

# Quantitative detection of circulating NfL and GFAP during the follow-up of stroke patients for the prediction of clinical outcome

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The evaluation of patients after stroke remains a major unmet clinical need. Neurofilament light chain (NfL) and glial fibrillary acidic protein (GFAP) are intermediate filament proteins released upon brain injury by neurons and astrocytes, respectively and they draw interest in clinics as biomarkers of patient's damage and recovery. This prospective longitudinal multicenter study aimed to quantify serum concentrations of NfL and GFAP at different time points after ischemic stroke and to evaluate their relationship with patient's outcome.

Ischemic stroke cases were enrolled within 24 hours from symptom onset. Blood samples were collected in the acute phase (D1) and after 7 (D7), 30 (M1), 90 days (M3) from onset. Serum concentration of NfL and GFAP were analyzed by Single Molecule Array (SiMoA<sup>®</sup>) immunoassay. For each observation period, neurological deficit, residual disability and neurorehabilitation scales were assessed by clinical evaluation.

Thirty-six patients were enrolled (43.2% with mild stroke, 45.9% with moderate stroke, 10.8% with severe stroke). The analysis revealed a significant increase in serum NfL and GFAP levels in the acute phase following the ischemic event ( $p < 0.0001$  vs. healthy controls) and a progressive decline at M1 and M3. Serum NfL levels were associated with GFAP concentrations at the time points analyzed, especially at D7 (Spearman's  $\rho = 0.89$ ,  $p < 10^{-5}$ ). A prospective correlation was found between both NfL and GFAP levels at D7 and the clinical/rehabilitation scales (NIHSS, TCT, FAC, FIM) at M1 and M3.

Preliminary results indicate that NfL and GFAP correlate with motor and cognitive functional recovery in stroke patients, supporting a prognostic role for these biomarkers. As perspective, the development of new biosensor platforms able to identify and quantify simultaneously multiple brain biomarkers in blood and with high sensitivity would be a precious tool to improve the clinical management of stroke patients.