Current Allergy and Asthma Reports (2019) 19: 20
https://doi.org/10.1007/s11882-019-0852-8

ANAPHYLAXIS AND DRUG ALLERGY (DAVID A. KHAN & MARIANA CASTELLS, SECTION EDITORS)

BAT in the Diagnosis of Drug Allergy: a Novel Tool in Clinical Daily Practice?

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Published online: 11 March 2019

Abstract

Purpose of Review The aim of this study is to critically review the relevant literature published on basophil activation test, presenting the current knowledge and future perspectives.

Recent Findings Basophil activation test (BAT) results varied accordingly to the class of the drug studied, and have promising results in immediate hypersensitivity reactions to pyrazolone (selective reactors), neuromuscular blockers, beta-lactams, and platinum compounds, all examples of classical IgE-mediated hypersensitivity drug reactions.

Summary Currently, BAT is applied in research settings, but based in the results of our review, the test can be considered as a diagnostic tool for daily practice for selected patients and selected drugs, when the test is available, particularly for patients who experienced severe reactions and when diagnosis cannot be stablished by serum-specific IgE and skin testing, in order to avoid unnecessary drug provocations tests.

Keywords Basophil activation test · BAT · Hypersensitivity reactions · Drug allergy

Introduction

Drug hypersensitivity reactions (DHRs) constitute an important health issue and economic burden on society. DHRs are usually underestimated, but there are also misdiagnoses leading to unnecessary drug substitutions. They account for approximately 15% of all adverse drug reactions and may be classified according to the time interval between drug intake and reaction onset as immediate, until 1–6 h after drug intake, or non-immediate reactions [1]. Immediate drug hypersensitivity reactions [2] manifest as urticaria, angioedema, rhinitis, conjunctivitis, bronchospasm, nausea, vomiting, diarrhea, or anaphylaxis, with or without cardiovascular collapse. Non-immediate reactions often affect the skin, with variable cutaneous manifestations such as delayed urticaria and maculopapular rashes [3••]. DHRs can be further classified as allergic, in which there is a specific immune response to the involved drug mediated by immunoglobulins and/or T cells, or non-allergic, without a specific immune response.

The diagnosis of the IDHR is based upon clinical history, immediate-reading skin tests, serum-specific immunoglobulin E (IgE), if available, and provocation tests, if necessary. Skin tests are standardized for a limited number of drugs and their sensitivity and specificity are usually low according to the ENDA/EAACI Drug Allergy Interest Group position paper. Drug provocation tests (DPTs) are still the gold standard [4••]. DPTs can be risky to patients and sometimes are contraindicated, depending on the severity of the initial reaction and other risk factors such as beta-blockers intake, for example. Regarding in vitro tests in the diagnoses of IDHRs, specific IgE have been used for a limited number of drugs, e.g., some

This article is part of the Topical Collection on *Anaphylaxis and Drug Allergy*

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beta-lactams antibiotics, neuromuscular blockers, and chlorhexidine. Their diagnostic predictive values vary according to the drug involved, but serum-specific IgE is generally less sensitive than skin tests [3••, 4••].

In this scenario, the development and validation of accurate in vitro tests are very important and needed. These tests are safer as diagnostics tools than in vivo tests and can provide deeper insights regarding the pathologic mechanisms involved in the IDHRs. The basophil activation test (BAT) has been applied in the diagnosis of IDHRs and has been validated to several compounds, including neuromuscular blocking agents (NMBAs), beta-lactams antibiotics, iodinate radiocontrast media (RCM), non-steroidal anti-inflammatory drugs (NSAIDs), chemotherapeutic agents, and monoclonal antibodies. The aim of this study is to critically review the relevant literature published on BAT, presenting the current knowledge and future perspectives.

Search Methods

A systematic search strategy was adopted to access the available literature on BAT. Searches of the MEDLINE and EMBASE databases were performed, from 1990 to September 2018, using the following keywords: "basophil activation test" associated with "adverse drug reaction," "drug allergy," "drug hypersensitivity," "NMBAs," "beta-lactams antibiotics," "RCM," "NSAIDs," "chemotherapeutic agents," and "monoclonal antibodies." Each article was reviewed for suitability, and only full-text available articles were included. Articles with no relevant clinical information or review articles were excluded.

BAT in IDHRs

Basophils are leukocytes that comprise less than 1% of the circulating white blood cells They share similar features with tissue mast cells such as expression of high-affinity IgE receptors and release of cytokines and inflammatory mediators when stimulated, and can be activated by IgE-mediated and non-IgE-mediated pathways [5]. When the reaction is IgE-mediated, there is cross-linking of IgEs bound to their membrane surface high-affinity receptors, generally, by proteins (allergens). In non-IgE-mediated pathways, activation may result from coupling of cell receptors with endogenous (e.g., cytokines, anaphylatoxines, chemokines, IgG, and neuropeptides) or exogenous substances. Degranulation of basophils can also result from other mechanisms, similar to mast cell degranulation, such as direct activation by opioids, iodinate contrast media, vancomycin, and quinolones [5].

