Novel Blood-derived Extracellular Vesicle-based Biomarkers in Alzheimer's Disease by the Proximity Extension Assay

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to characterize the EVs of AD patients as a biomarker for disease progression.

Methods: Blood samples were collected after obtaining signed informed consent (No. 0462-14-RMB) from 39 AD patients at three stages of disease severity and from 14 healthy controls (HC). Cerebrospinal fluid was collected from five patients and three HC. EV size and concentration were studied by Nano-tracking analysis. Membrane antigens were characterized by their cell origin as defined by flow cytometry. EV protein contents were screened by protein array, and miRNA content was screened by Nano-string technology and validated by RT-PCR.

Results: The AD patients' EVs were significantly smaller and the levels of neural cell markers were higher than EVs obtained from HC. Moderate or severe AD patients' EVs had a significantly higher level of the Myelin oligodendrocyte glycoprotein (MOG), compared to the EVs obtained from patients with mild AD (P = 0.0002 and P = 0.036). Levels of the EVs that expressed the axonal glycoprotein CD171 were significantly higher in the patients with severe AD compared to HC (P = 0.0066), possibly indicating injured apoptotic neural cells. There was also a significant increase in EVs originating from endothelial cells (labelled with CD31+ CD41-, P = 0.0115 and with CD144, P = 0.0276) in patients with moderate AD compared EVs obtained from the HC. A >2-fold increase was measured in the content of inflammatory cytokines (TNFα, IL8, IL-2, IFNγ) as was a >50% reduction in growth factors (FGF, EGF VEGF) and their receptors in the EVs of moderate AD patients. miR-146a-5p and several other miRNAs obtained from the EVs of severe AD patients had significantly low levels compared to HC.

Summary/Conclusion: The neural and endothelial damage severity as reflected by AD patients' EVs (antigen profiles cytokine and miRNA) may serve as a biomarker for disease dynamics.

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Novel Blood-derived Extracellular Vesicle-based Biomarkers in Alzheimer's Disease by the Proximity Extension Assay

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Introduction: Biomarkers capable of identifying complex pathways contributing to neuropathological development, especially in the early stages of Alzheimer's disease (AD), are lacking. Such biomarkers could be present in easily available fluids, such as blood, due to the breakdown of the blood–brain barrier (BBB) early in AD. However, the identification of specific and sensitive blood-based biomarkers is a challenging task. Therefore, extracellular vesicles (EVs) may provide a window into AD etiology and therapeutic targets, as brain-derived EVs have been shown to cross the BBB and are present in blood. As biomarkers, proteins are a potential source of relevant information relating to biological function. Thus, we investigated a subset of proteins hypothesized to be involved in neurological processes in plasma and EV samples using the Proximity Extension Assay (PEA).

Methods: EVs were isolated from platelet poor plasma from 10 healthy controls (HC), 10 patients with Mild Cognitive Impairment (MCI) and 10 patients with mild/moderate AD. Isolation was performed using centrifugation at 20.000 xg, 1 h, 4°C with a subsequent washing of the pellet at the same g-force. For the characterization of the EV isolates, Nanosight and western blotting (CD9) are performed. A neurology panel of 92 biomarkers were assessed in plasma and EVs using the PEA. Written informed consent was obtained from all study participants and the study was approved by The North Denmark Region Committee on Health Research Ethics (N-20150010).

Results: PEA showed no significant difference of protein levels comparing the three groups for the plasma samples. Interestingly, EV samples showed four statistically significant proteins; Siglec-9, CLM-1, CLM-6 and CD38, which were less expressed in the MCI and AD groups compared with the HC group with a false discovery rate adjusted p-values of 0.014, 0.024, 0.035 and 0.031, respectively. These proteins have been documented to be involved in neurotoxicity protection and inflammatory regulation.

Summary/Conclusion: Our preliminary results demonstrate that EVs, compared to plasma, hold potential as candidate diagnostic biomarkers in AD.

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Proteomic and transcriptomic profiting of extracellular vesicles isolated from immune-stimulated human primary astrocytes

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Introduction: Astrocytes are abundant glial cells in the central nervous system that provide supportive neuronal functions. They have critical roles in regulating