The clinical utility of basophil activation testing in diagnosis and monitoring of allergic disease

The Clinical Utility of Basophil Activation Testing in Diagnosis and Monitoring of Allergic Disease

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Abstract

The basophil activation test (BAT) has become a pervasive test for allergic response through development of flow cytometry, discovery of activation markers such as CD63 and unique markers identifying basophil granulocytes. BAT measures basophil response to allergen crosslinking IgE on between 150 and 2000 basophil granulocytes in less than 0.1 ml fresh blood. Dichotomous activation is assessed as the fraction of reacting basophils. In addition to history, skin prick testing and specific IgE determination, BAT can be part of the diagnostic evaluation of patients with food-, insect venom-, and drug allergy and chronic urticaria. It may be helpful in determining the clinically relevant allergen. Basophil sensitivity may be used to monitor patients on allergen immunotherapy, anti-IgE treatment or in the natural resolution of allergy. BAT may use fewer resources and be more reproducible than challenge testing. As it is less stressful for the patient and avoids severe allergic reactions, BAT ought to precede challenge testing. An important next step is to standardize BAT and make it available in diagnostic laboratories..

The nature of basophil activation as an ex vivo challenge makes it a multifaceted and promising tool for the allergist. In this EAACI Task force position paper we provide an overview of the practical and technical details as well as the clinical utility of BAT in diagnosis and management of allergic diseases.

Introduction

The biological framework of BAT

Basophils and mast cells are key effector cells in immediate type allergic reactions, and the clinical impact of BAT is due to the unique ability of these cells to degranulate upon cross-linking of the specific IgE (sIgE) bound on membrane-bound high affinity IgE-receptor (FcεRI) by allergen exposure. Basophils are estimated to have a half-life of less than a week (1) whereas mast cells persist for months in tissue (2). The density of FcεRI-IgE complexes on basophils and mast cells is determined by the free IgE concentration in blood (3). Following the discovery of the quantal upregulation of CD63 during basophil activation in 1991(4), the BAT was developed in the 90's (5). CD63 is a membrane protein localized to the same secretory lysosomal granule that contains histamine. Translocation of CD63 to the cell membrane during degranulation can be measured by flow cytometry. As flow cytometers are now commonly available, BAT has become a widely used measure of allergic activity. Compared with the determination of sIgE in serum, BAT reflects a functional response as basophil activation can be induced by cross-linking of FcεRI, which requires more than binding of sIgE to allergen (6).

Comparing CD63 BAT with the Basophil Histamine Release assay

Blood basophil granulocytes contain and release histamine on stimulation with an allergen they are sensitised to (7). CD63 is the first tetraspanin identified (8). It is located in the same secretory lysosome as histamine (4), and may be a more convenient marker for degranulation. Allergen activation of blood basophils can thus be assessed as either histamine release or as upregulation of CD63, which is the focus of this article. Histamine release and upregulation of CD63 correlate well during activation of both blood basophil activation (4,9) as well as in mast cell activation (10,11).

Histamine release is determined by measuring histamine in the supernatant by either ELISA or other fluoro-spectroscopic methods, and expressing it as a fraction of the total cellular histamine determined from a cell lysate. These tests have not been reviewed systematically for their clinical implication, but have been frequently used in clinical diagnosis of allergy. Technically, the determination of histamine is in general more cumbersome, due to potential crossreactivity of histamine antibodies to i.e. methyl-histamine (12) and or technical challenges and effects of other leukocytes in whole blood in the fluorometric analysis (13,14). Where histamine is thought to be released both by piecemeal degranulation as well as anaphylactic degranulation, CD63 is a precise marker of anaphylactic degranulation through regulated exocytosis after allergen mediated activation of mast cells and basophils (9).

Flowcytometric analysis in the CD63 expression on basophils in BAT can be performed in virtually all routine and research laboratories equipped with a flow cytometer. The MFI for CD63 can be assessed in addition to the fraction of activated basophils. Although this has not yet resulted in additional benefit, flow cytometric assessment also allows for detailed phenotyping of the activated basophils. CD203c has frequently been measured in addition to CD63, and appears to co-express with CD63 even though the pathways for upregulation differ (9).

