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POSITION PAPER

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

H. J. Hoffmann[1], A. F. Santos[2,3,4], C. Mayorga[5], A. Nopp[6], B. Eberlein[7], M. Ferrer[8], P. Rouzaire[9], D. G. Ebo[10], V. Sabato[10], M. L. Sanz[8], T. Pecaric-Petkovic[11], S. U. Patil[12], O. V. Hausmann[13,14], W. G. Shreffler[12], P. Korosec[15] and E. F. Knol[16]

[1]Department of Respiratory Diseases and Allergy, Department of Clinical Medicine, Aarhus University, Aarhus, Denmark; [2]Department of Paediatric Allergy, Division of Asthma, Allergy and Lung Biology, King's College London; [3]MRC & Asthma UK Centre in Allergic Mechanisms of Asthma, London, UK; [4]Immunoallergology Department, Coimbra University Hospital, Coimbra, Portugal; [5]Research Laboratory and Allergy Service, IBIMA-Regional University Hospital of Malaga, UMA, Malaga, Spain; [6]Clinical Immunology and Allergy Unit, Department of Medicine Solna, Karolinska Institutet, Karolinska University Hospital, Stockholm, Sweden; [7]Department of Dermatology and Allergy Biederstein, Technische Universität München, Munich, Germany; [8]Department of Allergy and Clinical Immunology, Clínica Universidad de Navarra, Pamplona, Spain; [9]Department of Immunology, University Hospital, Clermont-Ferrand and ERTICa Research Group, University of Auvergne, Clermont-Ferrand, France; [10]Department of Immunology-Allergology-Rheumatology, University of Antwerp and Antwerp University Hospital, Antwerp, Belgium; [11]Adverse Drug Reactions-Analysis and Consulting (ADR-AC) GmbH, Bern, Switzerland; [12]Center for Immunology and Inflammatory Diseases, Massachusetts General Hospital and Harvard Medical School, Boston, MA, USA; [13]Department of Rheumatology, Immunology and Allergology, Inselspital, University of Bern, Bern; [14]Loewenpraxis, Luzern, Switzerland; [15]Laboratory for Clinical Immunology & Molecular Genetics, University Clinic of Respiratory and Allergic Diseases Golnik, Golnik, Slovenia; [16]Department of Immunology and Department of Dermatology/Allergology, University Medical Center Utrecht, Utrecht, The Netherlands

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Correspondence
Professor Hans Jürgen Hoffmann, B.Sc. (Hons.), PhD, Department of Respiratory Diseases and Allergy, Department of Clinical Medicine, Aarhus University, Nørrebrogade 44, DK 8000 Aarhus C, Denmark.
Tel.: +45-28188147
Fax: +45 78 46 21 10
E-mail: hjh@clin.au.dk

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Abstract

The basophil activation test (BAT) has become a pervasive test for allergic response through the development of flow cytometry, discovery of activation markers such as CD63 and unique markers identifying basophil granulocytes. Basophil activation test measures basophil response to allergen cross-linking IgE on between 150 and 2000 basophil granulocytes in <0.1 ml fresh blood. Dichotomous activation is assessed as the fraction of reacting basophils. In addition to clinical history, skin prick test, and specific IgE determination, BAT can be a 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. Basophil activation test 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 basophil activation test (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 1990s (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 sensitized 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) and 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 cross-reactivity of histamine antibodies to, e.g. methylhistamine (12) or technical challenges and effects of other leukocytes in whole blood in the fluorometric analysis (13, 14). Where histamine is thought to be released by both piecemeal degranulation and 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 Mean fluorescence intensity (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 1 year of the most recent exposure to allergen (18–20). It is possible to use blood samples within 24 h 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 performed with whole blood are most commonly utilized. Basophil function is mostly assessed in heparin-stabilized blood. Basophils do not degranulate in EDTA or acid-citrate dextrose stabilized blood, but blood stabilized 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).

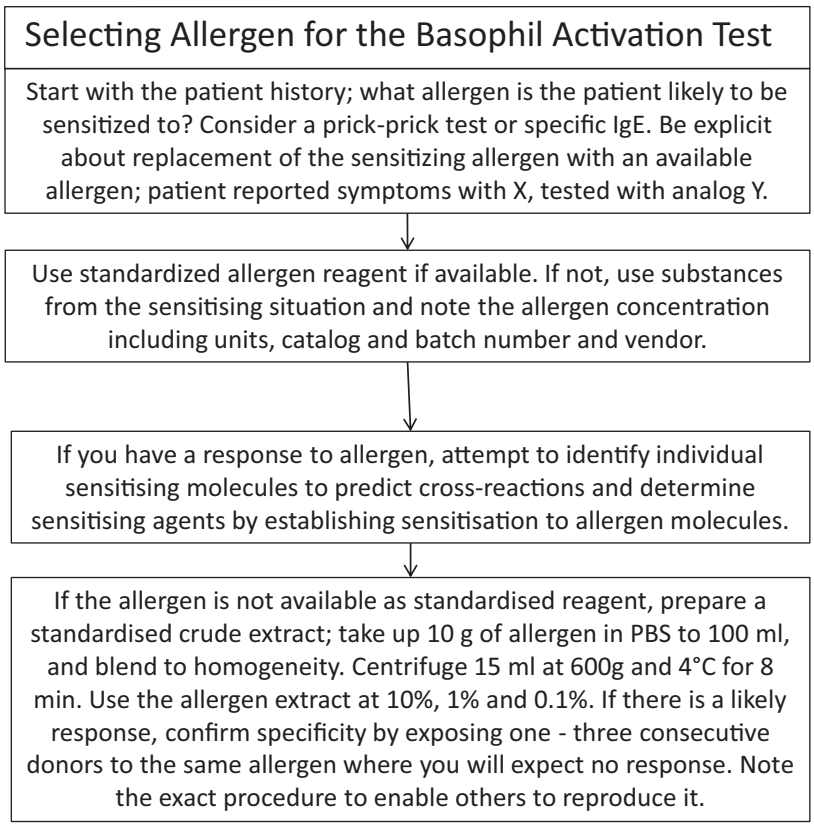
Interleukin-3 (IL-3) enhanced the allergen-specific upregulation of CD63 (15, 26) but unspecifically upregulates CD203c (27). IL3 synergized with stimulation through FcεRI to enhance degranulation of basophils by 30% (28). IL3 may act at a step preceding MEK and Erk, independently of the early events in signaling through FceRI (29). IL3 enhanced kinetics, reactance, and sensitivity of blood basophils to FceRI-mediated activation independently of extracellular calcium (30). This effect appears to be more significant in nonatopics than in atopic patients, which may limit its significance in allergy diagnosis (31). Maximal CD63 response was marginally higher with 10 ng/ml IL3 (32), and a twofold increase in sensitivity and 25% increase in reactivity to allergen was recorded with 4.5 ng/ml IL3 (15).

