

ISSN: 1784-3286 (Print) 2295-3337 (Online) Journal homepage: <http://www.tandfonline.com/loi/yacb20>



A bar chart with the x-axis labeled 'Age' and the y-axis labeled 'Number of people'. The x-axis has four categories: 18-24, 25-34, 35-44, and 45-54. The y-axis has a scale from 0 to 100 in increments of 20. The bars represent the following values: 18-24 is 20, 25-34 is 40, 35-44 is 30, and 45-54 is 50.

Age	Number of people
18-24	20
25-34	40
35-44	30
45-54	50



Date: 25 November 2017, At: 07:24

Original Article

THE BASOPHIL ACTIVATION TEST
IN IMMEDIATE DRUG ALLERGYD.G. Ebo¹, M.M. Hagendorens², C.H. Bridts¹, L.S. De Clerck¹, W.J. Stevens¹**Key words:** allergy, anaphylaxis, CD63, CD203c, diagnosis, drugs, p38 MAPK, sIgE

ABSTRACT

Diagnosis of drug allergy is not always straight forward for several reasons. First, a broad spectrum of drugs can elicit various immune-mediated diseases with distinct pathomechanism, secondly, although exact epitope identification is not mandatory for clinical diagnosis, the epitope that causes the reaction is frequently unknown, thirdly *in vitro* or *in vivo* test results might not be predictive of a clinical situation, and finally the gold standard or reference test for diagnosis, the drug challenge, is a complicated and sometimes dangerous endeavour.

Upon challenge with specific allergens that cross-link membrane-bound IgE antibodies, basophils upregulate the expression of different

activation markers such as CD63 and CD203c. These immunophenotypic alterations can be detected on a single-cell basis by multicolour flow cytometry using specific monoclonal antibodies in the basophil activation test (BAT).

This review intends to summarise our current experience with the BAT in the diagnostic management of immediate-type allergy to drugs and related compounds that are generally (but not always) mediated by drug-specific IgE antibodies.

INTRODUCTION

As already addressed in this journal (1), allergic adverse drug reactions (ADR) constitute an important and underestimated health issue in both outpatient and inpatient clinical settings. Diagnosis of drug allergy is difficult, as a broad spectrum of different drugs can elicit various (serious) immune-mediated diseases with distinct (sometimes unclear) pathomechanism, the exact structure (epitope) that causes the reaction is frequently unknown, the *in vitro* or *in vivo* test results might not be predictive of a clinical situation, and the gold standard or reference test for diagnosis, the drug challenge, is a difficult and sometimes hazardous procedure. Consequently, drug provocation tests can only be performed in specialised centres under rigorous surveillance conditions. Therefore, *in vitro* tests are of tremendous interest in order to identify the offending agent(s) and to avoid challenges, especially in patients that have suffered from a severe reaction or that were exposed to multiple allergens.

This review intends to summarise the application of a functional *in vitro* test, called the basophil activa-

¹ Department of Immunology,
Allergology and Rheumatology,
University Hospital Antwerp,
University Antwerp;

² Department of Paediatrics,
University Hospital Antwerp,
University Antwerp, Belgium

Address for Correspondence:

Prof. Dr. Wim Stevens
Department Immunology, Allergology, Rheumatology
University Antwerp
Campus Drie Eiken
Universiteitsplein 1
2610 Antwerpen
Belgium
Tel: +32 (0)3 820 25 95
Fax: +32 (0)3 820 26 55
E-mail: immuno@ua.ac.be

tion test (BAT), in the diagnostic management of immediate-type allergy to drugs and related compounds. These reactions are generally (but not always) mediated by specific IgE (sIgE) antibodies.

The BAT relies upon flow cytometric quantification of alterations of specific basophilic activation markers (2-4). In the context of an allergic reaction for drugs or related compounds, the technique has already been validated in the diagnosis of an IgE-mediated allergy from neuromuscular blocking agents (NMBA), beta-lactam antibiotics, the anti-septic chlorhexidine and gelatine-based plasma expanders. Moreover, as addressed below, in some of these studies, the technique has proven to be complementary to skin tests in the assessment of clinically relevant cross-reactivity and tailoring individual safe alternative regimens. In an increasing number of isolated cases the BAT constituted the sole or valuable additional test for agents such as the solvent cremophor, low molecular weight heparins, the dye patent blue, starch colloids, the enzyme hyaluronidase, the locally applied antibiotic rifamycin, recombinant hepatitis b vaccine, the antiviral valacyclovir and methylprednisolon.