Considerations when taking a blood sample for BAT

Antihistamines do not influence BAT (15,16), but systemic steroids (15) and cyclosporin A (17) should be avoided. Blood samples should preferentially be taken within one year of the most recent exposure to allergen (18–20). It is possible to use blood samples within 24 hours to document sensitization (21), even though basophils may lose reactivity. As there is diurnal variation in the reactivity to CD203c (22), timing of blood sampling may be important. This still has to be confirmed for CD63. For serial sensitivity measurements the sampling procedure should be consistent (23–25). Tests done with whole blood are most commonly utilized. Basophil function is mostly assessed in heparin-stabilised blood. Basophils do not degranulate in EDTA or acid-citrate dextrose stabilised blood, but blood stabilised by these agents can be converted to release after adding calcium (21). Separation of cells from protective elements found in plasma may optimize activation through cell-bound sIgE (25)

Interleukin-3 (IL-3) enhanced the allergen specific up-regulation of CD63 (15,26) but unspecifically upregulates CD203c (27). IL3 synergised with stimulation through FceRI to enhance degranulation of basophils by 30% (28). IL3 may act at a step preceding MEK and Erk,

156 independently of the early events in signalling through FceRI (29). IL3 enhanced kinetics, reactance
157 and sensitivity of blood basophils to FceRI mediated activation independently of extracellular
158 calcium (30). This effect appears to be more significant in non-atopics than in atopic patients, which
159 may limit its significance in allergy diagnosis (31). Maximal CD63 response was marginally higher
160 with 10 ng/ml IL3 (32), and a two-fold increase in sensitivity and 25% increase in reactivity to
161 allergen was recorded with 4.5 ng/ml IL3 (15).

162 ***Selection of the source of allergen extracts for BAT***

163 The allergen described in patient history should be used in BAT (BOX A). Optimized
164 concentrations for a wide range of allergens, allergen sources and allergen extracts are listed
165 in table S1. Optimized allergen preparations are also available from vendors. Drug allergens
166 are typically active in the mg/ml range, and can be diluted 5- to 25-fold. Pure active ingredients or
167 injectable intravenous drug preparations should be used when possible since solubilized tablets are
168 complex mixtures of drugs and excipients. Some drugs are unstable and metabolise in solution; thus
169 allergens must be prepared fresh for each test. Light exposure is a critical factor in BAT results
170 when photo labile drugs such as moxifloxacin are used (33). A negative test with a parent drug does
171 thus not rule out that the patient reacts to a metabolite of the drug (34). Toxicity and non-specific
172 activation should be evaluated for each tested substance.

173 Protein allergens are often used in concentrations starting in the µg/mL range, and may be diluted
174 up to 5 – 15 log concentrations to ng/ml - pg/ml before reactivity is lost. The molar concentration of
175 allergens enables very precise analyses if recombinant allergen preparations or purified allergens
176 are used for BAT (35). Basophil reactivity to selected peanut (36,37) and insect venom (38,39)
177 allergens has higher predictive value than measurement of reactivity with allergen extracts. The
178 only carbohydrate allergen known is α-Gal (40). Increasing numbers of purified and recombinant
179 allergens are commercially available, which allows further standardization of BAT. The thresholds
180 for basophil reactivity and sensitivity may vary from geographical region to region so a critical
181 approach remains essential.

182 Standardized allergen preparation is essential when comparing basophil sensitivity data (for
183 example during immunotherapy, anti-IgE treatment or natural resolution of food allergy); failing
184 this a given test can only give a dichotomous result; reactive or not reactive.

Flow Cytometry in BAT

Determination of activation of basophils by flow cytometry was first described with CD63 (4). Since then CD203c (27) and a number of other activation markers have been identified (41). Currently, BAT with CD63 is the best clinically validated test (32,42–44), but the BAT based on CD203c has been shown to be a reliable test (45–47).

