

Exploring the use of cICAT for the discovery of biomarkers in plasma
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Rapid development in protein separation and mass spectrometry technologies has created increasing interest in use of these tools for identification of disease markers in human plasma. Such markers offer the possibility to rapidly carry out multiple non-invasive tests compared to current diagnostic approaches for many disorders. The cleavable ICAT (cICAT) reagent allows enrichment and quantitative analysis of peptides for comparison of control and disease samples. We have used plasma from stroke patients to assess the advantages and disadvantages of this technique and for subsequent identification of disease markers. We also report a directed MS/MS approach to fragment target ions of interest and a normalisation method for the quantitative data obtained.

The abundance of albumin and immunoglobulins within plasma presents a considerable analytical challenge, as biomarkers of interest are expected to circulate at concentrations several orders of magnitude below the level of these proteins. To address this issue we have depleted the albumin and IgG from stroke and control patient plasma using the Aurum Kit (BioRad) and compared depleted samples with non-depleted samples. cICAT was carried out on plasma from stroke patients. Up to 60 different proteins were identified by nLC/MS/MS and three proteins exhibited quantitative changes in abundance in stroke patients compared to controls. These proteins all being involved in the blood clotting cascade. Two of these proteins were also found to be increasing in stroke using SELDI-TOF.

Direct comparisons of crude and depleted samples show at least 50% removal of albumin and IgG. However, this still has limitation for detection of very low abundant proteins due to the presence of dominant classical plasma proteins. Nevertheless, we were able to obtain quantitative data by cICAT. The number of protein identifications can be significantly increased, for example by increasing the amount of plasma used, by depleting more of the abundant plasma proteins, and optimising the separation prior to MS. Preliminary results of our recent work focusing on using the Agilent depletion column and 1D SDS PAGE will be presented.