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Article type : Correspondence

Correspondence: Basophil activation test in the diagnosis of hypersensitivity reactions to quinolones by Loli-Ausejo et al.

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This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the [Version of Record](#). Please cite this article as [doi: 10.1111/CEA.13853](https://doi.org/10.1111/CEA.13853)

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Dear Editor,

We have read with interest the paper by Loli-Ausejo and colleagues about the basophil activation test (BAT) in the diagnosis of hypersensitivity to fluoroquinolones (FQs) ¹. The authors report on a heterogenous group of 19 patients who experienced a hypersensitivity reaction reported as immediate, accelerated/non-immediate or of unknown time of onset. Based on clinical history, the authors posit these reactions to have resulted from levofloxacin, moxifloxacin or ciprofloxacin and stratified the patients in three severity grades according to Brown ². All together, these patients had 49 BATs with up to four different FQs in some cases and the authors concluded the BAT to FQs to be helpful in the diagnosis of FQ allergy and to be of great value in comparison to skin tests, even in mild reactions. It is also reported BAT to be especially useful when the reactions were more severe and when the fourth generation FQ moxifloxacin was involved.

However, we would like to express some comments and concerns. Correct diagnosis of immediate FQ hypersensitivity can pose a significant challenge, mainly because of unknowns and uncertainties of the underlying pathomechanisms. Since 2015, evidence has emerged that FQ hypersensitivity might result from occupation of the Mas-related G protein coupled receptor X2 (MRGPRX2) ³. This receptor is constitutively expressed on the surface of some mast cells (MCs), especially tryptase and chymase containing skin MC_{TC} ⁴. In contrast, MRGPRX2 is barely expressed by resting basophils that are used as a starting point in traditional BATs ⁵. Moreover, we recently speculated on the significance of negative CD63-BAT results in an attempt to discriminate between IgE- and probable MRGPRX2-mediated drug hypersensitivity reactions ^{6,7}.

As indicated higher, in the series by Loli-Ausejo et al ¹, diagnosis of FQ hypersensitivity relied upon clinical history without individual details, except severity grade and time of onset of the index reaction. However, in about a fifth of the patients, the time of onset equaled/exceeded 4 hours or was simply unknown. Therefore, together with the absence of appropriate drug challenges ^{3,8}, it cannot be excluded that some patients did not experience a basophil- and/or MC-related reaction and/or their reaction not to involve the used FQ. In this respect, problems are compounded by the lack of data about other drugs the patients could have been exposed to.

Therefore, the basis for confirming FQs as cause is inadequate, and reactions in some patients may have experienced a reaction to (an) alternative cause(s).

We also would like to comment on the execution of the BAT and interpretation of the results. As shown in table 1 of their manuscript ¹, many patients had different BATs including various FQs that were not administrated at the time of the index reaction. This approach entails a significant risk for generation of clinically irrelevant results and overdiagnosis. As acknowledged by the authors, only 4 out of 19 (21%) patients had a “positive” BAT with the alleged culprit. The remainder 5 patients had a “positive” BAT with a different FQ, which was considered diagnostic. Furthermore, the authors do not present essential data enabling correct appraisal of their findings ⁹. Information from drug-specific dose-finding studies, optimal stimulation conditions, variation coefficients and non-responders is missing. Furthermore, the authors did not study exposed control individuals but used a pre-defined arbitrary decision threshold. Actually, BAT was considered positive when the percentage of CD63⁺ cells was > 5% and the stimulation index was > 2 in at least one of the three dilutions. Unfortunately, table 1 of their manuscript ¹ does not indicate which dilution(s) resulted in a “positive” BAT, nor does it show the individual stimulation indices. Thereby, together with the absence of representative plots, hampering correct interpretation of the data and comparison with other studies that already indicated that the outcome of BAT depends on clinical entity and culprit FQ ¹⁰.

With respect to moxifloxacin, it seems that the authors overlooked two publications about skin testing and BAT and showing opposite outcomes ^{11, 12}. Skin prick and intradermal tests with moxifloxacin were shown to be useless ¹¹ and traditional CD63-based BAT proved to poorly discriminate between patients and uneventfully challenged control individuals, with only two positive tests in 15 patients ¹². The explanation for these observations has likely to be sought in the fact moxifloxacin, like other FQs, are potent MRGPRX2 activators that can trigger IgE-independent MC activation resulting in clinically irrelevant skin responsiveness and go undetected in traditional BAT.

