Giulia et al. World Allergy Organization Journal (2024) 17:100998
http://doi.org/10.1016/j.waojou.2024.100998

WORLD ALLERGY ORGANIZATION JOURNAL

REVIEW

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Applications of basophil activation test in paediatric allergic diseases

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ABSTRACT

Basophilic granulocytes, containing and releasing histamine after a specific allergy stimulation, are directly involved in IgE-mediated allergic reactions. CD63 is a transmembrane protein of secretory lysosomes of basophils and its upregulation is related with the release of histamine to the extracellular space during IgE-mediated allergic reactions. Basophil activation test (BAT) measures the activation of circulating basophils upon the *in vitro* stimulation of living blood cells with specific allergens. Such a test is particularly safe and reproducible and has recently emerged as a new promising diagnostic tool for allergic diseases.

BAT can be used to diagnose food allergy and represents a promising alternative to oral food challenge tests, especially in children as it is less invasive, safer, and cheaper than the gold standard tests. As a biomarker of tolerance and reactivity, it is also useful to monitor natural resolution and clinical response to immune-modulatory treatments. Regarding drug allergies, BAT is even the only possible applicable diagnostic tool for allergy reactions to some drugs, because of the lack of alternative test, or given that those commonly used are unreliable, or equivocal. Additionally, BAT allows to screen patients with more active urticarial and identify Hymenoptera-allergic patients with negative venom-specific immunoglobulin (Ig)E. In respiratory allergic diseases, BAT can facilitate the diagnosis of local allergic rhinitis and evaluate basophil allergen sensitivity in allergic asthma. Although IgE-sensitization in allergic asthma is usually demonstrated by skin prick test and specific IgE, those tests do not predict the clinical allergy contribution to asthma pathogenesis. To date, the potential of BAT in the diagnostic work-up of allergic diseases is well established, but a better standardization of its use is needed. This narrative review summarizes the state-of-the-art BAT technology and applications in pediatric allergic diseases, focusing on immune-related mechanisms and the BAT real clinical utility.

Keywords: Basophil activation test, Pediatrics, Allergic disease, Venom allergy, Spontaneous chronic urticaria

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http://doi.org/10.1016/j.waojou.2024.100998

Received 17 January 2024; Received in revised from 18 September 2024; Accepted 1 November 2024
Online publication date xxx

INTRODUCTION

In the algorithm for allergy diagnosis, collecting patient history, paying attention to the understanding of the potential allergen sources and the severity of the allergic reactions represents the first crucial step.[1] Further, the allergic response needs to be confirmed using an objective test, ideally within 1 year after the last symptomatic exposure.[2] Skin prick tests (SPTs), specific immunoglobulin (Ig)E and intradermal tests are well established first-line assays used in the diagnosis of allergies. Recently, the Basophil Activation Test (BAT), a functional test resembling an *ex vivo* provocation, has emerged as a new promising diagnostic tool, given that it is particularly safe and reproducible. It is usually coupled with the sIgE measurements, and in general precedes the *in vivo* provocation tests.[3] The BAT is a functional flow cytometry assay based on the measurement of the activation of circulating basophils upon the *in vitro* stimulation of living blood cells with specific allergens. Since its development, the BAT has progressively gained a role in the diagnosis and monitoring of allergic diseases. It assesses the IgE cross-linking, being more precise than the direct measurement of the concentration of circulating allergen specific IgE.[4] Being totally assayed *in vitro*, it represents a promising alternative to the provocation test, given that it is less invasive, safer, and cheaper. Nowadays, BAT is administrated when routine clinical (SPTs) and laboratory (sIgE) analyses give ambiguous or discordant results with respect to the patient anamnesis, and/or if the allergen administration represents a relevant risk for the patient. Therefore, the BAT has an enormous potential in the diagnosis of clinically relevant food and drug allergies, as well as in patients suffering from chronic urticaria; it has been also successfully applied in the follow-up of allergen immunotherapies.[3] Integrating the use of BAT in the algorithm of allergy diagnoses represents another relevant point to be implemented. In this narrative review, we will cover the state-of-the-art BAT technology and applications, focusing on the role of this test in food, venom, and respiratory allergies, as well as in drug adverse reactions and urticaria to explore immune related mechanisms and to assess the BAT real clinical utility. To this end, we assessed the research on PubMed, SCOPUS and Google Scholar using the following search terms and logic: "basophil activation test and allergy" OR "basophil activation test and clinical practice" OR "basophil activation test methods" OR "basophil activation test and drug" OR "basophil activation test urticaria" OR "basophil activation test and children". Furthermore, references of identified papers were also included, using their titles and abstracts as eligibility criteria. Only articles written in English, narrative and systemic reviews, longitudinal and retrospective studies and randomized control trials were included. Studies involving pediatric but also adults were included. Case reports, expert opinions and manuscripts published in languages other than English were excluded.