MECHANISMS OF DRUG ALLERGY

Drugs can elicit all types of immune reactions. Actually, all immune-mediated adverse drug reactions

are T-cell orchestrated, but the final effector functions depend mainly on antibody-dependent reactions (type I – III from the revised Gell and Coombs classification) or T-cell/cytokine-dependent reactions (type IV). Obviously, the BAT is only applicable in those cases where the final effector function relies upon activation of mast cells and/or basophils. The fact that the BAT does not discriminate between IgE-dependent (bridging of adjacent allergen-specific membrane-bound IgE molecules) and other mechanisms of cell activation (complement, short-sensitising IgG or even non-immunologic direct histamine release) should not be considered as a drawback. Alternatively, it is to note that the BAT is only applicable for drugs or excipients existing in a galenic formulation that is soluble in the stimulation buffer.

PRINCIPLES OF THE BAT

Upon encounter of specific allergens that cross-link membrane-bound sIgE molecules, basophils not only secrete and generate a variety of quantifiable bioactive mediators (e.g. histamine and leukotrienes), but also upregulate the expression of different intracellular as well as cell membrane markers such as phosphorylated p38 MAPK (mitogen-activated protein kinase), CD63 and CD203c (figure 1). These changes can be detected and quantified on a single-cell basis by

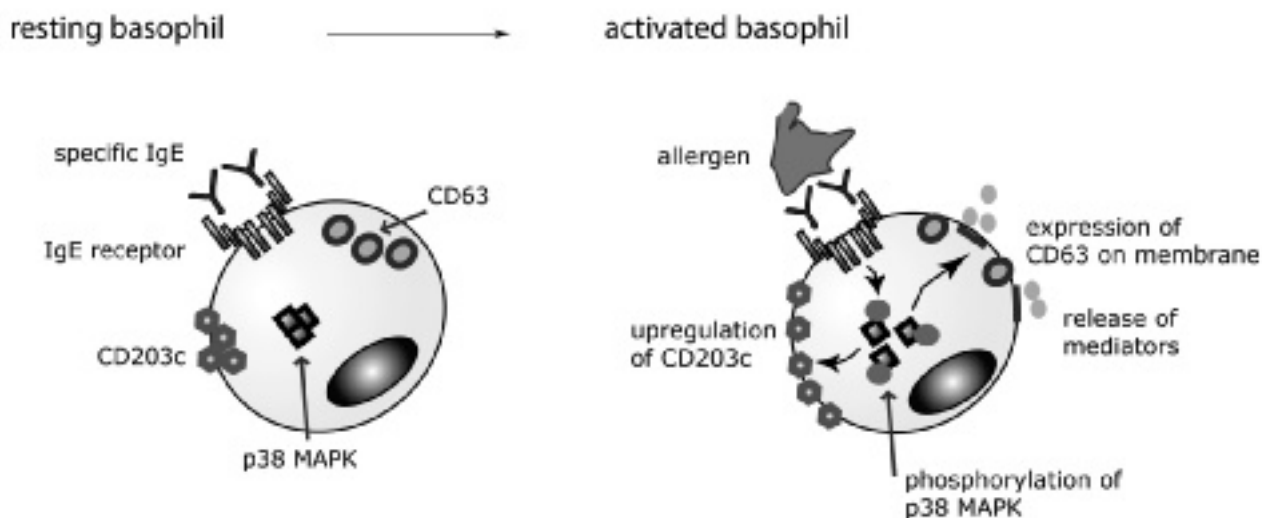


Figure 1: Upon cross-linking of membrane-bound IgE, basophils not only secrete particular mediators, but will also upregulate the expression of specific activation markers such as CD63 and CD203c. The exact mechanism(s) that govern basophil degranulation remain elusive but it has been demonstrated phosphorylation of p38 MAPK to exert a pivotal role in cell activation.

