

The Basophil Activation Test in Immediate-Type Drug Allergy

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KEYWORDS

• Drug allergy • Basophil activation test • Passive sensitization
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Allergic adverse drug reactions (ADRs) are important and underestimated health issues leading to considerable comorbidity and additional health costs.¹ Diagnosis of drug allergy is difficult for several reasons:

Type of immune reaction—drugs can elicit a broad range of immune reactions, each of which may require different tests to prove an involvement.

Epitope—drugs can act as haptens, and they may bind to different proteins. The exact epitope (hapten-carrier complex) causing the reaction is frequently unknown.

Clinical correlation—the outcome of in vitro or in vivo tests with different drug concentrations might not correlate with situations in clinical routine.

Comedication and comorbidity—the eliciting drug often is given in combination with other drugs in patients with different diseases. This comedication and the underlying disease may influence an immune reaction to a drug or the clinical picture.

Drug challenge—the gold standard or reference diagnostic test is a time-consuming and potentially dangerous endeavor limited to specialized centers.

In view of these limitations, many efforts have been made to enlarge the armamentarium to diagnose drug allergic reactions, one of them being the basophil activation test (BAT). This article summarizes the use of BAT as a functional in vitro test in immediate-type drug allergy. These immediate-type hypersensitivity reactions are orchestrated by T cells and mediated by specific IgE (sIgE) antibodies on the surface of

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two related but autonomous groups of effector cells: the basophils in peripheral blood and the tissue-resident mast cells. They show similar activation pathways by means of membrane-bound IgE (via Fc ϵ RI) produced by plasma cells in the bone marrow. These plasma cells were stimulated by allergens in the periphery with the help of T cells (TH2 cells).² Even if basophils and mast cells might share a common bone marrow progenitor,^{3,4} mast cells are no simple tissue counterparts of the basophils. Basophils can migrate into different tissues (eg, peribronchial basophils in asthma) and drive the inflammatory process side by side with mast cells and eosinophils.⁵ Mast cells are more abundant than basophils, which are actually the smallest leukocyte species by number in peripheral blood. But in contrast to mast cells, basophils are easily accessible in the peripheral blood and therefore are favored for experimental approaches and future routine diagnostics.

BAT relies on flow cytometric quantification of alterations of specific activation markers^{6–8} on the surface (CD63, CD203c) or inside the basophils (phosphorylated p38 mitogen-activated protein kinase, P-p38MAPK) as illustrated in **Fig. 1**. These changes can be detected and quantified on a single-cell basis by flow cytometry using specific monoclonal antibodies coupled to a fluorochrome. Obviously, BAT is only applicable in those cases where the final effector function relies on activation of basophils (eg, IgE-mediated immediate type hypersensitivity).

BAT may depend on bridging of adjacent allergen-specific membrane-bound IgE molecules on the surface of basophils. One has to be aware, however, that other mechanisms of cell activation (complement system or even nonimmunologic, pharmacologic basophil activation) may occur. Consideration of this fact is particularly important in BAT with drugs. Actually, the use of BAT for drug reaction to nonsteroidal anti-inflammatory drugs (NSAIDs) may rely on a heightened sensitivity of in vivo activated basophils to NSAID and is not a sign of IgE-mediated reactions.

