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Recent applications of basophil activation tests in the diagnosis of drug hypersensitivity

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Immediate-type drug hypersensitivity is an increasingly significant clinical issue; however, the diagnosis is frequently hindered due to lack of safe and precise diagnostic tests. Flow cytometry-assisted basophil activation test is a safe *in vitro* diagnostic tool for assessing basophil activation upon allergen stimulation. In this review, we have summarized current literature on the diagnostic utilities, new indications, and methodological aspects of the basophil activation test for the diagnosis of drug hypersensitivity.

Key words: Drug hypersensitivity; Immunologic tests; Basophils; CD63; CD203c; Basophil activation test

INTRODUCTION

Flow cytometry-assisted basophil activation test (BAT) has been utilized in the diagnosis of immediate-type drug hypersensitivity from the early 1990s, when CD63 was discovered as a marker of basophil activation by Knol et al. [1]. This method has been further refined [2], owing to which the clinical applications of BAT have expanded [3].

However, immediate-type drug hypersensitivity is still a major diagnostic challenge to allergists and clinicians, e.g., penicillin allergy [4]. The challenge for diagnosis exists because there are insufficient methods to assess causal relationships. Drug provocation tests (DPTs) are the gold standards in hypersensitivity testing; however, they cannot always be administered due to the risks of systemic reactions [5]. Drug skin tests have recently been standardized and are reliable [6, 7]; however, except for a few well-known drugs, they have limited utility due to low sensitivity and specificity (e.g., skin irritations) [6]. *In vitro* allergen-specific IgE testing is another diagnostic option, but it may not be available for drugs other than beta-lactams.

In this review, we discuss the diagnostic potential of BAT in drug hypersensitivity. Although BAT is more expensive and technically challenging compared to conventional *in vitro* or *in vivo* tests, it can simultaneously and safely assess multiple drug responses. In addition, it directly measures basophil responses instead of immunoglobulin E (IgE) sensitization. Recent studies

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suggest that the applications of BAT can be extrapolated to additional drugs. The present review aims to summarize the current literature on the applications and methodological considerations of BAT in drug hypersensitivity.

Search strategy and study selection

A systematic search strategy was adopted, in order to summarize the currently available literature. PubMed (http://www.ncbi.nlm.nih.gov/pubmed/) searches were carried out using search terms *basophil activation* in titles and/or abstracts, for the period from January 1990 to August 2013. A manual search, using the same keywords, in Google Scholar (http://scholar.google.com/) was performed to identify additional papers. The search process followed the recommendations of the PRISMA statement (Fig. 1) [8], and was confined to articles with full-text accessibility. The present review includes analyses from 74 relevant papers, including original articles and case reports.

CLINICAL APPLICATIONS

Beta-lactam antibiotics and neuromuscular blocking agents (NMBAs) were the first drugs for which BAT was applied. Aspirin and non-steroidal anti-inflammatory drugs (NSAIDs) are another class of drugs for which BAT was utilized. Recently, applications of BAT have extended to fluoroquinolones, radiocontrast media (RCM), and novel drugs such as anti-neoplastic or biologic agents.

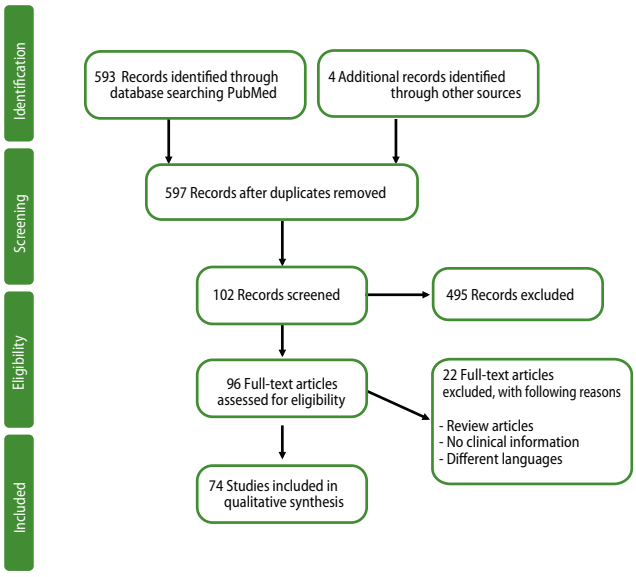

Fig. 1. Flowchart for the identification of relevant studies.

Beta-lactams

Conventionally, diagnoses of beta-lactam antibiotic hypersensitivities have been based on patient's clinical history and positive skin tests, or specific IgE antibody measurements [9]. To date, nine studies [10-18] have described the utility of BAT for diagnoses of beta-lactam allergies (Table 1). The sensitivities ranged from 28.6% to 55%; however, several large-scale studies have consistently demonstrated the sensitivity to be approximately 50%, in patients with positive clinical history and skin tests. Interestingly, the sensitivity of BAT was approximately 10% higher than that of the commercial specific IgE tests [14, 17, 18], and the specificity was more than 90%, clearly indicating that a positive BAT result was clinically significant. Importantly, BAT was positive in 25% of patients with positive provocation test and negative for specific IgE [17], and in 37% of patients with positive clinical history but negative skin tests [14]. These results suggest that BAT should be administered in cases where the diagnosis of drug allergy is highly suspected but is not supported by results of skin testing or *in vitro* IgE measurements. Because specific IgE tests are not available for most cephalosporins, BAT can be developed further for diagnosing allergies to a wider range of beta-lactams [9].

