ImmunoTools *FlowISiAM* Award 2024

Juan Manuel Maler, MD, Privatdozent

Universitätsklinikum Erlangen, Psychiatrische und Psychotherapeutische Klinik, Schwabachanlage 6, 91054 Erlangen, GERMANY

Analysis of intracellular β-Amyloid, PhosphoTau and synaptophysin in activated blood monocytes as potential biomarkers for Alzheimer's disease.

Alzheimer's disease (AD) is a common, slowly progressive neurodegenerative disorder affecting at least 40 million people worldwide. Its neuropathological hallmarks are the deposition of β-amyloid peptides in plaques, the aggregation of hyperphosphorylated tau protein (ptau) in intraneuronal neurofibrillary tangles and a variety of inflammatory processes involving microglia, but also cells of the peripheral immune system, in particular monocytes/phagocytes. Monocytes interact with β-amyloid deposits in cerebral vessel walls (amyloid angiopathy) or can cross the blood-brain barrier and migrate directly into plaque zones. The extent of these processes and the cellular involvement of monocytes appears to depend on the state of the blood-brain barrier and thus on age or progression of AD.

In this project we will investigate the extent to which monocytes that have phagocytosed β-amyloid or ptau neurofibrils or synaptic proteins recirculate into the bloodstream and how this correlates with blood-brain barrier impairment. As a next step, we will investigate whether a blood or monocyte-based diagnostic approach can be developed, that is less complex than the currently used cerebrospinal fluid-based AD diagnostics.

Using the *FlowISiAM* technique in collaboration with INVIGATE and *ImmunoTools* and building on many years of joint development and previous experience (Dr. Sebastian Krause) we will evaluate phagocytosed biomarkers of β-amyloid deposition (e.g. Aβ oligomers, fibrillar Aβ), tauopathy (e.g. ptau) and synaptic damage (e.g. synaptophysin) in defined monocyte subpopulations and correlate them with CSF biomarkers of AD (Aβ1-42, Aβ1-40,

phosphoTau181, totalTau) and biomarkers of blood-brain barrier dysfunction and vascular damage (e.g. albumin ratio, soluble platelet-derived growth factor receptor (PDGFRs)).

Experimental Design & Methods: We will compare a cohort of patients (n=20) from our memory clinic who have completed neuropsychological testing, MRI and neurochemical assessment and have an Erlangen Score of 3-4 (amyloidopathy (A+) and tauopathy (T+) according to CSF analysis) with a cohort of patients (n=20) with Erlangen Score 0 (A-, T-).
In addition, samples from our local biobank from a large cohort of well-characterised patients (n=900) can be used to compare soluble biomarkers.

Impact: The intended investigations will provide new insights into whether phagocytosed β-amyloid and synaptic epitopes can be detected by *FlowISiAM* analysis and how they correlate with individual levels in CSF. Furthermore, we look forward to obtain confirmation of recently obtained results for principal traceability of AD related markers by *FlowISiAM* in peripheral blood monocytes/macrophages.
If successful, we anticipate a promising foundation for a novel blood-based test concept early diagnosis of AD.

Cooperation partner: The group of Privatdozent Dr. Juan Manuel Maler will work together with *ImmunoTools* to adjust the experimental and instrumental set-up to conduct *FlowISiAM* analysis. Furthermore, *ImmunoTools* and INVIGATE will provide previously developed molecular probes from a proprietary collection of mAbs to AD-related antigens as well as other antibody reagents for *FlowISiAM* analysis. Privatdozent Dr. Manuel Maler and Dr. Sebastian Krause (INVIGATE) intend to further expand the work on the development of optimized monoclonal antibodies for detection of AD related molecular signatures within the *FlowISiAM* setting.

References

Berriat F, Lobsiger CS, Boillée S. The contribution of the peripheral immune system to neurodegeneration. Nat Neurosci. 2023 Jun;26(6):942-954. doi: 10.1038/s41593-023-01323-6. Epub 2023 May 25. PMID: 37231108.

Juul-Madsen K, Parbo P, Ismail R, Ovesen PL, Schmidt V, Madsen LS, Thyrsted J, Gierl S, Breum M, Larsen A, Andersen MN, Romero-Ramos M, Holm CK, Andersen GR, Zhao H, Schuck P, Nygaard JV, Sutherland DS, Eskildsen SF, Willnow TE, Brooks DJ, Vorup-Jensen T. Amyloid-β aggregates activate peripheral monocytes in mild cognitive impairment. Nat Commun. 2024 Feb 9;15(1):1224. doi: 10.1038/s41467-024-45627-y. PMID: 38336934; PMCID: PMC10858199.

Montagne A, Barnes SR, Sweeney MD, Halliday MR, Sagare AP, Zhao Z, Toga AW, Jacobs RE, Liu CY, Amezcua L, Harrington MG, Chui HC, Law M, Zlokovic BV. Blood-brain barrier breakdown in the aging human hippocampus. Neuron. 2015 Jan 21;85(2):296-302. doi: 10.1016/j.neuron.2014.12.032. PMID: 25611508; PMCID: PMC4350773.

Kurz C, Walker L, Rauchmann BS, Perneczky R. Dysfunction of the blood-brain barrier in Alzheimer's disease: Evidence from human studies. Neuropathol Appl Neurobiol. 2022 Apr;48(3):e12782. doi: 10.1111/nan.12782. Epub 2022 Feb 2. PMID: 34823269.

Ding R, Hase Y, Ameen-Ali KE, Ndung'u M, Stevenson W, Barsby J, Gourlay R, Akinyemi T, Akinyemi R, Uemura MT, Polvikoski T, Mukaetova-Ladinska E, Ihara M, Kalaria RN. Loss of capillary pericytes and the blood-brain barrier in white matter in poststroke and vascular dementias and Alzheimer's disease. Brain Pathol. 2020 Nov;30(6):1087-1101. doi: 10.1111/bpa.12888. Epub 2020 Aug 14. PMID: 32705757; PMCID: PMC8018063.