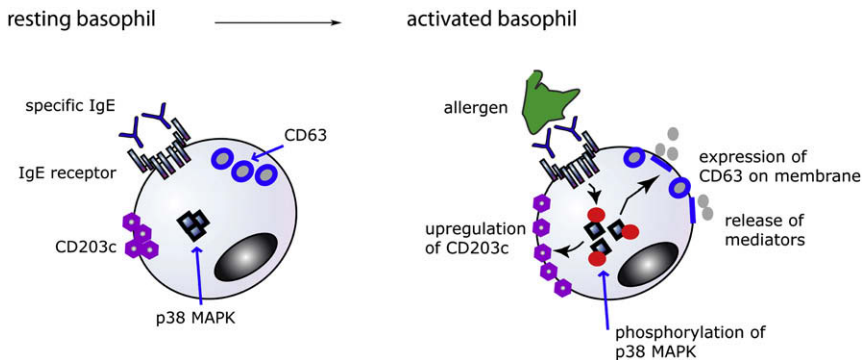


Fig. 1. Upon cross-linking of membrane-bound IgE, basophils not only secrete particular mediators, but also up-regulate the expression of specific activation markers such as CD63 and CD203c. A main difficulty of basophil activation test for drugs may be an insufficient IgE cross-linking by drugs with small molecular weight, so they are only able to bind to one IgE molecule; crosslinking and basophil activation may only occur, if the drug molecule is bivalent like eg, chlorhexidine and certain neuromuscular blocking agents or if more than two drug molecules bind to a larger protein, which then cross-links two adjacent IgE molecules. The exact mechanisms that govern basophil degranulation remain elusive, but it has been demonstrated that phosphorylation of p38 MAPK exerts a pivotal role in cell activation. (From Ebo DG, Hagendorens MM, Bridts CH, et al. The basophil activation test in immediate drug allergy. *Acta Clin Belg* 2009;64:129–35; with permission.)

In the context of an allergic reaction for drugs or related compounds, BAT has been validated in the diagnosis of an IgE-mediated allergy from neuromuscular blocking agents (NMBAs), beta-lactam antibiotics, and clavulanic acid as β -lactamase inhibitor, iodinated radiocontrast media (RCM), the antiseptic chlorhexidine, gelatine-based plasma expanders, and the infusion-excipient carboxymethylcellulose.

In some of these studies, BAT has proven to complement skin tests in assessing clinical relevant cross-reactivity and tailoring individual safe alternative therapeutic or diagnostic regimens. In an increasing number of case reports, BAT constituted the sole or a valuable additional test for agents such as the solvent Cremophor, low molecular weight heparins, the dye patent blue, starch colloids, platinum salts, the enzyme hyaluronidase, recombinant hepatitis B vaccine, the antiviral agent valacyclovir, and methylprednisolone.

PRINCIPLE OF THE BASOPHIL ACTIVATION TEST

Activation of basophils is a stepwise process starting with intracellular changes (signal transduction from Fc ϵ RI by means of phosphorylation of p38MAPK and calcium influx) followed by changes on the cell membrane (CD203c) with final degranulation of the basophils (CD63 detectable after fusion of intracellular granules with cell membrane and mediator release).⁶

BAT measures an *in vitro* activation of patients' basophils in the test tube. After blood sample preparations (whole-blood assay versus leukocyte isolation) and preincubation with interleukin (IL)-3 (depending on the allergen of interest) or prewarming of blood samples and reagents to 37°C, allergen is added, and basophils are incubated for 15 minutes to 1 hour at 37°C in the water bath (again depending on the allergen of interest). BAT is only applicable for drugs or excipients existing in a galenic formulation that is soluble in the stimulation buffer. After testing for cytotoxicity, usually one is not using a single concentration of the substance of interest but a concentration range spanning 3 to 7 log scales to demonstrate dose dependence of basophil activation. Afterwards, staining with monoclonal antibodies coupled to fluorochromes and lysis of contaminating erythrocytes follows. To select basophils from the whole leukocyte population, several positive and negative selection strategies are possible (eg, anti-CCR3/anti-CD3, anti-CD123/anti-HLA-DR). To determine the activation state, different markers, such as CD63, CD203c, or P-p38MAPK, are used. The minimal number of basophils to be analyzed should be 200 per test tube for accurate quantification of basophil activation, but it is preferred that 600 to 1000 basophils are evaluated to minimize variation of results caused by equipment measurement error alone.⁹ In the past, thresholds for positivity often were set arbitrarily (greater than 5% activated basophils with a stimulation index greater than 2, eg, less than 2.5% stimulation in negative control). These have been replaced by accurate receiver operating characteristics (ROC) analysis for the different allergens including nonallergic exposed controls.⁶

BAT is an established diagnostic test for different IgE-mediated allergies, including inhalant (eg, pollen, house dust mite, cat dander), food, hymenoptera venom, drug, and latex allergy.⁶⁻⁸ A major observation from these studies is that in contrast to protein allergens, chemicals such as drugs elicit less-pronounced *in vitro* basophil activation, resulting in a lower sensitivity of BAT for drugs as compared with inhalant and food allergens. New gating strategies and the combined use of extra- and intracellular activation markers such as phosphorylated p38 MAPK,¹⁰ however, might increase the sensitivity of BAT in this respect. For example, **Fig. 2** displays the BAT results for the different activation markers in a patient who had anaphylactic shock during intravenous therapy with cefuroxime.