BASOPHIL ACTIVATION TEST

Basophils express the tetrameric form of the high affinity IgE receptor (FcεRI) and when allergens interact with the IgE antibodies located on their surface, trigger basophil degranulation as well as the synthesis of different cytokines.[5] Therefore, basophils are thought to be directly involved in the IgE-mediated acute allergic reactions and anaphylaxis; indeed, blood basophil granulocytes contain and release histamine upon specific allergen stimulation.[3] BAT allows the detection of basophil activation, by paralleling the expression of basophil activation markers before and after specific *in vitro* stimulations. In other words, BAT measures the IgE function as its ability to induce the activation of basophils in the presence of an allergen. The activation of basophils is monitored by staining basophils before and after their specific allergen stimulation with dedicated flow cytometry panels. Basophils are a rare population (<2%) of circulating cells and, within the flow cytometry BAT gating strategy, they are identified as low side scatter events (between those of lymphocytes and monocytes), expressing specific basophil markers, such as CCR3, CD193, CD123, CD203c and high-affinity receptor for the Fc region of IgE (FcεRI).[6–8] Basophil activation is characterized by the upregulation of different surface proteins, also used as activation markers in BAT flow cytometry panels. Even if CD63 is the most widely used marker to identify activated basophils within BAT panels, some other molecules, such as CD203c, CD18/CD11b have been successfully used to this end. CD63 is a 4-transmembrane protein located in the secretory

lysosomes of basophils, related to the release of histamine, which relocates on the basophil surfaces upon the interaction with an allergen.[9] It seems to be associated with the reorganization of cell membrane and exosome formation. The relevance of CD63 in IgE-mediated allergic reactions is supported by its inverse correlation with intracellular diaminoxidase, which, in turn, is inversely correlated with the histamine released into the extracellular space.[10,11] Therefore, CD63 upregulation is used to monitor the allergen activation of blood basophils. Measuring CD63 up-regulation through BAT is easier than measuring histamine release, due to the technical challenges linked to histamine release detection and the effects produced on other leukocytes, as well as because of the potential cross-reactivity of histamine antibodies to, for example, methylhistamine.[12,13] Moreover, CD63 is expressed in case of anaphylactic degranulation through regulated exocytosis after allergen-mediated activation of mast cells and basophils, unlike the histamine release also occurs after both piecemeal and anaphylactic degranulation.[14]

Another widely used marker used within BAT panels is the CD203c, an enzyme constitutively expressed on the surface and in the cytoplasm of basophils, measurable within few hours from blood sampling.[9,15–17] These markers have been used alone or in combination, depending on the type of allergen used in the assay.[9,15–17]

According to the literature, CD203c, being co-expressed with CD63, is frequently used in combination with the CD63 itself.[14] Given that the upregulation of CD63 or CD203c may not always correlate with the total histamine release, it has been suggested that these 2 markers follow different pathways of basophil activation mechanisms, the CD63 reflecting the anaphylactic degranulation, while CD203c being linked to the piecemeal degranulation (Fig. 1).[14] Different gating strategies have been proposed for the correct analysis of the BAT results: SSClow/IgE positive, SSClow/CD203c positive/CD123positive/HLA-DR negative, CD45dim/CD123bright/HLA-DR negative, SSClow/CCR3 positive or SSClow/CRTH2 positive/CD3 negative events could be selected.[18–20] The use of larger numbers of markers, allows to better select a purer population with the disadvantage of increasing the costs and laboriousness of the assay. A comprehensive range of allergen concentrations is needed to assess the effect of the allergen on the basophil response;[21,22,34,36] at least 5 different allergen concentrations, for instance in 10-fold increments, are usually tested, whereas for drugs the dilution factor is often only 5.[11,23] The criteria to define the

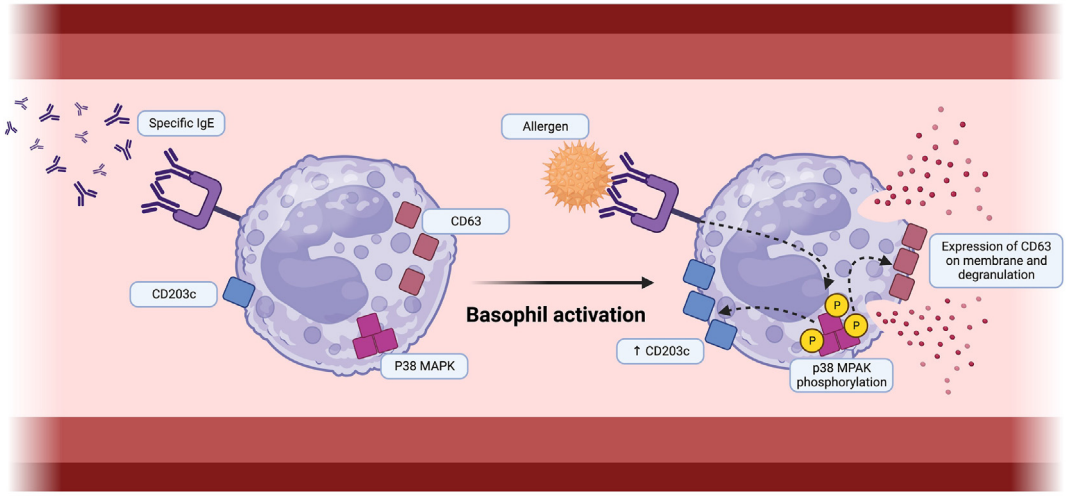

Fig. 1 CD63 and CD203c basophil activation mechanisms. CD63 appears to be involved in cell membrane re-organization and exosome formation. Histamine released into the extracellular space inversely affects its expression. This supports the role of CD63 in IgE-mediated allergies. Thus, the allergen activation of blood basophils can be measured by histamine release or CD63 upregulation. CD63 is expressed in anaphylactic degranulation through regulated exocytosis after allergen-mediated mast cell and basophil activation, unlike histamine release in piecemeal and anaphylactic degranulation. CD203c is often measured with CD63 and appears to be co-expressed. Since CD63 and CD203c upregulation may not always correlate with total histamine release, it has been suggested that these 2 markers follow different basophil activation pathways, with CD63 reflecting anaphylactic degranulation and CD203c piecemeal degranulation

negative gate and the cut-off for a positive BAT result have been also established.[17,20,24] In fact, whenever a BAT is assessed different negative and positive controls are used.[25]

