DOI:
10.1007/s40629-016-0116-2

Document Version
Peer reviewed version

Basophil Activation Testing in Diagnosis and Monitoring of Allergic Disease – an overview

Eberlein B[1*], Santos AF[2,3,4], Mayorga C[5], Nopp A[6], Ferrer M[7], Rouzaire P[8], Ebo D[9], Sabato V[9], Sanz ML[7], Pecaric-Petkovic T[10], Patil SU[11], Hausmann OV[12,13], Shreffler WG[11], Korosec P[14], Knol EF[15], Hoffmann HJ[16]

Affiliations:

1. Department of Dermatology and Allergy Biederstein, Technische Universität München, Munich, Germany

2. Department of Paediatric Allergy, Division of Asthma, Allergy and Lung Biology, King's College London, London, UK

3. MRC & Asthma UK Centre in Allergic Mechanisms of Asthma, London, United Kingdom

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 Allergy and Clinical Immunology, Clínica Universidad de Navarra, Pamplona, Spain

8. Department of Immunology, University Hospital, Clermont-Ferrand and ERTICa Research Group, University of Auvergne EA4677, Clermont-Ferrand, France

9. Department of Immunology-Allergology-Rheumatology, University of Antwerp and Antwerp University Hospital, Antwerp, Belgium

10. Adverse Drug Reactions-Analysis and Consulting (ADR-AC) GmbH, Bern, Switzerland

11. Center for Immunology and Inflammatory Diseases, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts, United States

12. Department of Rheumatology, Immunology and Allergology, Inselspital, University of Bern, Bern, Switzerland

13. Loewenpraxis, Luzern, Switzerland

14. Laboratory for Clinical Immunology & Molecular Genetics, University Clinic of Respiratory and Allergic Diseases Golnik, Golnik, Slovenia

15. Department of Immunology and Department of Dermatology/Allergology. University Medical Center Utrecht, Utrecht, The Netherlands

16. Department of Respiratory Diseases and Allergy, Department of Clinical Medicine, Aarhus University, Aarhus, Denmark

* Address correspondence to Bernadette Eberlein, M.D., Professor, Department of Dermatology and Allergy Biederstein, Technische Universität München, Biedersteiner Str. 29, D 80802 München, telephone + 49-8941403324, e-mail bernadette.eberlein@tum.de

Keywords
1. Basophil granulocyte
2. CD63
3. BAT, Basophil activation test
4. Allergy Diagnosis
5. Allergy monitoring
6. Allergen provocation /challenge testing

Abstract

The nature of basophil activation as an ex vivo challenge makes it a multifaceted and promising tool for the allergist. Through development of flow cytometry, discovery of activation markers such as CD63 and markers identifying basophil granulocytes the basophil activation test (BAT) has become a pervasive test. BAT measures basophil response to allergen crosslinking IgE on between 150 and 2000 basophil granulocytes with remarkable analytical sensitivity in less than 0.1 ml fresh blood. Dichotomous activation is assessed as the fraction of reacting basophils. In patients with food-, insect venom-, and drug allergy as well as with chronic urticaria BAT can be part of the diagnostic evaluation in addition to history, skin prick testing and specific IgE determination. BAT may be also 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. The test may use fewer resources and be more reproducible than oral, sting, nasal or bronchial challenge testing. BAT may be useful before challenge testing as it is less stressful for the patient and avoids severe allergic reactions. An important prospective step is to standardize BAT and make it available in diagnostic laboratories.

Introduction

The clinical impact of BAT is due to the unique ability of basophils to degranulate upon cross-linking of the specific IgE (sIgE) bound on membrane-bound high affinity IgE-receptor (FcεRI) by allergen exposure. After discovery of the quantal upregulation of CD63 during basophil activation in 1991(1), the BAT was developed in the 90's (2). 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. BAT (like SPT) reflects a functional response as basophil (or skin mast cell) activation can be induced by cross-linking of FcεRI.

In this overview, adapted from the EAACI Task force position paper (3) we provide an overview of the practical and technical details as well as the utility of BAT in diagnosis and management of allergic diseases.

Blood samples for BAT

Antihistamines do not interfere with BAT, but systemic steroids and cyclosporin A should be avoided (4,5). It is recommended to take blood samples within one year of the most recent exposure to the allergen source (6,7). Blood samples can be used within 24 hours (8), even though basophils may lose reactivity. As there is diurnal variation in the reactivity to CD203c (9), timing of blood sampling may be important. Tests done with whole blood are most commonly utilized, but separation of cells from protective elements found in plasma may optimize activation through cell-bound sIgE.

