Toward non-invasive stratification of neurodegenerative disorders through Raman analysis of salivary extracellular vesicles

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Neurodegenerative disorders (NDDs) need minimally invasive biomarkers capable of supporting earlier diagnosis, patient stratification, and disease monitoring. Salivary Extracellular Vesicles (SEVs) are gaining attention as a promising source of disease-related molecular information, since they carry biomolecules linked to neurodegeneration and neuroinflammatory processes. Within the MINERVA study (ClinicalTrials.gov Identifier: NCT06869135), we investigated Raman spectroscopy (RS) as a rapid, label-free, and valuable tool for the biochemical characterization of SEVs across neurodegenerative conditions.

For this purpose, SEVs were isolated from saliva samples collected from subjects with Parkinson's disease (PD), Atypical Parkinsonisms (AtP), and REM sleep behaviour disorder (RBD), a prodromal condition associated with α-synucleinopathies.

Isolation from saliva was performed by size exclusion chromatography coupled with ultracentrifugation, and the repeatability and robustness of the protocol were verified in accordance with the MISEV2023 guidelines prior to RS acquisition.

SEV samples were air-dried on calcium fluoride substrates and analyzed by RS following a previously optimized protocol.

Preliminary RS measurements yielded detailed biochemical fingerprints of the SEV cargo, with contributions from proteins, lipids, and further biomolecular components, and revealed biochemical differences among the investigated groups.

Multivariate analysis revealed partial overlap across groups, pointing to biochemical features shared among the spectra. Despite this, classification analysis reached good overall discrimination between the clinical groups, with higher accuracy for PD and AtP subjects. Misclassification concerned mainly RBD individuals, a result that may reflect the heterogeneous, prodromal nature of this condition.

Taken together, these preliminary findings support RS-based analysis of SEVs as a non-invasive strategy for the molecular stratification of neurodegenerative disorders and prodromal neurodegenerative states. Studies on larger cohorts are ongoing to validate the robustness and clinical applicability of the approach.