Basophils can be identified with different combinations of antibodies in flow cytometry. They were first identified as circulating IgE^+ cells. However, low side scatter in combination with $CD123^+/HLADR^-$, $CRTH2^+$, $CD203c^+$ or $CD193^+$ are commonly applied combinations. Cell surface expression of the basophil selection marker CD193 (CCR3) was more stable than IgE or CD123/HLA DR on resting basophils (48). IgE and CD123/HLA-DR showed somewhat more inter-individual variability in cell surface expression. Unfortunately the lineage marker CD203c for basophils (49) and stem cells was not included in this comparison. CD203c can be used for both identification and as an activation marker. Its expression on basophils rapidly increases upon manipulation of cells, or during non-degranulating stimulation of basophils (9).

Quality of blood basophils obtained is normally confirmed by stimulation with the bacterial peptide fMLP (50). Anti-IgE or anti-FcεRI antibodies must be used as IgE-mediated positive controls, and buffer is used as negative control. Initially, 1-3 consecutive not sensitized subjects can be used to ascertain the specificity of a response.

If standardized commercial tests are not used, the method used for testing has to undergo validation. Standardization of BAT procedures and allergen preparations would enable comparison of results of BAT in different centers both for clinical and for research purposes and would ensure consistency of BAT results in multicenter studies. Standardization requires multicenter studies where the same detailed description of the procedures, for example as defined by MiFlowCyt (51) (supplementary information), are followed, by using the same allergen preparations and sharing databases in which annotated raw data can be deposited for analysis by third parties.

Presentation and interpretation of BAT

There are two common measures of basophil activity; basophil reactivity (5), the number of basophils that respond to a given stimulus, and basophil sensitivity (1,4), the allergen

concentration at which half of all reactive basophils respond (Figure 1). Basophil reactivity depends on the priming state of the basophil and the cellular translation of the IgE signal within the cell (52). Basophil sensitivity is a function of reactivity and the compound affinity of cell-bound sIgE for allergen and free competing immunoglobulin (25,53). It is sufficient to measure reactivity at one or two concentrations, and assessment of basophil reactivity is important using a positive control before basophil sensitivity to allergen is measured. Positive responses must be interpreted in a clinical context.

Once reactivity is confirmed it may be useful to evaluate the basophil sensitivity (54–57). This requires measurement of reactivity at 6-8 allergen concentrations (25). The graded response to allergen is fitted to a curve of reactivity versus allergen concentration, and the eliciting concentration at which 50% of basophils respond (EC50) is determined. EC50 can be expressed as 'CD-sens' by inversion and multiplication by 100 (1).

More recently the area-under-the-dose curve (AUC) measurement attempts to combine reactivity and sensitivity into one; it is similar to a coordinate system of sensitivity and reactivity, but also incorporates partial anergy induced at high allergen concentrations and can be calculated even in cases where responses do not fit well to a typical dose-response curve (58). Oral and sublingual immunotherapy may induce anergy in a significant fraction of basophils (as well as mast cells), but may not change basophil sensitivity as much as subcutaneous immunotherapy (25). Considering this scenario, it may be important to combine reactivity and sensitivity into an AUC representation of basophil response. ROC curves are used in identification of novel allergens when ≥7 sensitized patients are available. This is difficult to achieve for rare allergens.

Basophil granulocytes of non-responders (6%-17% of population) can remain unresponsive to stimulation through FcεRI under standard BAT conditions (59–61). Results from non-responder patients should be regarded as false negatives when assessing test performance. No conclusions with regard to allergen-induced responses can be made. Non-responders can experience allergic symptoms and have positive skin prick test with relevant allergens. This feature is also present in healthy donors. It is attributed to differences in the intracellular signaling pathway (62,63).