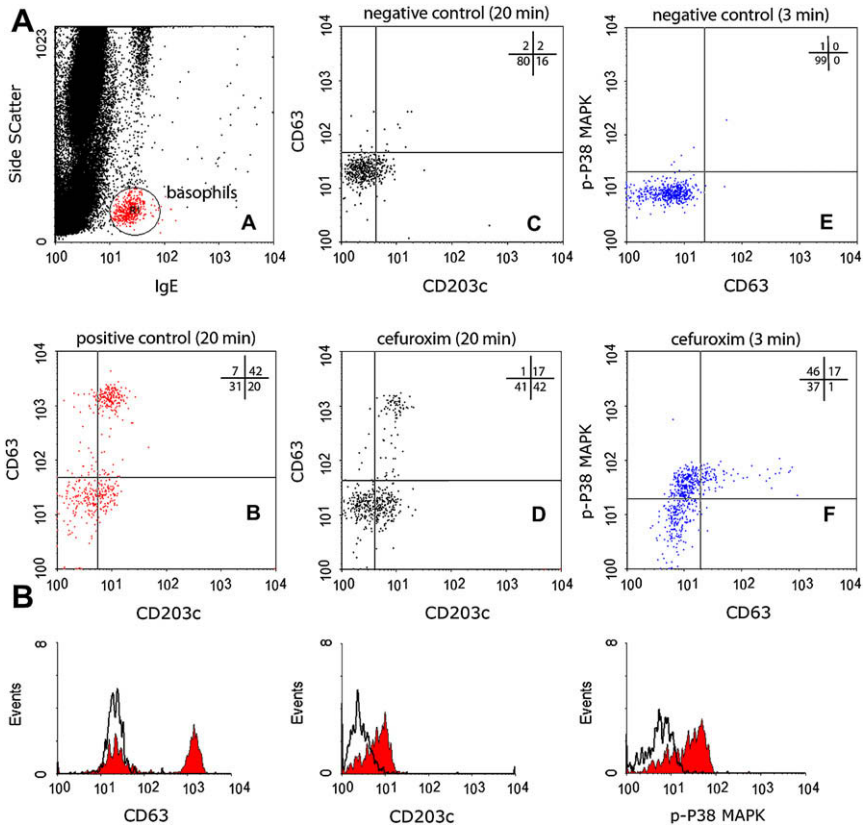


Fig. 2. Flow cytometric analysis using a dot plot (side scatter and fluorescence). Alexa 488-conjugated anti-IgE positive basophils are gated out in a circular region (*top left*). Pre-warmed basophils are stimulated for 3 or 20 minutes at 37°C with anti-IgE as positive control (*middle left*), washing solution to measure spontaneous CD63, CD203c, and phosphorylated p38 MAPK expression (negative control, *top middle and top right*) and cefuroxime (*central panel and middle right*). Note the clear bimodal up-regulation of CD63 and the more homogenous up-regulation of CD203c and phosphorylation of p38 MAPK in a larger proportion of the cells. A combination of these activation markers might increase the sensitivity of the test, particularly when only a low number of basophils can be analyzed. This is also clear from the histograms in the lower panel (open histogram spontaneous expression by resting cells, closed histogram expression after stimulation of cells with cefuroxime). (From Ebo DG, Hagendorens MM, Bridts CH, et al. The basophil activation test in immediate drug allergy. *Acta Clin Belg* 2009;64:129–35; with permission.)

The European Interest Group for the Evaluation of BAT in clinical routine (EuroBAT, part of the European Academy of Allergy and Clinical Immunology [EAACI] working group on allergy diagnosis) focuses on harmonization of the existing different BAT protocols (maybe even establishing a reference BAT protocol) to render results internationally comparable, so that as a final goal, multicenter studies are easier to conduct. This is especially important in the field of drug allergy, where the number of patients allergic to a specific drug or drug group per center is usually low, and conclusions of single-center studies are limited. Current limitations of and open questions in BAT, together with possible solutions, are shown in **Table 1**.