Neuromuscular blocking agents

Currently, data for evaluating BAT results from patients with a history of perioperative hypersensitivity are available from seven clinical trials [19-25]. The sensitivity of BAT varied from 36.1% to 91.7% (Table 2); however, there was considerable heterogeneity in the inclusion criteria and cutoff levels. In patients with proven NMBA anaphylaxis, the BAT sensitivity was primarily 36.1%, which increased to 85.7% when allergies with an onset of less than 3 years were separately considered [21]. In the same patients, BAT showed high correlations with skin prick tests [20, 23, 26], better sensitivity [23], and higher specificity (range, 93% to 100%). Therefore, the time elapsed between the anaphylaxis and *in vitro* basophil activation [21] is a significant parameter for analyzing BAT sensitivity. In addition, BAT also plays an important complementary role in identifying cross-reactivity and safe alternatives in these patients [19-21, 23, 27].

Aspirin/non-steroidal anti-inflammatory drugs

Aspirin or NSAIDs hypersensitivity is a heterogeneous disorder,

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Table 1. Summary of studies on the diagnostic utility of basophil activation tests in immediate type beta-lactam hypersensitivity

Reference	Drug	Diagnosis	Subjects	Activation marker	Reference test	Findings
Torres, 2011 [10]	Amoxicillin	Immediate hypersensitivity (anaphylaxis and urticaria)	30 Patients	CD63 (Basotest, Orpegen Pharma, Heidelberg, Germany)	Clinical history and skin tests	Sensitivity 50% (cutoff, SI ≥ 2)
Torres, 2010 [11]	Amoxicillin	Immediate hypersensitivity (anaphylaxis and urticaria)	32 Amoxicillin selective patients and 19 penicillin allergic patients	CD63 (Basotest)	Clinical history and skin tests	Sensitivity 50.9% (cutoff, SI ≥ 2)
Eberlein, 2010 [12]	Various beta-lactams	Immediate hypersensitivity	24 Patients and 16 controls	CD63 (Flow-CAST, Bühlmann Laboratories, Schönenbuch, Switzerland) and CD63/CCR3 (Flow2 CAST, Bühlmann Laboratories)	Clinical history and skin tests	Flow-CAST: sensitivity 53% and specificity 80% Flow2CAST: sensitivity 55% and specificity 80% (cutoff, activated basophils ≥ 5% and SI ≥ 2)
Garcia-Ortega, 2010 [13]	Amoxicillin	Anaphylaxis	14 Patients	CD63 (Basotest)	Clinical history	Sensitivity 28.6% (cutoff, activated basophils ≥ 5% and SI ≥ 2)
De Weck, 2009 [14]	Various beta-lactams	Immediate hypersensitivity	181 Patients and 81 controls	CD63 (Flow-CAST)	Clinical history and/or rechallenge	Sensitivity 48.3% (cutoff, activated basophils ≥ 5% and SI ≥ 2)
Abuaf, 2008 [15]	Amoxicillin	Immediate hypersensitivity (anaphylaxis and urticaria)	27 Patients, 14 tolerant controls, and 6 positive delayed controls	CD63 and CD203c	Clinical history and skin tests	CD63: sensitivity 22% and specificity 79% CD203c: sensitivity 52% and specificity 100% (cutoff, activated basophils ≥ negative controls plus 6%)
Torres, 2004 [16]	Various beta-lactams	Immediate hypersensitivity (anaphylaxis and urticaria)	70 Patients and 40 tolerant controls	CD63 (Basotest)	Clinical history and skin tests	Sensitivity 48.6% and specificity 91.3% (cutoff, activated basophils ≥ 5% and SI ≥ 2)
Gamboa, 2004 [17]	Penicillin G, ampicillin, and amoxicillin	Immediate hypersensitivity	23 Patients and 30 tolerant controls	CD63	Drug provocation test	Sensitivity 39.1% and specificity 93.3% (cutoff: activated basophils ≥ 5% and SI ≥ 2)
Sanz, 2002 [18]	Various beta-lactams	Immediate hypersensitivity (anaphylaxis and urticaria)	58 Patients and 30 tolerant controls	CD63	Clinical history and skin tests	Sensitivity 50% and specificity 93.3% (cutoff, activated basophils ≥ 5% and SI ≥ 2)

SI, stimulation index.

encompassing IgE-mediated allergic reactions and non-immunological intolerances. The results with BAT on aspirin/NSAIDs hypersensitivity are conflicting or inconclusive (Table 3) [28-40]. Aspirin intolerance is mediated by the pharmacological effects on cyclooxygenase enzyme inhibition; therefore, it may not be a usual indication for BAT. It was discovered that BAT was not useful in patients with mild or cutaneous reactions, but it could only be indicated for severe reactions [30, 31]. In patients with aspirin intolerance, the combination of CD63 and CD203c measurements did not enhance the test sensitivity, which remained at 33.3% [35]. De Weck et al. [41] have questioned the proper interpretation on two earlier positive reports [38, 39]. Release of tryptase and histamine in response to oral challenges with aspirin suggested that circulating basophils play a role in aspirin intolerance [42]. However, these relationships are dose-dependent and likely to be mediated by the pharmacological inhibition of synthesis of prostaglandin E2, a natural inhibitor of basophil activation [41]. Therefore, BAT in aspirin intolerance may have to be sophisticated further to enhance the differences in dose responses between patients and controls. As diclofenac and

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Table 2. Summary of studies in the diagnostic utility of basophil activation tests in immediate type neuromuscular blocking agent hypersensitivity