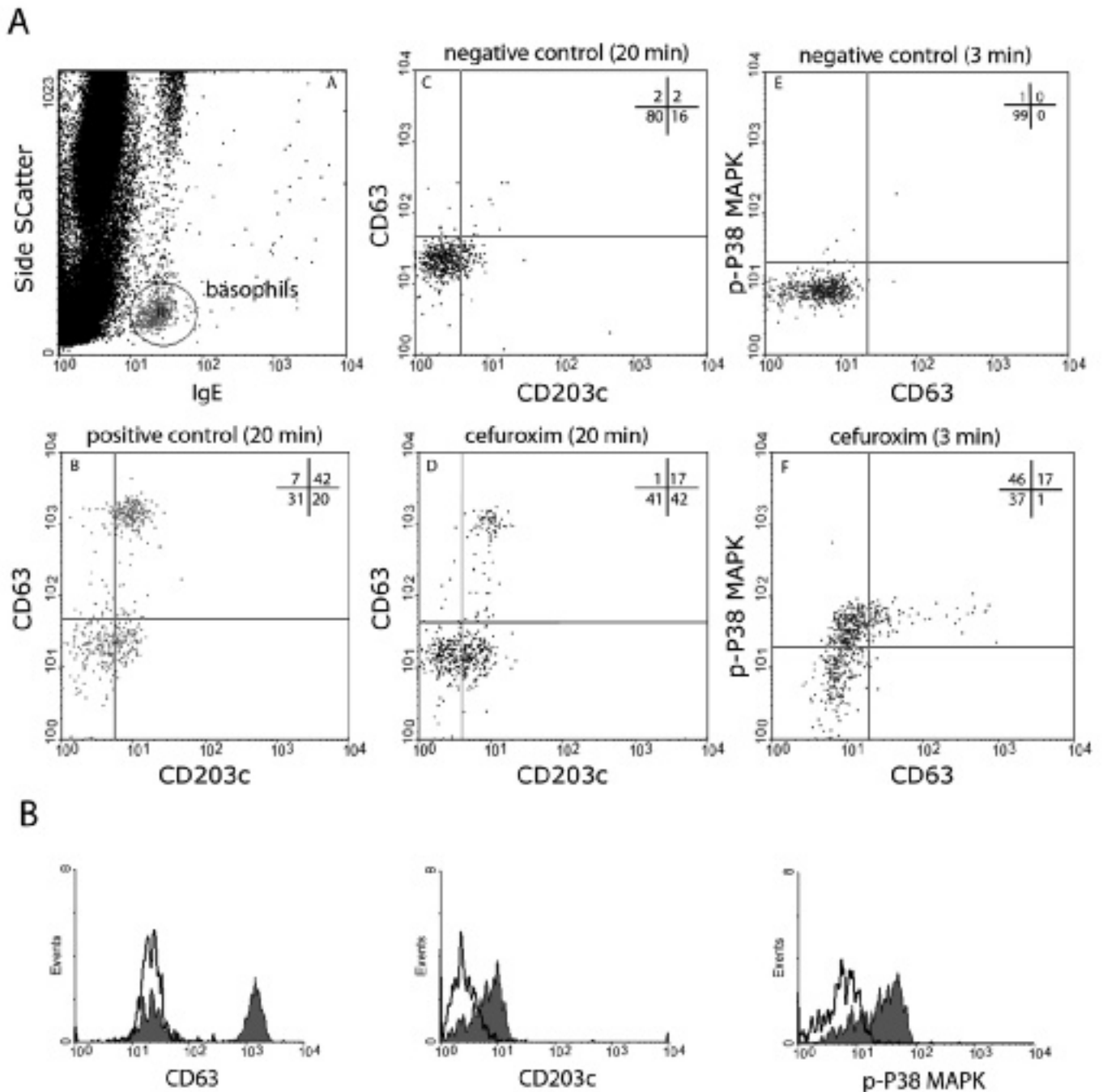


Figure 2: Flow cytometric analysis using a dot plot of side scatter and fluorescence. Alexa 488-conjugated anti-IgE positive basophils are gated out in a circular region (top left). Prewarmed basophils are stimulated for 3 or 20 minutes at 37°C with anti-IgE (BD, Biosciences Erembodegem, Belgium) as positive control (middle left), washing solution to measure spontaneous CD63, CD203c and phosphorylated p38 MAPK expression (negative control, top middle and top right) and cefuroxim (Cefurim®, TEVA Pharma, Wilrijk, Belgium) (central panel and middle right). Note the clear bimodal upregulation of CD63 and the more homogenous upregulation of CD203c and phosphorylation of p38 MAPK in a larger proportion of the cells. This is also clear from the histograms in the lower panel (open histogram spontaneous expression by resting cells, closed histogram expression after stimulation of cells with cefuroxim).