Selection of the source of allergen extracts for BAT

The allergen described in patient history should be used in BAT (Box 1). Optimized concentrations for a wide range of allergens, allergen sources, and allergen extracts are listed in Table S1. Optimized allergen preparations are also available from vendors. Drug allergens are typically active in the mg/ml range and can be diluted 5- to 25-fold. Pure active ingredients or injectable intravenous drug preparations should be used when possible as solubilized tablets are complex mixtures of drugs and excipients. Some drugs are unstable and metabolize in solution; thus, allergens must be prepared fresh for each test. Light exposure is a critical factor in BAT results when photo labile drugs such as moxifloxacin are used (33). A negative test with a parent drug does thus not rule out that the patient reacts to a metabolite of the drug (34). Toxicity and nonspecific activation should be evaluated for each tested substance.

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

Box 1:



Standardized allergen preparation is essential when comparing basophil sensitivity data (e.g., during immunotherapy, anti-IgE treatment or natural resolution of food allergy); failing 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 interindividual 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 both for identification and as an activation marker. Its expression on basophils rapidly increases upon manipulation of cells, or during nondegranulating 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) (Supporting information), are followed, 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 (Fig. 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 *vs* 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 nonresponders (6–17% of population) can remain unresponsive to stimulation through FcεRI under standard BAT conditions (59–61). Results from nonresponder patients should be regarded as false negatives when assessing test performance. No conclusions with regard to allergen-induced responses can be made. Nonresponders can experience allergic symptoms and have positive skin prick test (SPT) 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 1 year of the last symptomatic exposure (18–20). First-line tests include sIgE, SPT and, for insect venom and drug allergy, intradermal test. However, in very few patients (30 in 100 000) SPT and intradermal test 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.

Basophil activation test 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, for example, oral food, drug, or bronchial challenge that are time consuming, expensive, stressful, may be difficult to inter-

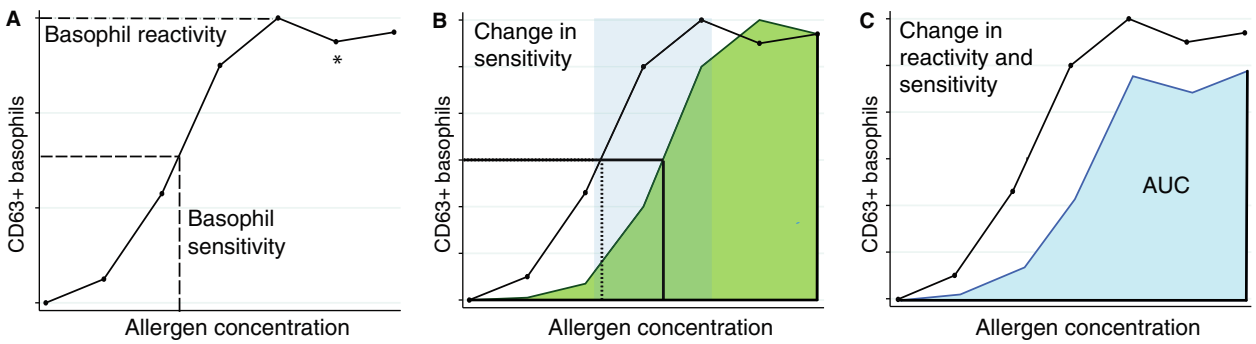
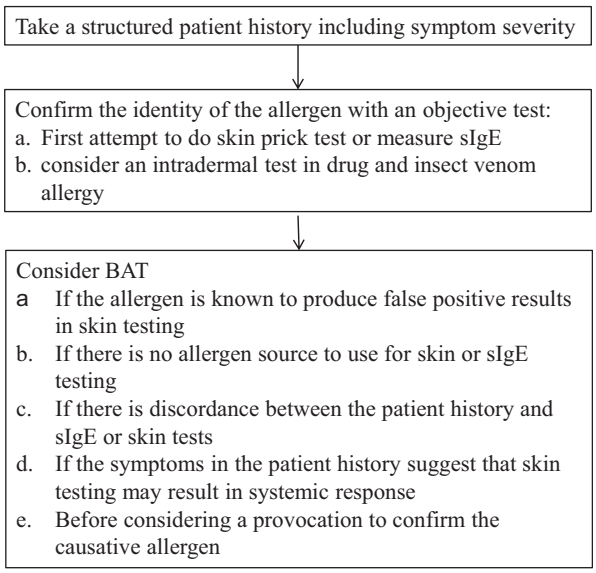


Figure 1 Assessing basophil response. The fraction of $CD63^+$ basophils is plotted against log allergen concentration. Adapted from (58) with permission from the authors. (A) *Basophil reactivity* is the dose (range) at which maximal response occurs. *Basophil sensitivity* is the dose at which half 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.

Box 2:



pret and may cause severe allergic reactions (Box 2). 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 skin tests, and sIgE is available only for a limited number of drugs, which 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. Basophil activation test can identify the culprit allergen in local allergic rhinitis (70). Basophil activation test can also be used in the follow-up of patients undergoing allergen immunotherapy (AIT) and treatment with anti-IgE (Box 3).

In the diagnosis of chronic urticaria, BAT was proposed as a specific, sensitive, and safe *in vitro* alternative for the autologous serum skin test (ASST) for the detection of 'autoreactive' serum components (26, 70). 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 (71, 72) and their reproducibility can be questioned (23). Basophil activation test 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 (73). 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 (71, 72). Basophil activation test 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 (74, 75). Other autoimmune markers such as IgE and IgG antibodies against thyroid peroxidase are frequently found (76). CU sera activate resting basophils of normal donors to release histamine and upregulate CD63 and CD203c. Basophil activation test may replace the ASST that uses the patients' own serum as an intradermal skin test reagent (70, 77).

Assessing autoreactivity in patients with chronic urticaria

Basophil activation test with CD63 upregulation as an activation marker for CU was established as a specific, sensitive, and safe *in vitro* alternative to detect functional autoantibodies (26, 78–80). Results with CD203c are less homogeneous (79–81). The central problem is the heterogeneity of the results using different basophil donors. This can be normalized by titrated addition of IL-3 (26). Basophil activation test with autologous basophils should not be performed because CU patients are often nonresponders or poor responders to IgE cross-linking (82) and have diagnostic basopenia (83).

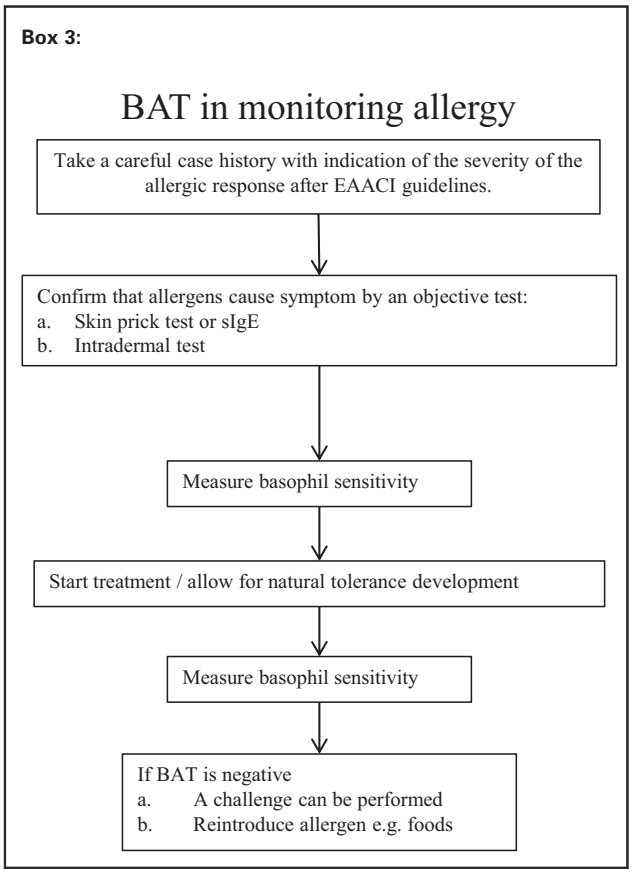
Current clinical research questions

- 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 nonantibody-mediated autoreactive CU subtypes.
- 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

- Basophil activation test 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.