Reference	Drug	Diagnosis	Subjects	Activation marker	Reference test	Findings
Leysen, 2011 [19]	Rocuronium	Perioperative anaphylaxis	59 IgE-mediated rocuronium allergic patients and 25 non-exposed controls	CD63	Positive reaction to any of skin test, basophil activation test, or ImmunoCAP specific IgE test	Sensitivity 80% and specificity 96% (cutoff, activated basophils ≥ 4%)
Ebo, 2006 [20]	Rocuronium	Perioperative anaphylaxis	14 Allergic patients and 8 tolerant controls	CD63	Clinical history and positive skin tests	Sensitivity 91.7% and specificity 100% (cutoff, activated basophils ≥ 4%)
Kvedariene, 2006 [21]	Suxamethonium, pancuronium, vecuronium, rocuronium, and atracurium	Perioperative hypersensitivity	47 Patients and 45 controls	CD63 (Basotest, Orpegen Pharma, Heidelberg, Germany)	Clinical history and skin tests	Sensitivity 36.1–85.7% and specificity 93.3% (cutoff, activated basophils ≥ 5% and SI ≥ 2)
Sainte-Laudy, 2006 [22]	Rocuroniun, succamethonium, vecuroniun, and cis-atracuriun	Perioperative anaphylaxis	10 Patients	CD63	Clinical history and skin tests	Sensitivity 57% (cutoff, predetermined index of 5)
Sudheer, 2005 [23]	Alcuronium, atracurium, mivacurium, rocuronium, suxamethonium, and vecuronium	Perioperative anaphylaxis	14 Patients and 10 controls	CD63 and CD203c	Clinical history	CD63: sensitivity 78.6% and specificity 100% CD203c: sensitivity 28.6% and specificity 100% (cutoff, two sequential dilutions induced greater than 10% in CD63 or CD203c expression)
Monneret, 2002 [24]	Atracurium, mivacurium, rocuronium, suxamethonium, and vecuronium	Perioperative immediate hypersensitivity	39 True allergic patients, 11 suspicious patients, and 17 controls	CD63	Clinical history and skin tests	Sensitivity 54% and specificity 100% (cutoff, two sequential dilutions induced greater than 10% in CD63 expression)
Abuaf, 1999 [25]	Vecuronium, suxamethonium, rocuronium, atracurium, pancuronium, and alcuronium	Perioperative allergy	28 Typical allergic patients, 5 atypical patients, 8 other drug allergic patients, 14 preanesthetic allergic patients, and 7 normal controls	CD63 or CD45	Clinical history and skin tests	CD63: sensitivity 64% and specificity 93% CD45: sensitivity 43% and specificity 93% (cutoff, changes more than 15% in CD63 or CD45 expression)

SI, stimulation index.

naproxen have stronger *in vitro* pharmacological activity than aspirin, their inclusion has been suggested for enhancing the sensitivity of BAT [41].

Specific allergy to dipyrone has been evaluated by BAT [28, 33, 40]. Sensitivity and specificity ranged from 42.3% to 70% and 85.7% to 100%, respectively, depending on the cutoff values. A propyphenazone allergy case, which was diagnosed by BAT after human serum albumin (HSA) conjugation, has been previously reported [43]. However, in patients with selective diclofenac allergies, either diclofenac- or HSA-conjugated metabolites did not trigger CD63 expression [32].

Fluoroquinolones

Fluoroquinolones, in addition to beta-lactams, cause one of the most common antibiotic allergies, and this hypersensitivity has become increasingly common with increased prescription rates of the drug [44]. BAT has gained considerable interest for testing fluoroquinolone hypersensitivities because the diagnostic utility of skin tests is very limited due to its skin-irritation properties in intradermal tests (88% false positives) [45]. To date, seven studies

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Table 3. Summary of studies in the diagnostic utility of basophil activation tests in aspirin/NSAIDs hypersensitivity

