anti-FcεRI solution (specific monoclonal antibody for the IgE receptor) and FMLP solution (N-Formyl-Met-Leu) as positive controls. Blood samples are exposed to the culprit drug.
3. Following basophil stimulation with the culprit drug, 20-μL staining reagent and two monoclonal antibodies are added in each tube. We used anti-CCR3-PE (human chemokine receptor coupled with phycoerythrin) and anti-CD63-FITC (a glycoprotein expressed on activated basophils coupled with fluorescein isothiocyanate) as staining reagents.
4. Incubation time is 15 min at 37°C. A prewarmed lysis solution of 2 mL is added to each tube and further incubation lasts for 10 min at room temperature. The samples are centrifuged. Afterwards, the cells are suspended in 300 μL wash buffer.
5. The upregulation of CD63 marker on the basophils is measured. The flowcytometry technique, which implies the detection of forward and side scatter, necessitates the analysis of at least 500 cells in drug allergy diagnosis. The basophils are characterized by low side scattering, and the percentage of CD63-positive cells compared to the total amount of gated basophils is calculated (**Figure 2**, adapted from [61]).
6. The results can be expressed as the percentage of activated basophils, the stimulation index (SI), or as the net percentage of activated basophils. SI is calculated as the percentage of activated basophils after stimulation with the culprit drug divided by the negative control. The net percentage of the activated basophils is calculated by subtracting the negative control from the value obtained with drug stimulation. An additional criterion

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necessitates the percentage of activated basophils to be >5% in order to avoid small, unspecific stimulation.