After basophil activation, signal transduction with phosphorylation of p32MAPK and calcium influx take place, followed by release of mediators. Two markers of basophil activation, CD203c and CD63, are expressed on the cell membrane surface and can be quantified by flow cytometry. There is low expression of CD203c in resting cells and it is upregulated after basophil activation. CD63 is normally expressed on the inner side of the granule membrane and it can be detectable after fusion of the intracellular granules with the cytoplasmic cell membrane during basophil degranulation and mediators release [6]. These mediators induce the IDHRs. BAT has been applied in the diagnosis of IDHRs, and despite the fact that it has been validated for a wide range of IgE-mediated reactions, there are still considerable variations in the performance of this test [7] (Fig. 1).

BAT Technique

The methodology of BAT varies, and several techniques have been described [8••, 9•, 10]. Here we describe the technique developed to perform BAT in allergic patients to platinum compounds. A whole blood aliquot is incubated at 37 °C for 45 min with the culprit drug, using previously standardized concentrations. The highest concentration used in the test should not exceed the highest plasma concentration observed during drug regular infusion. Polyclonal anti-IgE antibody and saline solution (NaCl 0.9%) are used as positive and negative controls, respectively. All experiments should be done in duplicate.

Samples need to be stained at 4 °C, in the dark, for 30 min with the following antibodies: anti-CD123, anti-HLADR, anti-CD63, and anti-CD203c. Basophils are identified in flow cytometry by characteristic forward and side scatter distribution and as CD123 + HLADR- cells. Activated basophils are identified by the expression of CD63 and/or CD203c, which can be quantified by geometric mean fluorescence intensity (gMFI) and percentage of expressing cells. The BAT can be considered positive if a twofold increase in the stimulation index (SI) is observed [11]. The SI is calculated by dividing the gMFI of the CD203c and/or CD63 expression on basophils after stimulation with the drug by the gMFI of these markers on basophils stimulated with saline.

BAT in the Diagnosis of IHRs to NSAIDS

NSAIDs are the medicines most frequently involved in IDHRs [12], being also the major cause of drug induced anaphylaxis [13]. The most common clinical manifestations are urticaria and bronchospasm, and it is known that the majority of those reactions are non-selective to one specific NSAID (cross-hypersensitivity) and they are non-IgE-mediated. The mechanism involved in these reactions is non-immunologic, caused by the inhibition of cyclo-oxygenase-1 [14], with decrease of prostaglandin E2 and increase of leukotrienes,

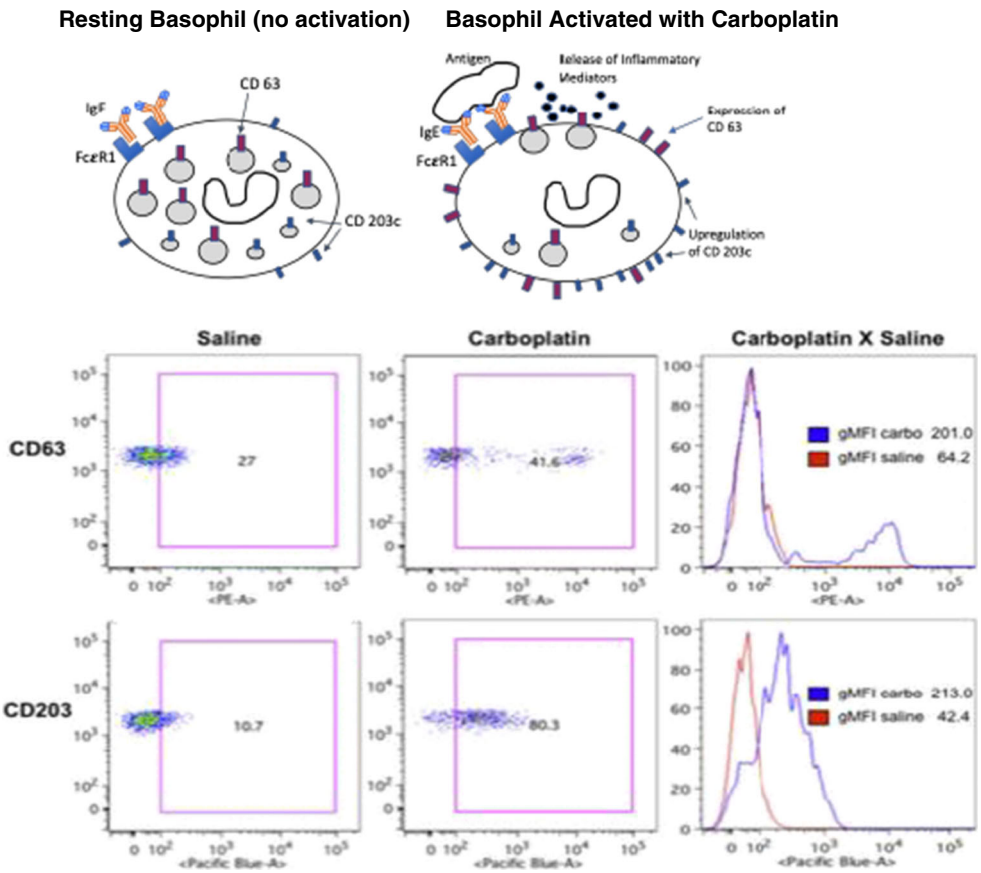

Fig. 1 Basophil activation test—Resting Basophil (no activation) Basophil Activated with Carboplatin CD63 and CD203c expression on basophils from a carboplatinallergic patient after saline and carboplatin incubation. The expression of CD63 and CD203c is shown in percentage of positive cells (first squares) and as gMFI (last square). (adapted from Giavina-Bianchi 2017)

leading to mast cells and basophils degranulation [15••, 16, 17]. Alternatively, patients with immediate hypersensitivity reactions [18] to NSAIDs can only react to one specific class of NSAID and tolerate the other classes. In those selective responders that account for less than 25–30% of all reactions, an IgE-mediated mechanism was proposed, and pyrazolones are the most frequent drug involved in this category [15••, 16, 17, 19].