Reference	Drug	Diagnosis	Subjects	Activation marker	Reference test	Findings
Hagau, 2013 [28]	Dipyrone	Immediate hypersensitivity	20 Patients and 10 tolerant controls	CD63 (Flow2-CAST, Bühlmann Laboratories, Schönenbuch, Switzerland)	Clinical history and skin tests	Sensitivity 70% and specificity (cutoff, SI > 1.73 on the basis of ROC analyses)
Kim, 2012 [29]	Aspirin, ibuprofen, naproxen, piroxicam, isopropylantipyrine, diclofenac, and Joins	Immediate hypersensitivity (anaphylaxis 17%)	18 Patients and 18 tolerant controls	CD63 (Flow-CAST, Bühlmann Laboratories)	Clinical history	Positive only to aspirin but not to culprits: sensitivity 61% and specificity 91% (cutoff, activated basophils ≥ 5% and SI ≥ 2)
Abuaf, 2012 [30]	Aspirin, diclofenac, ketoprofen, celecoxib, and acetaminophen	Nonallergic hypersensitivity	60 Patients and 12 controls	CD63	Clinical history	For aspirin; sensitivity 37% and specificity 90% (cutoff, activated basophils ≥ negative controls + 6%)
Korosec, 2011 [31]	Aspirin	Aspirin intolerance	19 Intolerant patients and 40 tolerant controls	CD63	Drug provocation test	For anaphylactoid reactions; sensitivity 80% and specificity 83% For asthma/rhinitis reactions; sensitivity 78% and specificity 50% (cutoff, SI > 2.18 by ROC analyses)
Harrer, 2010 [32]	Diclofenac	Acute hypersensitivity to diclofenac	15 Patients and 3 tolerant controls	CD63 (Flow2-CAST)	Clinical history (mostly drug provocation test)	Sensitivity 0% (cutoff, activated basophils > 5% and SI > 2)
Gomez, 2009 [33]	Dipyrone	Immediate allergic reactions to dipyrone	51 Patients and 56 controls	CD63	Clinical history, skin tests and/or drug provocation test	Sensitivity 54.9% and specificity 85.7% (cutoff, activated basophils ≥ 5% and SI ≥ 2)
Çelik, 2009 [34]	Lysine aspirin	Aspirin exacerbated respiratory disease	10 Patients and 10 tolerant controls	CD63, CD69 and CD203c	Clinically documented asthmatic responses after ingestion of aspirin	CD63: sensitivity 30% and specificity 40% CD203c: sensitivity 70% and specificity 45% CD69: sensitivity 80% and specificity 34% (cutoff: SI ≥ 2)
Bavbek, 2009 [35]	Aspirin	Aspirin intolerance	18 Aspirin sensitivity patients, 12 aspirin tolerant controls, and 12 healthy controls	CD63 (Flow-CAST) and CD203c (Allergenicity Kit; Beckman Coulter, Miami, FL, USA)	Drug provocation test	CD63: sensitivity 33.3% and specificity 79.2% CD203c: sensitivity 16.7% and specificity 100% (cutoff, activated basophils ≥ 5% and SI ≥ 2)
Rodriquez-Trobado, 2008 [36]	Aspirin, ibuprofen, metamizol, diclofenac, paracetamol, and ketorolac	Aspirin/NSAIDs hypersensitivity (both of selective hypersensitivity and intolerance)	16 Selective hypersensitive patients, 27 intolerant patients, and 29 tolerant controls	CD63 (Basotest, Orpegen Pharma, Heidelberg, Germany)	Clinical history and drug provocation test	Sensitivity 42.9% and specificity 100% (cutoff, SI ≥ 2)
Malbran, 2007 [37]	Diclofenac	Immediate reaction (asthma exacerbation, urticaria, angioedema, and anaphylaxis)	14 Patients and 12 controls	CD63	Clinical history	Sensitivity 0% and 100% (cutoff, mean + 2 standard deviation in controls)
Sanz, 2005 [38]	Aspirin, paracetamol, metamizol, diclofenac, and naproxen	Aspirin/NSAIDs hypersensitivity	60 Patients and 30 controls	CD63	Recurrent clinical history and/or drug provocation test	Aspirin: sensitivity 76.2% and specificity 89.5% (cutoff, activated basophils ≥ 5% and SI ≥ 2)
Gamboa, 2004 [39]	Aspirin, paracetamol, metamizol, diclofenac, and naproxen	Aspirin/NSAIDs hypersensitivity	60 Patients and 30 aspirin tolerant controls	CD63	Drug provocation test	Aspirin: sensitivity 43.3% and specificity 100% Aspirin + paracetamol + metamizol + diclofenac: se nsitivity 63.3% and specificity 93.3% (cutoff, activated basophils ≥ 5% and SI ≥ 2)
Gamboa, 2003 [40]	Metamizol (same with dipyrone)	Metamizol specific allergy (anaphylaxis, or urticaria/angioedema)	26 Patients and 30 controls (aspirin intolerance was excluded)	CD63	Drug provocation test	Sensitivity 42.3% and specificity 100% (cutoff, activated basophils ≥ 5% or SI ≥ 5)

SI, stimulation index; ROC, receiver operating curve, NSAIDs, non-steroidal anti-inflammatory drugs.

[46-52] have investigated the diagnostic utility of BAT (Table 4). The first study reported no positive BAT results in four DPT-proven patients [52]. Similarly, negative findings were reported in another study (n = 4, 0% positivity) [50]; however, larger scale studies performed later contradicted these findings. Another group discovered 70%–83% up-regulation of CD203c upon drug stimulation in all five participants with a history of anaphylaxis [51]. Other studies confirmed these findings by showing 71.1% sensitivity in 38 patients [49], and 36% sensitivity in 66 patients [47]. The excellent negative predictive value for DPT outcomes advocates the high utility of BAT in patients with suspected history of fluoroquinolone hypersensitivity [48].

Radiocontrast media

RCM hypersensitivity is a commonly encountered adverse drug reactions, and is the most common cause for anaphylaxis at a referral hospital in Korea [53]. Despite the introduction of non-ionic contrast media, the incidence of immediate hypersensitivity and severe reactions still appear as frequent as 2.1% and 0.01% per exposure, respectively [54]. Although skin testing is a relevant diagnostic method to determine the cause of hypersensitivity, it was meaningful only among patients with a history of moderate to severe hypersensitivity (40% positive in intradermal tests) [55]. Moreover, skin testing cannot detect non-IgE mediated RCM reactions.

Several studies [56-59] so far have analyzed the diagnostic value of RCM BAT (Table 5). Initial studies by Pinnobphun et al. [57] found

Table 4. Summary of studies in the diagnostic utility of basophil activation tests in immediate type fluoroquinolone hypersensitivity