The negative control usually displays percentages of spontaneously activated basophils in the range 1.5–2.5%, therefore the threshold for the percentage of activated basophils ($CD63^+$) in acceptable negative controls has been assessed at 2.5%.[16,20]

Notably, positive controls need to be acquired anytime a BAT is carried out. Samples activated using anti-IgE or anti-FcεRI or N-formyl-methionyl-leucyl-phenylalanine (fMLP) are usually used as positive controls. Anti-IgE antibodies directly cross-link with the IgEs on the surface of basophils, producing a downstream signalling inducing their degranulation.[26] Anti-FcεRI antibody binds directly to the receptor for the Fc fragment of IgE (C3 domain) triggering the signal transduction.[27] The bacterial chemotactic peptide fMLP activate basophils in a non-IgE-mediated manner through the interaction with G-protein coupled fMLP receptors.[28] It is also known that, in a significant percentage of subjects (<10%), basophils do not respond to the FcεRI receptor stimulation, and therefore they are defined as "non-responders" to the BAT.[29] This could be due to their reported low levels of Syk phosphatase,[30] involved in the activation of phospholipase C and in the signalling mediated by FcεRI 20 or low levels of CD45, that plays an active role on mast cells responses.[31] In detail, Syk phosphatase is part of the mechanisms that modulate basophil and mast cell secretion.[32] Syk down-regulation contributes to terminate the IgE-mediated response. On the other hand, IgE-mediated stimulation results in a loss of Syk expression, probably through the ubiquinylation of Syk during activation.[33] It is also known that FcεRI and IgE vary with the IgE plasma concentration and basophil degranulation.[34]

Whenever a BAT test has to be optimized, basophil activation is measured after allergen stimulation and a dose-response curve for the monitoring of the frequency of CD63 positive basophils is obtained by gradually increasing the concentration of allergens.[17] Basophil reactivity and basophil sensitivity are obtained from the related dose-response curves.[18,24] The basophil reactivity is defined by either the percentage of $CD63^+$ basophils at a given concentration or the CD-max, i.e. the concentration at which maximal basophil activation occurs. Basophil reactivity depends on the priming state of the basophil and the cellular translation of the IgE signal within the cell.[35] Basophil sensitivity is a function of the reactivity and the compound affinity of cell-bound sIgE for allergen and free competing Ig.[36,37] To calculate basophil sensitivity it is necessary to measure the basophil reactivity at 6–8 allergen concentrations.[21] It is assessed through either EC50 (the concentration at which 50% of maximal basophil response occurs) or CD-sens (defined as the inverse of EC50 multiplied by 100) which can be calculated from the slope of the dose-response curve).[21] More recently, the area under the dose-response curve has been used to assess basophil reactivity and sensitivity simultaneously.[38] It also includes the monitoring of the partial anergy induced at high allergen concentrations and can be calculated even in cases when the responses do not fit well to a typical dose–response curve (i.e. during oral or sublingual immunotherapy).[3]

The choice of allergen used in the test is also crucial. Allergen stimulants include crude extracts, purified single allergen sources or recombinant allergens, which have the greatest stability and consistency.[17,39] Standardized preparations are recommended to compare data over time and among different laboratories.[3]

Currently, there are companies that provide kits for performing the Basophil Activation Test. These kits include antibodies for flow cytometry panels, controls, and allergens necessary for the assay. They are optimized to guide the user on the optimal concentrations of each allergen and the corresponding positivity thresholds for the obtainment of accurate results.[40]

The BAT is carried out using either whole blood or isolated peripheral blood mononuclear cells. The use of whole blood is preferred because it is more physiological, preserving the *in vivo* environment of blood basophils which includes the blocking antibodies.[20,41] The collection of whole blood for BAT is usually carried out using heparin as an anticoagulant. However, the only kit approved for clinical application uses the EDTA,[25] in particular it

	Sensibility	Specificity
Cow's milk allergy bat		
• cd63 cm 2%	97	51
• cd63 cm 6%	91	90
• cd63 cm 15%	45	94
Cow's milk allergy spt	100	22
Cow's milk allergy SiGe		
• 0.35 kUA/l	94	21
• 2 kua/l	67	67
Egg white allergy bat		
• cd63 ovoalbumin	77.4	100
• cd203c ovoalbumin	57.7	96.6
• cast ovoalbumin	77.8	96.6
Egg white allergy spt	93.5	84.6
Egg white allergy SiGe	93.5	92.6
Peanut allergy bat		
• cd63 peanut	81.3	95.7
• cd203c peanut	89.5	97.2
• cast peanut	76.0	94.6
Peanuts allergy spt	90	83.7
Peanuts allergy SiGe	90.6	76.7

