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Plasma mass spectrometry-based proteomic biomarker discovery for endometriosis

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Background & Aim:

Endometriosis diagnosis requires surgical visualisation of lesions and histopathology. To date, no non-invasive diagnostic test has been validated. Discovery research using sensitive high-throughput mass spectrometry (MS) has the potential to improve identification of clinically-relevant plasma proteins.

The aim of this study was to undertake MS-based proteomic biomarker discovery using minimally-invasive plasma specimens from patients with and without endometriosis.

Method:

Blood was collected from patients attending the pelvic pain clinic (Royal Women's Hospital) for laparoscopy (investigation/treatment of suspected endometriosis) (HREC #10-43/16-43). Information was collected from patients surveys (demographics/symptoms), medical records and surgeon/pathology reports. Endometriosis status was confirmed using histopathology.

Plasma (EDTA-treated, stored at -80°C) from 60 endometriosis cases and 20 non-endometriosis controls were included in the study. Following trypsin digestion, plasma peptides were analysed using a timsTOF Pro (Bruker Daltonics) mass spectrometer. MaxQuant and Perseus were used for data processing and analysis (**Fig 1**).

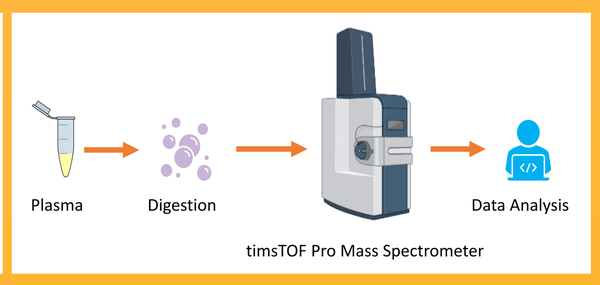

Figure 1: Schematic diagram showing the workflow of plasma proteomics.

Results:

A total of 470 proteins were quantified and identified* from plasma samples.

Compared to non-endometriosis controls, 26 proteins were significantly altered with endometriosis (FDR <0.05).

- 17 proteins demonstrated a higher fold-change in the endometriosis group (**Figure 2**).
- 9 proteins demonstrated reduced abundance in the endometriosis group compared to controls (**Fig 2**).