Placing BAT in the diagnostic algorithm for allergic disease

In the general algorithm for diagnosis of allergy (Box 2), patient history should be taken with an attempt to identify the allergen source and assess the severity of the allergic reaction (64,65). The allergic response should be confirmed by an objective test, ideally within one year of the last symptomatic exposure (18–20). First line tests include sIgE, skin prick testing and, for insect venom and drug allergy, intradermal testing. However, in very few patients (30 in 100 000) skin prick testing and intradermal testing might induce systemic symptoms if the allergic response was particularly severe (66,67). Measurement of sIgE may not be possible if the allergen is not available as a routine reagent, and may be of limited value depending on the performance of the available reagents. sIgE measurements and skin tests indicate sensitization and do not prove clinical relevance on their own.

BAT is a functional test resembling an *ex vivo* provocation. It can be measured at the same time as sIgE, and in general precedes *in vivo* provocation tests e.g. oral food, drug or bronchial challenge that are time consuming, expensive, stressful, may be difficult to interpret and may cause severe allergic reactions (Box B). Provocation testing is associated with additional risk if the patient is taking ACE-inhibitors that may increase the risk of anaphylaxis, or β-blockers that complicate the treatment of an anaphylactic reaction. The combination of ACE Inhibitors and β-blockers is associated with increased risk of anaphylaxis (68). Insect venom allergy can be diagnosed accurately and safely with BAT in patients with mastocytosis (69).

In the diagnosis of drug hypersensitivity, measurement of sIgE is available only for a limited number of drugs and skin tests, generally display low sensitivity, and are thus well complemented by BAT. In the diagnosis of venom allergy, the culprit allergen is sometimes difficult to select by sIgE or skin testing. Here BAT or component resolved diagnosis by sIgE may help in identifying the correct allergen. BAT can identify the culprit allergen in local allergic rhinitis (71). BAT can also be used in the follow-up of patients undergoing allergen immunotherapy (AIT) and treatment with anti-IgE (Box C).

In the diagnosis of chronic urticaria, BAT was proposed as a specific, sensitive and safe *in vitro* alternative for the autologous skin test (ASST) for the detection of "autoreactive" serum components (26,71). In contrast to the classical BAT procedure using patients' blood, here basophils from healthy donors are challenged with patients' serum.

In the diagnosis of food allergy, oral food challenges (OFC) are the gold standard but can cause

severe reactions (72,73) and their reproducibility can be questioned (23). BAT closely resembles the clinical phenotype of food allergic patients. It can be used in addition to sIgE and thus may reduce the need for OFC (74). Overall, provocation testing should be the last resort to document clinically relevant sensitization. Severe reactions recorded in the patient history are an important contraindication when contemplating provocation testing (72,73). BAT can be considered before provocation testing in most cases.

Chronic Urticaria

The underlying mechanism of chronic spontaneous urticaria (CU) is still incompletely understood. About half of the patients have autoantibodies against FcεRI and a few against IgE (75,76). Other autoimmune markers such as IgE and IgG antibodies against thyroid peroxidase are frequently found (77). CU sera activate resting basophils of normal donors to release histamine and upregulate CD63 and CD203c. BAT may replace the autologous serum skin test (ASST) that uses the patients' own serum as an intradermal skin test reagent (71,78).

Assessing autoreactivity in patients with chronic urticaria

BAT with CD63 upregulation as an activation marker for CU was established as a specific, sensitive and safe *in vitro* alternative to detect functional autoantibodies (79–81,26). Results with CD203c are less homogeneous (80–82). The central problem is the heterogeneity of the results using different basophil donors. This can be normalized by titrated addition of IL-3 (26). BAT with autologous basophils should not be performed because CU patients are often non responders or poor responders to IgE crosslinking (83) and have diagnostic basopenia (84).

Current clinical research questions

- Several issues remain to be addressed, especially methodological differences among laboratories and the lack of a gold standard test to compare results. An optimized and reproducible form of BAT should be developed and agreed upon to distinguish antibody and non-antibody mediated autoreactive CU subtypes.
- In order to elucidate the exact nature of the degranulating factors in patient serum, three major approaches should be investigated:

    1. Cellular approach modifying the response of the donor basophils (blocking of different signaling pathways etc.).

2. In spite of persistent failure to do so, a cell line should be characterized that could substitute the need of a basophil donor.
3. Serological approach aiming on an optimal serum protein separation to identify the nature of the factors leading to degranulation in donor basophils.