Table 1. The sensibility and specificity of BAT test, SPT and sIgE for allergies to cow's milk, egg white, and peanuts (adapted from Rubio et al.[48] and Ocmant et al.[49]). *CMP: cow milk proteins; SPT: skin prick test; BAT: basophil activation test; sIgE: specific Immunoglobulin E.*

converts EDTA blood to heparin with a buffer that also contains Ca and heparin, because blood stores up to 48 h in EDTA. The BAT requires in <0.1 ml fresh blood to measure the basophil response to allergen cross-linking IgE and the acquisition of 150–2000 basophil granulocytes/sample is mandatory.[3] Given that basophil reactivity considerably decreases over time,[41] BAT must be ideally executed within 4 h from blood collection.[42,43] If a longer time is needed, blood samples must be processed within 24 h from bleeding.[44] However, Mukai et al[45] demonstrated that carrying out the BAT on blood stored in heparin at 4 °C for 4 h or for 24 h following the blood collection did not significantly affect the results. Antihistamines do not influence BAT results,[45] but the use of systemic steroids[45] and cyclosporin should be avoided within 48 h before the BAT execution.[46]

The BAT seems to closely replicate *in vitro* IgE-mediated reactions which develop *in vivo* when allergic individuals are exposed to the allergen, and therefore it could be a useful tool in the diagnosis and follow up of allergic patients. Indeed, SPTs and sIgE show well known limitations: they both detect sensitization and not clinical allergy and SPTs also require intact skin and antihistamine cessation.[47]

In addition, the BAT allows the determination of a dose-response for the activation, the response to patient's use of histamine blockers[43] and the assessment of reactivity in the presence of non-IgE allergen-specific antibodies.[48]

Several studies demonstrated that BAT is more accurate than IgE sensitization tests with specificity between 75 and 100% and sensitivity between 77 and 98%.[48–51] Furthermore, BAT could be an alternative method to perform a challenge test which is expensive, time-consuming, and potentially dangerous for the child.[48–51] Table 1 summarizes the sensitivity and specificity of different allergy tests used for the most common allergens in children.

BAT & FOOD ALLERGY

Oral food challenge (OFC) is the gold standard for the diagnosis of food allergy (FA) even if it can cause severe reactions[51,52] and its reproducibility is still a matter of debate.[52,53] The application of the BAT in the diagnosis and monitoring of the FA is a topic of growing interest; as a functional test, the basophil activation is a biomarker of reactivity and tolerance and can be used for the monitoring of the FA.[54] The diagnosis of IgE-mediated food allergy is based on detailed clinical and dietary history, SPTs and/or sIgE dosage.[55–58] However, some patients have detectable food-sIgE or positive SPTs without a clear and recent history of an allergic reaction against the suspected food or alternatively a clear history of tolerating age-appropriate portions of the food, making the execution of OFC necessary.[54–59]

For example, Commins et al[60] observed the basophil activation in 12 adult patients with allergy to red meat during the execution of the

OFC and showed that the activation overlapped with the development of the symptoms.

The first publication that assessed the usefulness of the BAT to diagnose allergies was published in 1999.[61] Several studies further addressed the same topic, including different food such as cow's milk,[62–64] wheat,[65–68] egg,[62] peanut,[69,70] hazelnut.[71–75] Some of these studies relied in small sample size and particularly not all used the OFC in comparison with BAT. In a larger study, Santos et al[24] assessed 169 children for possible peanut allergy, including a primary population of 104 patients to identify the optimal diagnostic cut-offs and a second population of 65 patients to externally validate the findings. It was demonstrated that the BAT sensitivity and specificity to diagnose peanut allergy were 98% and 96% in the primary population and 83% and 100% in the second population. Therefore, the authors suggested that positive BAT to peanut confirmed peanut allergy with a 67% of reduction in the need of the OFC. Noteworthy, the reduction in the OFC involved particularly positive OFC, supporting the idea that the BAT might be useful to avoid the potential adverse reactions and the patients' discomfort.[24,76] Recently, European Academy of Allergy Asthma and Clinical Immunology (EAACI) guidelines included the execution of the BAT in patients with an equivocal diagnosis of IgE-mediated allergy to peanut or sesame, to further support diagnosis.[77] This suggestion is based on the evidence that the BAT for peanut and sesame showed 86% and 89% of sensitivity, and 90% and 93% of specificity, respectively, as demonstrated by a recent meta-analysis of diagnostic accuracy of tests in IgE-mediated food allergy.[78]

Additionally, in the case of tree nuts and hazelnut allergy, the BAT seems more specific than SPTs and sIgE to individual allergens.[79] Moreover, in most of the studies including peanut and cow's milk-allergic patients, the basophil reactivity has been directly correlated with the severity of symptoms observed during the OFC test.[49,77,80] A greater proportion of activated basophils were found in patients with more severe reactions; indeed, basophils start reacting at lower allergen concentrations in patients reacting to trace amounts of the allergen in their clinical history.[17]

In the context of the BAT execution, the use of individual allergens was also tested in comparison with food extracts to diagnose allergy to some foods as the casein for cow's milk allergy. BAT to cow's milk was also used to identify patients who had outgrown their cow's milk allergy.[49] Molecular allergens, such as Ara h 2 and Ara h 6, were also used in BAT to diagnose peanut allergy.[44,69,80,81] However, some patients might not be sensitized to the allergens which were used as stimulants in the BAT, potentially leading to false negative findings.

Furthermore, some Authors[81,82] carried out the BAT to monitor the acquisition of oral tolerance to foods over time, either naturally or under immunomodulatory interventions. Wanich et al[82] showed that children tolerating heated milk had intermediate degree of basophil reactivity between that of patients allergic to all forms of cow's milk and patients who had outgrown their cow's milk allergy. They defined 4 groups: allergic (n = 13, age range, 3.6–16.5 years), heated milk-tolerant (n = 32, 2.8–16.3 years), outgrown (n = 10, 4.8–10.4 years) and healthy donors (n = 13, 1.8–13.4 years) based on oral food challenges and performed BAT stimulating with a range of milk allergens. They found that heated milk tolerant subjects' basophils were significantly less responsive to milk allergen stimulation at all doses than were basophils from allergic individuals.