Interleukin-3 (IL-3) enhanced kinetics, reactance and sensitivity of blood basophils to FceRI mediated activation independently of extracellular Calcium. It enhances also the allergen specific up-regulation of CD63 (4,10), but unspecifically upregulates CD203c (11).

Selection of the source of allergen extracts (Box 1)

Optimized concentrations for a wide range of allergens, allergen sources and allergen extracts are listed in the original position paper in table S1 (3). Furthermore, optimized allergen preparations are 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 since solubilized tablets are complex mixtures of drugs and excipients.

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. If recombinant allergen preparation or purified allergens are used for BAT, the molar concentration of allergens enables very precise analyses.

This standardized allergen preparation is essential when comparing basophil sensitivity data.

Flow Cytometry in BAT

At the moment BAT with CD63 is the best clinically validated test (,12,13,14), but the BAT based on CD203c has been shown to be a reliable test (15,16).

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 (17). IgE and CD123/HLA-DR showed somewhat more inter-individual variability in cell surface expression. CD203c can be used for both identification and as an activation marker. Quality of blood basophils obtained is usually confirmed by stimulation with the bacterial peptide fMLP. Anti-IgE or anti-FcεRI antibodies are used as IgE-mediated positive controls, buffer as negative control.

If standardized commercial tests are not used, the method used for testing has to undergo validation.

Presentation and interpretation of BAT

There are two common measures of basophil activity; basophil reactivity (2), the number of

basophils that respond to a given stimulus, and basophil sensitivity (1, 18), the allergen concentration at which half of all reactive basophils respond (Fig. 1A). Basophil reactivity depends on the priming state of the basophil and the cellular translation of the IgE signal within the cell (19). 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.

Basophil sensitivity is a function of reactivity and the compound affinity of cell-bound sIgE for allergen and free competing immunoglobulin. It requires measurement of reactivity at 6-8 allergen concentrations. 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 (Fig. 1 B). EC50 can be expressed as 'CD-sens' by inversion and multiplication by 100 (20).

More recently the area-under-the-dose curve (AUC) measurement attempts to combine reactivity and sensitivity into one (Fig. 1C); 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 (21). ROC curves are used in identification of novel allergens when ≥7 sensitized patients are available.

Basophil granulocytes of non-responders (6%-17% of population) can remain unresponsive to stimulation through FcεRI. It is attributed to differences in the intracellular signaling pathway. Results from non-responder patients should be regarded as false negatives.

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. The allergic response should be confirmed by measurement of sIgE, skin prick testing and, for insect venom and drug allergy, intradermal testing. Measurement of sIgE may not be possible if the allergen source is not available as a routine reagent, and may be of limited value depending on the performance of the available reagents. BAT is a functional test resembling

an *ex vivo* IgE-mediated cellular response . It can be measured at the same time as sIgE, and in general precedes *in vivo* provocation tests.

Chronic Urticaria

The mechanism of chronic spontaneous urticaria (CU) is still incompletely understood. About half of the patients have autoantibodies against FcεRI and a few against IgE. CU sera activate resting basophils of normal donors to upregulate CD63 and CD203c. BAT may replace the autologous serum skin test (ASST) (22).

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 (10, 23, 24). The central problem is the heterogeneity of the results using different basophil donors. This can be normalized by titrated addition of IL-3 (10). BAT with autologous basophils should not be performed because CU patients are often non responders or poor responders to IgE crosslinking and have diagnostic basopenia.

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 (25). Here BAT is an additional tool that is safer, gentler and cheaper than a challenge and, in some instances, is the only available diagnostic tool. The sensitivity of BAT in diagnosis of drug allergy is about 50%, and the specificity up to 93%.

There are several studies including BAT in drug allergy diagnosis for beta-lactams, NMBA, quinolones, radio contrast media and pyrazolones with good sensitivity and specificity.

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. BAT has also a good negative predictive value, useful in the decision to perform the provocation test as demonstrated with quinolones. Furthermore, it has a complementary role to skin tests for different drug hypersensitivities and can be particularly useful in the study of cross-reactivity between NMBA, for the identification of safe alternatives for future surgery (25, 26).

.

