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...
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.

**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 N MBA 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,
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

### 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       | 30 Patients                       | CD63 (Basotest, Orpegen Pharma, Heidelberg, Germany) | Clinical history and skin tests | Sensitivity 50% (cutoff, SI ≥ 2) |
| Torres, 2010 [11] | Amoxicillin    | Immediate hypersensitivity       | 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, Schonenbuch, 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       | 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 | 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       | 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.
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

### 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, 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] | Rocuronium, succamethonium, vecuronium, and cis- atracurium | Perioperative anaphylaxis  | 10 Patients                                                             | CD63              | Clinical history and skin tests                      | Sensitivity 57% (cutoff, predetermined index of 5) |
| Sudheer, 2005 [23] | Alcuronium, atracurium, mivacurium, rocuronium, succamethonium, 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, succamethonium, 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, succamethonium, 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.
### 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 |
|-----------|------|-----------|----------|------------------|---------------|----------|
| Hagauf, 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, proxicam, isopyphenantipyrine, 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 | Sensitivity 42.3% and specificity 100% (cutoff, activated basophils ≥ 5% and SI ≥ 2) |
| 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: specificity 80% and specificity 94% (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) |
| Rodriguez-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 (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 48.3% and specificity 100% Aspirin + paracetamol + metamizol + diclofenac sensitivity 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.
Basophil activation test for drug hypersensitivity

[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

| 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, 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 (FlowCAST, Buhlmann Laboratories, Schönenbuch, Switzerland) | Drug provocation test | Sensitivity 0% (cutoff, activated basophils ≥ 5%)

SI, stimulation index.
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], alfoqualone [80], cremophor EL [81], hydrochlorothiazide [82, 83], 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    | Iobitrol, iomeplor, 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 | 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 | 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    | Iopamidol, iopromide, iomeplor, 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.
Basophil activation test for drug hypersensitivity

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 83% (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/mL (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) |
| Soniano 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-formylmethionyl-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.

If the authors require any specific inputs, they are free to provide them. However, the provided text is a faithful representation of the document content as per the guidelines.
| 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                            | Negative                                                                 |
| Anders, 2013 [78]              | Enoxaparin                                | Anaphylaxis                            | Negative                                                                 |
| Caballero, 2003 [79]           | Heparin                                   | Acute urticaria (n = 2)                | Positive                                                                 |
| Hu, 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                            | Positive in patients (n = 3) and negative in controls (n = 3), (cutoff, activated basophils > 10%) |
| Garcia-Ortega, 2010 [13]       | Metamizol (same with dipyrene)            | Anaphylaxis (n = 5)                    | Positive (n = 5)                                                        |
| Nuñez, 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 in the patient, but negative in 2 controls                     |
| 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                     |
| Vardot-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  | Positive (possibly false negative reaction as skin prick test was positive) |
| Coors, 2005 [84]               | Polyoxethylene-sorbitan-20-monoooleate (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]       | Dechlorpheniramine                        | 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                                                                |
Basophil activation test for drug hypersensitivity

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; penicillloyl-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 ceftazolin 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 × 10⁻⁵ 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 1:0, 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 to 1⁵ dilutions, were first tested on 3 × 1⁰⁵ peripheral blood mononuclear cells [57]. Cell viability was measured by staining for annexin-V, and the optimal dilution of RCM was determined to be...
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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