

Salvatore Chirumbolo

Laboratory of Physiopathology of Obesity, Department of Medicine-University of Verona

Basophil activation tests (BAT): wheat and chaff

Test aktywacji bazofilów (BAT): jak oddzielić ziarno od plew

The author declares no financial disclosure

Pneumonol Alergol Pol 2015; 83: 243–244

To the Editor

The recent article by Gawinowska et al., on the latest issue of „Polish Pneumology and Allergology” addressed the issue of the application of basophil activation test (BAT) in the diagnosis of drug hypersensitivity [1]. The Authors stressed on the commonly recognized role exerted by CD63 and CD203c markers in BAT, while existing further suggestions [2, 3] but they never addressed the critical issue of basophil electronic capture in flow cytometry (FC), i.e. cell phenotyping protocols [4, 5]. Advantages of BAT in allergy diagnosis are closely related to the simple fact that this assay is a cell based investigation procedure [6] and therefore, while its major value is the ability in diagnosing either an IgE- or non IgE-mediated allergy, immunotherapy and anaphylaxis and to prevent the ethical issue associated to skin tests [1], a main concern is represented by the interpretation of activation markers in a good phenotyping FC approach [7]. The Authors reported, particularly in Table 1 of their paper, that BAT false negatives might be due mainly to the existence of non releaser subjects [1]. Non releaser individuals have usually reduced expression of basophil membrane FcεRI, besides to a decrease in released molecules such as histamine and serum IgEs [8]. The non releaser subject, otherwise incorrectly known as non responder, has reduced Lyn and Syk kinases expression, besides a possible reduced response to IL-3 [9, 10].

However, it is particularly difficult to diagnose a non releaser subject through the performance of a BAT, mainly because of the many issues related to CD63 evaluation [5, 7], besides to effects caused by the differential expression of membrane FcεRI/IgE complexes, circulating IgEs and relationships with FcγRs [11], downregulation and intracellular recycling of Fc receptors upon activation [12, 13], doses and strength of polyclonal anti-IgEs or serum IgEs in inducing an activation response from basophils in a BAT. Non releaser subjects, unless an intracellular FC evaluation of Syk, might be misled among people performing different causes to their basophils responsiveness. These issues may compressively hamper the promising feasibility of BAT, without a respectable expertise in the field, suggesting therefore that the debate needs to be further expanded to address possible bias and criticism.

BAT potentially may discriminate between IgE-mediated and IgE-non mediated allergy, although, as suggested by the Authors [1], non IgE-mediated allergy seems to be more frequently reported for drug hypersensitivity, for which sensitivity evaluation should be highly influenced. Interestingly, the Authors described a case report, a 52-years old patient, in whom hypersensitivity to NSAIDs was reported, in the absence of other allergologic signs, based on the CD63% FC expression in BAT ($\geq 22\%$, c.o. 10%) [1]. The use of BAT in diagnosing NSAIDs allergy, including acetylsalicylic acid (ASA) or aspirin[®], is a major

Address for correspondence: Salvatore Chirumbolo, MD, PhD, Laboratory of Physiopathology of Obesity Department of Medicine-University of Verona, LURM Est Policlinico GB Rossi, Piazzale AL Scurio 10, 37134 Verona-Italy, Tel +390458128456, Fax +390458027403 e-mail: salvatore.chirumbolo@univr.it
DOI: 10.5603/PiAP.2015.0040

Received: 11.03.2015

Copyright © 2015 PTChP

ISSN 0867–7077

concern in the diagnosis of drug hypersensitivity but any analytical evaluation, which should include the calculation of parameters such as sensitivity, specificity and predictive values, fundamentally through ROC curves, have to be fitted to a defined FC protocol, properly suited to investigate basophils *in vitro*. In a more general way, values of sensitivity and specificity should be adapted to different technical approaches in evaluating basophils by FC. Performances of the different gating protocols and activation markers used to optimize BAT in diagnosing allergy, should be investigated in order to suggest the optimal technical approach and reduce bias and mis-interpretations. While the attempt to reduce the number of markers in BAT is always welcome, yet methods need to be reappraised, probably a Consensus Panel is necessary in order to achieve the wider agreement about the best protocol to study basophils in a BAT.

