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Towards the first antibody-free serum biomarker assay for NAFLD

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Background & Aim: Liver biopsy is the reference standard for NAFLD diagnosis and staging. NAFLD can be assessed less invasively using immunoassays to detect serum biomarkers. However, biomarkers can degrade due to sample storage conditions and therefore may not be detected using these antibody-based assays. Detection of biomarkers by mass spectrometry (MS) overcomes this disadvantage.

Methods: MS and two dimensional gel electrophoresis (2DE) were used to find differences in the abundance of proteins among serum samples from patients with varying NAFLD and fibrosis stages (NAFL, NASH F0, NASH F1, NASH F3 and healthy individuals). The identified proteins are potential NAFLD biomarkers. Mass spectrometry was used to assay for biomarkers by detecting their tryptic peptides and fragments. We are the first and only lab to use a novel quantitation method with clinical samples. Unlike current liver disease biomarker tests, our approach is the only biomarker assay using a universal calibration mix.

Results: Using MS and 2DE, we identified several potential biomarkers for NAFLD which were checked by both targeted MS and Western blotting using serum from patients with varying stages of NAFLD. Our novel biomarkers look promising when compared to cytokeratin-18 and the protein markers used in NashTest. A mass spectrometry assay was developed for the most promising novel liver fibrosis biomarkers.

Conclusion: We are working towards the first ever antibody-free biomarker assay for NAFLD. Our assay is nine times faster than conventional quantitation by MS making our approach for absolute biomarker quantitation applicable for clinical use. In all current liver disease biomarker assays, different calibrations curves are required for each biomarker with each point on the curves being read separately and samples could be analyzed several hours after establishing a calibration curve by which time there is the possibility of instrument drift. Our assay overcomes these disadvantages since it is the only assay which can analyze all points of the calibration curve and determine the absolute concentration of the NAFLD biomarker in a single acquisition. Our assay may help reduce the need for invasive liver biopsies in NAFLD patients.

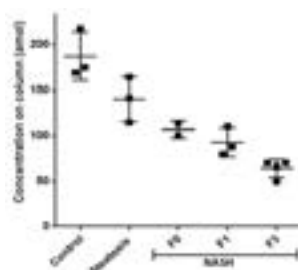


Figure 1: Quantitation of a novel NAFLD biomarker by MS

Biography

Bevin Gangadharan is a Research Associate in the Oxford Antiviral Drug Discovery Unit, University of Oxford headed by Professor Nicole Zitzmann. The main focus of his current research is to use proteomics to identify and validate novel serum biomarker candidates for NAFLD. He carried out his DPhil with Professor Nicole Zitzmann where he identified serum biomarker candidates for liver fibrosis in hepatitis C patients.

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