Key messages

- BAT may replace ASST as the standard diagnostic procedure to identify autoreactive serum factors in CU with a quantifiable result that may be used to monitor treatment.
- BAT removes the risk of accidental infection
- In contrast to ASST, there is no need to suspend antihistamines, as they do not influence the result of BAT.

Drug Allergy

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. This is particularly important in diagnosis of allergy to drugs used in anesthesia, where challenge testing is impossible, impractical or unethical. Moreover, in the evaluation of many drug reactions the determination of sIgE is not available since binding the molecules or their metabolites into a solid phase is often not possible (85). BAT is an additional tool in the diagnosis of drug allergy that is safer, gentler and cheaper than a provocation test and, in some instances, is the only available diagnostic tool. Table S2 lists an overview of the performance of BAT in the diagnosis of major drug allergens. The sensitivity of BAT in diagnosis of drug allergy is about 50%, and the specificity up to 93%. Non-IgE-mediated anaphylactic reactions may be due to complement-mediated or direct activation (86). This response to radio contrast media may involve the G-protein-coupled activation pathway and elevated Il-1β (87). Involvement of the FcεRI-mediated pathway can be confirmed by inhibition with PI3Kinase inhibitors such as wortmannin (59,88).

Validated Drug classes

There are several studies including BAT in drug allergy diagnosis for beta-lactams (50,89,90), NMBA (91–93), quinolones (88,94), radio contrast media (95,96) and pyrazolones (20,97) with good sensitivity and specificity.

Importantly, BAT provides positive results in 40% of the patients with immediate-type systemic reaction and negative skin test and confirmed by provocation that constitute about 25% of all beta-lactam-allergic patients (98). BAT has a good negative predictive value, useful in the decision to perform the provocation test as demonstrated with quinolones (94). It has a complementary role to skin tests for different drug hypersensitivities (20,98) and can be particularly useful in the study of cross-reactivity between NMBA, for the identification of safe alternatives for future surgery (99).

BAT appears particularly useful for drugs where other *in vitro* tests are lacking and skin tests are unavailable or unreliable or where they provide equivocal results. These cases include carboplatin (100), chlorhexidine (101), atropine (102), glatiramer acetate (103), methylprednisone (20), gelatines, carboxymethylcellulose, hydroxyl ethyl starch, cremophor, opiates (104) and bovine serum albumin (105).

Current clinical research questions

- Further studies are needed to depict the clinical relevance of the different degranulation processes.
- The usefulness of other activation markers needs to be explored.
- Pathways that lead to basophil activation in non-IgE mediated immediate drug hypersensitivity need to be described.

Key Messages

- For a number of drugs, BAT is the only available test to confirm a hypersensitivity response.
- A negative test does not rule out that the patient reacts to a metabolite of the drug.
- Exposure to drugs is infrequent, and for this reason it may be difficult to confirm the clinical history of hypersensitivity if the evaluation is >18 months from the most recent clinical reaction.

  Once the hypersensitivity is established, cross-reacting drugs and safe alternatives may be suggested by BAT

Food allergy

The performance of BAT in the diagnosis of different food allergies including pollen-food syndromes has been assessed in various studies (Table S3-1). The reported sensitivity of BAT in diagnosis of food allergy ranges from 77-98%, and the specificity 75-100%. BAT in these studies was more accurate than sSPT and sIgE (60,61,74). In a recent study in peanut allergy, for the first time BAT diagnostic cut-offs were validated in an independent prospective population. BAT significantly improved clinical diagnosis over the use of SPT and sIgE and reduced the number of OFC required (74). BAT showed 100% specificity, suggesting that in patients with a positive BAT the OFC could be deferred (74).

