Towards Immuno-profiling of Complex Biological Fluids in Patients Recovering from Major Surgery

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I certify that all material in this thesis which is not my own work has been identified and that no material has previously been submitted and approved for the award of a degree by this or any other University.

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Abstract

The conventional biochemical diagnosis of disease using isolated blood biomarkers must be revisited by clinicians, replacing it with a multi-biomarker, personalised profile of human health. A label-free nanoparticle array technology, Liscar, has been developed. It is capable of performing rapid, multi-biomarker assays from complex biological samples which, if employed to assay the Complement cascade of the innate immune system, has the potential for a novel systemic profile of patient health.

An assay for IgG has been developed on the Liscar platform with a detection limit of 380 ± 100 ng/mL IgG in model sera. Furthermore, addition of a chaotropic agent to the complex sample is shown to improve the accuracy of the IgG assay. Competitive binding between nonspecific interfering proteins and specific target analytes (IgG) at the sensor surface is studied, and a quantitative mathematical model is developed to analyse the data, yielding evidence for the active displacement of albumin by IgG-antigen binding.

With a sensitive, accurate multi-biomarker detection platform, a systemic profile of patient health may be possible by examination of the Complement cascade. The Complement system can be activated by a variety of immunological challenges, causing large numbers of activation biomarkers to be produced quickly. Assays for three activation markers, C3d, TCC and Bb are developed, with detection limits of 0.864 ACS Units, 2.32 ng/ml and 54.7 ng/ml respectively.

Complement activation was tested in a prospective cohort study of 45 patients undergoing major abdominal surgery. Patient recovery was monitored from admission to ~60 hours postoperatively by Complement activation and consumption using C3d, TCC, Bb, C3 and C4 as biomarkers. A response profile was obtained for the entire cohort for C3 and C4 assays by normalising with respect to individual analyte levels on admission, against which individual responses are compared. 22% of patients in the study suffered postoperative complications, and 73% showed Complement activation by increased levels of C3d, as expected from the initial trauma of surgery. Expansion of the trial is needed to establish clinical significance and utility, especially in relation to the presymptomatic diagnosis of disease.

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