multicolour flow cytometry using specific monoclonal antibodies. Currently, the technique proved reliable for the diagnosis of different IgE-mediated allergies including classical inhalant allergens (e.g. pollen, house dust mite, cat), primary and secondary food allergies (e.g. pollen-associated food syndromes), hymenoptera venom allergy, drug allergies and *Hevea* latex allergy (for review: (2-4)). A major observation from these studies is that in contrast to proteinaceous allergens, chemicals such as drugs may elicit less pronounced *in vitro* basophilic stimulation, resulting in lower sensitivity of BAT for drugs as compared to inhalant and food allergens. However, the advent of new gating strategies and intracellular activation markers such as phosphorylated p38 MAPK, a pivotal regulator of basophil activation following stimulation with various secretagogues (5-6), might increase (considerably) sensitivity of the assay. For example, figure 1 displays the BAT results for CD63, CD203c and intracellular phosphorylated p38 MAPK in a patient that suffered from an anaphylactic shock from the beta-lactam antibiotic cefuroxim. From this figure it emerges that, at least in individual cases, the upregulation of CD203c and phosphorylation of p38 MAPK might be more homogeneous involving a larger proportion of cells than the expression of CD63 that is rather bimodal with only upregulation in a part of the cells. This might increase the sensitivity of the test, particularly when only a low number of basophils can be analysed.

APPLICATIONS

- Neuromuscular blocking agents

Currently, 5 studies have investigated the diagnostic performances of BAT test in allergy from curarising neuromuscular blocking agents (NMBA) (table 1, (7-11)) These studies demonstrate a specificity over 90% and a variable sensitivity (36%-92%) to diagnose allergy from NMBA. However, as already addressed elsewhere (11), the rather disappointing sensitivity observed in some of these publications merits reflection. First, considering history as "reference test" in NMBA allergy is questionable. Currently, skin tests are considered the gold standard for diagnosis. Second, the lowest sensitivity was observed in surveys that failed to enrol well-characterised control individuals that were uneventfully exposed to the drug(s) under investigation. Thereby disabling precise calculation of the optimal discriminative cut-off value between patients and control individuals. Actually, in these publications, deci-

sion thresholds were set arbitrarily relative to spontaneous CD63 expression by resting basophils and were based upon experience with potent proteinaceous allergens that are known to elicit stronger *in vitro* basophil responses than small chemicals. Third, the optimal stimulation concentration might differ considerably from one NMBA to another (7), rendering the proposal to adopt a universal decision threshold for all NMBA difficult to justify and probably obsolete. Finally, sensitivity of the test might decrease over time (10).

From some of these studies and case reports (7, 9, 11-12) it also emerges that the BAT constitutes an additional tool that allows simultaneous testing of different potentially cross-reactive drugs and to tailor individual safe alternative regimens for future anaesthesia. Actually, we demonstrated the BAT (11) but not sIgE-inhibition assays (13), to be complementary to skin tests to identify clinically relevant cross-reactivity between different NMBA.

- Beta-lactam antibiotics.

The diagnostic approach for immediate allergic reactions to beta-lactam antibiotics has recently been standardized under the aegis of the European Network for Drug Allergy (ENDA) and the European Academy of Allergy and Clinical Immunology (EAACI) interest group on drug hypersensitivity. From this position paper it emerges that a considerable number of patients

Table 1: Basophil activation test in drug allergy (adapted from (3))

Stimulus	Reference test	Sens	Spec	N	Reference
Neuromuscular blocking agents (NMBA)					
NMBA	H	64	93	50	(7)
NMBA	H + ST	54	100	60	(8)
NMBA	H	79	100	24	(9)
NMBA	H + ST	36-86	93	92	(10)
Rocuronium (NMBA)	H + ST	92	100	22	(11)
Antibiotics					
β-Lactam	H + ST	50	93	88	(14)
β-Lactam	H + ST + IgE + PT	49	91	110	(15)
β-Lactam (amoxicillin)	H + ST	67 (CD203c) 33 (CD63)	100 (CD203c) 79 (CD63)	41	(17)
Non-steroidal anti-inflammatory drugs (NSAID)					
Metamizol	H+ PT	42	100	55	(18)
Aspirin and NSAID	H + PT	15-55	74-100	90	(19)