Reference	Drug	Diagnosis	Subjects	Activation marker	Reference test	Findings
Mayorga, 2013 [46]	Ciprofloxacin and moxifloxacin	Immediate hypersensitivity	15 Ciprofloxacin patients, 13 moxifloxacin patients, and 20 tolerant controls	CD63	Drug provocation test (mostly)	Sensitivity to ciprofloxacin: 33.3% in light and 40% in dark conditions Sensitivity to moxifloxacin: 15.4% under light conditions and 46.2% under dark conditions Specificity 90% to both (cutoff, SI ≥ 3)
Blanca-Lopez, 2013 [47]	Norfloxacin, ciprofloxacin, moxifloxacin, and levofloxacin	Immediate hypersensitivity	66 Retrospectively confirmed patients	CD63	Drug provocation test and/or basophil activation test	Sensitivity 36% (cutoff, activated basophils ≥ 5% and SI ≥ 2)
Rouzaire, 2011 [48]	Levofloxacin, ofloxacin, ciprofloxacin, moxifloxacin, lomefloxacin, flumequin, norfloxacin, and pipemidic acid	Immediate hypersensitivity	34 Patients with suspected history (16 patients underwent drug provocation tests)	CD203c	Drug provocation test	Specificity 100% (cutoff, at least two sequential drug dilutions induced more than 10% CD203c above the negative control)
Aranda, 2011 [49]	Ciprofloxacin, moxifloxacin, and levofloxacin	Immediate hypersensitivity	38 Patients and 25 tolerant controls	CD63	Anaphylaxis by clinical history; urticaria by drug provocation test	Sensitivity 71.1% and specificity 88% (cutoff, activated basophils ≥ 5% and SI ≥ 2)
Lobera, 2010 [50]	Ciprofloxacin, levofloxacin, moxifloxacin, and norfloxacin	Immediate hypersensitivity	6 Tested patients and 12 controls	CD63	Drug provocation test	Sensitivity 0% and specificity 100% (cutoff, activated basophils ≥ 5% and SI ≥ 2)
Ben Said, 2010 [51]	Levofloxacin, moxifloxacin, and ofloxacin	Immediate hypersensitivity, moderate to severe grade	5 Patients and unclear number of controls	CD203c	Clinical history	Sensitivity 100% and specificity 100% (activated basophils, 70%–83% in patients and 1%–2% in controls)
Seitz, 2009 [52]	Levofloxacin, moxifloxacin, and ciprofloxacin	Anaphylaxis	4 Patients	CD63 (Flow-CAST, Bühlmann Laboratories, Schönenbuch, Switzerland)	Drug provocation test	Sensitivity 0% (cutoff, activated basophils ≥ 5%)

SI, stimulation index.

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the sensitivity to be 46.2%-61.5% and specificity 88.4%-100%, depending on the cutoff values. Recent studies reported the BAT sensitivity to be 62.5% compared to the outcome from intravenous challenges (n = 8), thereby confirming previous findings [56]. Interestingly, the skin test positivity did not correlate with BAT results, and BAT positivity did not correlate with the severity of reactions [57]. These findings suggest complementary roles for BAT in the diagnosis of RCM hypersensitivity. Further studies are necessary to understand its negative predictive values and to identify the precise mechanism for predicting safe alternative RCM in high-risk patients.

Antineoplastics and others

Recent studies [60-64] examined the outcome of BAT in patients with hypersensitivities to antineoplastic, biologic agents, or other drugs (Table 6). L-Asparaginase allergies were assessed using CD203c expression and were found to have high sensitivity (75%) and negative predictive value (96%) [60]. One case study also reported the potential utility of BAT in cisplatin hypersensitivity [65]. Because patients with malignancies may frequently have comorbidities or conditions that hamper skin testing, administering BAT will be advantageous in these cases.

Hypersensitivity to other biologic agents such as rituximab [61] or infliximab [66] were examined by BAT, although the results warrant further confirmation. Among corticosteroids, methylprednisolone [62, 67] and succinylated corticosteroids [68-70] have been tested. Hypersensitivity to anti-histamines such as cetirizine, desloratadine, ebastine, fexofenadine, or dexchlorpheniramine was also assessed by BAT [71-74]. Other reports included testing for pholcodine [75], glatiramer [63], gelofusine [64], amidotrizoate [76], pristinamycin [77], enoxaparin [78], heparin [79], afloqualone [80], cremophor EL [81], hydrochlorothiazide [82, 83], polyoxyethylene (20) sorbitan monooleate [84, 85], chlorhexidine [86], ophthalmic atropine [87], and carboxymethylcellulose [88] in allergic or non-immunologic adverse reactions (Table 7) [13, 27, 61, 66-93]. Further studies are required for determining the causal relationships and identifying safe alternatives in patients with hypersensitivity to drugs that are not evaluated until date.

METHODOLOGY

The theoretical and technical details of BAT have been extensively discussed before [2, 3, 41, 94-98]. Briefly, BAT is a flow cytometry-based cellular assay that measures the activation of basophils upon allergen stimulation. The activation response can be measured at a single-cell level by using fluorochrome-bound monoclonal antibodies (mAbs) to specific activation

Table 5. Summary of studies in the diagnostic utility of basophil activation tests in immediate type radiocontrast media hypersensitivity

Reference	Drug	Diagnosis	Subjects	Activation marker	Reference test	Findings
Salas, 2013 [56]	Iobitridol, iomeprol, iodixanol, iohexol, ioversol, iopromide, and ioxaglate	Immediate hypersensitivity	8 Patients confirmed by drug provocation test and 20 controls	CD63 (Basotest, Orpegen Pharma, Heidelberg, Germany)	Drug provocation test (intravenous administration of cumulative dose 100 cc)	Sensitivity 62.5% and specificity 100% (cutoff, activated basophils ≥ 5% and SI ≥ 2)
Pinnobphun, 2011 [57]	Ioxithalamate, iopromide, iohexol, iopamidol, and iobitridol	Immediate hypersensitivity	26 Patients and 14 controls	CD63 (Flow2-CAST, Bühlmann Laboratories, Schönenbuch, Switzerland)	Clinical history	Sensitivity 61.5% and specificity 76.7% with 1:10 RCM; sensitivity 50% and specificity 90.7% with 1:100 RCM (cutoff, activated basophils ≥ 5% and SI ≥ 2)
Javaloyes, 2012 [58]	Gadobutrol	Anaphylaxis	3 Patients and 5 controls	No information	Clinical history	Sensitivity 100% and specificity 100% (cutoff, activated basophils ≥ 5% and SI ≥ 2)
Trcka, 2008 [59]	Iopamidol, iopromide, iomeprol, and iopentol	Anaphylaxis	3 Patients with positive intradermal tests and unknown number of non-allergic controls	CD63 (Flow2-CAST)	Intradermal tests	Sensitivity 100% and specificity 100% (cutoff, activated basophils ≥ 5% and SI ≥ 2)

SI, stimulation index.

