

LC-MS biomarker diagnostics for neuroinflammatory disorders



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In a pilot study published in *eBioMedicine*, Bandothkar and colleagues aimed to develop and validate a diagnostic panel of inflammatory biomarkers for neuroinflammatory diseases by use of LC-MS/MS.¹ The adopted methodology is meticulously substantiated and the CSF-based panel of nine tryptophan-kynurenine and four nitric oxide pathway metabolites performs well in terms of sensitivity and accuracy to discriminate patients with acute encephalitis from healthy individuals.

Mirroring similar efforts in Alzheimer's disease,² this work aspires to enrich the chiefly clinical outcome assessment (COA)-based diagnostic instrumentarium for neuroinflammatory diseases with biological parameters. Hence, it addresses a highly pressing need in neuropsychiatry.

Immune-related aberrations are known to instigate and / or maintain neuropsychiatric disease states.^{3,4} Thus, scrutinizing a batch of inflammatory markers for their potential in differential diagnostics is an auspicious and valid research strategy. Several metabolites of the kynurenine pathway have modulating effects on glutamatergic signaling, which in turn plays a role in the pathophysiology of psychiatric and neurological disease.⁵ As such, this pathway is considered to instigate altered neurotransmission following immune dysregulation, hypothetically rendering kynurenine metabolites to be more promising biomarkers than nonspecific inflammatory markers like acute phase proteins (e.g. C-reactive protein) or cytokines (e.g. IL-6). Up to now however, none of the attempts to validate the diagnostic proficiency of individual inflammatory biomarkers have been fruitful. Bandothkar et al. aimed to bypass this

impasse by directing their research efforts towards multiplex biomarker panels which hypothetically reflect a 'biological fingerprint' more specific to the disparate illnesses.

A good biomarker is of diagnostic use in clinical practice when it discriminates between different but similar diagnostic categories with sufficient sensitivity and specificity and is thereby reliably, precisely, and repeatably measurable at a low cost.⁶ Indeed, Bandothkar et al. robustly demonstrate their candidate biomarker panel to be both reliable and precise when comparing their combined patient sample to controls. Whether the composed 13-compound panel also proves discriminant amongst diverging neuropsychiatric pathologies should be further examined. Demonstrating this differentiating power may however prove to be challenging given that (1) different neuropsychiatric illnesses show substantial similarities in the alterations observed in these pathways and (2) these metabolites are also heavily vulnerable to lifestyle factors, as evidenced by modulating effects of BMI, glucose and lipid profiles and other metabolic markers, smoking habits and alimentation status; all of which are themselves affected in neuropsychiatric patients.⁷

On top of the diagnostic potential of the panel, other interesting research ventures should be considered. In both neurological and psychiatric conditions, tryptophan-kynurenine metabolites and nitric oxide pathway markers deviate over time dependent on age and duration of illness⁵ and as such could inform on illness phase and outcome. So, it would be enticing to delineate the panel's power as both prognostic and predictive biomarker by quantifying prediction accuracy of respectively disease outcome and treatment response. Furthermore, the panel would ideally prove to be equally adept for biomarking in peripheral blood as it is in CSF.

Due to relatively high acquisition, operational and maintenance costs, LC-MS does not lend itself to high-throughput diagnostic screening as easily as other automated analyses like immunoassays. Future work on the Bandothkar panel should thus demonstrate its diagnostic potency to excel to that level that it justifies such sizeable expenditures.

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Other predominant criticisms regarding application of LC-MS in biomarker discovery are the high false discovery rates that inevitably stem from simultaneously investigating thousands of features, and the lack of validation efforts. As the authors rightfully point out, their heterogeneous sample of $n = 10$ patients limits conclusiveness on the inflammatory panel's clinical applicability, discriminative power and diagnostic scope. That being said, it deserves merit in terms of validation as this pilot study sequels the author's previous work on untargeted metabolomics biomarker discovery in CSF of a homogeneous patient population with acute encephalitis.⁸ Here, 35 metabolites significantly differed in $\geq 75\%$ of the 14 acutely ill encephalitic patients vs. healthy control individuals, as defined by analysis of variance (ANOVA) with a p-value cut-off of 0.001. Cave, caution is warranted as also the mother study does not stand out in terms of sample size (while $n = 50$ cases vs. $n = 50$ is advised to detect a 1.2 fold change with 80% power and $p < 0.05^9$), nor does it apply the advised state-of-the-art statistics like Storey's q -values to control for the false discovery rate (FDR)¹⁰ for LC-MS based biomarker discovery endeavors.

To conclude, Bandodkar et al. present a meticulously detailed LC-MS/MS methodology to differentiate patients with various forms of encephalitis from healthy individuals, and validate their previously discovered panel of 13 kynurenine and nitric oxide pathway metabolites as discriminative case-control biomarkers. They thereby lead by example in ensuing preliminary and false-positivity prone untargeted -omics data with a targeted validation trajectory in a clinically less homogeneous patient sample. Thorough and elaborate follow-up research still needs to reveal the panel's proficiency in a substantially larger, randomized patient sample. Crucially, discriminative power should be punctiliously established amongst different neuroinflammatory diagnoses. It would further be of interest to investigate usefulness of the approach in differential diagnostics in the field of psychiatry and to evaluate the panel's performance as prognostic and / or predictive biomarker.

Contributors

Both authors contributed equally to this manuscript.

Declaration of interests

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