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.
- It may be difficult to confirm the clinical history of hypersensitivity by BAT after 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 food allergies has been assessed in various studies. The reported sensitivity of BAT ranges from 77-98%, and the specificity from 75-100%. BAT in these studies was more accurate than sSPT and sIgE (27, 28). For single individuals BAT sensitivity seems to allow a risk estimation for severe clinical reactions: In peanut allergy, BAT significantly improved clinical diagnosis over the use of SPT and sIgE and reduced the number of OFC required. BAT showed 100% specificity, suggesting that in patients with a positive BAT the OFC could be deferred (29).

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. Although OFC and basophil sensitivity both identified all clinically sensitized children, only basophil sensitivity was reproducible at two consecutive visits (r^2=0.94). In a recent publication, BAT reactivity reflected the allergy severity and BAT sensitivity reflected the threshold of response to the allergen source in an OFC (30).

It has been shown that basophil reactivity distinguish patients that tolerate extensively heated forms of cow's milk and egg from patients who do not. BAT may be useful in assessing the natural resolution of food allergies that are commonly outgrown over time, such as cow's milk allergy (28), and in determining when the food can safely be reintroduced in the diet. BAT has also been used to monitor clinical response to immune-modulatory treatment of food allergy.

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 (31). Also improvement in basophil sensitivity to milk of milk allergic children treated with Omalizumab predicted tolerance in a milk challenge test.

Key messages

- BAT can improve the diagnosis of food allergy in addition to 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% (13,14,15). 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. BAT allows the identification of about two thirds of those patients (32).

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. Basophil reactivity has the lowest rate of double positivity of diagnostic tests for hymenoptera allergy (33) and repeatedly shows a positive result to only one venom in about one-quarter to one-third of patients with double sIgE positivity (13 34). In the case of patients with double positive BAT, the venomto which the patient is markedly more sensitive might represent the primary sensitizing allergen source, but this requires further research. BAT adds more clinically relevant information about the culprit insect than component-resolved sIgE testing with single recombinant allergens (32, 34). However, recombinant venom allergens applied to BAT might represent a step forward in developing better in vitro tests for specific diagnosis of Hymenoptera allergy.

Monitoring the effect of venom immunotherapy with basophil sensitivity: A clear decrease in basophil sensitivity is found up to 4 years after initiation of VIT, without a change in basophil reactivity. 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 (35). Some studies suggest that side effects during the build-up phase of VIT are predicted by a high basophil sensitivity (36).

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.
- The utility of basophil sensitivity as the tool of choice to monitor the effect of VIT should be explored.

Inhalant allergens

Determination 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 (37). Crude allergen sources as well as modified and recombinant allergens have been tested with good outcomes, Basophil sensitivity correlates with the nasal provocation titer in allergic rhinitis (38), the allergen specific bronchial provocation threshold in allergic asthma and the asthma control test. This indicates that basophil allergen threshold sensitivity (CD-sens or EC50) may accurately reflect clinical allergen sensitivity (39).

Monitoring the effect of allergen immunotherapy and anti-IgE treatment effect: Basophil sensitivity 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 and timothy (18,19), and showed reduced allergen sensitivity already during the up-dosing stage. Basophil sensitivity has also successfully been used to identify patients who respond to the humanized monoclonal anti-IgE antibody Omalizumab and to assess treatment efficacy (40).

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

Different methods of reporting results of BAT may be useful when asking different clinical questions; stimulation index and % positive basophils are used. When reactivity is measured in clinical settings, the aim is usually to identify an allergen concentration at which change in sensitivity is optimally identified. Basophil sensitivity is used to monitor change in allergic disease. 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.

A threshold for basophil reactivity is often set 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.

Major applications of BAT are summarized in Box 4. Laboratory procedures and allergen concentrations in BAT should be standardized e.g by the use of industry standards like MiFlowCyt or purchase of standardized material from CE-approved vendors. An important next step is the standardization and automation of analysis of BAT. Then it will be possible to perform large multicenter trials to characterize the diagnostic performance of BAT and broaden its use as a clinical tool. Such studies should also address the relationship of measures of BAT and sensitivity to sIgE, clinical symptoms and symptom severity.

References

1. 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

2. 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

3. Hoffmann HJ, Santos AF, Mayorga C, Nopp A, Eberlein B, Ferrer M et al. The clinical utility of basophil activation testing in diagnosis and monitoring of allergic disease. Allergy 2015; doi: 10.1111/all.12698. [Epub ahead of print]

4. 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

5. Iqbal K, Bhargava K, Skov PS, FalkencroneS, 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

6. 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

7. 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

8. 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

9. 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

10. 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.