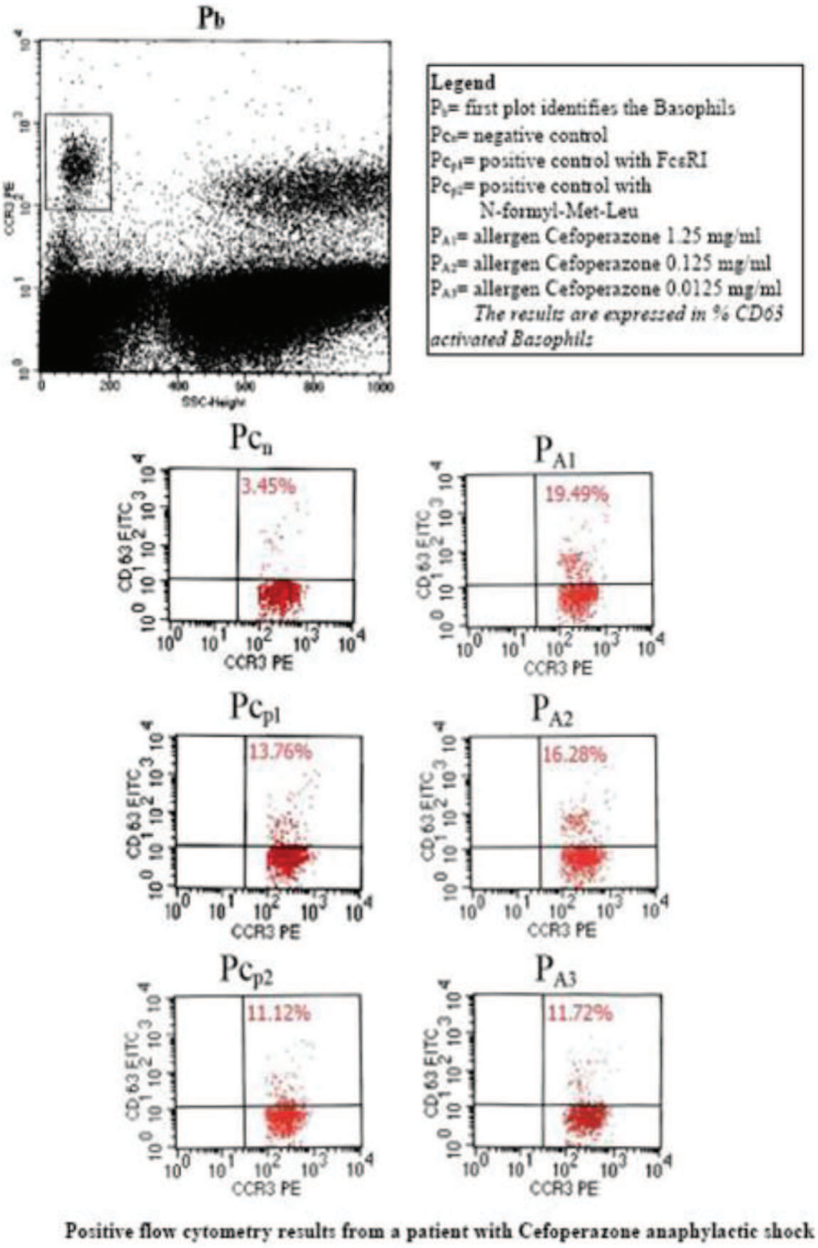

Figure 2. The basophil activation test results in a patient with previous intraanesthetic anaphylactic shock caused by cefoperazone. Adapted from [61].

5.4. Potential benefits and technical challenges in flowcytometry techniques

BAT is a rapid test (results are available within 3 h), and the amount of blood that is required is minimal. BAT reproduces in vitro the immunological hypersensitivity reactions that are induced in vivo when the patient is exposed to the culprit drug. Thus, potentially

dangerous re-exposures during the skin tests or the challenge tests are avoided. BAT allows the simultaneous evaluation of several drugs, including therapeutic alternatives and cross-reactive compounds. It can be used in pregnant patients and in those with dermographism, when in vivo tests are contraindicated or the results cannot be interpreted. It is useful for the investigation of clinical reactions that were suspected to be caused by histamine-releasing drugs such as atracurium and morphine, for which the skin tests are false positive due to the local release of histamine. BAT could confirm IgE- and non-IgE-mediated immediate-type hypersensitivity reactions.

The technical challenges of BAT, which restrict its use to specialized centers, are as follows:

- There is no activation marker with 100% specificity for the basophils.
- There is wide interindividual variability regarding the density of IgE receptors on the surface of the cells.
- The minimum number of gated basophils is 200, as this cell population can present variable responses to stimulation.
- The flow analysis is temperature dependent, requires an optimal incubation period (15–20 min), and requires specific buffer solutions and drug concentrations.
- The use of anti-IgE antibodies can stain other cells such as monocytes, eosinophils, and dendritic cells, which present the IgE receptors on their surface.
- Anti-IgE and IL-3, which is used for priming, can activate basophils in high doses.
- The standardization of the techniques is difficult to achieve.

5.5. Current knowledge on the performance of BAT for drug hypersensitivity in view of the STARD criteria

The evaluation of the performance of diagnostic tests, expressed in terms of sensitivity and specificity, can be done using the receiver operating characteristics curves (ROC curves) (**Figure 3**, adapted from [18]) [62]. Using this first method, an optimal threshold is identified, together with the test's sensitivity and specificity [26]. Another method to estimate the diagnostic accuracy would be to calculate sensitivity by dividing number of patients with positive BAT by the total number of investigated patients, while specificity is calculated as the ratio between the number of controls with negative BAT divided by the total number of healthy controls, assuming that the threshold for positivity is well-known and accepted. Both methods have been used in the diagnosis of immediate-type hypersensitivity reactions triggered by drugs. The latter method has been used for basophil activation test, using the cutoff identified for other allergens and antibiotics in the studies on other drugs, which rarely cause drug hypersensitivity, such as the NMBAs [8, 25]. The diagnostic accuracy of the tests could be improved by applying specific cutoffs. For drug allergy diagnosis, it is essential that an adequate number of allergic patients and healthy controls are included in the ROC analysis to determine the most discriminative thresholds for positivity [40, 42].

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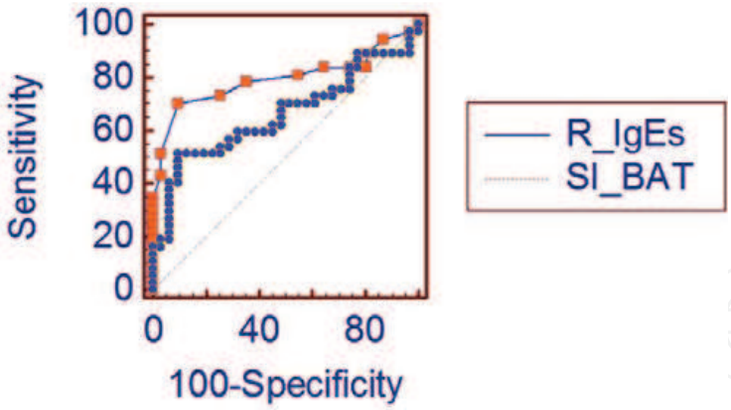

Figure 3. Receiver operating characteristics (ROC) curves for two in vitro techniques: radioimmunoassay (R) and flow-cytometry (BAT) for β-lactam antibiotic hypersensitivity. Adapted from [18].