Box 3:



- Basophil activation test 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 as binding the molecules or their metabolites into a solid phase is often not possible (84). Basophil activation test 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 (85). This response to radio contrast media may involve the G protein-coupled activation pathway and elevated Il-1β (86). Involvement of the FcεRI-mediated pathway can be confirmed by inhibition with PI3Kinase inhibitors such as wortmannin (59, 87).

Validated drug classes

There are several studies including BAT in drug allergy diagnosis for beta-lactams (50, 88, 89), Neuromuscular blocking agent (NMBA) (90–92), quinolones (87, 93), radio contrast media (94, 95), and pyrazolones (20, 96) 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 (97). Basophil activation test has a good negative predictive value, useful in the decision to perform the provocation test as demonstrated with quinolones (93). It has a complementary role to skin tests for different drug hypersensitivities (20, 97) and can be particularly useful in the study of cross-reactivity between NMBA, for the identification of safe alternatives for future surgery (98).

Basophil activation test 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 (99), chlorhexidine (100), atropine (101), glatiramer acetate (102), methylprednisone (20), gelatines, carboxymethylcellulose, hydroxyl ethyl starch, cremophor, opiates (103), and bovine serum albumin (104).

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% to 98%, and the specificity 75–100%. Basophil activation test in these

studies was more accurate than sSPT and sIgE (60, 61, 73). In a recent study in peanut allergy, for the first time BAT diagnostic cutoffs were validated in an independent prospective population. Basophil activation test significantly improved clinical diagnosis over the use of SPT and sIgE and reduced the number of OFC required (73). Basophil activation test showed 100% specificity, suggesting that in patients with a positive BAT the OFC could be deferred (73).

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 Fig. 1B (73, 105, 106). 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 (107).

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 (105, 108, 109). 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.

Basophil activation test 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). Basophil activation test 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 (106, 110) and egg (111), basophil reactivity to the respective food allergens decreased during treatment. In the study of egg oral immunotherapy (OIT) by Burks, there was a correlation between basophil suppression and clinical desensitization, but not with long-lasting clinical tolerance (111). Basophil CD203c expression has shown to decrease during treatment with omalizumab and to return to pretreatment levels after cessation of therapy in patients with peanut allergy (112). Improvement in basophil sensitivity to milk in milk-allergic children treated with omalizumab predicted tolerance in a milk challenge test (113).

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 AIT 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

- Basophil activation test can improve the diagnosis of food allergy over SPT and sIgE and may be able to reduce the number of OFC.
- Basophil activation test 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. Basophil activation test allows the identification of about two-thirds of those patients (114–116). However, in patients with systemic mastocytosis (with elevated serum tryptase levels) the diagnosis of venom allergy should be performed 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 venom immunotherapy (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 (117) and repeatedly shows a positive result to only one venom in about one-quarter to one-third of patients with double sIgE positivity (43, 117, 118). In patients with double sIgE positivity, a positive BAT can help to identify the primary sensitizing allergen (117–120). 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 (116, 119), but this requires further research. Basophil activation test 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. (116, 119). 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, 121–123). 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 (123). Some studies suggest that side-effects during the build-up phase of VIT are predicted by a high basophil sensitivity (122, 124).

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 (125). 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

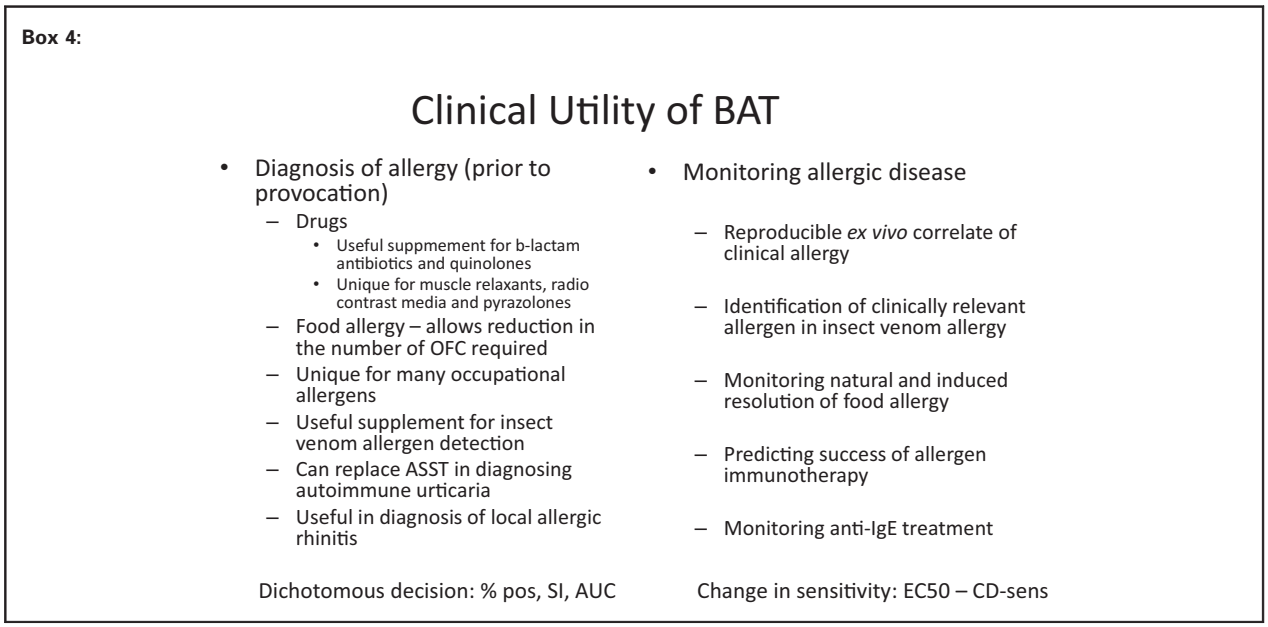
Since its discovery in 1991 (4) and the first clinical applications in 1994 (131), BAT has been developed as a diagnostic aid in allergy.

Cellular changes in basophil granulocytes and mast cells may be as important as, but are still more elusive than change in sIgE (25, 123, 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 (123, 134, 135). When reactivity is measured in clinical settings, the aim was usually to identify an allergen concentration at which change in sensitivity is optimally identified (114, 119, 124, 136). Basophil sensitivity is used to monitor change in allergic disease during natural development (23) and during treatment with AIT (25) or anti-IgE (137), 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 carried out 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, nonresponders would fail at this stage, as the threshold could not be set. The usefulness of such a threshold could be evaluated in a

Box 4:



retrospective trial, in which participating laboratories contribute data from consecutive tests that are analyzed as one would do in their laboratory or by this new method.