Basophil activation changes during allergen-specific immunotherapy and they were reduced in patients undergoing oral immunotherapy to foods, such as cow's milk, peanut and egg.[83–87]

The BAT was also used to assess if basophil anergy occurs during chronic allergen exposure in the setting of a clinical oral immunotherapy trial (OIT).[85–87] Thyagarajan et al[87] obtained samples of peripheral blood from 28 adults during a placebo-controlled clinical trial of peanut OIT and performed BAT with peanut allergen, showing that the upregulation of CD63 following the stimulation of the IgE receptor was strongly suppressed by active OIT.

It is still unknown whether basophil suppression persists following a discontinuation of allergen-specific immunotherapy. Burks et al[84] found a correlation of basophil suppression with clinical

desensitization by using egg oral immunotherapy, but not with long-lasting clinical tolerance.

Therefore, the BAT is useful to improve the diagnosis of food allergy over SPT and sIgE and allows to reduce the number of OFC. In addition, it is also used to monitor the natural resolution and clinical response to immune-modulatory treatments.[3]

BAT AND DRUG ALLERGY

Drug hypersensitivity reactions (DHRs) mediated by immunological mechanisms are a part of adverse drug reactions (ADRs): noxious, unintended responses to a drug that are triggered at a dose that is usually safe for prophylaxis, diagnosis, or therapy. The diagnostic work-up of drug hypersensitivity reactions (DHR) aims to identify the culprit agent, identify cross-reactive drugs and to determine a safe alternative drug. As well as in food allergy, challenge testing is also the gold standard for DHRs diagnosis, even if drug provocation tests are impractical and even unethical in some cases (e.g.in the diagnosis of allergy to drugs used in anaesthesia).[3] At the same time, sIgE is a useful diagnostic tool, although it shows low sensitivity,[88] given that sIgE testing cannot be carried out to both the native drug and all its metabolites, many of which may be responsible of the allergic reaction. Therefore, in the literature, attempts have been made to understand whether BAT could represent a supplementary tool for DHR diagnosis or not.[88,89] For example, Marraccini et al[89] enrolled 204 adults with DHR history who performed specific IgE, BAT and challenging test. The authors found that BAT presented high specificity, but low sensitivity for antibiotics and non-steroidal anti-inflammatory drugs (NSAIDs): 90.0% (95% CI 85.8–93.3) and 95.1% (95% CI 92.6–96.9); 33.6% (95% CI 25.3.8–42.7) and 22.4% (95% CI 15.2.8–31.1), respectively. Among negative patients for both *in vitro* tests, no one displayed positive challenging test. The authors concluded that subjects with negative clinical history and negative BAT or patients with positive clinical history and positive BAT results do not need to undergo challenging tests.[89] The reliability of the BAT was assessed in the amoxicillin[90] and cephalosporin allergy diagnosing,[91] showing lower sensitivity when compared to the skin tests. However, there are 5 studies in adults[90–94] which evaluated the BAT usefulness in the diagnosis of beta-lactam allergic reactions, showing its higher sensitivity (about 50%) and specificity (about 90%) than sIgE. Recently, Heremans and co-authors[90] investigated the reliability of the BAT in the diagnostic algorithm of amoxicillin allergies. They performed the BAT for amoxicillin in 70 controls and 66 patients, measuring the upregulation of both CD63 and CD203c; they obtained 13% and 100% of sensitivity and specificity for CD63, and 23% and 98% for CD203c upregulation, therefore they concluded that the sensitivity of BAT is too low to be used to diagnose amoxicillin allergy.[90] On the other hand, regarding amoxicillin reactions, Céspedes et al[93] found that CD63 and CD203c showed similar sensibility values (48.6% and 46.7%, respectively) and different specificity values: in particular CD63 showed 81.1% and CD203 showed 94.6%.[95]

Regarding the allergic reactions to NSAIDs, an important aspect is that allergic reactions to drugs are not exclusively dependent of IgE/FceRI cross-linking, but more often they depend on different mechanisms, such as the inhibition of cyclo-oxygenase-1 isoenzyme and involve not only basophils, but also mast cells and eosinophils. Therefore, the low sensitivity of the BAT (about 20–40%) in this case does not make it a useful diagnostic tool in this contest.[96] Some studies confirm that the BAT produces acceptable specificity, but highly variable sensitivity for the diagnosis of NSAIDs allergy.[2,97–99]

To date, the BAT is even the only possible applicable diagnostic test for some drugs, because of the lack of unreliable or equivocal *in vitro* test. The European Academy of Allergy and Clinical Immunology (EAACI) included in this group bovine serum albumin, chlorhexidine, carboplatin, atropine, methylprednisone, gelatines, and carboxymethylcellulose.[3]

Recently, a position paper by Mayorga et al[100] provided the recommendations for using the BAT in case of drug allergy. The authors strongly recommend the BAT in severe cases of drug allergy when STs and quantification of sIgE yield negative results, and a drug challenge test is contraindicated due to life-threatening anaphylaxis or strong suspicion in the context of a convincing clinical history. Additionally, in allergy

to beta-lactams BAT has shown usefulness for evaluating allergy to clavulanic acid, which is not possible in other *in vitro* tests[101][It was also efficient to diagnose neuromuscular blocking agents and chlorhexidine allergy.[100]

