ImmunoTools *FlowISiAM* Award 2024

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Glycans as biomarkers for Parkinson's disease

Parkinson's disease (PD) is the second most common neurodegenerative disease [1]. Despite efforts towards understanding the neuropathological mechanisms involved in neuronal degeneration, the pathways involved are still not fully understood in detail. For this reason, there is still no therapy that can cure or slow down the progression of the disease. The only therapeutic approaches available merely alleviate symptoms but come with adverse side effects, especially as the dosage increases with disease progression stages. Furthermore, there is still no biomarker available to aid in clinical diagnosis. As PD shares some similarities with other neurological disorders with common symptomatology, the absence of a biomarker sometimes delays appropriate medication, leading to a decrease in the quality of life for patients.

Glycosylation is the most common post-translational modification, affecting protein function and cell-cell recognition. It is a vital modification since aberrant glycosylation can condition the pathological role of proteins, as well as alter cellular communication and induce inflammatory responses, including in the brain [2, 3]. Recently, with the advancement of new technologies and analytical methodologies, there has been growing interest in studying glycosylation in neurodegenerative diseases. The few studies published to date show that there may be a pattern of aberrant glycosylation in PD patients, both in the brain and in the blood [4-8]. However, the translation of these findings into the identification of a biomarker has not yet been achieved.

Here, we aim to assess the expression of glycans by mass spectrometry and/or lectin binding assays on peripheral blood cells membranes to identify a new biomarker for the disease. In parallel, we will evaluate the expression of key enzymes responsible

for the glycosylation patterns under study, by western blot and qRT-PCR. For this, we will select a cohort of patients and matched controls from Lisbon area Hospitals. Patients' selection will be defined according to the UK Brain Bank Clinical Diagnostic Criteria, and patients will be stratified according to the disease stage. Age- and gender-matched healthy subjects will also be selected by the medical team.

The presence of glycans in peripheral blood lymphocytes and monocytes from patients with PD will be evaluated using flow cytometry-based staining or *FlowISiAM* technology (ImmunoTools). Glycans expression will be correlated with peripheral indicators of pathology and inflammation. We will perform flow cytometry with blood leukocytes to detect for example CD11c, CD8a or B220 in combination with CD11b, CD86, MHC II and glycans, for the analysis of cell type/inflammation marker/glycan. Expression of fucosyltransferases, sialidase, fucosidase, and cytokines in leukocytes will also be determined, by flow cytometry or ELISA, together with Brain-derived neurotrophic factor (BDNF), Glial cell-derived neurotrophic factor (GDNF). PD markers of pathology such as α-synuclein and Tau will also be determined.

The possibility that monocytes may contain cell debris from the central nervous system could provide valuable insights into glycan expression and correlation with inflammation in the brain through a non-invasive method.

The results obtained will be stratified according to age, gender, disease progression and medication. We hope to identify glycans as a potential complementary biomarker for PD, offering supplementary insights when integrated with other markers and clinical assessment. The correlation of glycans levels in the blood with disease progression will add depth to the overall diagnostic or prognostic assessment in PD.

Cooperation partner: Prof. Castro Caldas group will cooperate with ImmunoTools to establish *FlowISiAM* analysis at the iMed.Ulisboa. ImmunoTools will share specific know-how for computer-aided scoring from *FlowISiAM* raw data for optimal test resuts.

References

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ImmunoTools *FlowISiAM* AWARD for **Margarida Castro-Caldas** includes antibodies for *FlowISiAM*, know how transfer and protocol, support regarding selection of specific antibodies against specific biomarkers from INVIGATE, expert assistance in evaluating the results obtained, and integration into the ImmunoTools *FlowISiAM* network.

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