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Table 6. Summary of studies on the outcomes of basophil activation tests in various drug reactions

Reference	Drug	Diagnosis	Subjects	Activation marker	Reference test	Findings
Hino, 2013 [60]	L-asparaginase	Allergic reaction within several hours after injection	8 Allergic patients and 24 tolerant controls	CD203c (Allergenicity Kit; Beckman Coulter, Miami, FL, USA)	Clinical history	Sensitivity 75% and specificity 82% (cutoff, activated basophils ≥ 14.4%, showing the area under the curve 0.81)
Piva, 2011 [61]	Rituximab	Immediate hypersensitivity despite premedication	5 Hypersensitivity patients, 13 tolerant controls and 18 healthy controls	CD63	Clinical history	No results on sensitivity and specificity; activated basophils: 6.75 ± 3.79 in patients and 1.92 ± 1.16 in controls at 0.25 μg/μL ($p < 0.001$)
Aranda, 2010 [62]	Methylprednisolone	Anaphylaxis and urticaria	4 Patients and 10 tolerant controls	CD63	Skin test and drug provocation test	Sensitivity 75% and specificity 100% (cutoff, SI ≥ 2)
Soriano Gomis, 2012 [63]	Glatiramer	Anaphylaxis	3 Patients and 6 controls	No information	Clinical history	Sensitivity 66.7% and specificity 83.3% (no information on the cutoff)
Apostolou, 2006 [64]	Gelofusine (succinylated gelatin)	Perioperative anaphylaxis	6 Clinically gelofusine anaphylaxis patients, 3 healthy controls, and 5 controls with confirmed sensitivity to NMBA	CD63	Clinical history	Sensitivity 100% and specificity 87.5% (cutoff, activated basophils ≥ 3.6%)

SI, stimulation index; NMBA, neuromuscular blocking agent.

markers. Currently, two activation markers, CD63 and CD203c, are commonly used for diagnostic purposes. Upon basophil activation, these two markers are commonly upregulated with similar kinetics, but they have distinct characteristics from each other. CD63 has been better validated for drug allergies; however, CD203c is increasingly utilized in recent studies [15, 23, 34, 35, 48, 51, 60]. Upon anaphylactic stimulation, there is degranulation that causes CD63 to appear at the cell surface during the process of fusion of main granules with plasma membranes [2]. Although CD63 is also expressed on platelets, eosinophils, and monocytes; its expression on basophils can be identified using additional stains for basophil markers such as IgE, CD123, CCR3, CRTH2, and CD203c [3]. CD203c can also be used as an identification marker since it is exclusively expressed on basophils, and this expression is related to piecemeal degranulation of basophils [2]. Unlike CD63, CD203c is constitutively expressed on resting basophils at low levels, but it is highly expressed upon activation [99].

Because CD63 and CD203c activation markers do not show the same responses to stimulation, some commercial kits measure both markers simultaneously, to increase the sensitivity of the tests. In some clinical studies, CD203c showed better sensitivity (52%) than CD63 (22%) in patients with amoxicillin allergy [15]; however, other studies reported better sensitivity of CD63 [23, 35]. Another difference between the two markers is their response to IL-3 priming. In commercial kits, IL-3 is often used for increasing BAT sensitivity; its addition results in the enhancement of CD63 expression but a blunted CD203c response to allergen stimulation [3].

For the BAT procedure, fresh whole blood is withdrawn (100 μL per tube) and processed within 4 h, because the basophil reactivity starts to decline after 4 h from sampling [96]. Anti-FcεRI mAb and N-formyl-methionyl-leucyl-phenylalanine (fMLP) are used as positive controls, and stimulation buffer alone as a negative control. If subjects do not respond to anti-FcεRI (called non-responders), then their BAT results cannot be interpreted and have to be rejected for analysis. The response to fMLP is utilized for assessing cellular viability and the ability to express activation markers. Laboratory protocols differ with different commercial kits and between institutions. For diagnostic purposes, researchers may either set up their own in-house protocols, or utilize commercially available BAT kits that are designed to enhance the sensitivity of the tests.

Drug preparation and dose determination

The preparation of drugs and their dose determination is one of the most challenging steps of BAT because they have a narrower range of testing concentrations than inhalant or food allergens [98]. Several varieties of drug allergens are commercially available, but they are expensive, and selection is frequently a limiting factor.