However, it must be considered that a negative test does not rule out that the patient reacts to a metabolite of the drug. Moreover, larger studies, mostly in children, are needed to understand the clinical relevance of the different degranulation processes, and the potential of activation markers other than CD63, as well as basophil activation in non-IgE-mediated immediate drug hypersensitivity.[3]

BAT & URTICARIA

Chronic spontaneous urticaria (CSU) is characterized by recurrent itchy wheals, angioedema or both, that persist for longer than 6 weeks. Though CSU is often idiopathic, some patients have an autoimmune pathophysiology with mast cells and basophils-activating autoantibodies mostly directed against FcεRI.[3,17] In this case, the presence of autoantibodies towards IgE or its high affinity receptor FcεRI is reported[102] as well as anti-DsDNA antibodies, IgE and IgG targeted towards thyroid peroxidase.[103,106,104,105] The autologous serum skin test (ASST) is the most used diagnostic tool, even if it presents the risk of an accidental infection and it does not always correlate with other *in vitro* assays.[105,106] The BAT was proposed as an *in vitro* alternative test instead of the ASST for the detection of 'autoreactive' serum components.[107,108] In this case, basophils from healthy donors are challenged with patients' serum. In this case, after the stimulation with CSU patients' sera, both CD63 and CD203c expression increased.[109–119]

In a longitudinal study, D'Auria et al[111] analysed data from 16 children with CSU, aged from 3 to 16 years (median age 8.81 yrs), comparing the BAT with the gold standard ASST. For indirect BAT they mean the *in vitro* stimulation of heterologous basophils from peripheral blood donors mediated by the serum of CSU patients, followed by the flow cytometric determination of the basophil activation. They used 5% of $CD63^{+}$ basophil as cut-off to define positive sera and found no difference between the BAT results obtained from negative controls on the basophil donors and patients' sera that gave negative basophil activation; 37.5% of patients showed a positive indirect BAT on at least 1 donor basophils. They concluded that the serum is not activating basophil per se, confirming the high BAT specificity. Moreover, the patients with negative ASST showed a positive result by using an indirect BAT, while 1 patient who had a positive ASST, was also positive to the BAT [1118].

BAT was also used to identify patients with more active disease. ASST and BAT were compared and their relationship with disease activity was investigated in 139 adult patients with CSU.[112] In this case, 56.8% of patients presented positive ASST, 31.6% of which were positive to BAT. ASST and BAT were paralleled and compared with the activity of the disease, measured with the Urticaria Activity Score from the day before, the 7 days before and the 3 weeks before the baseline clinical assessment. Patients with positive ASST and BAT presented higher Urticaria Activity Score at 7 days and at 3 weeks than patients with positive to ASST and negative to BAT (mean and standard deviation: 26.57, 10.56 versus 18.40,12.05 for the Urticaria Activity Score of 7 days wit p value = 0.004 and 56.47, 23.78 versus 39.88, 25.44 for the Urticaria Activity Score of 3 weeks with p value = 0.004). The specificity of this study is of 98.3%, while the sensitivity is very low (31.6%). CSU patients with positive ASST whose serum induced the expression of CD63 showed the higher UAS scores, therefore the authors concluded that the BAT is also useful for the screening of patients with more active diseases.[112]

BAT & VENOM ALLERGY

Specific serum IgE of wasp venom present a sensitivity of only 60–80%,[109] while the BAT seems to have both high sensitivity (85–100%) and specificity (83–100%) to diagnose Hymenoptera venom allergy.[113,114]

Erdmann et al[113] compared CD63-BAT with SPTs and measurement of sIgE in a sample of 50 adult patients with wasp venom allergy and 20 controls. They found that the sensitivity of SPTs, sIgE and BAT were 100, 76, and 92%, respectively, while specificity of sIgE and BAT were 85 and 80%,

respectively. There was also a positive correlation between IgE reactivity to wasp venom and the percentage of $CD63^{+}$ basophils.[113] Eberlein-König et al[114] carried-out a cross-sectional study including 43 adults with a history of insect venom anaphylaxis and 25 controls to investigate the accuracy of BAT compared with SPTs and sIgE. They found that the specificity of BAT using CD63 or CD203c as activation markers were 100% and 89%, respectively, when compared with controls with negative history and negative sIgE. However, CD203c staining showed a slightly higher sensitivity than CD63 monitoring (97% vs 89%).[114]

Some reports suggest the usefulness of the BAT for Hymenoptera-allergic patients with negative venom-specific IgE antibodies.[115–117] In the clinical practice, a small proportion of patients with a clinical history of venom allergy report undetectable sIgE and negative SPTs and there is no shared indication on how to diagnose allergy in these patients, mostly because sting provocation tests might be unethical. Korošec et al[115] used BAT and intradermal skin testing in 21 patients with anaphylactic reactions to Hymenoptera sting and negative venom specific IgE diagnosing 81% and 57% of patients, respectively.[115]

Another interesting aspect to consider is the double positivity to both wasp and bee venom. In that case, indeed, the determination of the responsible allergen is mandatory to start venom immunotherapy. Up to 59% of the patients with Hymenoptera venom allergy revealed positive results for both bee and wasp venom at SPTs and sIgE.[116] Noteworthy, the BAT showed the lowest levels of double positivity when compared with a different diagnostic test. In a population of 117 adult patients with a history of bee or vespid allergy, sIgE, SPTs, and BAT were performed. Among patients, double sensitization was observed in 60% of cases by using sIgE, 47.9% by using SPTs and only in 17.1% by using BAT.[117]

