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### Multiplex proximity extension assay of 425 candidate biomarkers in the sera of bladder cancer patients: Correlation with stage and outcome

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Abstract Title: **Multiplex proximity extension assay of 425 candidate biomarkers in the sera of bladder cancer patients: Correlation with stage and outcome**

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Body:

**Introduction & Objectives** Serum protein biomarkers that inform on UBC stage and outcome could accelerate and improve clinical decision making but this requires a thoroughly validated high-performance biomarker or panel of biomarkers. Unbiased discovery of serum biomarkers by shotgun proteomics is challenging due to their low abundance in a protein-rich milieu and candidate based discovery is slow and expensive due to the number of individual immunoassays required. In this study we make use of a recently developed multiplex assay platform to measure 425 proteins in the serum of bladder cancer patients with the aim of identifying novel staging and prognostic biomarkers.

**Material & Methods** All sera were collected as part of the Bladder Cancer Prognosis Programme (ethics approval 06/MRE04/65). Patients whose diagnostic cystoscopy indicated primary UBC were recruited to the study and blood collected prior to TURBT. Ultimately, some of the patients were diagnosed with non-malignant conditions and these serve as non-cancer controls. All sera were prepared following a standard operating procedure and stored at -80°C. The patients used in this study comprised 10 non-cancer controls, 10 G1pTa, 10 G3pTa, 30 G3T1 and 30 G3T2+. There were no significant differences in age and gender between the groups. Proseek multiplex immunoassays (OLINK Proteomics) were used to measure 444 proteins of which 425 passed QC. The statistical analysis was done using R statistical software 3.2.5. Five samples were excluded from the analysis using PCA analysis. Multiple testing correction was done with the Benjamini-Hochberg method and an adjusted p-value < 0.05 was considered significant. We used parametric tests and examined any significant hits for heteroscedasticity by evaluation of QQ and box-plots.

**Results** 425 proteins were successfully measured in the serum of 9 non-cancer controls and 76 UBC patients. 10 proteins were significantly associated with UBC and are listed in order of p-value (smallest first): nectin-4, syndecan-1, kidney injury molecule 1, macrophage colony-stimulating factor 1, matrilysin, thrombopoietin, latency-associated peptide transforming growth factor beta-1, C-C motif chemokine 23, heat shock protein beta-1 and protein AMBP. The serum levels of these proteins all showed a positive association with increasing stage although none showed absolute discrimination between stages. High levels of nectin-4, syndecan-1, macrophage colony-stimulating factor 1 were significantly associated with shorter UBC specific survival times.

**Conclusions** We have studied the relationship between bladder cancer and the serum concentrations of 425 proteins. Those which reach statistical significance include known biomarkers and new candidates that may warrant further investigation. Bladder cancer causes many biologically plausible changes in the serum proteome which may aid in UBC staging and prognosis.