In addition, the Authors suggest the use of BAT as a confirmatory test, as it may fulfil the suspicion of an allergy response but, in this case, the physician/allergist or the practitioner should have previous symptoms or signs about the existence of an allergic or hypersensitivity reaction. As in the case report they described, the investigation about NSAID allergy with a BAT allowed to reach a diagnostic evaluation and to forward a medical decision. How much current BATs are able to help physicians to address a medical decision? The evaluation of CD63%, based on an arbitrary non responding threshold, is particularly cumbersome, despite its apparent feasibility [5, 7] and may oblige operators to add further activation markers or interpretation algorithms in BATs [7]. Very few physicians are experienced with BATs and this represents a main education concern in laboratory and clinics. Several available tests are purchasable on the market but a consensus lacks to warrant for an authoritative guidance in order to advice and suggest better approaches and less expensive assay protocols.

In conclusion, while fully in agreement that BAT is a formidable tool to investigate allergy, many further insights are requested to elucidate the many concerns and issues related to the application and performance of this *in vitro* assay.

Conflicts of interest

The author declares no conflict of interest.

References

1. Gawinowska M. Application of basophil activation test in diagnosing aspirin hypersensitivity. *Pneumonol Alergol Pol* 2015; 83: 66–73. doi: 10.5603/PiAP.2015.0010.
2. MacGlashan DW Jr. Basophil activation testing. *J Allergy Clin Immunol* 2013; 132: 777–787. doi: 10.1016/j.jaci.2013.06.038.
3. Chirumbolo S. CD164 and other recently discovered activation markers as promising tools for allergy diagnosis: what's new? *Clin Exp Med* 2011; 11: 255–257. doi: 10.1007/s10238-011-0132-y.
4. Chirumbolo S, Ortolani R, Vella A. CCR3 as a single selection marker compared to CD123/HLADR to isolate basophils in flow cytometry: some comments. *Cytometry A* 2011; 79: 102–106. doi: 10.1002/cyto.a.21008.
5. Chirumbolo S. Major pitfalls in BAT performance may be caused by gating protocols and CD63% cut off evaluation. *Cytometry A* 2014; 85: 382–385. doi: 10.1002/cyto.a.22466.
6. Song WJ, Chang YS. Recent applications of basophil activation tests in the diagnosis of drug hypersensitivity. *Asia Pac Allergy* 2013; 3: 266–280. doi: 10.5415/apallergy.2013.3.4.266.
7. Chirumbolo S. Basophil activation test in allergy: time for an update? *Int Arch Allergy Immunol* 2012; 158: 99–114. doi: 10.1159/000331312.
8. Kumar P, Singh B, Lal R, Rembhotkar GW, Singh AB. Association between reduced levels of total serum IgE and FcεsilonRI expression in non-releaser basophils. *Immunobiology* 2009; 214: 377–383. doi: 10.1016/j.imbio.2008.09.008.
9. Kumar P, Singh B, Lal R, Rembhotkar GW, Singh AB. Histamine releasability and expression of Lyn and Syk kinases in Indian subjects and role of less potent IL-3 in non-releaser basophils. *Cytokine* 2007; 37: 200–205.
10. Kepley CL, Youssef L, Andrews RP, Wilson BS, Oliver JM. Syk deficiency in nonreleaser basophils. *J Allergy Clin Immunol* 1999; 104: 279–284.
11. Chirumbolo S, Olivieri M. Increase in human basophils IgE-mediated stimulation by omalizumab: a role for membrane FcγRs? *J Allergy Clin Immunol* 2014; 133: 1493–1494. doi: 10.1016/j.jaci.2013.12.1094.
12. MacGlashan DW Jr. Endocytosis, recycling, and degradation of unoccupied FcεsilonRI in human basophils. *J Leukoc Biol* 2007; 82: 1003–1010.
13. MacGlashan DW Jr, Xia HZ, Schwartz LB, Gong J. IgE-regulated loss, not IgE-regulated synthesis, controls expression of FcεsilonRI in human basophils. *J Leukoc Biol* 2001; 70: 207–218.