The significance of the methodology by which we evaluate BAT performance is remarkable:

- The diagnostic tests for drug-induced immediate-type hypersensitivity reactions need a high sensitivity. A low sensitivity would mean a high number of false-negative results, and consequently the patients could subsequently be at risk upon re-exposure to the culprit drug. False-negative diagnosis can put these patients at risk for severe allergic reactions [17].
- As drug hypersensitivity is a low-prevalence disease, the specificity has to be high as well. A high specificity rarely generates false-positive results and guarantees that a positive result has clinical significance. False-positive results would be disadvantageous as they would result in drug avoidance.

5.6. Allergological survey for the retrospective diagnosis of drug-induced immediate-type hypersensitivity reactions: the reference tests

The retrospective diagnosis of immediate-type hypersensitivity reactions caused by drug agents is established after the performance of a complex allergological survey that includes the history, the allergologic tests, and drug-specific IgE dosing [2].

The history is not always reliable and the differential diagnosis is complex, especially for intra-anesthetic hypersensitivity reactions, when several drugs are administered simultaneously. Skin testing is the most widely used method to determine sensitization [2]. The skin tests have not been evaluated regarding their diagnostic accuracy and need to be performed in an optimal time frame after the reaction [42]. Testing within 4–6 weeks might give false-negative results, as well as testing after several years. The sensitivity and specificity of skin testing can be variable [2, 20]. Thus, the optimal time frame for in vivo studies would be 6 weeks to 6 months after the clinical reaction. Moreover, several categories of patients including pregnant women cannot be skin tested due to the severity of the reaction (patients with previous severe anaphylaxis might not even give consent), as in vivo studies always carry the risk of re-exposure to the culprit drug. The challenge tests cannot be recommended for daily routine, as they are difficult to perform, the standardization is difficult, and they carry the risk of a

potentially dangerous re-exposure. Their use is hampered by ethical and practical limitations [40].

The evaluation of the serum tryptase concentration can be carried out in the first few hours following the clinical reaction. While an immediate-type hypersensitivity reaction can be confirmed when the clinical signs are highly suggestive, there are some issues regarding the interpretation of serum tryptase measurement: there is no definitive agreement upon the cutoff value, there can be false-positive results in mastocytosis or severe hypoxemia, as well as false negative when there is sudden cardiac arrest with no or limited cardiac output and blood flow to bring tryptase from the peripheral tissues in the central circulating blood volume [63, 64].

The detection of IgE-type drug-specific antibodies is currently recommended by the clinical guidelines, even though their absolute value is not 100%. They are the most widely used in vitro tests to confirm drug hypersensitivity retrospectively. For β-lactam antibiotics, the results of the tests are altered by the value of the total serum IgE [5], while for NMBAs, specific IgE antibodies were identified in the serum of normal healthy controls, with no clinical reaction upon exposure [6, 40, 65].

None of the available diagnostic methods demonstrates absolute accuracy and the lack of a perfect gold standard test is one of the major problems in drug allergy diagnosis. Therefore, it is difficult to investigate the performance of BAT [8, 27, 42].

Due to the limitations of these diagnostic methods, the performance of cellular functional studies has been introduced in the last two decades. Over 50% of the drug allergy centers use the basophil activation tests in the diagnostic algorithms [51].

5.7. BAT for antibiotics hypersensitivity

Characteristic of antibiotics, loss of sensitivity naturally occurs over time. First, several years after a clinical reactions, the results of the skin tests may be negative and the patient might need to undergo challenge tests. Second, the in vitro diagnosis is challenging as the determination of specific IgE antibodies is only available for a limited number of antibiotics and the immunoassays do not show an absolute accuracy [15, 40].