The diagnosis of non-selective reactions is often based in a reported history of reactions induced by NSAIDs from different classes. In suspicious cases, a drug provocation test can be necessary to exclude or confirm IHRs. Diagnosis of selective reactions is based in detailed history associated with evidence of IgE antibodies. Skin testing (prick and intradermal) is only validated for pyrazolones, with sensitivity not higher than 50% [15••], and in vitro methods to measure serum-specific IgE have also poor sensitivity and are not used in clinical practice [19, 20•, 21].

Studies assessing BAT as a diagnostic test to IHRs induced by NSAIDs are very heterogeneous in terms of sensitivity and specificity. A total of 18 studies were reviewed, and, apparently, the test is more predictive in subjects with selective IHRs to NSAIDs than in subjects with hypersensitivity to multiple NSAIDs.

BAT in Pyrazolone Selective IHRs

The predictive values of BAT to diagnose selective IHRs to pyrazolones was assessed by Gomez and cols [15••]. The study included 51 patients with selective immediate allergic reactions to pyrazolones and 56 controls. BAT was positive in 54.9% of the cases, and BAT sensitivity was higher in those who were skin test positive (85.7%) compared with those who were skin test negative (33.3%), concluding that BAT could be a useful complement to skin testing. Follow-up of BAT-positive patients showed decrease in positivity over time, suggesting that time to perform the test is critical. Gamboa also studied a similar group of subjects ($N = 56$) with selective immediate allergic reactions to dipyrone (metamizole), showing similar results, with sensitivity of 42.3% and specificity of 100%, concluding that BAT may complement skin testing and it is a reasonable alternative in patients with negative skin test and history of severe reactions. BAT detected a larger number of cases when patients were assessed within the first 6 months after the initial reaction. The association skin testing and BAT detected 69.2% of patients allergic to dipyrone [22].

In order to standardize BAT, Hagau and cols tested three low dipyrone concentrations. Thirteen of 20 patients had positive results for at least one of the tested concentrations, and all

healthy controls presented negative tests, showing that BAT can be a useful method to diagnose dipyrone allergy [21]. Blaca-López evaluated 137 patients with selective IHR to dipyrone, of whom 60% had anaphylaxis. Skin testing was positive in 62.04% of all cases. In patients with negative skin testing, BAT was positive in 14 subjects (28%). In this study, the overall sensitivity of BAT could not be calculated, because the test was not performed in all patients [23].

Dipyrone has four major metabolites described in the literature: 4-methylaminoantipyrine, 4-aminoantipyrine (AA), 4-formylaminoantipyrine [24•], and 4-acetylaminoantipyrine [24•]. These metabolites may contribute to induce IDHRs, fact that could explain the low sensitivity of the diagnostic tests [22]. Based in this hypothesis, BAT with dipyrone and its four metabolites (MAA, AA, AAA, and FAA), which were purified from human urine samples, was performed in 16 patients with positive history and skin testing for IDHR. The aim of this study was to analyze whether dipyrone metabolites could be recognized by IgE bound to basophil surface and induce cell activation. BAT with MAA was positive in 50% of the cases and elicited more positive results than native dipyrone (37.5%), with all positive cases to dipyrone also being positive to MAA. However, low or non-positive responses were detected for the other metabolites tested. This was the first study demonstrating the role of dipyrone metabolites in IHRs and supporting the hypothesis that drug metabolites may underlie allergic reactions [24•]. Using only the parent drug may be a major cause of the lack of sensitivity observed in BAT (Table 1).

Conclusions

- Sensitivity varies from 42 to 70% and specificity from 85 to 100%.
- BAT is a useful complement to skin testing and may allow DPT avoidance.
- For patients with negative skin testing, BAT can be a reasonable alternative in severe IDHRs.
- One reason for negative results can be a longer time interval between initial reaction and the realization of the assay.
- Drug metabolites can contribute to IHRs and can be the major cause of lack of sensitivity of diagnosis tests.

BAT in NSAIDs Non-Selective IHRs

A total of 10 publications regarding BAT in NSAIDs IHRs were reviewed, being 3 studies with acetylsalicylic acid (ASA), 2 with diclofenac, and 7 with various AINES. The studies of BAT in non-selective IHRs to aspirin/NSAIDs are conflicting or inconclusive, and generally show insufficient sensitivity (about 20–40%) in the diagnosis of these reactions.