Table 7. Summary of case reports on the application of basophil activation tests in immediate type drug allergy

Reference	Drug	Diagnosis	Findings
Philipse, 2013 [89]	Iomeprol	Anaphylaxis	Positive
Dewachter, 2009 [76]	Amidotrizoate	Anaphylaxis	Positive for CD203c but negative for CD63 in the patient, and negative for both markers in 2 controls
Longo, 2008 [90]	Amoxicillin/clavulanic acid	Urticaria and angioedema (n = 2)	Positive to clavulanic acid, but negative to amoxicillin
Rodriguez Trabado, 2006 [91]	Cloxacillin	Anaphylaxis	Positive
Bensaid, 2009 [77]	Pristinamycin	Anaphylaxis (n = 3)	Positive (n = 3)
Anders, 2013 [78]	Enoxaparin	Anaphylaxis	Negative
Caballero, 2003 [79]	Heparin	Acute urticaria (n = 2)	Positive (n = 1)
Hur, 2012 [80]	Afloqualone	Anaphylaxis	Positive
Renauld, 2011 [92]	Atracurium	Perioperative anaphylaxis	Negative (suggesting the diagnosis of mastocytosis)
Sudheer, 2007 [27]	Vecuronium	Anaphylaxis	Positive
Monneret, 2000 [93]	Rocuronium, and suxamethonium	Perioperative anaphylaxis (n = 4)	Positive (n = 4)
Leysen, 2013 [75]	Pholcodine	Anaphylaxis (n = 3)	Positive in patients (n = 3) and negative in controls (n = 3), (cutoff, activated basophils ≥ 10%)
Garcia-Ortega, 2010 [13]	Metamizol (same with dipyrone)	Anaphylaxis (n = 5)	Positive (n = 5)
Nucera, 2011 [69]	Hydrocortisone sodium succinate	Urticaria	Undetermined due to no adequate basophil responses to positive controls
Walker, 2011 [68]	Succinylated corticosteroids	Immediate hypersensitivity (n = 2)	Positive (n = 2) to succinylated corticosteroids but negative to non-succinylated corticosteroids
Ben Said, 2010 [67]	Methylprednisolone	Anaphylaxis	Positive in the patient, but negative in 2 controls
Lehmann, 2008 [70]	Prednisolone-21-hydrogen succinate	Anaphylaxis	Positive
Ebo, 2001 [81]	Cremophor EL (polyethoxylated castor oil) in cyclosporine intravenous preparation	Anaphylaxis after intravenous cyclosporine injection	Positive in the patient, but negative in 2 controls
Viardot-Helmer, 2008 [65]	Cisplatin	Anaphylaxis	Positive
Manso, 2010 [66]	Infliximab	Malaise, flushing, palpitation, and urticaria	Negative (suggesting no involvements of IgE mechanisms)
Manso, 2010 [82]	Hydrochlorothiazide	Noncardiogenic pulmonary edema (n = 2)	Positive (n = 2)
Gamboa, 2005 [83]	Hydrochlorothiazide	Acute lung edema	Negative (suggesting no involvements of IgE mechanisms)
Badiu, 2012 [85]	Polysorbate 80	Anaphylaxis after Gardasil® injection	Negative (possibly false negative reaction as skin prick test was positive)
Coors, 2005 [84]	Polyoxyethylene-sorbitan-20-monooleate (also known as polysorbate 80 and Tween 80)	Anaphylaxis after multivitamin product injection	Positive in the patient, but negative in 2 controls
Ebo, 2004 [86]	Chlorhexidine	Anaphylaxis after urethral catheterisation	Positive in the patient, but negative in 2 controls
Bobadilla-Gonzalez, 2011 [71]	Cetirizine and desloratadine	Acute urticaria	Positive to desloratadine, cetirizine, ebastine, and hydroxyzine
Sanchez Morillas, 2011 [72]	Ebastine and fexofenadine	Urticaria	Negative
Lee, 2011 [73]	Fexofenadine	Urticaria	Positive
Cáceres Calle, 2004 [74]	Dexchlorpheniramine	Anaphylaxis	Positive in the patients, and negative in 8 controls
Cabrera-Freitag, 2009 [87]	Ophthalmic atropine	Erythema and generalized edema	Positive in the patient, and negative in 3 controls
Dumond, 2009 [88]	Carboxymethylcellulose	Anaphylaxis	Positive

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In the case of drugs that are not commercially available, dose response curve analyses and cytotoxicity assays are mandatory steps for determination of optimal concentrations [100]. In this section, we have summarized the methods and results from previous studies, as a reference point. Higher drug concentrations can be used for diagnostic purposes since they provide enhanced sensitivity; however, they should be tested in tolerant controls due to the risk of cellular toxicity and nonspecific basophil activation.

Beta-lactams

Previous dose-response and cytotoxicity studies provided a range of drug concentrations that can be used for stimulation. Beta-lactams, in general, were reconstituted at 0.01, 0.1, and 1 mg/mL in the dilution buffer [15]; and specifically, benzylpenicillin at 0.4 and 2 mg/mL; penicilloyl-polylysine at 0.005 and 0.025 mg/mL; penicillin minor determinant mixture at 0.1 and 0.5 mg/mL; ampicillin at 0.25 and 1.25 mg/mL [14, 16]; clavulanic acid at 0.156 and 0.625 mg/mL [90]; cefuroxime at 0.83 and 1.2 mg/mL; and cefazolin at 0.16 and 0.4 mg/mL [18]. In the case of amoxicillin, 1.25 mg/mL and a range of 0.25–0.31 mg/mL final concentrations were utilized [11, 13, 14].

Neuromuscular blocking agents

Several studies successfully tested varying concentrations of NMBAs, ranging from 1:1000 to 1:10 dilutions [21, 23, 25]. At a dilution of 1:10,000, no significant basophil activation was observed [21]. Other studies have reported 5×10^2 μg/mL NMBA concentration as optimal [20]. However, it should be noted that there might be different optimal concentrations required for stimulation [20, 25].