Major applications of BAT are summarized in Box 4. Basophil activation test has been established as a routine diagnostic test with standardized allergen preparations in a number of service laboratories. The routine application of BAT for established allergens is quite different to that of identifying and characterizing novel allergens or monitoring allergy intensity. To strengthen the use of BAT as diagnostic test, laboratory procedures and allergen concentrations in BAT should be standardized. This can be made possible with the use of industry standards like MiFlowCyt (51) or purchase of standardized material from CE-approved vendors. An important next step is the standardization and automation of analysis of BAT. Once that is achieved, it will be possible to do large multicenter trials to characterize the diagnostic performance of BAT and broaden its use as a clinical tool. These multicenter studies should also address the relationship of measures of BAT and sensitivity to sIgE, clinical symptoms, and symptom severity.

Author contributions

HJH and EK drafted the introduction and compiled the entire manuscript; 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.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1 Optimal allergen concentrations.
Table S2 Drug allergy.
Table S3 Food allergy.
Table S4 Hymenoptera venom allergy.
Table S5 Inhalant allergens.
MiFlowCyt Experimental Example 1
MiFlowCyt Experimental Example 2

References

1. Johansson SGO, Nopp A, van Hage M, Olofsson N, Lundahl J, Wehlin L et al. Passive IgE-sensitization by blood transfusion. *Allergy* 2005;**60**:1192–1199.
2. Savage JH, Courneya J-P, Sterba PM, Macglashan DW, Saini SS, Wood RA. Kinetics of mast cell, basophil, and oral food challenge responses in omalizumab-treated adults with peanut allergy. *J Allergy Clin Immunol* 2012;**130**:1123–1129.
3. Sihra BS, Kon OM, Grant JA, Kay AB. Expression of high-affinity IgE receptors

(Fc epsilon RI) on peripheral blood basophils, monocytes, and eosinophils in atopic and nonatopic subjects: relationship to total serum IgE concentrations. *J Allergy Clin Immunol* 1997;**99**:699–706.
4. Knol EF, Mul FP, Jansen H, Calafat J, Roos D. Monitoring human basophil activation via CD63 monoclonal antibody 435. *J Allergy Clin Immunol* 1991;**88**:328–338.
5. Sainte-Laudy J, Vallon C, Guérin JC. [Analysis of membrane expression of the CD63 human basophil activation marker. Applications to allergologic diagnosis]. *Allerg Immunol (Leipz)* 1994;**26**:211–214.
6. Knol EF. Requirements for effective IgE cross-linking on mast cells and basophils. *Mol Nutr Food Res* 2006;**50**:620–624.
7. Ishizaka T, De Bernardo R, Tomioka H, Lichtenstein LM, Ishizaka K. Identification of basophil granulocytes as a site of allergic histamine release. *J Immunol* 1972;**108**:1000–1008.
8. Pols MS, Klumperman J. Trafficking and function of the tetraspanin CD63. *Exp Cell Res* 2009;**315**:1584–1592.
9. MacGlashan D Jr. Expression of CD203c and CD63 in human basophils: relationship to differential regulation of piecemeal and anaphylactic degranulation processes. *Clin Exp Allergy* 2010;**40**:1365–1377.
10. Hoffmann HJ, Frandsen PM, Christensen LH, Schiøtz PO, Dahl R. Cultured human mast cells are heterogeneous for expression of the high-affinity IgE receptor FcεRI. *Int Arch Allergy Immunol* 2012;**157**:246–250.
11. Krohn IK, Lund G, Frandsen PM, Schiøtz PO, Dahl R, Hoffmann HJ. Mast cell FcεRI density and function dissociate from dependence on soluble IgE concentration at very low and very high IgE concentrations. *J Asthma* 2013;**50**:117–121.
12. Andersson M, Nolte H, Olsson M, Skov PS, Pipkorn U. Measurement of histamine in nasal lavage fluid: comparison of a glass fiber-based fluorometric method with two radioimmunoassays. *J Allergy Clin Immunol* 1990;**86**:815–820.
13. Siraganian RP. Refinements in the automated fluorometric histamine analysis system. *J Immunol Methods* 1975;**7**:283–290.
14. Siegel PD, Lewis DM, Olenchock SA. Neutrophil derived interference in the fluorometric determination of histamine. *Int Arch Allergy Appl Immunol* 1990;**93**:80–82.
15. Sturm GJ, Kranzelbinder B, Sturm EM, Heinemann A, Groselj-Strele A, Aberer W. The basophil activation test in the diagnosis of allergy: technical issues and critical factors. *Allergy* 2009;**64**:1319–1326.
16. Sturm EM, Kranzelbinder B, Heinemann A, Groselj-Strele A, Aberer W, Sturm GJ. CD203c-based basophil activation test in allergy diagnosis: characteristics and differences to CD63 upregulation. *Cytometry B Clin Cytom* 2010;**78**:308–318.