Table 1
Limitations and open questions in the basophil activation test

Open Questions	Possible Solutions
Optimal drug concentration	Dose–response curve with patients and healthy (exposed) controls for exclusion of unspecific stimulation
Thresholds for positivity	Receiver operating characteristics analysis for the different drugs in question with confirmed drug-allergic patients and healthy (exposed) controls instead of arbitrarily chosen thresholds
Minimal basophil number to be analyzed (600–1000 per tube)	Multicenter studies for the different drugs in question
Drug solubility	Contact experienced centers and test for unspecific stimulation by solvent (eg, EtOH) if needed
Blood sample storage and preparation (whole blood versus isolated leukocytes)	EDTA as anticoagulant at 4°C for a maximum of 24 h or passive sensitization of donor basophils with patient sera (IgE stabile for months)
Optimal preincubation conditions (interleukin 3 addition versus prewarming) and incubation time (10–40 min)	Multicenter studies for the different drugs in question

PRINCIPLE OF PASSIVE SENSITIZATION OF DONOR BASOPHILS WITH PATIENT SERUM

The proof of an IgE-mediated mechanism in drug allergy is of utmost importance for the individual patient concerning prognosis and avoidance strategies. BAT, in combination with passive sensitization of donor basophils, offers an *in vitro* correlate to the famous *in vivo* Prausnitz-Kuestner reaction or so-called passive cutaneous anaphylaxis (PCA).¹¹ This wheal-and-flare reaction on the forearm of a nonallergic subject (eg, Carl Prausnitz) 24 hours after intradermal injection of the serum of his fish-allergic colleague Kuestner at the same site, was the first proof of a serum factor transferring hypersensitivity, which later became known as IgE.¹² Because of the obvious risk of infection, this test was abandoned from clinical practice. Nevertheless, an *in vitro* Prausnitz-Kuestner approach using the BAT in combination with passive sensitization of healthy donor basophils would prove an IgE-mediated mechanism. Especially if the sensitizing capacity of patient's serum is lost after heating to 56°C for 30 minutes (with disintegration of immunoglobulins) or immunoglobulin elimination by affinity column, the IgE dependence of the reaction is proven.^{13,14}

The principle steps of passive sensitization are:

- Peripheral blood mononuclear cells (PBMC) isolation of a healthy donor, whose basophils are well-characterized in the ability of stimulation
- Removal of receptor-bound IgE with lactic acid (pH 3.9), so-called stripping
- Incubation with patient serum (1 hour, 37°C)
- Regular BAT as described previously, including negative control (eg, unstripped and stripped), but unsensitized donor basophils

This multistep procedure represents an *in vitro* Prausnitz-Kuestner or *in vitro* passive anaphylaxis test. It depends on the exact and cautious handling of the basophils to avoid unspecific stimulation or cell death. A protocol elaborated by Pruzansky

and colleagues,¹⁵ which has been modified by different groups,^{16,17} is working well in the authors' hands.

CURRENT APPLICATIONS
Neuromuscular Blocking Agents

Six larger studies have investigated the diagnostic performance of BAT in allergy from curarising neuromuscular blocking agents (NMBAs) (Table 2).^{18–23} The widely varying sensitivity (36% to 92%, specificity greater than 90%) reported in some of these publications merits reflection. First, the lowest sensitivity was observed in surveys that failed to enroll well-characterized control individuals who were exposed but did not react to the drug(s) under investigation. Therefore, a ROC analysis for discriminative cut-off calculation between patients and controls was not possible, and an arbitrarily chosen decision threshold was set relative to spontaneous CD63 expression by resting basophils. These thresholds were based upon experience with potent protein allergens that are known to elicit stronger in vitro basophil responses than drugs. Second, the optimal concentration for basophil activation might differ considerably from one NMBA to another,¹⁸ rendering the proposal to adopt a universal decision threshold for all NMBAs difficult to justify and probably obsolete. Finally, sensitivity of the test might decrease over time.²¹

In contrary to the in vivo drug challenge, BAT allows simultaneous testing of different potentially cross-reactive drugs (with subsequent drug challenge to document

Table 2 Basophil activation test in drug allergy					
Stimulus	Reference Test	Sens	Spec	N	Reference
Neuromuscular blocking agents (NMBA)					
NMBA	H	64	93	62	18
NMBA	H ± ST	54	100	60	19
NMBA	H	79	100	31	20
NMBA	H + ST	36–86	93	92	21
Rocuronium (NMBA)	H + ST	92	100	22	22
NMBA	H ± ST ± IgE	68	100	49	23
Antibiotics					
β-lactam	H + ST	50	93	88	27
β-lactam	H ± ST ± IgE ± PT	49	91	110	28
β-lactam (amoxicillin)	H + ST	67 (CD203c) 33 (CD63)	100 (CD203c) 79 (CD63)	41	30
Nonsteroidal anti-inflammatory drugs (NSAID)					
Metamizol	H ± PT	42	100	55	36
Aspirin and NSAID	H ± PT	15–55	74–100	90	37
NSAID	H	43	100	72	38