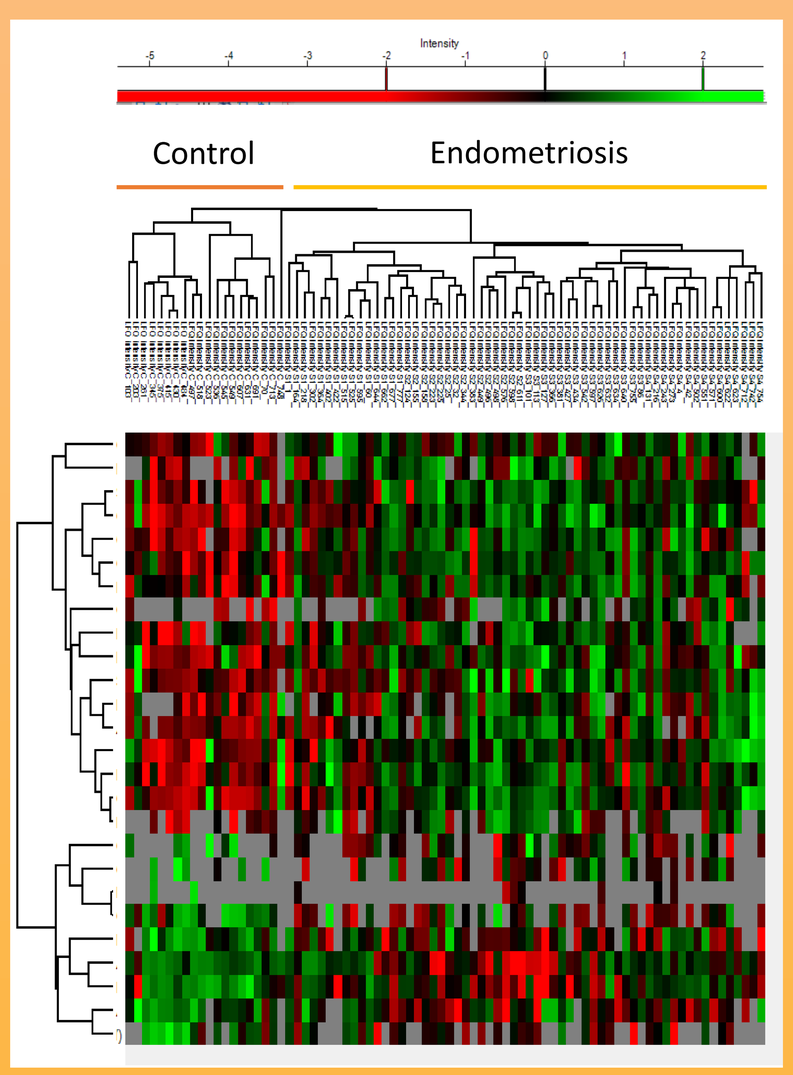

Figure 2: Heatmap visualisation (Z-Score normalised) of the plasma protein profiles for control and endometriosis groups. A dendogram of patient groups is shown on top. On the left, hierarchical clustering of protein expression profiles identified 17 proteins increased and 9 protein decreased in the endometriosis group.

We also identified* 15 proteins that were absent in controls, but present in endometriosis cases (**Figure 3a**).

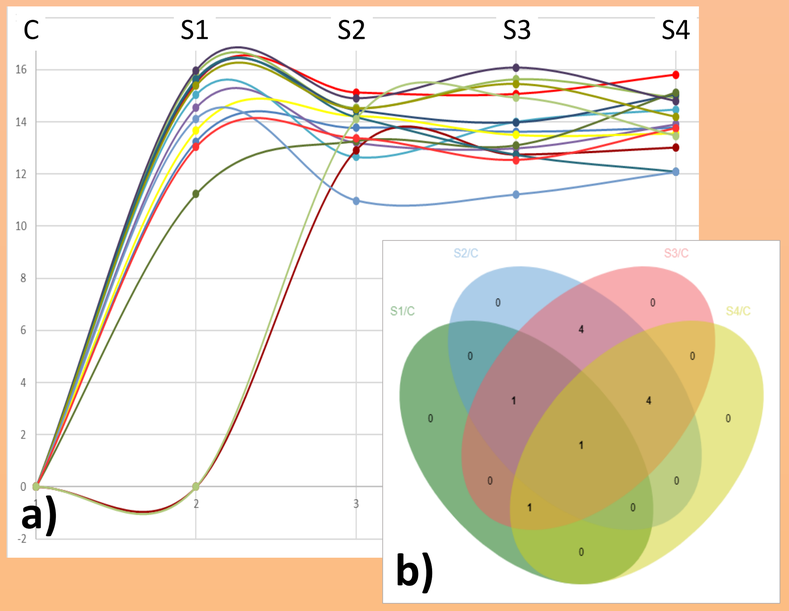

Figure 3: **a)** A plot displaying 15 plasma protein profiles that were absent in control (C) samples, but were present in endometriosis groups (S1, S2, S3, S4). **b)** A Venn diagram splitting the number of significantly different proteins by individual stages of disease compared to control (green: S1 - Control, blue: S2 - C, pink: S3 – C, yellow: S4 - C).

By grouping endometriosis cases by stage of disease, unique protein signatures were revealed (**Figure 3b**).

The proteins of interest belonged to biological processes associated with immunity (complement system), angiogenesis, proliferation and cholesterol metabolism.

Conclusions:

High-throughput MS-based proteomic biomarker discovery successfully identified 470 proteins in an endometriosis cohort. Case-control analysis revealed significantly different protein expression profiles in association with endometriosis. Further validation in an independent sample set is underway. Biomarker discovery using plasma proteomics offers a minimally-invasive approach to endometriosis diagnosis.

**Protein identifications have intentionally been left out for protection of intellectual property.*

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