Aspirin/non-steroidal anti-inflammatory drugs

Aspirin intolerance is usually dose dependent; therefore, the dose determination in this case is extremely critical. According to some functional cytotoxicity studies, only diclofenac showed *in vitro* cytotoxicity at levels higher than 1.25 mg/mL [38]. The concentrations recommended for stimulation are as follows: aspirin at 0.3, 1.25, and 5 mg/mL; paracetamol at 0.3, 1.25, and 5 mg/mL; dipyrone at 0.6, 5, and 20 mg/mL; and diclofenac at 0.08 and 0.3 mg/mL. Interestingly, high concentrations of aspirin (5 mg/mL) enhanced the sensitivity of the test but also lowered its specificity (to 89.5%). Diclofenac at a high concentration (1.25 mg/mL) resulted in false-positive reactions in 36.8% of controls, but it gave acceptable results at lower concentrations. Naproxen at 5 mg/mL resulted in up-regulation of CD63 in controls, giving rise to increased false positives (85.2%); therefore, it was not routinely recommended for use in BAT. The test concentrations determined by other researchers were sometimes quite low [36] but mostly within the range as for aspirin [29-31, 35].

Fluoroquinolones

Fluoroquinolones are known to have skin-irritating properties [45]. Recent studies have reported contrasting but interesting results. Two studies have shown negative BAT results in patients. In the first study, 4 patients were administered 1:10, 1:100, and 1:1,000 dilutions of levofloxacin, moxifloxacin, or ciprofloxacin, ranging from 1.6 to 5 mg/mL parenteral preparations [52]; and in the second study, 6 patients were administered ciprofloxacin at 0.05–0.1 mg/mL, levofloxacin at 0.05–0.1 mg/mL, and moxifloxacin at 0.125–0.25 mg/mL [50]. Aranda et al. [49] were the first to report the dose response analyses for fluoroquinolones in a large group of patients (n = 38), and they have provided an optimal stimulation concentration range (ciprofloxacin at 0.2–2 mg/mL; moxifloxacin at 0.1–0.2 mg/mL; and levofloxacin at 2–4 mg/mL) in their subsequent studies [46, 47].

One important point to note is the potential difference in immunogenicity between fluoroquinolones. Researchers have found that in patients with moxifloxacin hypersensitivity, moxifloxacin was the most frequent culprit drug *in vivo* [47, 49], but it had a lower sensitivity than ciprofloxacin in inducing basophil activation *in vitro* [49]. These results demonstrated the cross-reactive nature of fluoroquinolone hypersensitivity, and highlighted the involvement of specific critical factors related to *in vitro* moxifloxacin allergenicity. Recently it was discovered that moxifloxacin underwent photo-degradation, which critically decreased *in vitro* basophil responses, thus resulting in lower BAT positivity under light (17.9%) than under dark (35.7%) conditions [46]. In contrast, ciprofloxacin did not have different outcomes between light and dark conditions (both 46.4%). It is not confirmed whether these observations are applicable to other kinds of drugs, but they emphasize the importance of accurate drug preparations for conducting *in vitro* drug assays.

Radiocontrast media

The effects of a wide range of RCM concentrations, from 10^0 to 10^5 dilutions, were first tested on 3×10^5 peripheral blood mononuclear cells [57]. Cell viability was measured by staining for annexin-V, and the optimal dilution of RCM was determined to be

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1:10 and 1:100. Later studies confirmed the optimal dilutions for RCM at 1:10 [56].

Cutoff points for positive BAT

A sufficient number of well-defined cases and controls are necessary for determining appropriate cutoff points for each drug. Based on these, researchers perform receiver operating characteristic (ROC) curve analyses to locate optimal points. However, the prevalence of drug allergies is low, and drug allergens are more varied than inhalant or food allergens.

The cutoff points are usually based on the percentage of activated basophils, e.g., > 15% above background for inhalant or food allergens, and > 10% above background for latex or hymenoptera venoms [41]. However, in the case of drug allergens, the basophil response is usually lower than that of inhalant or food allergens; therefore, the cutoff is set at > 5% above background, or determined specifically for individual drugs. The stimulation index (defined as the percentage of activated basophils after allergen stimulation per negative control stimulation) of ≥ 2 is additionally adopted, to decrease the chances of false positivity resulting from the low cutoff levels.

Further considerations

Leysen et al. [99] recently summarized several factors that should be considered while carrying out the drug BAT. The maximum recommended time interval between the anaphylactic reaction and its testing was 12 months. Effects of medications such as antihistamines and corticosteroids on *in vitro* basophil reactivity warranted further studies and should be taken into account while testing. Oral intake of 10 mg desloratadine, an antihistamine, did not influence CD63 expression in basophils upon anti-IgE stimulation, even after 3 h. However, a 30-min *in vitro* pretreatment of basophils with dimethindene (antihistamine) or prednisolone significantly influenced their activation at concentrations 50-fold higher than the therapeutic level, but not at 10-fold higher concentrations [96].

CONCLUSIONS

Drug hypersensitivity is an increasingly significant clinical issue; however, diagnosis is difficult because the underlying pathomechanisms are still unclear and allergenic structures are mostly unknown. Although DPT is the gold standard for diagnosis of drug allergies, there are potential risks of systemic reactions. Moreover, polypharmacy frequently confounds identification of the culprit drugs. BAT has several advantages over conventional diagnostic tools; it can assess multiple drugs simultaneously, safely, and specifically. As summarized in this review, BAT is being validated for diagnosing hypersensitivity with beta-lactams, NBMAs, aspirin/NSAIDs, fluoroquinolones, and RCM. In addition, the applications of BAT are rapidly extending into diagnosing allergies caused by various other drugs. In conclusion, we suggest that BAT is a promising diagnostic tool for clinical decisions regarding patients with drug hypersensitivities.

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