On the other hand, sIgE against both bee and wasp venom might be due to a sensitivity either to insect venoms or to cross-reactive carbohydrate determinants (CCDs). The execution of the BAT with both venoms as well as with bromelain and horseradish peroxidase (HRP) or recombinant allergen–based IgE testing can improve the diagnostic procedure. Specifically, Eberlein et al[118] found in a group of 22 adult patients with insect venom allergy and double positivity to bee and wasp venom that up to 60% of patients with Hymenoptera venom allergy had positive results in the skin test or for sIgE antibodies to both bee and wasp venom. The BAT was positive in 9 patients with bromelain and in 15 patients with HRP and the BAT was significantly higher in patients with HRP than those with bromelain at all concentrations ($p < 0.5$), therefore the authors concluded that BAT with HRP was a good method to determine sensitivity to CCDs.[119]

When there is the presence of a double positivity, patients are more than 10-fold more sensitized to primary sensitising allergen.[120] In this case, BAT could allow to identify the "real" allergen. In a population of 14 adult patients with history of reactions to Hymenoptera stings and doubtful SPTs and sIgE, BAT and SPT were concordant in 42.9%, BAT and sIgE in 57.1%. In a few cases, the BAT led to a more reliable diagnostic results concerning the relevant insect and was always negative in controls.[118]

In conclusion, in complicated cases of Hymenoptera allergy, where history, SPTs and sIgE are not conclusive, BAT might be helpful to better identify the "real" allergen involved.

BAT & RESPIRATORY ALLERGY

Typical tools to diagnose respiratory allergy are sIgE and SPTs, even if allergic reactions against inhalant allergens are heterogeneous and can be complex because of both diversity of the potential allergens and the multiplicity of the possible sites affected.[121]

Despite suggestive symptoms of allergic rhinitis, some patients have a negative diagnostic test for atopy; a considerable number of patients previously given a diagnosis of non-allergic rhinitis have local allergic rhinitis (LAR) or ''entopy".[121,122] Rondón et al[122] compared the inflammatory response, sIgE to Dermatophagoides pteronyssinus (Df Pt), and the response to a nasal allergen provocation test with Df Pt in patients with persistent nonallergic rhinitis ($n = 50$), patients with persistent allergic rhinitis ($n = 30$) and healthy controls ($n = 30$). They found that patients with persistent nonallergic rhinitis (PNAR group) had a similar leukocyte-

lymphocyte phenotype in nasal lavage than patients with persistent allergic rhinitis, while their nasal lavage was different than 1 control subject. Within the PNAR group, 54% showed a positive nasal provocation test (NPT) with DF Pt and 22% of these also presented nasal sIgE to Df Pt.[122] Currently, LAR is characterized by a positive response to specific nasal provocation test (NPT) with potential allergens, with a T helper 2 response in the absence of systemic atopy (negative SPTs/sIgE). To date, NPT is the gold standard to diagnose LAR, but it is invasive for the patient and time consuming whereas, the measurement of local sIgE levels is non-invasive and extremely specific, although the sensitivity is rather low.[123–125] For these reasons, in the diagnosis of LAR, BAT could be useful. Gomez et al[126] evaluated the activation of basophils in a group of 55 adult patients with confirmed LAR caused by Df Pt BAT was able to diagnose at least 50% of cases of LAR to Df Pt and was more sensitive than the detection of nasal sIgE and less time-consuming than NPT.[126]

BAT was also used to evaluate basophil allergen sensitivity in allergic asthma. In fact, although in allergic asthma IgE-sensitization is usually demonstrated by SPTs and sIgE, these tests do not directly predict if clinically the allergy contributes to asthma pathogenesis.[126,127] In their study, Dahlén et al[127] tested 26 adults with stable, intermittent allergic asthma with SPTs, spirometry, methacholine (provocative dose causing a 20% drop in forced expiratory volume in 1 s (methacholine PD-20) and allergen inhalation challenges (allergen PD-20). Their findings were related to basophil allergen threshold sensitivity (CD-sens) for the same allergen obtained from BAT and serological parameters (i.e. specific IgE- and IgG4 antibodies). Additionally, the authors found a statistically significant correlation between CD-sens and allergen PD-20 ($r = 0.49$; $p = 0.010$), as well as between CD-sens and the ratio of allergen PD-20 to methacholine PD-20 ($r = 0.52$; $p = 0.007$). Therefore, CD-sens obtained from BAT could be an objective marker of airway allergen sensitivity in stable allergic asthmatics allowing to predict airway responsiveness without performing dangerous bronchial challenge tests.[127,128] BAT applications are depicted in Fig. 2.