Bavbek and cols studied patients with ASA hypersensitivity, patients with ASA tolerance, and healthy volunteers. The highest BAT sensitivity was 33.3% (CD63) to ASA and 22.2% to diclofenac [25]. Korosek and cols observed higher sensitivity (80%) and specificity (83%) in patients with anaphylaxis. In patients with respiratory manifestations, BAT sensitivity and specificity was 78% and 50%, respectively [26]. BAT with ASA was also evaluated by Celik and cols, who measured drug-induced expression of CD63, CD69, and CD203c in patients with aspirin-exacerbated respiratory disease. BAT showed low clinical value in identifying aspirin-induced respiratory reactions [27].

Harrer and cols [28] and Malbran and cols [29•] investigated patients with acute history of IHRs to diclofenac (DF) and showed an extremely low sensitivity of BAT (CD63). The first group performed BAT with DF/DF-metabolite in 22 selected patients within less than 2 years interval from the initial IHDR and observed negative results in all patients and controls. The second group compared BAT with human basophil degranulation test (HBDT) using light microscopy (toluidine blue staining mononuclear cells). While BAT was negative in almost all patients, HBDT was positive. Those results suggest that diclofenac activates basophils in hypersensitive individuals in a way that do not induce CD63 expression, but it promotes basophil degranulation as assessed by disappearance of metachromatic-stained cytoplasmic granules on light microscopy. The study corroborate the existence of two different degranulation mechanisms: anaphylactic degranulation with rapid extrusion of granules to the extracellular milieu, during an IgE-mediated immune responses; and piecemeal degranulation, which includes the release of focal and complete granule contents in the absence of granule-granule or granule cytoplasmatic membrane fusions, described by Dvorak e cols [30, 31]. The studies suggest that DF-sensitive patients may present piecemeal degranulation.

Table 1 BAT for pyrazolones selective reactions

Author	Reference test	Activation marker 1	% patients with anaphylaxis	Sensitivity	Specificity	*N*	Time interval
Gamboa 2003		CD 63	57.70%	42.3	100	56	16.9 months
Gomez 2009	H + ST	CD 63	74.50%	54.9	85.71	107	8 months
Hagau 2013	H + ST	CD 63	100%	70	100	30	NA
Ariza 2016	H + ST	CD63	50%	NA	NA	16	NA
Blanca-Lopez 2016	H + ST	CD63	60%	NA	NA		NA

ST skin testing, *H* history, *DPT* drug provocation test, *NA* not applicable

Abuaf et al. [32] assessed 60 patients with IHRs to NSAIDs and observed a low overall BAT sensitivity of 21%, but sensitivity increased to 64% in the severe reactions. BAT may be useful in the evaluation of severe IHRs to NSAIDs, but it has limited value in assessing milder non-IgE-mediated reactions. Gamboa and cols [33••, 34] also studied patients with multiple NSAIDs hypersensitivity, which was confirmed by clinical history of at least two reactions to two or more different NSAIDs or by positive oral provocation challenge. BAT with aspirin showed sensitivity of 43.3% and specificity of 100%. For the other NSAIDs, sensitivity and specificity were 11.7% and 100% for paracetamol, 15% and 100% for metamizole, 43.3% and 93.3% for diclofenac, and 54.8% and 74.1% for naproxen. Joining all the results, the global sensitivity raised to 63.3% and even to 88% when the tests were performed within 1 month of the last clinical drug exposure and reaction.

Ariza and cols studied 46 patients with cross-intolerance to NSAIDs and 45 tolerant controls. BAT was performed with acetyl salicylic acid, paracetamol, diclofenac, dipyrone, naproxen, and ibuprofen. The best results were found with dipyrone and the worst with paracetamol followed by diclofenac. Considering BAT positivity to at least one NSAID, 100% sensitivity could be obtained in the patient group, but the specificity dramatically decreased to 31.1%. These data may indicate that the main pitfall of BAT to evaluate NSAIDs hypersensitivity is the false-positive results, once the drug interacts with basophils in both NSAID-intolerant and NSAID-tolerant subjects [24•]. De Weck [35••] and Rodríguez-Trobado e cols [36] also found similar results, with good positive predictive value but low negative predictive value. The phenomenon is clearly dose-related, and hypersensitivity patients seem to react to lower NSAID concentrations.

Overall, those results suggest that BAT is not a reliable method in the diagnosis of multiple NSAIDs hypersensitivity. The severity of the reaction and the time to perform the test may impact in the sensitivity of BAT, but more studies are necessary to confirm the utility of the assay in this specific patient phenotype (Table 2).

Conclusions

- BAT studies are controversial, and the test is not reliable to diagnose non-IgE-mediated mild to moderate IHRs to multiple-NSAIDs.
- BAT may have a higher sensitivity to evaluate patients who had severe IHRs to NSAIDs.
- Timing to perform the test is important; the lower interval between the initial reaction and the assay favors higher sensitivity rates.

BAT and IHRs to Neuromuscular Blocking Agents

Neuromuscular blocking agents (NMBAs) represent the most common cause of IHRs during anesthesia [21]. Skin testing associated with clinical history remain the main tools for the diagnosis of an IgE-mediated IHR, but though reliable they are not infallible. Eleven studies were reviewed to assess the performance of BAT in the diagnosis of IHRs to NMBAs. Sensitivity of BAT for NMBAs varies between 36 and 92%, while the specificity from 93 to 100% [37–41, 42••, 43].