β-Lactam antibiotics are the drugs that most frequently produce immediate-type hypersensitivity reactions [13, 17]. This explains the fact that the studies on BAT sensitivity and specificity for β-lactam antibiotics include a large number of patients and do not show extensive heterogeneity, as for NMBAs. For example, most of the studies, except one, used an optimal SI >2 and Ba% >5% as positivity cutoffs, as identified by the performance of the ROC curve analysis (**Table 1**). The first multicenter European study that addressed in vitro drug allergy diagnosis using BAT was performed for β-lactams [14].

BAT sensitivity is close to that of IgE immunoassays, but the use of both diagnostic tests improves sensitivity [14, 16]. BAT and IgE are complementary and allow the increase of the allergologic survey sensitivity when decision algorithms are used. The number of patients with positive history and negative diagnostic tests is reduced when several diagnostic methods are used [14, 18]. Although BAT has moderate sensitivity, it could allow the avoidance of challenge

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tests in patients with negative skin tests and positive BAT results [40]. The use of BAT in patients with negative skin tests might allow the avoidance of challenge tests in patients with hypersensitivity, but whose skin sensitivity has diminished in time.

Few reports have also been published on allergies to antibiotics, other than β-lactams, for instance, quinolones [66, 67]. In these cases, BAT was performed for the retrospective confirmation of a clinical reaction using the cutoffs identified for the β-lactams.

5.8. BAT for NMBAs hypersensitivity

Muscle relaxants are the main cause of anaphylaxis during anesthesia [63, 68]. The diagnosis of NMBA allergy remains particularly difficult: during anesthesia, several drugs are administered in a short period of time; the histamine-releasing NMBAs might show false-positive skin tests results if the testing concentrations are higher than recommended, while it is difficult to perform challenge tests due to ethical and methodological issues [2, 40]. Even though the skin tests have no absolute predictive values, they remain the reference standard for the diagnosis in this context [24]. In previous studies, inclusion criteria varied from history alone to history and skin test results and even positive IgEs (**Table 2**). This inclusion variability could be in part responsible for the differences in the reported BAT sensitivity and specificity. NMBA allergy is a low-prevalence disease, making the recruitment of well-defined patients difficult. Thus, in the first studies, patients with hypersensitivity reactions caused by NMBAs were included in the analysis, and the assessment of BAT performance was done for this class of drugs using arbitrarily defined cutoffs [20, 22–24]. Only in recent years, few drug-specific studies used the ROC curve analysis, which included a limited number of patients and identified optimal thresholds for positivity [21, 26, 27]. The manufacturers recommend the use of an SI >2 as the cutoff for positivity, by extrapolating from the antibiotic and NSAID studies on drug hypersensitivity, which included a larger number of patients. However, it is difficult to justify the use of a single cutoff for all drugs, as the stimulation conditions might differ considerably from NMBA to NMBA [21]. The methodology and optimal decision thresholds for BAT vary considerably from one study to another [26]. Among all the drugs, BAT for NMBAs displays the highest degree of variability regarding the inclusion criteria and positivity thresholds.

We have identified an optimal SI >1.76 for NMBAs as a class and an optimal SI >1.85 for atracurium [25, 26]. By lowering the threshold for positivity, the sensitivity of the assay might be increased. As anaphylaxis from NMBAs can be a threat to the patients' life, it is critical to establish a sensitive cutoff [21]. The time frame for testing was also important; higher sensitivities were achieved when the tests were performed 6 weeks to 3.5 years after the clinical reaction and when the reaction was severe [22].

Moreover, the concentrations for the tested NMBAs varied considerably in the studies, and it seems that each drug might have different optimal concentrations [40].

There was no perfect correlation between the clinical assessment of the disease and the biological tests such as BAT and IgE, suggesting that they investigate potentially different immunological mechanisms and the need to be used complementary [24]. BAT can add to the

diagnosis of NMBA allergy especially when the skin test results are negative and inconclusive [40].

BAT confirms previous reactions caused by drug agents and might allow the clinician chose a safe alternative for future anesthesia [27, 40].

5.9. BAT for NSAIDs hypersensitivity

NSAIDs are the second group of drugs responsible for anaphylaxis [34, 69]. The evaluation of BAT performance for the diagnosis of NSAID hypersensitivity is even more complex due to the clinical spectrum of the reactions. Several types of hypersensitivity reactions have been defined. First, an immediate-type hypersensitivity reaction is related to COX-1 inhibition, which is induced by multiple NSAIDs and displays cross-reactivity in the same patient for several drugs from this class. Second, single-drug-induced immediate-type hypersensitivity reaction was defined, which is mediated by IgE [69].