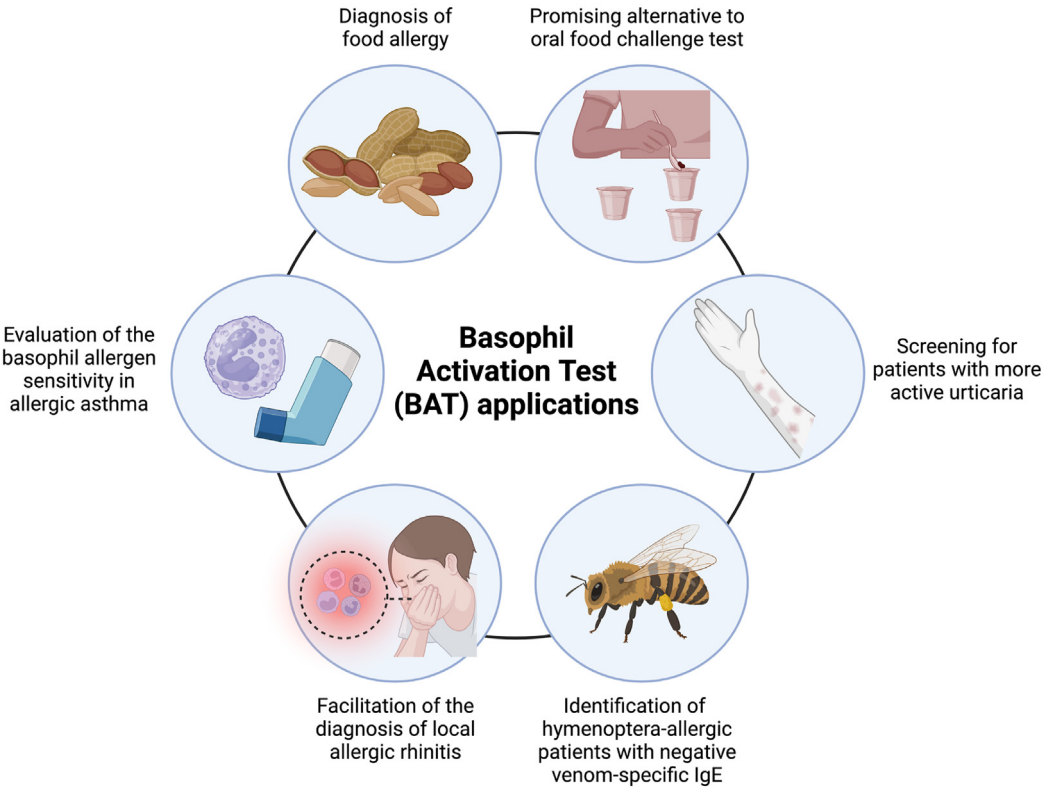

Fig. 2 Basophil Activation Test applications.

BAT LIMITATIONS

In clinical practice, the use of BAT is possible if the standardization, the quality of the laboratory and the training of flow cytometry operators are ensured, in addition to clinical validation of the diagnostic performance of the BAT to different allergens.[129,130] Currently, EAACI has gathered experts with extensive experience in the clinical application of BAT to start addressing its quality assurance.[3] However, some limitations of BAT for its routine use are needed to be discussed. Firstly, since BAT requires fresh blood, it must be carried out within 24 h of blood collection.[45] The allergen standardization as well as skilled operators are mandatory. Secondly, 17% of subjects present basophils selectively unresponsive to FcERI-mediated signalling.[57] Thirdly, BAT is more expensive than SPT or sIgE, but much cheaper and safer than OFC.[17]

However, given the heterogeneity in the current studies, further studies with larger and well-characterized patients are needed to better understand the relevance of its clinical applications.

CONCLUSIONS

In this narrative review we described the accepted procedure to perform BAT and its field of application in allergic diseases. We conclude that it is useful in food allergies particularly for the assessment of tolerance, in the diagnosis of equivocal venom allergy and drug adverse reactions. Additionally, it also could have a role in urticaria and respiratory allergy when classical tools of diagnosis failed.

This review is a starting point for increasing the applications of BAT in our clinical practice. Being an *in vitro* tool, surely, it is less stressful for children and its specificity could be essential for example in reintroducing foods, and avoiding the use of OFC test, which are expensive and dangerous for children. As described in this review, most of the studies involve adult patients and the use of BAT in pediatric population need further research to better understand its strengths and limitations.

Abbreviations

BAT, Basophil Activation Test; SPTs, Skin Prick Tests; sIgE, specific Immunoglobulin E; FcεRI, high affinity IgE receptor; fMLP, N-formyl-methionyl-leucyl-phenylalanine; FcεRI, Fc region of IgE; OFC, Oral Food Challenge; FA, Food Allergy; OIT, Oral Immunotherapy Trial; DHRs, Drug Hypersensitivity Reactions; ADRs, Adverse Drug Reactions; EAACI, European Academy of Allergy and Clinical Immunology; CSU, Chronic Spontaneous Urticaria; CCDs, Cross-Reactive Carbohydrate Determinants; LAR, Local Allergic Rhinitis; PNAR, Persistent Nonallergic Rhinitis; NPT, Nasal Provocation Test; Df Pt, Dermatophagoides pteronyssinus.

Availability of data and materials

Not applicable.

Authors' contributions

GD and PDF wrote the manuscript. ADL created the figures. SP, DDB, FDA participated in the writing of the manuscript. MA, PL, and FC revised the manuscript.

Ethics approval

Not applicable.

Authors' consent for publication

Consent for publication was obtained from all authors.

Funding

No funds, grants, or other support was received. See "Acknowledgments".

Declaration of competing interest

The authors have no conflicts of interest to declare that are relevant to the content of this article.

Acknowledgments

We thank all the authors for their contributions. This study was in part supported by the Italian Ministry of University and Research (MUR), grant Fondo per il Programma Nazionale Ricerca (PNR) e progetti di Ricerca di Significativo Interesse Nazionale (PRIN) CUP: D53D23010640006. Domenico De Bellis PhD fellowship has been found by the framework of PON RI 2014/2020, I.1-"Innovative PhDs with industrial characterization" (code: n. 1353889), Italian Ministry of University and Research (MUR), Italy, FSE-FESR").

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