



Commentary

Metabolomics for the diagnosis of influenza

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In this article of EBioMedicine [1], Hogan and colleagues uncover a panel of potential biomarkers (a top-20 ion feature signal) for influenza using untargeted metabolomics, which suggest new avenues for rapid diagnosis of the disease. The study design is good with a well-chosen and matched population; diagnosis of Influenza was performed on the ePlex Respiratory Pathogen panel, which is the method used for clinical diagnosis in their setting. After assignment of the 20-feature panel using the discovery cohort, biomarkers were verified on a validation cohort using a targeted tandem mass spectrometry method. Pyroglutamic acid was observed to be lower in nasopharyngeal swabs from influenza infected individuals. The authors then developed a fully quantitative LC-MS/MS method utilising deuterated pyroglutamic acid as an internal standard. This study is an excellent showcase of the opportunities and challenges involved in the use of metabolomics for translational science.

Metabolomics is split between the sub-fields of 'targeted' and 'untargeted'. In targeted metabolomics, a panel of small molecules is selected and instrumentation is tuned to detect only these molecules, which is generally performed with very high specificity, with the drawback that one only finds what one is looking for. In untargeted metabolomics, a broad-based detection is performed, with the aim of providing an unbiased assessment of the metabolome.

The aim of untargeted metabolomics is to discover something new: a previously undiscovered metabolite, or one that failed to fit into the hypothesis that the panel of metabolites assigned for a targeted analysis would test. In this paper, Hogan and colleagues have detected a panel of features that are associated with disease. The metabolite standards initiative [2] requires a match to an authentic standard (via a minimum of a match to mass to charge ratio (m/z) and chromatographic retention time) to be considered an 'identification'. The challenge of identifying other compounds is significant and

an accurate mass measurement alone, while it can provide clues as to the empirical chemical formula of a compound, provides no information about the *structure* of an unknown metabolite. Metabolites for use as a biomarker must be positively identified in order to avoid misleading interpretations [3]. Unambiguous characterisation of an unknown biomarker is extremely challenging. Thus, while the panel discovered provides enticing leads for future biomarker discovery, pyroglutamate represents a dramatic refinement from what was originally a relatively wide panel.

In terms of translation to the clinic, the authors acknowledge there is a long road ahead. The difficulty in translating biomarkers into the clinic is well known [4]. As yet, no biomarkers identified using metabolomics have been translated to the clinic [5].

For a biomarker to be applicable to clinical use the key considerations below must be met. The population, as in this study, must be well chosen in order to reflect the population the test is expected to be used in [6]. The participants must be well controlled in order to avoid confounding variables; the study size should be chosen so that there is sufficient power to detect true biomarkers (although this is difficult in metabolomics studies in the discovery phase as it is difficult to estimate the clinically significant differences required [7]. Outcomes need to be well chosen: hard outcomes (in terms of patient outcomes) rather than surrogate outcomes (e.g. reduction of a disease marker) and properly adjudicated endpoints according to clinical trial guidelines [8]. The biomarker must be robustly identified, and absolute quantification is essential [6]. Procedures for collection, storage, preparation and analysis must be fully standardised, particularly in multi-centre studies to avoid artefactual results. The assays themselves must be subject to good Quality Control procedures, particularly if batch to batch variation is likely to be an issue, and this is exemplified by the Metabolomics Quality Assurance and Control Consortium guidance [9]. Care should be taken to avoid over-fitting of models. Any novel biomarkers found in one study should be validated in an independent cohort to ensure that results are robust and generalizable [6]. When these requirements are met, this has the benefit of also allowing comparison between studies and improved biological interpretation.

Before translation into the clinic, comparison with gold standard methods and routine biochemistry is essential in order to demonstrate real improvements in clinical utility compared to the methods already in use, particularly in terms of turnaround time, availability, robustness, accuracy and precision. Finally, a health economics analysis is critical to determine if any increase in costs introduced by any new tests is justified in terms of patient outcomes [6]. So, there are

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E-mail addresses: karl.burgess@ed.ac.uk (K. Burgess), naomi.rankin@lanarkshire.scot.nhs.uk (N. Rankin).<https://doi.org/10.1016/j.ebiom.2021.103599>2352-3964/© 2021 Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>)

many barriers to cross before a putative biomarker can make a difference in clinical settings – it is therefore unsurprising that the lack of translation noted by Poste [4] still stands. This study was performed with samples obtained pre-COVID-19 pandemic and it will be interesting to see if these biomarkers are able to distinguish Influenza from SARS-CoV in future validation studies.

Contributors

KB and NR contributed equally to this manuscript. KB performed the literature search and writing of the metabolomics portion. NR performed the literature search, wrote the clinical metabolomics portion and provided the clinical perspective. KB and NR jointly contributed to revision of the manuscript.

Declaration of Competing Interest

KB had full access to all data referred to in the study and had final responsibility for the decision to submit for publication. No funding sources are relevant for this manuscript.

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