ImmunoTools *FlowISiAM* Award 2024

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Macrophage-mediated α-synuclein processing: a biomarker for Parkinson's disease progression

Background: The diagnosis of Parkinson's disease (PD) currently relies on clinical diagnostic criteria and neuroimaging. Additionally, rating scales related to motor and nonmotor features are used for monitoring. However, these scales are often subjective and influenced by periodic fluctuations in symptoms and the effectiveness of symptomatic therapies. While neuroimaging techniques like dopamine transporter-single photon emission CT offer a quantifiable measure of disease progression, they are limited by practicality and cost. Furthermore, protein biomarkers based on cytokines and dopamine metabolic products have yielded inconsistent results.

Alpha-synuclein (SNCA) is an abundant presynaptic protein that functions as a SNARE-complex chaperone involved in regulating synaptic neurotransmission. Converging evidence implicates SNCA in the pathogenesis of PD and other alpha-synucleinopathies. Point mutations, gene duplications, and triplications at the SNCA locus have been identified in several families with autosomal dominant early-onset PD [1-3]. Viral-mediated overexpression of wild-type or mutant SNCA within nigral neurons of rodents and non-human primates resulted in progressive motor dysfunction resembling the motor symptoms observed in PD patients [4-6]. Moreover, SNCA is a major component of neuronal cytoplasmic deposits known as Lewy bodies (LB) found in sporadic PD, dementia with Lewy bodies (DLB), and other proteinaceous inclusions in both glial and neuronal cells in multiple system atrophy (MSA) [7]. Misfolded, post-translationally modified, and aggregated forms of SNCA are released from neurons, contributing to the spread of pathology in a prion-like manner.

While the exact causes of SNCA neurodegeneration remain unknown, SNCA-induced neuroinflammation is believed to play a significant downstream role. Specifically, SNCA has been shown to lead to an expansion and unique damage-associated activation of a specific

subset of CNS-resident macrophages, the border-associated macrophages. These activated macrophages further contribute to pathology by promoting T-cell recruitment, infiltration, and antigen presentation [8].

Objectives: This study proposes a novel blood macrophage-based approach to investigate its potential for diagnosing PD at both the initial disease stage and, crucially, during the preclinical phase. We further aim to evaluate its utility in monitoring disease progression. To achieve this objective, we will utilize the *FlowISiAM* technique in collaboration with ImmunoTools and INVIGATE. This technique will allow us to evaluate circulating immune cell ratios, phagocytosed biomarkers specific to SNCA epitopes, and other endogenous proteins directly implicated in PD pathogenesis.

Experimental Design & Methods: This study will initially employ a case-control design to compare a cohort of patients with PD (n=20) and healthy controls (n=20) following established methodologies [9-11]. All patients and control subjects will be recruited from the local University Hospital clinic. PD diagnosis will be established by two movement disorder specialists following the Movement Disorder Society (MDS) criteria. For all participants, essential demographic and clinical information will be collected, including a standardized study questionnaire for motor and nonmotor manifestations of the disease, along with established rating scales to assess disease severity. Additionally, patients will undergo brain MRI or CT scans to exclude any brain vascular lesions that could mimic the clinical phenotype of PD. The control group will be age-matched to the PD cohort comprising spouses or unrelated companions of the PD patients. These individuals will be screened to ensure they have no known neurological diseases, significant comorbidities, or family history of PD.

Cooperation partners: This study will leverage collaborations with two key partners to achieve its goals. ImmunoTools will provide expertise in setting up the *FlowISiAM* assay in-house. Additionally, they will supply essential antibodies and reagents for cytometric immunophenotyping of blood samples. We will utilize our antibodies targeting SNCA and other PD-associated proteins alongside those developed by ImmunoTools. Furthermore, INVIGATE will play a crucial role by preparing custom monoclonal antibodies targeting the specific epitopes we have identified as most informative.

Impact: This study aims to develop a non-invasive blood test for PD diagnosis, potentially enabling earlier intervention and monitoring disease progression. The ability to identify preclinical cases could revolutionize PD management and reduce the healthcare burden.

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