Abbreviations: H, history; N, total number of patients and control individuals; PT, provocation tests; Sens, sensitivity; Spec, specificity; ST, skin test.
Data from Ebo DG, Sainte-Laudy J, Bridts CH, et al. Flow-assisted allergy diagnosis: current applications and future perspectives. Allergy 2006;61(9):1028–39.

tolerance) to find safe alternative regimens for future anesthesia. This is another advantage of this *in vitro* test.^{18,20,22,24} The authors demonstrated that BAT²² (not sIgE-inhibition assays)²⁵ is complementary to skin tests in identifying clinically relevant cross-reactivity between different NMBA.

Beta-lactam Antibiotics

The diagnostic approach for immediate allergic reactions to beta-lactam antibiotics recently was standardized under the aegis of the European Network for Drug Allergy (ENDA) and the European Academy of Allergy and Clinical Immunology (EAACI) interest group on drug hypersensitivity.²⁶ This position paper highlights the importance of drug challenge for diagnosis in a considerable number of patients as quantification of sIgE and skin test yields equivocal, contradictory, or false-negative results. In a comparison between quantification of sIgE and basophilic CD63 expression including 58 patients suffering from skin test-proven beta-lactam allergy and 30 healthy control individuals, sensitivity and specificity of the assays approximated 38% and 87% for sIgE and 50% and 94% for the basophil activation test, respectively.²⁷ Comparable results were observed in a survey by Torres and colleagues,²⁸ as shown in **Table 2**. Whether other (more upstream-located) activation markers such as down-regulation of membrane-bound IgE will increase sensitivity of the BAT²⁹ and whether CD203c is a more sensitive and specific marker than CD63 to diagnose allergy from beta-lactam antibiotics remains to be established.³⁰ It is of note that again in all these studies on antibiotics, as for NMBA, decision thresholds were set arbitrarily or relative to spontaneous expression of the applied activation marker.

Radiocontrast Material

RCM application generally is tolerated well. Besides the pharmacologic adverse effects (flush, nausea, vomiting), the associated risk of a severe anaphylactic reaction (angioedema, bronchospasm, hypotension) has been estimated to be between 0.02% (nonionic RCM) and 0.4% (ionic RCM).^{31,32} Around 4% of these are highly suggestive of an IgE-mediated mechanism (positive skin test and BAT), which is therefore not the general rule in RCM allergy.^{14,33} Direct histamine release by means of nonspecific RCM binding to membrane receptors on mast cells or basophils and indirect mast cell or basophil activation by means of the complement or kinin cascade outweigh the genuine IgE-mediated reactions.³⁴ Taking into account that in the United States RCM is applied in more than 10 million radiologic procedures, the relevance of these seemingly small percentages becomes obvious.³⁵ Especially because preventive medication with antihistamines and steroids does not avoid systemic reactions in these cases, IgE-mediated reactions need to be identified.³³ Besides identifying the culprit RCM and cross-reactive substances, BAT in combination with skin test results can help define a safe alternative for future radiologic diagnostics while rendering the choice for preventive provocation tests as safe as possible.

Aspirin and Nonsteroidal Anti-inflammatory Drugs

Besides case reports, three larger studies from Spain have assessed BAT in the diagnosis of hypersensitivity to aspirin, metamizol, and other NSAIDs.^{36–38} The sensitivity for the different NSAIDs varied from 15% for metamizol to 55% for naproxen, whereas specificity exceeded 90%. With positive predictive values up to 100%, authors classified BAT a reliable instrument for diagnosing hypersensitivity from NSAIDs. In contrast, Erdmann and colleagues (20 patients, different NSAID)³⁹ and Malbran and colleagues (14 patients, diclofenac)⁴⁰ concluded that the value of CD63-based BAT

in NSAID intolerance remains unclear, and especially with a negative BAT result, a drug challenge cannot be omitted. Currently, the authors do not recommend BAT in immediate hypersensitivity because of NSAIDs and believe that additional studies with consistent correlation to provocation tests are mandatory to confirm the Spanish data, particularly because NSAID-induced respiratory and cutaneous reactions are generally IgE-independent adverse events resulting from inhibition of cyclooxygenase-1 (COX-1), which results in depletion of prostaglandin E2 with unrestrained synthesis of cysteinyl leukotrienes and mediator release from mast cells and eosinophils.⁴¹