Hagau and cols tested, in vitro *and* in vivo, 22 patients with an intra-anesthesia IHR caused by NMBAs, comparing with 34 surgical control patients [43]. Their data suggest that BAT for different NMBAs may have different sensitivities, but the limited number of patients tested for each drug does not allow a definitive conclusion. The overall performance of BAT showed sensitivity of 68% and specificity of 100%. Ebo and cols studied 14 patients with perioperative anaphylaxis with positive skin test for rocuronium and 8 individuals enrolled as controls, who tolerated rocuronium and had negative skin tests. Sensitivity and specificity of BAT for rocuronium was 91.7% and 100%, respectively. BAT was also positive to vecuronium in 58.3% of the patients. All controls presented negative BAT for NMBAs. The study concludes that BAT is a reliable instrument to diagnosis anaphylaxis induced by rocuronium [39].

Uyttebroek and cols [41] assessed the utility of BAT to identify atracurium sensitization and to investigate cross-reactivity between muscle relaxants. Eight patients with perioperative anaphylaxis to atracurium, 5 reactors to other NMBAs, and 7 individuals experiencing perioperative anaphylaxis, but not exposed to NMBAs, were studied. BAT sensitivity and specificity was 63% and 100%, respectively. Two atracurium-exposed individuals with negative atracurium skin testing had clear positive BATs. The BAT with atracurium was positive in one cisatracurium-sensitized patient and negative in all cisatracurium-exposed patients with negative ST to the drug. The rocuronium- and suxamethonium-sensitized patients had negative BAT with atracurium. These results suggest that BAT can be a useful diagnostic method for atracurium-induced anaphylaxis and may be complementary to STs. The technique enables quick and simultaneous testing of potentially cross-reactive NMBA and the identification of safe alternatives for future surgery.

In patients with proven NMBA anaphylaxis, BAT sensitivity was primarily 36.1%, which increased to 85.7% when IHRs with an onset of less than 3 years were considered separately [44, 45••]. In the same patients, BAT showed high correlation with skin prick test

Table 2 BAT and NSAIDs non-selective reactions

Author	Drug	Reference test	Activation marker	Sensitivity	Specificity	*N*
Gamboa 2004	Various NSAIDs	H + DPT	CD63	15–55	74–100	90
Sanz 2005	Various NSAIDs	H + DPT	CD63	76.2	89.5	90
Malbran 2007 (Diclofenac)	Diclofenac	H	CD63	0–100	NA	26
Rodriguez-Trobado 2008	Various NSAIDs	H	CD63	4.8	100	72
Bavbek 2009	Asprin	H + DPT	CD63/CD203c	16.7–33.3	79.2–100	42
Celik 2009 (DREA)	Asprin	H + DPT	CD63/CD203c/CD69	30/70/80	40/45/34	20
Harrer 2010 (Diclofenac)	Diclofenac	H + DPT	CD63	0		18
Korosec 2011 (Asprin)	Asprin	H + ST +/− DPT	CD63	79	70	59
Abuaf 2012	Various NSAIDs	H + ST	CD63	37/64	90/90	85
Kim 2012	Various NSAIDs	H + DPT	CD63	61	91	36
Ariza 2014	Various NSAIDs	H + DPT	CD63	100	31	91
De Weck 2010	Various NSAIDs	H	CD63	NA	NA	67

ST skin tests, *H* history, *DPT* drug provocation test, *NA* not applicable

[39, 40, 43], with higher sensitivity and specificity (range, 93% to 100%) [40]. Dewachter and cols [46] evaluated 31 patients experiencing IHRs to NMBA and compare skin testing and BAT, and BAT had lower sensitivity, concluding that BAT does not replace skin test in the assessment of NMBA allergy (Table 3).

Conclusions

- BAT is a valuable method to diagnose NMBA immediate hypersensitivity reactions.
- The technique enables quick and simultaneous testing of potentially cross-reactive NMBA and the identification of safe alternatives for future surgeries.
- Time between the reaction and the test is an important parameter, and lower intervals can increase BAT sensitivity.

BAT and IHRs to Antibiotics

Allergy to antibiotics is an important worldwide problem, with an estimated prevalence of up to 10% of the population [47]. Most of the allergic reactions to antibiotics have been reported for beta-lactams (BLs), followed by quinolones and macrolides and, to a lesser extent, to others, such as metronidazole, clindamycin, and sulfonamides [12]. The diagnostic algorithm includes a detailed clinical history, followed by cutaneous tests, quantification of serum-specific IgE, when available, and drug provocation test. Risk stratification is essential in each algorithm step, because the in vivo procedures are not risk-free [4••].