Sens: sensitivity, spec: specificity, N: total number of patients and control individuals
H: history, ST: skin test, PT: provocation tests

need drug provocation tests as quantification of sIgE and skin test yield equivocal, contradictory or false negative results. In a comparison between quantification of sIgE and basophilic CD63 expression including 58 patients suffering from skin test proven beta-lactam allergy and 30 healthy control individuals, sensitivity and specificity of the assays approximated 38% and 87% for sIgE and 50% and 94% for the basophil activation test, respectively (14). From table 1 it emerges that comparable results were observed in a survey by Torres et al. (15). Whether other (more upstream located) activation markers such as down-regulation of membrane-bound IgE will increase sensitivity of the BAT (16) and whether CD203c is a more sensitive and specific marker than CD63 to diagnose allergy from beta-lactam antibiotics remains to be established (17). It is of note that in all these studies on antibiotics, as for NMBA, decision thresholds were set arbitrarily or relative to spontaneous expression of the applied activation marker.

- Aspirin and non-steroidal anti-inflammatory drugs.

At present, 2 studies from the same group of investigators have assessed flow cytometric analysis of *in vitro* activated basophils in the diagnosis of hypersensitivity to aspirin, metamizol and other non-steroidal anti-inflammatory drugs (NSAID) (18-19). In the first study, sensitivity and specificity of the test for metamizol was 42% and 100%, respectively. In the second study, the sensitivity for the different NSAID varied from 15% for metamizol and 55% for naproxen, whereas specificity generally exceeded 90%. In contrast, Erdmann et al. (20) and Malbran et al. (21) did not find the CD63-based BAT to be a reliable instrument for the diagnosis of hypersensitivity from NSAID. Currently, we do not recommend BAT in immediate hypersensitivity from NSAID and believe that additional studies are mandatory to confirm the Spanish data, particularly because NSAID-induced respiratory as well as cutaneous reactions are generally IgE-independent adverse events resulting from inhibition of cyclooxygenase-1 (COX-1) that results in depletion of prostaglandin E2 and unrestrained synthesis of cysteinyl leukotrienes and mediator release from mast cells and eosinophils (22).

- Chlorhexidine

Chlorhexidine is a cationic bisguanide antiseptic and disinfectant that is used as the (di)acetate or (di)gluconate salt. Chlorhexidine salts can trigger irritant dermatitis, allergic contact dermatitis, IgE-mediated

reactions (including life-threatening anaphylaxis) and combined IgE-mediated reactions and allergic contact dermatitis. For long diagnosis of IgE-mediated chlorhexidine allergy has relied upon skin prick tests. However, recently it was demonstrated that BAT (23) as well as quantification of sIgE (23,24) for chlorhexidine constitute valuable and safe alternative diagnostic instruments, particularly for severe cases in which skin tests to chlorhexidine are deemed unethical (25).

- Gelatine

Anaphylaxis to gelatine in vaccines and gelatine-based colloids is well known (26). Although diagnosis can generally easily be established on quantification of sIgE and/or appropriate skin tests, the BAT has recently proven to constitute a valuable additional diagnostic tool (27).

- Miscellaneous

In an increasing number of cases, the technique helped to identify the plasma expander hydroxyl ethyl starch (28), chemophor (the emulsifier of the intravenous cyclosporine formulation) (29), bovine serum albumin present in a semen culture medium for artificial insemination (30), omeprazole (31), different low-molecular-weight heparins (32,33), viscotoxins of mistletoe (*Viscum album*) (34), the dye patent blue (35) the enzyme hyaluronidase (36), platinum salts (37), recombinant Hepatitis B vaccine (38), and the antiviral valacyclovir (39) as the causative compound in patients with sometimes life-threatening allergic reactions. Moreover, in some of these patients the technique has contributed to establish the individual therapeutic alternative and/or allowed identification of potentially cross-reactive structures.

SUMMARY

It is evident that flow-assisted analysis and quantification of *in vitro* activated basophils provides the physician with a novel instrument in the diagnostic management of immediate drug allergy that involves IgE-dependent or independent mast cell and basophil activation, particularly for those cases where no alternative tests are available and confirmation of clinical suspicion would require a potential dangerous drug provocation test. However, currently the technique is mostly applied for research settings and additional clinical studies are mandatory to allow its entrance in

mainstream clinical use. In such studies correct selection of patients and appropriate control groups will be an absolute prerequisite in order to establish optimal drug-specific thresholds and to abandon arbitrarily chosen decision thresholds for determination of sensitivity, specificity and predictive values. Alternatively, the technique might further benefit by application of novel basophil gating strategies and activation markers such as phosphorylation of the upstream located p38 MAPK. Moreover, as these novel markers might become activated through alternative routes, it seems theoretically justified to anticipate that the BAT might be applicable for the diagnosis of IgE-independent basophil-mediated adverse drug reactions.

