ABSTRACT

Endometriosis is a chronic, estrogen-dependent gynaecological disease characterized by ectopic growth of endometrial-like tissue and associated with chronic inflammation, immune dysregulation, and extensive tissue remodelling. Despite significant advances in research, its diagnosis still relies primarily on invasive procedures, highlighting the urgent need for reliable, non-invasive biomarkers.

This doctoral thesis aimed to comprehensively characterize and directly compare the proteomic profiles of extracellular vesicles (EVs) derived from peritoneal fluid (PF) and plasma of patients with endometriosis and controls, in order to elucidate both local and systemic molecular mechanisms of the disease and to identify potentially diagnostic biomarker. EVs were isolated using size-exclusion chromatography (SEC) and rigorously characterized in accordance with the Minimal Information for Studies of Extracellular Vesicles (MISEV2023). High-resolution, quantitative proteomics based on Tandem Mass Tags (TMT), combined with imaging flow cytometry (IFCM), enabled multidimensional and cross-platform analysis of EV cargo and surface phenotypes.

Proteomic analysis identified over 3,500 proteins in PF-derived EVs and 2,600 proteins in plasma-derived EVs, including a subset of differentially expressed proteins (DEPs) distinguishing endometriosis patients from controls. PF-derived EVs demonstrated a markedly stronger discriminatory capacity, reflecting localized pathological processes within the peritoneal microenvironment, including immune activation, oxidative stress, iron metabolism, extracellular matrix (ECM) remodelling, and cellular adhesion. In contrast, plasma-derived EVs captured more subtle yet consistent systemic alterations associated with immune modulation, vesicle trafficking, and epithelial remodelling.

Importantly, a shared subset of EV-associated proteins was consistently detected across both PF and plasma, revealing a common molecular signature linking local disease activity with its systemic manifestations. Key proteins included inflammatory mediators (e.g., S100 family), ECM components (e.g., COL1A1/2, VCAN), and adhesion-related molecules (e.g., SDC4), suggesting coordinated and multi-compartmental regulation of endometriosis-related processes.

Integration of proteomic profiling with IFCM enabled cross-platform validation of selected EV markers, including CD81 and MUC1 in plasma and HLA-DR in PF, supporting their robustness and translational potential. Furthermore, dynamic changes in EV-associated

markers following surgical treatment indicate their potential utility for monitoring disease activity.

Overall, this study demonstrates that EVs serve as biologically informative carriers of disease-associated signals and, importantly, provide a unique interface between local and systemic aspects of endometriosis. The applied integrative analytical strategy establishes a comprehensive framework for EV-based biomarker discovery and highlights EV-associated proteins as promising targets for the development of non-invasive diagnostic approaches.