11. 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

12. 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

13. 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

14. 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

15. 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

16. 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

17. 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

18. 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.

19. 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

20. Nopp A, Cardell LO, Johansson SGO, Oman H. CD-sens: a biological measure of immunological changes stimulated by ASIT. Allergy 2009;64:811–814

21. 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

22. 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

23. Wedi B, Novacovic V, Koerner M, Kapp A. Chronic urticaria serum induces histamine release, leukotriene production, and basophil CD63 surface expression--inhibitory effects of anti-inflammatory drugs. J Allergy Clin Immunol 2000;105:552–560

24. 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

25. Mayorga C, Sanz ML, Gamboa PM, García BE, Caballero MT, García JM et al. In vitro

449 diagnosis of immediate allergic reactions to drugs: an update. J Investig Allergol Clin Immunol
450 2010;20:103–109
451
452 26. Hausmann OV, Gentinetta T, Bridts CH, Ebo DG. The basophil activation test in immediate-
453 type drug allergy. Immunol Allergy Clin North Am 2009;29:555–566
454
455 27. Ocmant A, Mulier S, Hanssens L, Goldman M, Casimir G, Mascart F et al. Basophil activation
456 tests for the diagnosis of food allergy in children. Clin Exp Allergy J Br Soc Allergy Clin
457 Immunol 2009;39:1234–1245
458
459 28. Rubio A, Vivinus-Nébot M, Bourrier T, Saggio B, Albertini M, Bernard A. Benefit of the
460 basophil activation test in deciding when to reintroduce cow's milk in allergic children.
461 Allergy 2011;66:92–100
462
463 29. Santos AF, Douiri A, Bécares N, Wu S-Y, Stephens A, Radulovic S et al. Basophil activation
464 test discriminates between allergy and tolerance in peanut-sensitized children. J Allergy Clin
465 Immunol 2015 Jan;135(1):179-86. doi: 10.1016/j.jaci.2014.09.001.
466
467 30. Santos AF, Du Toit G, Douiri A, Radulovic S, Stephens A, Turcanu V et al. Distinct
468 parameters of the basophil activation test reflect the severity and threshold of allergic
469 reactions to peanut. J Allergy Clin Immunol 2015;135:179–186
470
471 31. Gernez Y, Tirouvanziam R, Yu G, Ghosn EEB, Reshamwala N, Nguyen T et al. Basophil
472 CD203c levels are increased at baseline and can be used to monitor omalizumab treatment in
473 subjects with nut allergy. Int Arch Allergy Immunol 2011;154:318–327
474
475 32. Korošec P, Šilar M, Eržen R, Čelesnik N, Bajrović N, Zidarn M et al. Clinical routine utility of
476 basophil activation testing for diagnosis of hymenoptera-allergic patients with emphasis on
477 individuals with negative venom-specific IgE antibodies. Int Arch Allergy Immunol
478 2013;161:363–368
479
480 33. Sturm GJ, Jin C, Kranzelbinder B, Hemmer W, Sturm EM, Griesbacher A et al. Inconsistent
481 results of diagnostic tools hamper the differentiation between bee and vespid venom allergy.
482 PloS One 2011;6:e20842
483
484 34. Eberlein B, Krischan L, Darsow U, Ollert M, Ring J. Double positivity to bee and wasp
485 venom: Improved diagnostic procedure by recombinant allergen-based IgE testing and
486 basophil activation test including data about cross-reactive carbohydrate determinants. J
487 Allergy Clin Immunol 2012; 130: 155-161
488
489 35. Eržen R, Košnik M, Silar M, Korošec P. Basophil response and the induction of a tolerance
490 in venom immunotherapy: a long-term sting challenge study. Allergy 2012;67:822–830
491
492 36. Kosnik M, Silar M, Bajrovic N, Music E, Korosec P. High sensitivity of basophils predicts
493 side-effects in venom immunotherapy. Allergy 2005;60:1401–1406
494
495 37. 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.

38. 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

39. 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

40. 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

Figure 1: Assessing basophil response.

The fraction of $CD63^+$ basophils is plotted against log allergen concentration. Adapted from (20) 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 reduced 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 (grey box).

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.

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 confirm that there are no conflicts of interest.