For NSAIDs, the oral challenge tests are the reference standards for drug allergy diagnosis and there are no commercialized in vitro assays to detect specific IgE, which is not the mechanism in multiple drug hypersensitivity [33]. Thus, reliable in vitro tools are required to confirm this type of drug-induced hypersensitivity. BAT is not the first test that is recommended in the allergological workup for NSAID hypersensitivity, but it can be used in addition to other diagnostic methods, even though heterogenous results have been obtained regarding its performance for this class of drugs [37].

Whether BAT is useful for multiple NSAID-induced hypersensitivity syndrome has been debated in previous studies, as the sensitivity of the test was generally low and varied widely, in part due to different methodologies and drug concentrations that were used. Thus, the reliability of NSAID-induced basophil activation determined by flowcytometry has been questioned. Clinically validated and standardized studies, that include a large number of patients, are still awaited for BAT in this field of multiple NSAID-induced hypersensitivity diagnosis and further development is required to increase the sensitivity of the test [33, 39]. Up to date, no universal in vitro test, which would be applicable to the diagnosis of all types of NSAID hypersensitivity, can be recommended [69].

The three studies on metamizole hypersensitivity diagnosis are not comparable as the drug dilutions used in BAT differed, as well as the threshold for positivity which was obtained by ROC analysis. We have shown that by lowering the threshold and using lower concentrations of metamizole, the sensitivity of the test can be improved, without diminishing the specificity [36]. As drug anaphylaxis can be severe, any improvements in BAT methodology, which have a positive impact on the sensitivity of the test, deserve attention.

5.10. BAT for miscellaneous drugs and rare drug allergies

Several case reports on BAT confirm immediate-type hypersensitivity reactions caused by biological drugs, radiocontrast media, and methylprednisolone [70–73]. In these cases, the extremely low incidence of the disease will not allow the identification of optimal incubation conditions, concentrations, and cutoffs, to estimate the performance of BAT.

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6. Conclusions

The current limitations for the use of basophil activation tests in the retrospective diagnosis of immediate-type hypersensitivity reactions caused by drugs arise from the low prevalence of the disease, limiting the number of patients in the clinical studies, and from the lack of a gold standard test with absolute accuracy, together with the use of surrogate reference tests in the studies. There was a wide variability in the methodology of BAT for drugs concerning the concentrations used in the test and the optimal thresholds for positivity chosen and how these were identified. BAT for drug hypersensitivity has good specificity, but only moderate sensitivity, which needs to be improved in future. BAT could be improved using other activation markers, by new gating strategies to capture basophils and possibly by building a consensus guideline for its standardization [74].

Despite the shortcomings of the currently available BAT techniques and the methodological differences, the benefits of using rapid cellular tests in patients with severe reactions, thus avoiding challenge tests or the potential to increase the allergologic survey sensitivity by the combined use of several in vitro tests, have led to their widespread use in clinical practice and warrant their future standardization. Larger study populations from multicenter studies and a standardized methodological approach are needed to better assess the performance of BAT in terms of sensitivity and specificity for drug-induced immediate-type hypersensitivity reactions.

7. Executive summary

1. The use of BAT allows the rapid confirmation of the previous reaction to the culprit drug in patients with drug hypersensitivity.
2. The main characteristics of the retrospective diagnostic tests for drug hypersensitivity should be high sensitivity to identify and confirm the previous clinical reaction in order to avoid further re-exposure and high specificity as a guarantee that a positive result has clinical significance.
3. None of the available diagnostic methods demonstrates absolute accuracy, and the lack of a perfect gold standard test is one of the major problems in drug allergy diagnosis. In previous studies, inclusion criteria varied from history alone to history and skin test results, and even positive IgEs.
4. The testing methodology and drug concentrations varied from one study to another.
5. Few drug-specific studies used the ROC curve analysis for identifying the optimal threshold for positivity and these included a limited number of patients due to the low prevalence of the disease.
6. Due to methodological differences, previous studies are not comparable.
7. Overall, BAT for drug hypersensitivity has good specificity, but only moderate sensitivity.

8. BAT sensitivity needs to be improved in the future, possibly using new gating strategies to capture the reactive basophils, by adding other activation markers, and by achieving BAT standardization after the performance of multicenter studies that include larger number of patients.

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