Mass spectrometric quantification of amyloid-beta in cerebrospinal fluid and plasma – Implications for Alzheimer's disease

Akademisk avhandling

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av

Josef Pannee

Fakultetsopponent:
Andrew N. Hoofnagle, MD, Associate Professor
Department of Laboratory Medicine, University of Washington, Seattle, WA, USA.

Avhandlingen baseras på följande arbeten:

I. Pannee J, Portelius E, Oppermann M, Atkins A, Hornshaw M, Zegers I, Hojrup P, Minthon L, Hansson O, Zetterberg H, Blennow K, Gobom J. A selected reaction monitoring (SRM)-based method for absolute quantification of Abeta38, Abeta40, and Abeta42 in cerebrospinal fluid of Alzheimer's disease patients and healthy controls. *Journal of Alzheimer's Disease. 2013;33(4):1021-32.*

II. Leinenbach A, Pannee J, Dulffer T, Huber A, Bittner T, Andreasson U, Gobom J, Zetterberg H, Kobold U, Portelius E, Blennow K, on behalf of the IFCC Scientific Division Working Group on CSF proteins. Mass spectrometry-based candidate reference measurement procedure for quantification of amyloid-beta in cerebrospinal fluid. *Clinical Chemistry. 2014;60(7):987-84.*

III. Pannee J, Tornqvist U, Westerlund A, Ingelsson M, Lannfelt L, Brinkmalm G, Persson R, Gobom J, Svensson J, Johansson P, Zetterberg H, Blennow K, Portelius E. The amyloid-beta degradation pattern in plasma-A possible tool for clinical trials in Alzheimer's disease. *Neuroscience Letters. 2014;573:7-12.*

IV. Pannee J, Gobom J, Shaw LM, Korecka M, Chambers EE, Lame M, Jenkins R, Mylott W, Carrillo MC, Zegers I, Zetterberg H, Blennow K, Portelius E. Round robin test on quantification of Aβ42 in CSF by mass spectrometry. *Alzheimer's & Dementia. In press, published online July 21th 2015.*

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Mass spectrometric quantification of amyloid-beta in cerebrospinal fluid and plasma – Implications for Alzheimer's disease

Josef Pannee

Department of Psychiatry and Neurochemistry, Institute of Neuroscience and Physiology, Sahlgrenska Academy at University of Gothenburg, Gothenburg, Sweden

ABSTRACT

Alzheimer's disease (AD) is the most common neurodegenerative disease among the elderly and accounts for 60-80% of all cases of dementia. Currently, the diagnosis of AD is based on cognitive tests and mental state exams, but the peptide amyloid-beta (Aβ) in cerebrospinal fluid (CSF) is increasingly used in clinical trials and settings. As for most protein and peptide biomarkers, quantification is performed using antibody-based techniques such as enzyme-linked immunosorbent assay (ELISA). However these immunoassays suffer from high variability in measurements of Aβ concentrations, hampering its use as a diagnostic marker.

The aim of this thesis was to develop an antibody independent method for absolute quantification of Aβ in human CSF, free of the specificity and reproducibility issues associated with antibody-based quantification. The method was based on solid-phase extraction (SPE) and liquid chromatography (LC)-tandem mass spectrometry (MS/MS). Stable isotope labeled Aβ peptides were used as internal standards, enabling absolute quantification. The method was first tested in a pilot study with CSF samples from AD patients and controls. As expected, the level of the 42 amino acid variant of Aβ ($Aβ_{1-42}$) was decreased in AD CSF as compared to controls ($p<0.01$). The results were similar to those obtained with conventional ELISA, and an even better separation between the groups was obtained when using the $Aβ_{1-42}/Aβ_{1-40}$ ratio. To investigate whether the antibody independent method would give similar results across different research centers, an inter-laboratory study was initiated which included three other laboratories using similar LC-MS/MS methods. Results showed good agreement and highlighted the importance of a certified reference material (CRM) to further increase the agreement between laboratories and MS methods. The method was further optimized, validated and published as a candidate reference measurement procedure (RMP). An RMP is required to set the value of a CRM used as a 'gold standard' to harmonize CSF Aβ measurements. To investigate if the large number of Aβ peptides in addition to $Aβ_{1-38}$, $Aβ_{1-40}$ and $Aβ_{1-42}$ found in CSF could also be found in human plasma, an immunoprecipitation-based method for enrichment of Aβ peptides was developed. Sixteen N- or C-terminally truncated Aβ peptides were reproducibly detected using matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) MS. While quantification of $Aβ_{1-38}$, $Aβ_{1-40}$ and $Aβ_{1-42}$ using LC-MS/MS showed no AD association, the method may be useful in clinical trials of drugs affecting amyloid precursor protein (APP) processing or Aβ homeostasis.

In summary, absolute quantification of $Aβ_{1-42}$ using the developed LC-MS-MS method overcomes many of the issues associated with antibody-based methods. The method is currently being considered for formal certification as a RMP to determine the absolute concentration of $Aβ_{1-42}$ in a CRM to harmonize CSF $Aβ_{1-42}$ measurements across techniques and analytical platforms.

Keywords: Alzheimer's Disease, Mass Spectrometry, Biological markers, Cerebrospinal fluid, Amyloid beta-Peptides

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