Integrating Raman micro-spectroscopy and Machine Learning to uncover biochemical signatures of hypertensive nephropathy in human kidney tissue

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Raman micro-spectroscopy (RS) is a non-destructive, label-free analytical technique that provides detailed molecular information with micrometer-scale spatial resolution. Owing to its high chemical specificity, RS has been increasingly applied in biomedical research to investigate disease mechanisms and support diagnostic applications[1]. Its use on formalin-fixed, paraffin-embedded (FFPE) tissues is particularly promising, as clinical biobanks contain vast archives of well-annotated FFPE samples linked to patient outcomes. Although residual paraffin presents a technical challenge for Raman analysis, recent advances in spectral processing and machine learning enable the extraction of biologically relevant information[2]. In this context, a workflow combining RS and machine learning is proposed to analyze FFPE renal biopsy specimens, focusing on second-derivative spectral features to mitigate paraffin interference. This approach is applied to the study of hypertensive nephropathy (HN), a common chronic kidney disease caused by long-standing arterial hypertension. The disease is characterized by progressive vascular, glomerular, and tubulointerstitial damage, including arteriolosclerosis, glomerulosclerosis, microvascular rarefaction, epithelial–mesenchymal transition, and interstitial fibrosis, ultimately leading to chronic kidney disease and end-stage kidney failure. Because the disease often remains clinically silent in its early stages, improved tools for early diagnosis and prognostic stratification are critically needed[3]. By integrating RS with computational analysis of FFPE biopsy samples, this approach has the potential to reveal subtle biochemical alterations associated with disease progression, identify novel molecular biomarkers, and improve diagnostic accuracy, thereby enhancing both retrospective research and clinical evaluation of hypertensive kidney disease.

The study cohort was established in collaboration with the Department of Medicine, Haukeland University Hospital, University of Bergen (Norway). It comprised twenty hypertensive patients who underwent diagnostic kidney biopsy due to suspected renal damage, indicated by altered serum creatinine, reduced eGFR, and proteinuria or albuminuria. Renal tissues were processed as FFPE sections and analyzed by RS. Patients were stratified according to disease stage and progression into five groups: healthy controls (C), early stable (ES), early progressive (EP), late stable (LS), and late progressive (LP) disease. Late-stage disease was defined by an eGFR $\leq$45 ml/min/1.73 m² at biopsy, while disease progression (stable or progressive) was assessed over a median follow-up of nine years based on annual eGFR decline or initiation of renal replacement therapy. By integrating RS-derived molecular profiles with long-term clinical outcomes and three different machine learning algorithms, specifically k-nearest neighbors (KNN), random forest (RF), and support vector machines (SVM), this workflow aims to identify biochemical markers associated with disease stage and progression, offering new insights into hypertensive nephropathy pathogenesis and supporting the development of improved diagnostic and prognostic tools.

References

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