BAT and IHRs to Beta-Lactams

Hypersensitivity reactions to beta-lactams can be immediate or non-immediate, generally IgE or T cell–

Table 3 BAT and IHRs to NMBAs

Author	Drug	Reference test	Activation marker	Sensitivity	Specificity	*N*
Abuaf 1999	Various NMBAs	H	CD63/CD45	43–64	81–96	26
Monneret 2002	Various NMBAs	H + ST	CD63	54	100	56
Sudheer 2005	Various NMBAs	H	CD63/CD203c	28.6–78.6	100	24
Kvedariene 2006	Various NMBAs	H + ST	CD63	36.1–85.7	93.3	92
Ebo 2006	Rocuronium	H + ST	CD64	91.7	100	22
Leysen 2011	Rocuronium	H + ST	CD66	80	96	104
Hagau 2013	Various NMBAs	H + ST	CD63	68	100	56
Uittebroek 2014	Atracurium	H + ST	CD63	71	100	75
Dawachter 2018	Various NMBAs		CD63/CD203c	80	91.7	31

ST skin tests, *H* history, *DPT* drug provocation test, *NA* not applicable

mediated, respectively. History of allergic reaction to BL antibiotics is very common, being reported in approximately 10% of hospitalized patients [47]. However, the diagnosis of allergy is confirmed in less than 5% of these cases. Frequently, the reaction has occurred many years ago and patients may not be able to describe it properly, making the diagnosis even more challenging. Skin reactivity to BLs also declines with time in allergic patients. Serum-specific IgE measurement is available for amoxicillin [48], penicillin G, penicillin V, ampicillin, and cefuroxime, and its sensitivity depends on the BL involved, but it is rather low and variable (0–75%) [49]. The serum-specific IgE can decline rapidly in allergic patients, usually, in 6 months to 3 years after the last exposure [20•]. In case of negative cutaneous tests and serum-specific IgE, drug challenge can be indicated.

Eleven studies were reviewed to evaluate the accuracy of BAT as a diagnostic tool for IHR to BLs. BAT showed sensitivity between 22 and 55% and specificity from 80 to 97% [6, 50–53, 54••, 55–57].

De Week and cols conducted a multicenter study in 10 European centers, assessing a total of 178 BLs-allergic patients. Of 121 skin test–positive patients, 65 (53.7%) were BAT positive. Of 45 skin test–negative patients, 17 (37.0%) were BAT-positive. When only a single allergen was assessed, the rate of positivity varies from 16% for PPL to 33% for amoxicillin. However, when all 5 BLs allergens were used, an overall sensitivity of 48.3% was reached. These results emphasize the need to test more than one allergen, and with at least two concentrations, in order to obtain optimal results. Interestingly, BAT was positive in 37% of 45 patients with positive clinical history for allergy but negative skin tests. Of 13 skin test and serum-specific IgE–negative and BAT-positive patients challenged with BLs, all had positive results. The study supports the advantage of BAT over skin tests and specific IgE in this subgroup of patients [54].

In a recent trial, BAT was positive in 9 of 12 cases with a positive clinical history but negative skin test results. Furthermore, all patients who reported severe IHRs (anaphylactic reaction grade 2 and above) showed positive BAT (5/5), while only three of these five cases had positive skin testing. The authors concluded that although skin testing remains the most important part of the primary diagnostic investigation, BAT is an additional valuable and sensitive in vitro test in the diagnostic algorithm of immediate allergic reactions to antibiotics [58].

Torres and cols also demonstrated that in patients with cephalosporin reactions, BAT to the culprit cephalosporin was positive in 77.7%, and although further studies are required, BAT results in cephalosporin allergy seem very promising. The test did not help to differentiate between selective reactors and cross-reactors [52].

BAT is the only available in vitro assay for diagnosing patients with IHR to clavulanic acid [59••]; however, few studies have been published. Salas and cols established the sensitivity and specificity of BAT to amoxicillin and CLV in a trial with 115 patients with immediate allergic reactions induced by AX-CLV treatment. The overall sensitivity was 55%, specificity 89%, and positive predictive value [22] 96% [59••] (Table 4).

Conclusions

- BAT is more sensitive than serum-specific IgE measurement. The use of both in vitro tests allows the diagnosis of a higher number of patients.
- BAT is a very important diagnostic tool in patients with IgE-mediated allergy to beta-lactams and negative skin tests, avoiding the performance of potentially dangerous oral provocation tests in a high percentage of cases.
- Skin testing remains the main diagnostic procedure, although BAT has a useful complementary role, and both in vivo and in vitro tests must be performed.

BAT and IHRs to Quinolones

The incidence of IHRs to quinolones has been increasing in the last years, likely due to increased prescription. Diagnosis is particularly difficult, since skin testing can induce false-positive results, and commercial in vitro test are not well validated. Therefore, drug provocation test, which is not a risk-free procedure, is considered the gold standard to establish diagnosis. Cross-reactivity between quinolones is difficult to predict due to the small number of patients included in the few published studies [60].

BAT has been used in the diagnosis of IHR to quinolones, but variable results have been reported regarding its sensitivity (15–100%) [61–66]. Demir and cols [67] evaluated 19 patients with IHR to different quinolones and BATs with the culprit drugs were positive in only 2 patients (10%). BAT was not found useful in the studies published by Seitz and cols [68] and Lobera and cols [61]. Controversially, Ben-Said showed BAT (CD203c) sensitivity of 100%, concluding that the test may be useful in the diagnosis of quinolones allergy [64]. Promising results were also observed by Aranda and cols in a study with 38 patients with confirmed IHRs to quinolones. BAT was positive in 27 patients (71.05%) [62].