REFERENCES

1. Ebo DG, Hagendorens MM, Bridts CH, Stevens WJ. Immediate-type allergy to drugs and related compounds: evaluation and management. *Acta Clin Belg* 2005; 60:350-61.
2. Ebo DG, Hagendorens MM, Bridts CH, Schuerwegh AJ, De Clerck LS, Stevens WJ. In vitro allergy diagnosis: should we follow the flow? *Clin Exp Allergy* 2004; 34:332-9.
3. Ebo DG, Sainte-Laudy J, Bridts CH, et al. Flow-assisted allergy diagnosis: current applications and future perspectives. *Allergy* 2006; 61:1028-39.
4. de Weck AL, Sanz ML, Gamboa PM, et al. Diagnostic Tests Based on Human Basophils: More Potentials and Perspectives than Pitfalls. *Int Arch Allergy Immunol* 2008; 146:177-89.
5. Ebo DG, Dombrecht EJ, Bridts CH, Aerts NE, De Clerck LS, Stevens WJ. Combined analysis of intracellular signalling and immunophenotype of human peripheral blood basophils by flow cytometry: a proof of concept. *Clin Exp Allergy* 2007; 37:1668-75.
6. Ebo DG, Bridts CH, Stevens WJ. IgE-mediated large local reaction from recombinant hepatitis B vaccine. *Allergy* 2008; 63:483-4.
7. Abuaf N, Rajoely B, Ghazouani E, 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; 104:411-8.
8. Monneret G, Benoit Y, Debard AL, Gutowski MC, Topenot I, Bienvu J. Monitoring of basophil activation using CD63 and CCR3 in allergy to muscle relaxant drugs. *Clin Immunol* 2002; 102:192-9.
9. Sudheer PS, Hall JE, Read GF, Rowbottom AW, Williams PE. Flow cytometric investigation of peri-anaesthetic anaphylaxis using CD63 and CD203c. *Anaesthesia* 2005; 60:251-6.
10. Kvedariene V, Kamey S, Ryckwaert Y, et al. Diagnosis of neuromuscular blocking agent hypersensitivity reactions using cytofluorimetric analysis of basophils. *Allergy* 2006; 61:311-5.
11. Ebo DG, Bridts CH, Hagendorens MM, Mertens CH, De Clerck LS, Stevens WJ. Flow-assisted diagnostic management of anaphylaxis from rocuronium bromide. *Allergy* 2006; 61:935-9.
12. Sudheer PS, Appadurai IR. Anaphylaxis to vecuronium: the use of basophil CD63 expression as a possible screening tool to identify a safe alternative. *J Clin Anesth* 2007; 19:555-7.
13. Ebo DG, Venemalm L, Bridts CH, et al. Immunoglobulin E antibodies to rocuronium: a new diagnostic tool. *Anesthesiology* 2007; 107:253-9.
14. Sanz ML, Gamboa PM, Antepara I, 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; 32:277-86.
15. Torres MJ, Padial A, Mayorga C, et al. The diagnostic interpretation of basophil activation test in immediate allergic reactions to betalactams. *Clin Exp Allergy* 2004; 34:1768-75.
16. Sainte-Laudy J, Boumediene A, Touraine F, et al. Use of both CD63 up regulation and IgE down regulation for the flow cytometric analysis of allergen induced basophil activation. Definition of an activation index. *Inflamm Res* 2007; 56:291-6.
17. Abuaf N, Rostane H, Rajoely B, et al. Comparison of two basophil activation markers CD63 and CD203c in the diagnosis of amoxicillin allergy. *Clin Exp Allergy* 2008; 38:921-8.
18. Gamboa PM, Sanz ML, Caballero MR, et al. Use of CD63 expression as a marker of in vitro basophil activation and leukotriene determination in metamizol allergic patients. *Allergy* 2003; 58:312-7.
19. Gamboa P, Sanz ML, Caballero MR, et al. The flow-cytometric determination of basophil activation induced by aspirin and other non-steroidal anti-inflammatory drugs (NSAIDs). is useful for in vitro diagnosis of the NSAID hypersensitivity syndrome. *Clin Exp Allergy* 2004; 34:1448-57.
20. Erdmann SM, Ventocilla S, Moll-Slodowy S, Sauer I, Merk HF. (Basophil activation tests in the diagnosis of drug reactions). *Hautarzt* 2005; 56:38-43.
21. Malbran A, Yeyati E, Rey GL, Galassi N. Diclofenac induces basophil degranulation without increasing CD63 expression in sensitive patients. *Clin Exp Immunol* 2007; 147:99-105.
22. Mastalerz L, Setkowicz M, Sanak M, Szczeklik A. Hypersensitivity to aspirin: common eicosanoid alterations in urticaria and asthma. *J Allergy Clin Immunol* 2004; 113:771-5.
23. Ebo DG, Bridts CH, Stevens WJ. IgE-mediated anaphylaxis from chlorhexidine: diagnostic possibilities. *Contact Dermatitis* 2006; 55:301-2.
24. Garvey LH, Kroigaard M, Poulsen LK, et al. IgE-mediated allergy to chlorhexidine. *J Allergy Clin Immunol* 2007; 120:409-15.
25. Pham NH, Weiner JM, Reisner GS, Baldo BA. Anaphylaxis to chlorhexidine. Case report. Implication of immunoglobulin E antibodies and identification of an allergenic determinant. *Clin Exp Allergy* 2000; 30:1001-7.
26. Laxenaire MC, Charpentier C, Feldman L. (Anaphylactoid reactions to colloid plasma substitutes: incidence, risk factors, mechanisms. A French multicenter prospective study). *Ann Fr Anesth Reanim* 1994; 13:301-10.
27. Apostolou E, Deckert K, Puy R, et al. Anaphylaxis to Gelofusine confirmed by in vitro basophil activation test: a case series. *Anaesthesia* 2006; 61:264-8.
28. Ebo DG, Schuerwegh A, Stevens WJ. Anaphylaxis to starch. *Allergy* 2000; 55:1098-9.
29. Ebo DG, Piel GC, Conraads V, Stevens WJ. IgE-mediated anaphylaxis after first intravenous infusion of cyclosporine. *Ann Allergy Asthma Immunol* 2001; 87:243-5.
30. Orta M, Ordoqui E, Aranzabal A, Fernandez C, Bartolome B, Sanz ML. Anaphylactic reaction after artificial insemination. *Ann Allergy Asthma Immunol* 2003; 90:446-51.
31. Gamboa PM, Sanz ML, Urrutia I, et al. CD63 expression by flow cytometry in the in vitro diagnosis of allergy to omeprazole. *Allergy* 2003; 58:538-9.