17. Iqbal K, Bhargava K, Skov PS, Falkencrone S, Grattan CE. A positive serum basophil histamine release assay is a marker for ciclosporin-responsiveness in patients with chronic spontaneous urticaria. *Clin Transl Allergy* 2012;**2**:19.
18. Kvedariene V, Kamey S, Ryckwaert Y, Rongier M, Bousquet J, Demoly P et al. Diagnosis of neuromuscular blocking agent hypersensitivity reactions using cytofluorimetric analysis of basophils. *Allergy* 2006;**61**:311–315.
19. Fernández TD, Torres MJ, Blanca-López N, Rodríguez-Bada JL, Gomez E, Canto G et al. Negativization rates of IgE radioimmunoassay and basophil activation test in immediate reactions to penicillins. *Allergy* 2009;**64**:242–248.
20. Gómez E, Blanca-Lopez N, Torres MJ, Requena G, Rondon C, Canto G et al. Immunoglobulin E-mediated immediate allergic reactions to dipyrone: value of basophil activation test in the identification of patients. *Clin Exp Allergy* 2009;**39**:1217–1224.
21. Sousa N, Martínez-Aranguren R, Fernández-Benitez M, Ribeiro F, Sanz ML. Comparison of basophil activation test results in blood preserved in acid citrate dextrose and EDTA. *J Investig Allergol Clin Immunol* 2010;**20**:535–536.
22. Ando N, Nakamura Y, Ishimaru K, Ogawa H, Okumura K, Shimada S et al. Allergen-specific basophil reactivity exhibits daily variations in seasonal allergic rhinitis. *Allergy* 2015;**70**:319–322.
23. Glaumann S, Nopp A, Johansson SGO, Borres MP, Nilsson C. Oral peanut challenge identifies an allergy but the peanut allergen threshold sensitivity is not reproducible. *PLoS One* 2013;**8**:e53465.
24. Nopp A, Cardell LO, Johansson SGO. CD-sens can be a reliable and easy-to-use complement in the diagnosis of allergic rhinitis. *Int Arch Allergy Immunol* 2013;**161**:87–90.
25. Schmid JM, Würtzen PA, Dahl R, Hoffmann HJ. Early improvement in basophil sensitivity predicts symptom relief with grass pollen immunotherapy. *J Allergy Clin Immunol* 2014;**134**:741–744.
26. Gentinetta T, Pecaric-Petkovic T, Wan D, Falcone FH, Dahinden CA, Pichler WJ et al. Individual IL-3 priming is crucial for consistent in vitro activation of donor basophils in patients with chronic urticaria. *J Allergy Clin Immunol* 2011;**128**:1227–1234.
27. Bühring H-J, Streble A, Valent P. The basophil-specific ectoenzyme E-NPP3 (CD203c) as a marker for cell activation and allergy diagnosis. *Int Arch Allergy Immunol* 2004;**133**:317–329.
28. Hirai K, Morita Y, Misaki Y, Ohta K, Takaishi T, Suzuki S et al. Modulation of human basophil histamine release by hemopoietic growth factors. *J Immunol* 1988;**141**:3958–3964.
29. Vilariño N, Miura K, MacGlashan DW. Acute IL-3 priming up-regulates the stimulus-induced Raf-1-Mek-Erk cascade independently of IL-3-induced activation of Erk. *J Immunol* 2005;**175**:3006–3014.
30. Kurimoto Y, De Weck AL, Dahinden CA. The effect of interleukin 3 upon IgE-dependent and IgE-independent basophil degranulation and leukotriene generation. *Eur J Immunol* 1991;**21**:361–368.
31. Miadonna A, Salmaso C, Cottini M, Milazzo N, Tedeschi A. Enhancement of basophil histamine release by interleukin-3: reduced effect in atopic subjects. *Allergy* 1996;**51**:525–531.
32. Sainte-Laudy J, Sabbah A, Drouet M, Lauret MG, Loiry M. Diagnosis of venom allergy by flow cytometry. Correlation with clinical history, skin tests, specific IgE, histamine and leukotriene C4 release. *Clin Exp Allergy* 2000;**30**:1166–1171.
33. Mayorga C, Andreu I, Aranda A, Doña I, Montañez MI, Blanca-Lopez N et al. Fluoroquinolone photodegradation influences specific basophil activation. *Int Arch Allergy Immunol* 2013;**160**:377–382.
34. Brockow K, Garvey LH, Aberer W, Atanaskovic-Markovic M, Barbaud A, Bilo MB et al. Skin test concentrations for systemically administered drugs – an ENDA/EAACI Drug Allergy Interest Group position paper. *Allergy* 2013;**68**:702–712.
35. Canonica GW, Ansotegui IJ, Pawankar R, Schmid-Grendelmeier P, van Hage M, Baena-Cagnani CE et al. A WAO – ARIA – GA^{2}LEN consensus document on molecular-based allergy diagnostics. *World Allergy Organ J* 2013;**6**:17.
36. Mayorga C, Gomez F, Aranda A, Koppelman SJ, Diaz-Perales A, Blanca-López N et al. Basophil response to peanut allergens in Mediterranean peanut-allergic patients. *Allergy* 2014;**69**:964–968.
37. Moneret-Vautrin DA, Mayorga L. Basophil responses to peanut allergens. *Allergy* 2014;**69**:1701–1702.
38. Blank S, Seismann H, Bockisch B, Braren I, Cifuentes L, McIntyre M et al. Identification, recombinant expression, and characterization of the 100 kDa high molecular weight hymenoptera venom allergens Api m 5 and Ves v 3. *J Immunol* 2010;**184**:5403–5413.