Table 4 BAT and IHRs to beta-lactams

Author	Drug	Reference test	Activation marker	Sensitivity (%)	Specificity (%)	*N*
De Weck 2009	B-lactam	H + ST + IgE	CD63	50	89–97	262
Eberlein 2010	B-lactam	H + ST + IgE	CD63	53–55	80	39
Sanz 2002	B-lactam	H	CD63	50	93	88
Gamboa PM 2004	B-lactam	H + DPT	CD63	39	93	53
Torres MJ 2004	B-lactam	H +/−ST +/− IgE +/− DPT	CD63	49	91	110
Abuaf N 2008	Amoxicillin	H +/− ST	CD 203c/CD63	22–52	79–100	41
Garcia-Ortega P 2010	Amoxicillin	H	CD63	29	NA	14
Torres MJ 2010	Amoxicillin	H +/− ST +/− DPT	CD 63	50	NA	61
Torres MJ 2011	Amoxicillin	H +/− ST	CD 63	50	NA	30
Thinnes 2018	Antibiotics	H +/−ST +/− IgE +/− DPT	CD 63	50	97	82 (62 beta-lactams)
Salas 218	Amoxicillin-clavulanic	H +/− ST +/− IgE +/− DPT	CD 203c /CD63	55	89	115

ST skin tests, *H* history, *DPT* drug provocation test, *NA* not applicable

Recently, Fernandez and cols [69] studied 17 patients with IHRs to quinolones. BAT was performed with moxifloxacin and ciprofloxacin, and each drug showed different results regarding sensitivity–specificity and the best basophil activation marker used. The best sensitivity in moxifloxacin-allergic patients was obtained with CD203c (sensitivity = 36.4%; specificity = 94.4%), and in ciprofloxacin-allergic patients with CD63 (sensitivity = 83.3%; specificity = 88.9%) (Table 5).

Conclusions

- Further larger, multicenter studies are needed to assess BAT in the diagnosis of quinolone IHRs.
- BAT may be useful when skin testing is not suitable.
- The accuracy of BAT for quinolones IHRs may be different for each drug of this class of antibiotic, considering differences in the stimulatory mechanism that leads to the upregulation of different activation markers.

BAT and Platinum Compound Agents

There are promising data evaluating the use of BAT in the investigation of IHRs to platinum compound agents [8••, 9•, 70]. A case report showed a positive BAT in an IHR to cisplatin [71]. In 2012, a prospective study followed patients who were receiving carboplatin for the treatment of gynecologic cancers, assessing CD203c expression in these patients. Six of them became allergic to the drug during the study. The authors concluded that CD203c could be a biomarker of anaphylactic reactions, since the basophils from patients who reacted presented an increased expression of CD203c on the day prior to the reaction. Furthermore, IHR tended to be more severe in patients with positive BAT [8••, 9•, 70].

Recently, our group evaluated BAT as a biomarker in patients undergoing rapid drug desensitization to carboplatin and oxaliplatin. We performed BATs in 15 patients allergic to either carboplatin or oxaliplatin

Table 5 BAT and IHRs to quinolones

Author	Drug	Reference test	Activation marker	Sensitivity (%)	Specificity (%)	*N*
Seitz CS 2009	Quinolones	H + DPT	CD63	0		4
Aranda 2010	Quinolones	H + DPT (urticaria)		42–79	88	42
Lobera T 2010	Quinolones	H ± ST ± DPT	CD63	0	100	18
Ben Said B 2010	Quinolones	H	CD203c	100	100	5
Rouzaire P 2012	Quinolones	H + DPT	CD203c	na	100	34
Mayorga 2013	Cipro and moxifloxacin	DPT	CD63	15–46	90	48
Blanca-Lopez 2013	Quinolones	DPT + BAT (retrospective)	CD63	36		66
Fernandez 2016	Cipro and moxifloxacin	H +/− ST ± DPT	CD63/CD203c	36.4–83.2	94.4–88.8	16
Demir 2018	Quinolones	H +/− ST ± DPT	CD63	NA	NA	19

ST skin tests, *H* history, *DPT* drug provocation test, *NA* not applicable

Table 6 BAT and platinum compound agents

Author	Drug	Reference test	Activation marker	Sensitivity	Specificity	*N*
Giavina Bianchi P 2017	Platinum compound agents	H + ST	CD 203c or CD63	73	100	15

ST skin tests, *H* history, *DPT* drug provocation test, *NA* not applicable

undergoing RDD, six tolerant patients to platinum agents and six healthy controls. BAT was positive in 11 of 15 allergic patients (73.3%), with an increased expression of CD203c in 11/15 (73.3%) and CD63 in 6/15 (40.0%). Higher CD63 expression was observed in patients with severe initial IHRs. When assessing RDD outcomes, we observed that patients who presented with breakthrough reactions during desensitization, especially those with increased tryptase levels, had positive BAT, which points out to the BAT could be a predictive biomarker. All control patients had negative BATs. We also observed that BAT remained positive in multiple RDDs, reinforcing the notion that RDD does not induce persistent hypo-responsiveness of basophils [8••] (Table 6).

BAT and Monoclonal Antibodies

BAT may be an additional tool in the investigation of IHRs and injection site reactions secondary to monoclonal antibodies, and case reports have increasingly proved its value. Two patients with injection site reactions to etanercept with negative skin tests presented positive BATs and underwent successfully desensitization [72]. A case of severe anaphylaxis to pertuzumab, with increased tryptase levels, in a 38-year-old woman with breast cancer was investigated using skin testing and BAT. While prick and intradermal tests were both negative, the BAT using the drug in its regular infusion concentration was positive for both markers, CD63 and CD203c [73]. The patient was successfully desensitized.

