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Answer to: "Biomarkers in allergic asthma: Which matrix should we use?"

We appreciate comments from Dr. M. Maniscalco and A. Motta on our review article.[1] The comments made on the matter about which biological matrix(ces) should be used in the quest of biomarkers in allergic asthma are well accepted. However, we would like to open the discussion about the topics exposed by the authors. Definitely, asthma is a very complex disease which presents heterogeneous clinical symptoms and multiple causes. Presenting diverse subphenotypes, to date there is not enough information to characterize them in the clinical setting.[2,3] In this sense, we agree with the authors that as much information as possible from different types of samples will provide a better global picture of the molecular mechanism underlying this pathology. Whereas these significant molecules from any kind of biological matrix describe the molecular mechanism underneath, only a set of these should be chosen as potential biomarkers for the clinic. In this sense, these panels of biomarkers would predict accurately the diagnosis or the prognosis of the disease. Moreover, if this is the case the ideal biological sample should be easy to extract from the patient, and potential biomarkers should be measured using a simple laboratory methodology. Therefore, we suggest to use biological matrixes such as blood or urine to fulfil this purpose. As a proof of concept, Kong J.[4] found a set of significant metabolites in an animal model using plasma for peanut allergy, from which one of them was selected to validate their findings using urine in children.

Regarding exhaled breath condensate (EBC), this biological sample has shown very promising results in asthma[5-9] and other airway-related diseases (eg COPD[10]). EBC is a sample that can be easily taken and is non-invasive for the patient. It comprises volatile and non-volatile compounds from the airway[11] covering a variety of metabolites (eg inorganic compounds, isoprostanes, prostanoids, eicosanoids).[12-14] Up to date, the exploration and use of EBC had seemed to be limited by the lack of an optimal standard procedure for breath sampling. Sample variability, effect of pH over some metabolites and high dilution are mostly the challenges that EBC sampling is facing,[13] where currently great efforts are being made.[15] Despite EBC is not a sample used in the daily clinic routine compared to blood and urine, it has showed recently its potential into the clinical setting.[16] However, in this study, EBC was only successfully analysed from 185 samples from 242 in total, the rest of the samples included a strong contamination.[16] In summary, EBC is a very promising biological sample whose use is increasing nowadays but still facing problems with the sample collection, concentration of samples and dilution.

In conjunction, together with this kind of sample a variant from EBC, is the exhaled breath (EB). EB is also a biological sample that consists of volatile organic compounds (VOC). The EB volatilome has also shown great advantages especially for the discrimination between the groups, such as between COPD and asthma.[17,18] In addition, markers of EB have been found to distinguish between asthmatic and healthy children.[19-21] Sample collection of EB is also non-invasive, simple and inexpensive.[22] This sample has caught attention in respiratory diseases because of its discriminating power; however, one of the major questions around the compounds found in EB is the characterization of their origin. It is cited that these EB compounds are formed during the inflammatory processes in a pathology, but their origin can include the lung, liver, muscle, kidney.[23,24] The sentence in our review: "while EB is a relevant sample in respiratory affections because it shows the characteristic compounds from lung metabolism, its greatest handicap is the difficulty to obtain a reliable interpretation and correlation of these metabolites with the disease[1]", written as EB instead of EBC as authors state in their comment (page 1 line 48), is in agreement with the sentence: "the origin and pathways of biosynthesis of many VOCs are not known".[14] Speaking about the interpretation of the EB volatilome from metabolic precursors, a recent publication[14] has linked these EB compounds with the EBC metabolome. The outcome of this publication showed very interesting findings; first they demonstrate that VOC—precursors candidates in EBC showed a very specific correlation.

To sum up, the findings from EBC and EB volatilome matrices are promising and have contributed to the study of asthma. There is no doubt that the integration of these along with other biological types of samples (eg urine, blood, tissue biopsy, sputum and bronchoalveolar lavage fluid (BALF)) is important to obtain a more holistic picture of the biological situation. However, regarding to obtain potential biomarkers used in the clinic, there is no doubt that the research using easily extracted and straightforward biofluids is compulsory whenever is possible.

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