39. Balzer L, Pennino D, Blank S, Seismann H, Darsow U, Schnedler M et al. Basophil activation test using recombinant allergens: highly specific diagnostic method complementing routine tests in wasp venom allergy. *PLoS One* 2014;**9**:e108619.
40. Michel S, Scherer K, Heijnen IAFM, Bircher AJ. Skin prick test and basophil reactivity to cetuximab in patients with IgE to alpha-gal and allergy to red meat. *Allergy* 2014;**69**:403–405.
41. Hennersdorf F, Florian S, Jakob A, Baumgärtner K, Sonneck K, Nordheim A et al. Identification of CD13, CD107a, and CD164 as novel basophil-activation markers and dissection of two response patterns in time kinetics of IgE-dependent upregulation. *Cell Res* 2005;**15**:325–335.
42. De Weck AL, Sanz ML, Gamboa PM, Aberer W, Bienvenu J, Blanca M et al. Diagnostic tests based on human basophils: more potentials and perspectives than pitfalls. *Int Arch Allergy Immunol* 2008;**146**:177–189.
43. Sturm GJ, Böhm E, Trummer M, Weiglhofer I, Heinemann A, Aberer W. The CD63 basophil activation test in Hymenoptera venom allergy: a prospective study. *Allergy* 2004;**59**:1110–1117.
44. Erdmann SM, Sachs B, Kwiecien R, Moll-Slodowy S, Sauer I, Merk HF. The basophil activation test in wasp venom allergy: sensitivity, specificity and monitoring specific immunotherapy. *Allergy* 2004;**59**:1102–1109.
45. Eberlein-König B, Varga R, Mempel M, Darsow U, Behrendt H, Ring J. Comparison of basophil activation tests using CD63 or CD203c expression in patients with insect venom allergy. *Allergy* 2006;**61**:1084–1085.
46. Ocmant A, Peignois Y, Mulier S, Hanssens L, Michils A, Schandené L. Flow cytometry for basophil activation markers: the measurement of CD203c up-regulation is as reliable as CD63 expression in the diagnosis of cat allergy. *J Immunol Methods* 2007;**320**:40–48.
47. Abuaf N, Rostane H, Rajoely B, Gaouar H, Autegarden JE, Leynadier F et al. Comparison of two basophil activation markers CD63 and CD203c in the diagnosis of amoxicillin allergy. *Clin Exp Allergy* 2008;**38**:921–928.
48. Hausmann OV, Gentinetta T, Fux M, Ducrest S, Pichler WJ, Dahinden CA. Robust expression of CCR3 as a single basophil selection marker in flow cytometry. *Allergy* 2011;**66**:85–91.
49. Hauswirth AW, Natter S, Ghannadan M, Majlesi Y, Schernthaner G-H, Sperr WR et al. Recombinant allergens promote expression of CD203c on basophils in sensitized individuals. *J Allergy Clin Immunol* 2002;**110**:102–109.
50. Eberlein B, León Suárez I, Darsow U, Ruëff F, Behrendt H, Ring J. A new basophil activation test using CD63 and CCR3 in allergy to antibiotics. *Clin Exp Allergy* 2010;**40**:411–418.
51. Lee JA, Spidlen J, Boyce K, Cai J, Crosbie N, Dalphin M et al. MIFlowCyt: the minimum information about a Flow Cytometry Experiment. *Cytometry A* 2008;**73**:926–930.
52. MacGlashan DW. Releasability of human basophils: cellular sensitivity and maximal histamine release are independent variables. *J Allergy Clin Immunol* 1993;**91**:605–615.
53. Christensen LH, Holm J, Lund G, Riise E, Lund K. Several distinct properties of the IgE repertoire determine effector cell degranulation in response to allergen challenge. *J Allergy Clin Immunol* 2008;**122**:298–304.
54. Nopp A, Cardell LO, Johansson SGO, Oman H. CD-sens: a biological measure of immunological changes stimulated by ASIT. *Allergy* 2009;**64**:811–814.
55. Dahlén B, Nopp A, Johansson SGO, Eduards M, Skedinger M, Adédoyin J. Basophil allergen threshold sensitivity, CD-sens, is a measure of allergen sensitivity in asthma. *Clin Exp Allergy* 2011;**41**:1091–1097.
56. Konradsen JR, Nordlund B, Nilsson OB, van Hage M, Nopp A, Hedlin G et al. High basophil allergen sensitivity (CD-sens) is associated with severe allergic asthma in children. *Pediatr Allergy Immunol* 2012;**23**:376–384.
57. Glaumann S, Nopp A, Johansson SGO, Rudengren M, Borres MP, Nilsson C. Basophil allergen threshold sensitivity, CD-sens, IgE-sensitization and DBPCFC in peanut-sensitized children. *Allergy* 2012;**67**:242–247.
58. Patil SU, Shreffler WG. Immunology in the Clinic Review Series; focus on allergies: basophils as biomarkers for assessing immune modulation. *Clin Exp Immunol* 2012;**167**:59–66.
59. Knol EF, Koenderman L, Mul FP, Verhoeven AJ, Roos D. Differential activation of human basophils by anti-IgE and formyl-methionyl-leucyl-phenylalanine. Indications for protein kinase C-dependent and -independent activation pathways. *Eur J Immunol* 1991;**21**:881–885.
60. Ocmant A, Mulier S, Hanssens L, Goldman M, Casimir G, Mascart F et al. Basophil activation tests for the diagnosis of food allergy in children. *Clin Exp Allergy* 2009;**39**:1234–1245.
61. Rubio A, Vivinus-Nébot M, Bourrier T, Saggio B, Albertini M, Bernard A. Benefit of the basophil activation test in deciding when to reintroduce cow's milk in allergic children. *Allergy* 2011;**66**:92–100.
62. Knol EF, Mul FP, Kuijpers TW, Verhoeven AJ, Roos D. Intracellular events in anti-IgE nonreleasing human basophils. *J Allergy Clin Immunol* 1992;**90**:92–103.
63. Kepley CL, Youssef L, Andrews RP, Wilson BS, Oliver JM. Syk deficiency in nonreleaser basophils. *J Allergy Clin Immunol* 1999;**104**:279–284.
64. Brown SGA. Clinical features and severity grading of anaphylaxis. *J Allergy Clin Immunol* 2004;**114**:371–376.
65. Muraro A, Roberts G, Clark A, Eigenmann PA, Halken S, Lack G et al. The management of anaphylaxis in childhood: position paper of the European academy of allergology and clinical immunology. *Allergy* 2007;**62**:857–871.
66. Valyasevi MA, Maddox DE, Li JTC. Systemic reactions to allergy skin tests. *Ann Allergy Asthma Immunol* 1999;**83**:132–136.
67. Pitsios C, Dimitriou A, Stefanaki EC, Kontou-Fili K. Anaphylaxis during skin testing with food allergens in children. *Eur J Pediatr* 2009;**169**:613–615.
68. Nassiri M, Babina M, Dölle S, Edenharter G, Ruëff F, Worm M. Ramipril and metoprolol intake aggravate human and murine anaphylaxis: evidence for direct mast cell priming. *J Allergy Clin Immunol* 2015;**135**:491–499.
69. Bidad K, Nawijn MC, van Oosterhout AJM, van der Heide S, Elberink JNGO. Basophil activation test in the diagnosis and monitoring of mastocytosis patients with wasp venom allergy on immunotherapy. *Cytometry B Clin Cytom* 2014;**86**:183–190.
70. Konstantinou GN, Asero R, Maurer M, Sabroe RA, Schmid-Grendelmeier P, Grattan CEH. EAACI/GA(2)LEN task force consensus report: the autologous serum skin test in urticaria. *Allergy* 2009;**64**:1256–1268.
71. Perry TT, Matsui EC, Conover-Walker MK, Wood RA. Risk of oral food challenges. *J Allergy Clin Immunol* 2004;**114**:1164–1168.
72. Muraro A, Hoffmann-Sommergruber K, Holzhauser T, Poulsen LK, Gowland MH, Akdis CA et al. EAACI Food Allergy and Anaphylaxis Guidelines. Protecting consumers with food allergies: understanding food consumption, meeting regulations and identifying unmet needs. *Allergy* 2014;**69**:1464–1472.
73. Santos AF, Douiri A, Bécares N, Wu S-Y, Stephens A, Radulovic S et al. Basophil activation test discriminates between allergy and tolerance in peanut-sensitized children. *J Allergy Clin Immunol* 2014;**134**:645–52.