Chlorhexidine

Chlorhexidine is a cationic bisguanide antiseptic and can trigger irritant dermatitis, allergic contact dermatitis, and IgE-mediated reactions (including life-threatening anaphylaxis). For a long time, diagnosis of IgE-mediated chlorhexidine allergy has relied only upon skin prick tests, and additional confirmatory in vitro test were awaited. Recently, BAT⁴² and quantification of sIgE^{42,43} for chlorhexidine proved to be valuable and safe alternative diagnostic instruments, particularly for severe cases.⁴⁴

Gelatine

Anaphylaxis to gelatine in vaccines and gelatine-based colloids is known⁴⁵ and generally can be established on quantification of sIgE and/or appropriate skin tests. In certain cases with unequivocal or even contradictory results, BAT has been proven to constitute a valuable additional diagnostic tool.⁴⁶

Carboxymethylcellulose

Different groups have identified carboxymethylcellulose (CMC),^{47–51} a common infusion excipient, as the elicitor of anaphylactic reactions following minimally invasive interventions (eg, joint infiltration with local anesthetic and steroids) by means of skin testing, ELISA and BAT. Especially in situations where multiple drugs are given simultaneously and repetitive procedures are needed, a clarification of the elicitor is of paramount importance. When infusion excipients like CMC are involved, repetitive anaphylaxis during following interventions with structurally unrelated alternative drugs can occur, as they might contain the same excipient (CMC). Even some steroids (for intramuscular injection) that might be used to treat anaphylaxis contain CMC and can aggravate the patient's critical state.

Miscellaneous

In an increasing number of cases, BAT helped to identify the plasma expander hydroxyl ethyl starch,⁵² cremophor (the emulsifier of the intravenous cyclosporine formulation),⁵³ bovine serum albumin present in a semen culture medium for artificial insemination,⁵⁴ omeprazole,⁵⁵ different low-molecular-weight heparins,^{56,57} viscotoxins of mistletoe (*Viscum album*),⁵⁸ the dye patent blue,⁵⁹ the enzyme hyaluronidase,⁶⁰ platinum salts,^{61,62} recombinant hepatitis B vaccine,⁶³ clavulanic acid mimicking amoxicillin allergy,⁶⁴ and the antiviral valacyclovir⁶⁵ as the causative compound in patients with sometimes life-threatening allergic reactions. Moreover, in some of these patients, BAT has contributed to establish the individual therapeutic alternative or allowed identification of potentially cross-reactive structures.

SUMMARY

BAT provides the physician with a novel instrument in the diagnostic management of immediate-type drug allergy that involves IgE-dependent mast cell and basophil activation. It is of particular interest in those cases where current in vitro routine diagnostics do not provide alternative tests, and confirmation of clinical suspicion (despite negative skin test results) would require a potential dangerous drug provocation test. Currently, however, BAT mostly is applied for research settings, and additional clinical studies are mandatory to allow its entrance into clinical routine. Attempts to harmonize BAT protocols on an international scale under the aegis of the EAACI are ongoing. Correct selection of patients and appropriate exposed, nonallergic control groups are important prerequisites to establish optimal drug-specific positivity thresholds, replacing arbitrarily chosen decision thresholds, and should allow correct determination of sensitivity, specificity, and predictive values. Passive sensitization of donor basophils with patients' serum is another advantage of BAT, as this cannot be done in vivo (theoretical risk of infection) and provides an instrument to prove the IgE dependence of a drug reaction in question. This is important for prognosis and avoidance measurements, as IgE-mediated reactions are generally more dangerous and potentially life-threatening. The possibility to test several drugs at the same time helps in tailoring drug challenge for safe therapeutic alternatives. Additionally, novel basophil gating strategies and activation markers such as p38 MAPK might improve the diagnostic performance of BAT. Moreover, as these novel markers might become activated through alternative routes, it seems theoretically justified to anticipate that the BAT might be applicable for diagnosing IgE-independent basophil-mediated adverse drug reactions.

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