32. Caballero MR, Fernandez-Benitez M. Allergy to heparin: a new in vitro diagnostic technique. *Allergol Immunopathol (Madr)* 2003; 31:324-8.
33. Ebo DG, Haine SE, Hagendorens MM, et al. Hypersensitivity to nadroparin calcium: case report and review of the literature. *Clin Drug Investig* 2004; 24:421-6.
34. Bauer C, Oppel T, Rueff F, Przybilla B. Anaphylaxis to viscotoxins of mistletoe (*Viscum album*) extracts. *Ann Allergy Asthma Immunol* 2005; 94:86-9.
35. Ebo DG, Wets RD, Spiessens TK, Bridts CH, Stevens WJ. Flow-assisted diagnosis of anaphylaxis to patent blue. *Allergy* 2005; 60:703-4.
36. Ebo DG, Goossens S, Opsomer F, Bridts CH, Stevens WJ. Flow-assisted diagnosis of anaphylaxis to hyaluronidase. *Allergy* 2005; 60:1333-4.
37. Touraine F, Sainte-Laudy J, Boumediene A, et al. Exploration d'une allergie aux sels de platine. *Rev Mal Respir* 2006; 23:458-62.
38. Ebo DG, Bridts CH, Stevens WJ. IgE-mediated large local reaction from recombinant hepatitis B. *Allergy* 2008;63:483-4.
39. Ebo DG, Bridts CH, De Clerck LS, Stevens WJ. Immediate allergy from valacyclovir. *Allergy* 2008;63:941-2.