Piva et al evaluated the BAT in the diagnosis of five patients treated for lymphoproliferative diseases suspected of having IHR secondary to rituximab. CD63 expression was higher in patients presenting reactions compared to 18 healthy controls [74•].

Summary of BAT Results

In Table 7, we summarize the results of BAT in the diagnosis of IDHR.

Conclusion

Drug hypersensitivity reactions are an important health issue and their diagnosis is difficult, lacking trustful in vivo and in vitro diagnostic methods. A total of 47 trials were reviewed in order to assess BAT efficiency in the diagnosis of immediate drug hypersensitivity reactions. In the studies, different BAT methodologies were used, as well as drug concentrations and cutoffs for test positivity were not standardized. Harmonization of the existent protocols will be crucial to render comparable results between studies.

In the trials reviewed, the diagnosis of IDHR were made based upon clinical history, immediate-reading skin tests and provocation tests, if necessary, following the consensus guidelines published. Skin tests are the most commonly used procedure to confirm a sensitization in drug hypersensitivity but unfortunately, the sensitivity of skin tests to most drugs is low and appears to be moderate to high for immediate hypersensitivity reactions to beta-lactam antibiotics, perioperative drugs, heparins, platinum salts, radiocontrast media, but low for many other drugs. Therefore, in cases of negative reactions, drug allergy cannot be excluded.

BAT results varied accordingly to the class of the drug studied, and have promising results in immediate hypersensitivity reactions to pyrazolone (selective reactors), neuromuscular blockers, beta-lactams, and platinum compounds, all examples of classical IgE-mediated hypersensitivity drug reactions. The sensitivity was lower among NSAIDs (non-selective reactors) and quinolones, both groups of drugs that degranulate mast cells by mechanisms non-IgE mediated. The time between the test and the reaction can also impact the results, and lower intervals can increase BAT sensitivity. It has a complementary role to skin tests for different drug hypersensitivities and can be particularly useful in patients with negative skin tests, in order to avoid unnecessary drug provocations tests.

Currently BAT is applied in research settings, but based in the results of our review, the test can be considered as a diagnostic tool for daily practice for selected patients and selected drugs, when the test is available, particularly for patients who experienced severe reactions and when diagnosis cannot be stablished by serum-specific IgE and skin testing. Increasing the number of diagnostic tests used to confirm a suspected clinical history of allergy can improve diagnosis efficiency and accuracy.

Table 7 Results of BAT in the diagnosis of IDHR

Compound	# of publications included	Drugs	Reference test	Activation marker	Sensitivity	Specificity	*N*	Reference numbers
Pyrazolones	5 publications	Pyrazolones	H + ST	CD 63	42.3–70%	85.7–100	209	13, 18–21
	7	Various AI NES	H+ ST +/− DPI	CD63	15–100	31–100	531	9, 21, 29, 30, 31, 32, 35
	2	Diclofenac	H + DPT	CD63	0–100	NA	44	27, 28
	3	Asprin (ASA)	H + DPT	CD63/CD203c/CD69	16.7–80	40–100	121	24, 25, 26
Various AINES	12 publications	AINES (except Pyrazolones)	H+ ST +/− DPT	CD63/CD203c/CD69	15–100	31–100	696	9, 21, 24–32, 35
	6	Various NMBAs	H + ST+/− IgE	CD3/CD45/CD203c	36–86	81–100	285	36, 37, 39, 42, 44, 45
	1	Antracurium	H + ST	CD63	71	100	75	38, 41
	2	Rocuronium	H + ST	CD63	80–92	96–100	126	40
NMBAs	9 publications	NMBAs	H + ST+/− IgE	CD3/CD45/CD203c	36–92	93–100	486	36–42, 44–45
	5	Various beta-lactams	H +/− ST +/− IgE +/− DPI	CD63	39–55	80–97	552	49, 50, 51, 52, 53
	4	Amoxicillin	H +/− ST +/− DPT	CD 203c /CD63	22–50	79–100	146	6, 54, 55, 56
	1	Beta-lactams + other antibiotics	H +/−ST +/− IgE +/− DPI	CD63	50	97	62	57
	1	Amoxicillin + clavulanate	H +/− ST +/− IgE +/− DPI	CD 203c/CD63	55	89	115	58
Beta-lactams antibiotics	11 publications	Beta-lactams	H +/− ST +/− IgE +/− DPT	CD 203c/CD63	22–55	79–100	875	6, 49–56
Quinolones	9 publications	Quinolones	H +/− ST ± DPT	CD 203c or CD63	15–100	88–100	251	60–68
Platinum compound Agents	1 publication	Platinum compound agents	H + ST	CD 203c or CD63	73	100	15	7

ST skin tests, *H* history, *DPT* drug provocation test, *NA* not applicable

Statement of Contribution All authors meet the three conditions:
(1) Substantial contributions to conception of the study
(2) Drafting the article and revising it critically for important intellectual content
(3) Final approval of the version to be published

Compliance with Ethical Standards

Conflict of Interest The authors declare no conflicts of interest relevant to this manuscript.

I confirm that this paper has been read and approved by all the co-authors, and that no conflicts of interest exist regarding its publication.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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•• Of major importance

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