In conclusion, in our opinion, this study does not allow formulation of statements about the clinical utility of the BAT in the diagnosis of FQ hypersensitivity. The inadequate description of the clinics, together with the absence of a drug challenge, hinder to ascertain that the patients

had experienced from a basophil-mediated hypersensitivity to a FQ. Critical data about drug-specific experiments and validation of the BAT, which appear not to be performed according to the prevailing recommendations⁹, are missing. It is recommended to abandon arbitrary generic decision thresholds and to systematically perform tests with drugs the patients was not exposed to. Broader testing should be personalized and reserved to the exploration of cross-reactivity and identification of safe alternatives for the future.

Conflict of interest

The authors declare no conflict of interest.

Author contributions

All authors participated in writing the paper and in proofreading and revising the final text.

References

1. Loli-Ausejo D, Vílchez-Sánchez F, Cabañas R, Fiandor A, Lluch-Bernal M, González-Muñoz M, et al. Basophil activation test in the diagnosis of hypersensitivity reactions to quinolones. *Clin Exp Allergy* 2020.
2. Brown SG. Clinical features and severity grading of anaphylaxis. *J Allergy Clin Immunol* 2004; 114:371-6.
3. McGee EU, Samuel E, Boronea B, Dillard N, Milby MN, Lewis SJ. Quinolone Allergy. *Pharmacy (Basel)* 2019; 7.
4. Varricchi G, Pecoraro A, Loffredo S, Poto R, Rivellesse F, Genovese A, et al. Heterogeneity of Human Mast Cells With Respect to MRGPRX2 Receptor Expression and Function. *Front Cell Neurosci* 2019; 13:299.
5. Elst J, Sabato V, Hagendorens MM, van Houdt M, Faber MA, Bridts CH, et al. Measurement and Functional Analysis of the Mas-Related G Protein-Coupled Receptor MRGPRX2 on Human Mast Cells and Basophils. *Methods Mol Biol* 2020; 2163:219-26.
6. Ebo DG, Bridts CH, Mertens CH, Sabato V. Principles, potential, and limitations of ex vivo basophil activation by flow cytometry in allergology: A narrative review. *J Allergy Clin Immunol* 2020.

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7. Ebo DG, Van der Poorten ML, Elst J, Van Gasse AL, Mertens C, Bridts C, et al. Immunoglobulin E cross-linking or MRGPRX2 activation: clinical insights from rocuronium hypersensitivity. *Br J Anaesth* 2021; 126:e27-e9.
 8. Demir S, Gelincik A, Akdeniz N, Aktas-Cetin E, Olgac M, Unal D, et al. Usefulness of In Vivo and In Vitro Diagnostic Tests in the Diagnosis of Hypersensitivity Reactions to Quinolones and in the Evaluation of Cross-Reactivity: A Comprehensive Study Including the Latest Quinolone Gemifloxacin. *Allergy Asthma Immunol Res* 2017; 9:347-59.
 9. Mayorga C, Ebo DG, Lang DM, Pichler WJ, Sabato V, Park MA, et al. Controversies in drug allergy: In vitro testing. *J Allergy Clin Immunol* 2019; 143:56-65.
 10. Fernández TD, Ariza A, Palomares F, Montañez MI, Salas M, Martín-Serrano A, et al. Hypersensitivity to fluoroquinolones: The expression of basophil activation markers depends on the clinical entity and the culprit fluoroquinolone. *Medicine (Baltimore)* 2016; 95:e3679.
 11. Uyttbroek AP, Sabato V, Bridts CH, De Clerck LS, Ebo DG. Moxifloxacin hypersensitivity: Uselessness of skin testing. *J Allergy Clin Immunol Pract* 2015; 3:443-5.
 12. Van Gasse AL, Sabato V, Uyttbroek AP, Elst J, Faber MA, Hagendorens MM, et al. Immediate moxifloxacin hypersensitivity: Is there more than currently meets the eye? *Allergy* 2017; 72:2039-43.