Patients with clinical allergy that developed symptoms in an OFC to peanut had high basophil sensitivity to peanut, and patients who tolerated peanuts in a OFC had low basophil sensitivity to peanut (57). A similar message emerges in studies attempting to measure reactivity at allergen concentrations where a change in sensitivity results in a change in reactivity as illustrated in figure 1b (106,107,74). Although OFC and basophil sensitivity both identified all clinically sensitized children, only basophil sensitivity was reproducible at two consecutive visits (r^2=0.94) (23). In a recent publication, for the first time BAT reactivity reflected the allergy severity and BAT sensitivity reflected the threshold of response to allergen in an OFC (108).

Monitoring natural resolution and immune modulatory treatments of food allergy

Basophil reactivity has been shown to distinguish patients that tolerate extensively heated forms of cow's milk and egg from patients who do not (106,109,110). This has prognostic implications as the natural history of these groups varies: patients reacting to extensively heated milk or egg tend to have more persistent food allergy.

BAT may be useful in assessing the natural resolution of food allergies that are commonly outgrown over time, such as cow's milk allergy (61), and in determining when the food in question can safely be reintroduced in the diet (Table S3-2). BAT has also been used to monitor clinical response to immune-modulatory treatment of food allergy (Table S3-3). Overall, in studies of immunotherapy to foods such as peanut (107,111) and egg (112), basophil reactivity to the respective food allergens decreased during treatment. In the study of egg OIT by Burks there was a correlation between basophil suppression and clinical desensitization, but not with long-lasting clinical tolerance (112). Basophil CD203c expression has shown to decrease during treatment with Omalizumab and to return to pre-treatment levels after cessation of therapy in patients with peanut allergy (113). Improvement in basophil sensitivity to milk in milk allergic children treated with Omalizumab predicted tolerance in a milk challenge test (114).

Current clinical research questions

- The diagnostic utility of BAT needs to be validated for specific food allergens and in different populations.
- Changes in the basophil response during allergen immunotherapy and anti-IgE treatment in food allergy should be investigated.
- The predictive value of basophil suppression for treatment outcome has to be established.

Key messages

- BAT can improve the diagnosis of food allergy over SPT and sIgE and may be able to reduce the number of OFC.
- BAT can be used to monitor the natural resolution and clinical response to immune-modulatory treatments for food allergy.

Hymenoptera venom allergy

Overall, the diagnostic sensitivity of BAT with insect venoms referred to the history was found to be 85%-100%, the diagnostic specificity 83%-100% (32,43–45). Table S4 lists papers describing the use of BAT in hymenoptera venom allergy. Specific diagnostic problems can be resolved by measuring basophil reactivity and sensitivity.

BAT in patients with negative standard tests: A subset of patients (4-6%) with a history of systemic reactions after Hymenoptera stings have negative venom-specific IgE and skin test results. These patients can subsequently experience another severe or even fatal reaction to an insect sting. Diagnostic sting provocation tests are considered as unethical for such cases. BAT allows the identification of about two thirds of those patients (115–117). However, in patients with systemic mastocytosis (with elevated serum tryptase levels) the diagnosis of venom allergy should be done with care (69).

BAT in patients sensitized to bee and wasp venom "double positivity": Up to 60% of the patients with Hymenoptera venom allergy have sIgE to both bee and wasp venom. It is important to identify the relevant venom for VIT, especially if the patient has had an anaphylactic reaction to only one insect. The double positivity might be due to a true double sensitization to both venoms, irrelevant recognition of cross-reactive epitopes or cross-reactivity due to sequence homology among venom proteins. Basophil reactivity has the lowest rate of double positivity of diagnostic tests for hymenoptera allergy (118) and repeatedly shows a positive result to only one venom in about one-quarter to one-third of patients with double sIgE positivity (43,118,119). In patients with double sIgE positivity a positive BAT can help to identify the primary sensitizing allergen (118–121). In the case of patients with double positive BAT, the allergen to which the patient is markedly more sensitive might represent the primary sensitizing allergen (117,120), but this requires further research. BAT adds more clinically relevant information about the culprit insect than component-resolved sIgE testing with single recombinant allergens such as Api m 1, Ves v 5 and Ves v 1. (117,120). However, recombinant venom allergens applied to BAT might represent a step forward in developing better in vitro tests for specific diagnosis of Hymenoptera allergy (39).