74. Kikuchi Y, Kaplan AP. Mechanisms of autoimmune activation of basophils in chronic urticaria. *J Allergy Clin Immunol* 2001;**107**:1056–1062.
75. Hide M, Francis DM, Grattan CE, Hakimi J, Kochan JP, Greaves MW. Autoantibodies against the high-affinity IgE receptor as a cause of histamine release in chronic urticaria. *N Engl J Med* 1993;**328**:1599–1604.
76. Maurer M, Altrichter S, Bieber T, Biedermann T, Bräutigam M, Seyfried S et al. Efficacy and safety of omalizumab in patients with chronic urticaria who exhibit IgE against thyroperoxidase. *J Allergy Clin Immunol* 2011;**128**:202–209.
77. Konstantinou GN, Asero R, Ferrer M, Knol EF, Maurer M, Raap U et al. EAACI taskforce position paper: evidence for autoimmune urticaria and proposal for defining diagnostic criteria. *Allergy* 2013;**68**:27–36.
78. Ferrer M, Kinét JP, Kaplan AP. Comparative studies of functional and binding assays for IgG anti-Fc(epsilon)RIalpha (alpha-subunit) in chronic urticaria. *J Allergy Clin Immunol* 1998;**101**:672–676.
79. Wedi B, Novacovic V, Koerner M, Kapp A. Chronic urticaria serum induces histamine release, leukotriene production, and basophil CD63 surface expression–inhibitory effects ofanti-inflammatory drugs. *J Allergy Clin Immunol* 2000;**105**: 552–560.
80. Yasnowsky KM, Dreskin SC, Efaw B, Schoen D, Vedanthan PK, Alam R et al. Chronic urticaria sera increase basophil CD203c expression. *J Allergy Clin Immunol* 2006;**117**:1430–1434.
81. Szegedi A, Irinyi B, Gál M, Hunyadi J, Dankó K, Kiss E et al. Significant correlation between the CD63 assay and the histamine release assay in chronic urticaria. *Br J Dermatol* 2006;**155**:67–75.
82. Luquin E, Kaplan AP, Ferrer M. Increased responsiveness of basophils of patients with chronic urticaria to sera but hypo-responsiveness to other stimuli. *Clin Exp Allergy* 2005;**35**:456–460.
83. Grattan CEH, Dawn G, Gibbs S, Francis DM. Blood basophil numbers in chronic ordinary urticaria and healthy controls: diurnal variation, influence of loratadine and prednisolone and relationship to disease activity. *Clin Exp Allergy* 2003;**33**:337–341.
84. Mayorga C, Sanz ML, Gamboa PM, García BE, Caballero MT, García JM et al. In vitro diagnosis of immediate allergic reactions to drugs: an update. *J Investig Allergol Clin Immunol* 2010;**20**:103–109.
85. Genovese A, Stellato C, Marsella CV, Adt M, Marone G. Role of mast cells, basophils and their mediators in adverse reactions to general anesthetics and radiocontrast media. *Int Arch Allergy Immunol* 1996;**110**:13–22.
86. Böhm I, Speck U, Schild HH. Pilot study on basophil activation induced by contrast medium. *Fundam Clin Pharmacol* 2011;**25**:267–276.
87. Aranda A, Mayorga C, Ariza A, Doña I, Rosado A, Blanca-Lopez N et al. In vitro evaluation of IgE-mediated hypersensitivity reactions to quinolones. *Allergy* 2011;**66**:247–254.
88. Sanz ML, Gamboa PM, Antépara I, Uasuf C, Vila L, Garcia-Avilés C et al. Flow cytometric basophil activation test by detection of CD63 expression in patients with immediate-type reactions to betalactam antibiotics. *Clin Exp Allergy* 2002;**32**:277–286.
89. Torres MJ, Padial A, Mayorga C, Fernández T, Sanchez-Sabate E, Cornejo-García JA et al. The diagnostic interpretation of basophil activation test in immediate allergic reactions to betalactams. *Clin Exp Allergy* 2004;**34**:1768–1775.
90. Abuaf N, Rajoely B, Ghazouani E, Levy DA, Pecquet C, Chabane H et al. Validation of a flow cytometric assay detecting in vitro basophil activation for the diagnosis of muscle relaxant allergy. *J Allergy Clin Immunol* 1999;**104**:411–418.
91. Monneret G, Benoit Y, Debard AL, Gutowski MC, Topenot I, Bienvenu J. Monitoring of basophil activation using CD63 and CCR3 in allergy to muscle relaxant drugs. *Clin Immunol* 2002;**102**:192–199.
92. Ebo DG, Bridts CH, Hagendorens MM, Mertens CH, De Clerck LS, Stevens WJ. Flow-assisted diagnostic management of anaphylaxis from rocuronium bromide. *Allergy* 2006;**61**:935–939.
93. Rouzaire P, Nosbaum A, Denis L, Bienvenu F, Bérard F, Cozon G et al. Negativity of the basophil activation test in quinolone hypersensitivity: a breakthrough for provocation test decision-making. *Int Arch Allergy Immunol* 2012;**157**:299–302.
94. Pinnobphun P, Buranapraditkun S, Kampitak T, Hirankarn N, Klaewsongkram J. The diagnostic value of basophil activation test in patients with an immediate hypersensitivity reaction to radiocontrast media. *Ann Allergy Asthma Immunol* 2011;**106**:387–393.
95. Salas M, Gomez F, Fernandez TD, Doña I, Aranda A, Ariza A et al. Diagnosis of immediate hypersensitivity reactions to radiocontrast media. *Allergy* 2013;**68**:1203–1206.
96. Gamboa PM, Sanz ML, Caballero MR, Antépara I, Urrutia I, Jáuregui I et al. Use of CD63 expression as a marker of in vitro basophil activation and leukotriene determination in metamizol allergic patients. *Allergy* 2003;**58**:312–317.
97. Ebo DG, Leysen J, Mayorga C, Rozieres A, Knol EF, Terreehorst I. The in vitro diagnosis of drug allergy: status and perspectives. *Allergy* 2011;**66**:1275–1286.
98. Ebo DG, Venemalm L, Bridts CH, Degerbeck F, Hagberg H, De Clerck LS et al. Immunoglobulin E antibodies to rocuronium: a new diagnostic tool. *Anesthesiology* 2007;**107**:253–259.
99. Iwamoto T, Yuta A, Tabata T, Sugimoto H, Gabazza EC, Hirai H et al. Evaluation of basophil CD203c as a predictor of carboplatin-related hypersensitivity reaction in patients with gynecologic cancer. *Biol Pharm Bull* 2012;**35**:1487–1495.
100. Ebo DG, Bridts CH, Stevens WJ. IgE-mediated anaphylaxis from chlorhexidine: diagnostic possibilities. *Contact Dermatitis* 2006;**55**:301–302.
101. Cabrera-Freitag P, Gastaminza G, Goikoetxea MJ, Lafuente A, de la Borbolla JM, Sanz ML. Immediate allergic reaction to atropine in ophthalmic solution confirmed by basophil activation test. *Allergy* 2009;**64**:1388–1389.
102. Soriano Gomis V, Pérez Sempere A, González Delgado P, Sempere JM, Niveiro Hernández E, Marco FM. Glatiramer acetate anaphylaxis: detection of antibodies and basophil activation test. *J Investig Allergol Clin Immunol* 2012;**22**:65–66.
103. Leysen J, De Witte L, Sabato V, Faber M, Hagendorens M, Bridts C et al. IgE-mediated allergy to pholcodine and cross-reactivity to neuromuscular blocking agents: lessons from flow cytometry. *Cytometry B Clin Cytom* 2013;**84**:65–70.
104. Hausmann OV, Gentinetta T, Bridts CH, Ebo DG. The basophil activation test in immediate-type drug allergy. *Immunol Allergy Clin North Am* 2009;**29**:555–566.
105. Wanich N, Nowak-Wegrzyn A, Sampson HA, Shreffler WG. Allergen-specific basophil suppression associated with clinical tolerance in patients with milk allergy. *J Allergy Clin Immunol* 2009;**123**:789–794.
106. Thyagarajan A, Jones SM, Calatroni A, Pons L, Kulis M, Woo CS et al. Evidence of pathway-specific basophil anergy induced by peanut oral immunotherapy in peanut-allergic children. *Clin Exp Allergy* 2012;**42**:1197–1205.
107. Santos AF, Du Toit G, Douiri A, Radulovic S, Stephens A, Turcanu V et al. Distinct parameters of the basophil activation test reflect the severity and threshold of allergic reactions to peanut. *J Allergy Clin Immunol* 2015;**135**:179–186.
108. Sato S, Tachimoto H, Shukuya A, Kurosaka N, Yanagida N, Utsunomiya T et al. Basophil activation marker CD203c is useful in the diagnosis of hen's egg and cow's