Monitoring the effect of venom immunotherapy with basophil sensitivity

Importantly, a clear decrease in basophil sensitivity is found up to 4 years after initiation of VIT, without a change in basophil reactivity (44,122–124). A recent report about an 8-year follow up of patients submitted to VIT showed that the decrease in basophil sensitivity seemed to be also associated with the induction of tolerance (124). Some studies suggest that side effects during the build-up phase of VIT are predicted by a high basophil sensitivity (123,125).

Current clinical research questions

- The minimal difference in sensitivity between primary and cross-reacting allergens in patients with sIgE to several venoms needs to be defined.
- The utility of basophil sensitivity as the tool of choice to monitor the effect of VIT should be explored.

Key messages

Basophil reactivity and sensitivity (in that order) play an important role in the diagnosis of venom allergy, as they are effective tools to identify the primary sensitizing antigen.

Inhalant allergens

Measurements of sIgE or skin testing in combination with the clinical history are usually sufficient to diagnose allergy to inhalant allergens. However, in specific cases BAT can be helpful for diagnosis. Patients with local allergic rhinitis by nasal provocation who have no detectable sIgE or skin testing but have a positive BAT are a notable example (70). Crude (126,127) as well as modified (128) and recombinant allergens (46,49) have been tested with good outcomes, but more studies are needed for describing the advantage of using recombinant allergens. Basophil sensitivity correlates with the nasal provocation titer in allergic rhinitis (126), the allergen specific bronchial provocation threshold in allergic asthma (55) and the asthma control test (56). When using an allergen titration, the correlation of the outcome between BAT and bronchial allergen sensitivity was statistically significant. This indicates that basophil allergen threshold sensitivity (CD-sens or EC50) may accurately reflect clinical allergen sensitivity (55). Papers describing the use of BAT in diagnosing and monitoring allergy to inhalant allergens are listed in Table S5.

Monitoring the effect of allergen immunotherapy and anti-IgE Treatment effect

Basophil sensitivity is a stable and reproducible measure (23,24,57) and can be used to assess the efficacy of allergen-specific immunotherapy (AIT) to aeroallergens. It has been used to monitor patients treated with AIT for birch (128,129) and timothy (25,54), and showed reduced allergen sensitivity already during the up-dosing stage. Several studies reported that the reduction in basophil allergen sensitivity after AIT is due to serological allergen blocking/binding factors, competing with the cell bound sIgE for allergen. sIgG (especially IgG4) is the major competitor for allergen binding (54,128,129).

The humanized monoclonal anti-IgE antibody Omalizumab (Xolair) has been used for a decade to treat patients with severe allergic asthma. Basophil sensitivity has successfully been used to identify patients who respond to this treatment (130) and to assess treatment efficacy (126,130).

Current clinical research questions

- Basophil sensitivity tests have great potential to be used to determine patients sensitivity to inhalant allergens and to monitor treatment effect and could be considered as a supplement, and eventually possibly as replacement for allergen challenge tests.

Key message

- Basophil sensitivity has the unique ability to monitor a patient's inhalant allergen sensitivity over time, to measure natural progression of allergy, and may be developed to serve as a tool to measure the response to treatment with AIT and Omalizumab.

Perspectives

Since its discovery in 1991 (4) and the first clinical applications in 1994 (131), BAT has been developed as a diagnostic aid in allergy.

Cellular changes in basophil granulocytes and mast cells may be as important as, but are still more elusive than change in sIgE (25,124,132). This may be explored by passive sensitization using DARPins (133) where both patient and control blood may be stripped entirely of IgE under physiological conditions, and be resensitized with known IgE to evaluate the effect of sIgE and cellular response independently.