milk allergies in children. *Int Arch Allergy Immunol* 2010;**152**(Suppl 1):54–61.
109. Ford LS, Bloom KA, Nowak-Węgrzyn AH, Shreffler WG, Masilamani M, Sampson HA. Basophil reactivity, wheal size, and immunoglobulin levels distinguish degrees of cow's milk tolerance. *J Allergy Clin Immunol* 2013;**131**:180–186.
110. Jones SM, Pons L, Roberts JL, Scurlock AM, Perry TT, Kulis M et al. Clinical efficacy and immune regulation with peanut oral immunotherapy. *J Allergy Clin Immunol* 2009;**124**:292–300.
111. Burks AW, Jones SM, Wood RA, Fleischer DM, Sicherer SH, Lindblad RW et al. Oral immunotherapy for treatment of egg allergy in children. *N Engl J Med* 2012;**367**:233–243.
112. Gernez Y, Tirouvanziam R, Yu G, Ghosn EEB, Reshamwala N, Nguyen T et al. Basophil CD203c levels are increased at baseline and can be used to monitor omalizumab treatment in subjects with nut allergy. *Int Arch Allergy Immunol* 2011;**154**:318–327.
113. Nilsson C, Nordvall L, Johansson SGO, Nopp A. Successful management of severe cow's milk allergy with omalizumab treatment and CD-sens monitoring. *Asia Pac Allergy* 2014;**4**:257.
114. Ebo DG, Hagendorens MM, Bridts CH, De Clerck LS, Stevens WJ. Hymenoptera venom allergy: taking the sting out of difficult cases. *J Investig Allergol Clin Immunol* 2007;**17**:357–360.
115. Korosec P, Erzen R, Silar M, Bajrovic N, Kopac P, Kosnik M. Basophil responsiveness in patients with insect sting allergies and negative venom-specific immunoglobulin E and skin prick test results. *Clin Exp Allergy* 2009;**39**:1730–1737.
116. Korošec P, Šilar M, Eržen R, Čelesnik N, Bajrović N, Zidarn M et al. Clinical routine utility of basophil activation testing for diagnosis of hymenoptera-allergic patients with emphasis on individuals with negative venom-specific IgE antibodies. *Int Arch Allergy Immunol* 2013;**161**:363–368.
117. Sturm GJ, Jin C, Kranzelbinder B, Hemmer W, Sturm EM, Griesbacher A et al. Inconsistent results of diagnostic tools hamper the differentiation between bee and vespid venom allergy. *PLoS One* 2011;**6**:e20842.
118. Eberlein-König B, Rakoski J, Behrendt H, Ring J. Use of CD63 expression as marker of in vitro basophil activation in identifying the culprit in insect venom allergy. *J Investig Allergol Clin Immunol* 2004;**14**:10–16.
119. Eberlein B, Krischan L, Darsow U, Ollert M, Ring J. Double positivity to bee and wasp venom: improved diagnostic procedure by recombinant allergen-based IgE testing and basophil activation test including data about cross-reactive carbohydrate determinants. *J Allergy Clin Immunol* 2012;**130**:155–161.
120. Bokanovic D, Laiplod K, Pickl-Herg B, Griesbacher A, Aberer W, Vollmann J et al. Negative predictive value of the basophil activation test in hymenoptera venom allergy. In: *Insect Venom Hypersensitivity* 2013;**68**:491.
121. Mikkelsen S, Bibby BM, Dolberg MKB, Dahl R, Hoffmann HJ. Basophil sensitivity through CD63 or CD203c is a functional measure for specific immunotherapy. *Clin Mol Allergy* 2010;**8**:2.
122. Žitnik SEK, Vesel T, Avčin T, Šilar M, Košnik M, Korošec P. Monitoring honeybee venom immunotherapy in children with the basophil activation test. *Pediatr Allergy Immunol* 2012;**23**:166–172.
123. Eržen R, Košnik M, Silar M, Korošec P. Basophil response and the induction of a tolerance in venom immunotherapy: a long-term sting challenge study. *Allergy* 2012;**67**:822–830.
124. Kosnik M, Silar M, Bajrovic N, Music E, Korosec P. High sensitivity of basophils predicts side-effects in venom immunotherapy. *Allergy* 2005;**60**:1401–1406.
125. Gómez E, Campo P, Rondón C, Barrionuevo E, Blanca-López N, Torres MJ et al. Role of the basophil activation test in the diagnosis of local allergic rhinitis. *J Allergy Clin Immunol* 2013;**132**:975–976.
126. Nopp A, Johansson SGO, Ankerst J, Bylin G, Cardell LO, Grönneberg R et al. Basophil allergen threshold sensitivity: a useful approach to anti-IgE treatment efficacy evaluation. *Allergy* 2006;**61**:298–302.
127. Zidarn M, Košnik M, Silar M, Grahek A, Korošec P. Rhinitis symptoms caused by grass pollen are associated with elevated basophile allergen sensitivity and a larger grass-specific immunoglobulin E fraction. *Clin Exp Allergy* 2012;**42**:49–57.
128. Ceuppens JL, Bullens D, Kleinjans H, van der Werf J, PURETHAL Birch Efficacy Study Group. Immunotherapy with a modified birch pollen extract in allergic rhinoconjunctivitis: clinical and immunological effects. *Clin Exp Allergy* 2009;**39**:1903–1909.
129. Lalek N, Kosnik M, Silar M, Korosec P. Immunoglobulin G-dependent changes in basophil allergen threshold sensitivity during birch pollen immunotherapy. *Clin Exp Allergy* 2010;**40**:1186–1193.
130. Johansson SGO, Nopp A, Oman H, Ankerst J, Cardell LO, Grönneberg R et al. The size of the disease relevant IgE antibody fraction in relation to 'total-IgE' predicts the efficacy of anti-IgE (Xolair) treatment. *Allergy* 2009;**64**:1472–1477.
131. Sainte-Laudy J, Touraine F, Boumediene A, Bonnaud F, Cogné M. Clinico-biological characteristics of flow cytometry applied to hypersensitivity to NSAIDs. *Inflamm Res* 2007;**56**(Suppl 1):S63–S64.
132. Witting Christensen SK, Krohn IK, Thuraiaiyah J, Skjold T, Schmid JM, Hoffmann HJH. Sequential allergen desensitization of basophils is non-specific and may involve p38 MAPK. *Allergy* 2014;**69**:1343–1349.
133. Eggel A, Baravalle G, Hobi G, Kim B, Buschor P, Forrer P et al. Accelerated dissociation of IgE-FcεRI complexes by disruptive inhibitors actively desensitizes allergic effector cells. *J Allergy Clin Immunol* 2014;**133**:1709–1719.
134. Castells M. Rapid desensitization for hypersensitivity reactions to medications. *Immunol Allergy Clin North Am* 2009;**29**:585–606.
135. del Sancho-Serra M del C, Simarro M, Castells M. Rapid IgE desensitization is antigen specific and impairs early and late mast cell responses targeting FcεRI internalization. *Eur J Immunol* 2011;**41**:1004–1013.
136. Homšak M, Silar M, Berce V, Tomazin M, Skerbinjek-Kavalar M, Celesnik N et al. The relevance of basophil allergen sensitivity testing to distinguish between severe and mild peanut-allergic children. *Int Arch Allergy Immunol* 2013;**162**:310–317.
137. Nopp A, Johansson SGO, Ankerst J, Palmqvist M, Oman H. CD-sens and clinical changes during withdrawal of Xolair after 6 years of treatment. *Allergy* 2007;**62**:1175–1181.