Different methods of reporting results of BAT may be useful when asking different clinical questions; stimulation index and % positive basophils are used in the diagnosis of food, drug (42), venom and occupational allergy, but do not reflect improvement during venom immunotherapy (44). It has recently been shown that reactivity decreases with basophil (and mast cell?) desensitization (132) which may also occur during clinical treatment of allergy (134–136). When reactivity is measured in clinical settings, the aim is usually to identify an allergen concentration at which change in sensitivity is optimally identified (125,115,120,137). Basophil sensitivity is used to monitor change in allergic disease during natural development (23) and during treatment with AIT (25) or anti-IgE (138), and AUC may be useful in monitoring food allergy progression (58). Both reactivity and allergen sensitivity are measured when allergy severity is evaluated by basophil sensitivity, but a useful composite measure has yet to be designed.

For all reports of BAT, a threshold for basophil reactivity has to be set. This is often done at 2%, 10% or 15% of resting basophils. An alternative method would be to set the threshold halfway between the MFI of resting basophils and the positive control. With this practice, non-responders would fail at this stage, as the threshold could not be set. The usefulness of such a threshold could be evaluated in a retrospective trial, in which participating laboratories contribute data from consecutive tests that are analyzed as one would do in their lab, or by this new method.

Major applications of BAT are summarized in Box 4. BAT has been established as a routine diagnostic test with standardized allergen preparations in a number of service labs. The

509 routine application of BAT for established allergens is quite different to that of identifying and
510 characterizing novel allergens or monitoring allergy intensity. To strengthen the use of BAT as
511 diagnostic test, laboratory procedures and allergen concentrations in BAT should be
512 standardized. This can be made possible with the use of industry standards like MiFlowCyt
513 (51) or purchase of standardized material from CE-approved vendors. An important next step
514 is the standardization and automation of analysis of BAT. Once that is achieved, it will be
515 possible to do large multicenter trials to characterize the diagnostic performance of BAT and
516 broaden its use as a clinical tool. These multicenter studies should also address the
517 relationship of measures of BAT and sensitivity to sIgE, clinical symptoms and symptom
518 severity.

519

Author contribution

HJH and EK drafted the introduction, MF, TPP and OVH the section on chronic urticaria, AFS, AN, EK, SUP and WGS the section on food allergy, CM, BE, PR, DE, VS, MLS, and PK the section on drug allergy, BE, PK and HJH the section on hymenoptera allergy and AN, PK and HJH on the inhalant allergies section. All authors reviewed the entire final manuscript.

Conflict of Interest

AFS, BE, SUP, WGS, OVH obtained research funding, and BE and OVH received speaker honoraria from Bühlmann Laboratories, Schoenenbuch, CH. BE received research funding from BD Biosciences, Erembodegem, Belgium. AN received research funding from Novartis, Basel, CH. HJH, CM, MF, PR, DE, VS, MLS, TPP, PK and EFK have no conflict of interest to report.

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Figure 1: Assessing basophil response.

The fraction of $CD63^+$ basophils is plotted against log allergen concentration. Adapted from (58) with permission from the authors.

A. **Basophil reactivity** is the dose (range) at which maximal response occurs. **Basophil sensitivity** is the dose at which half of the maximal response occurs. *At high allergen concentrations, basophil response may be suppressed.

B. A Change in sensitivity toward higher allergen concentration is the most reproducible basophil biomarker for clinical sensitivity to allergen to date. Attempts to reduce the number of BAT tests required to determine a significant change in basophil response have focussed on identifying an allergen concentration at which a change in sensitivity can readily be assessed (blue box; typically close to the sensitivity of the investigated population).

C. Basophil response could also be assessed as area under the curve (AUC) with a log allergen axis, or a similar composite measure reflecting both reactivity and sensitivity. Variation in maximal basophil reactivity arises concurrently with, and may be inseparable from, a change in sensitivity.