MEDIZINISCHES LABOR BREMEN

Plasma based biomarkers for assignment to early Alzheimer treatment

A. Gerritzen[1], S. Gerritzen[1], B. Marcos Morgado[2], H. Esselmann[2], N. Hansen[2] and J. Wiltfang[2, 3]

[1]MVZ Medizinisches Labor Bremen GmbH, Haferwende 12, 28357 Bremen
[2]Universitätsmedizin Göttingen, Klinik für Psychiatrie und Psychologie, Von-Siebold-Straße 5, 37075 Göttingen
[3]Deutsches Zentrum für Neurodegenerative Erkrankungen (DZNE), Von-Siebold-Straße 3a, 37075 Göttingen

Abstract

Neurodegenerative disorders, esp. Alzheimer's disease, are a growing problem in a population, which is characterized by increasing average lifespan and urn-shaped age pyramid. So far, all therapeutic approaches revealed only limited benefits for the patients. Both the well-established NMDA antagonists (i.e. memantine) and acetylcholine esterase inhibitors (i.e. rivastigmine, galantamine, donepezil), but also the new antibody derived therapies capable to reduce intracerebral amyloid plaques (i.e. lecanemab, donanemab) show best efficacies when installed early in the progress of disease. This stresses the urgent need for diagnostic tools capable to identify those patients who will profit most from therapeutic measures, which are very expensive and hampered by adverse side effects. In recent studies plasma based biomarkers turned out as promising items to support favorably the classical functional tests, PET scans and CSF based biomarker assays, but high precision, sensitivity and specificity of the assays are mandatory. More over the best biomarker profile (i.e. ß-amyloid 1-40 and 1-42, phospho-Tau 181 and 217) and reliable interpreting rules for the results, based on the respective reference values, are just being established. Based on our long term knowledge in CSF based dementia diagnostics, we here describe the establishment and experiences with the chemiluminescence enzyme immuno assays (CLEIA) on the automated Lumipulse™ platform (Fujirebio) for the detection of plasma based biomarkers for Alzheimer's disease in a routine laboratory setting.

Enhanced Chemiluminescence: Lumipulse™ (Fujirebio)

Reliable investigation of plasma biomarkers for dementia requires assays with high sensitivity, specificity, accuracy and precision. These needs are out of reach for manually operated ELISA tests, and can be met only by fully automated systems with innovative detection systems like enhanced chemiluminescence (ECL). For our investigations we employed the well-established Lumipulse™ platforms (models 600 and 1200) by Fujirebio.

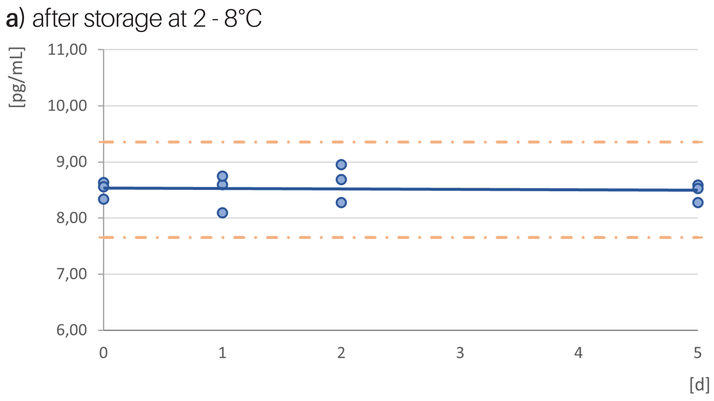
Fig. 1: Lumipulse™ 1200

Specimen Stability

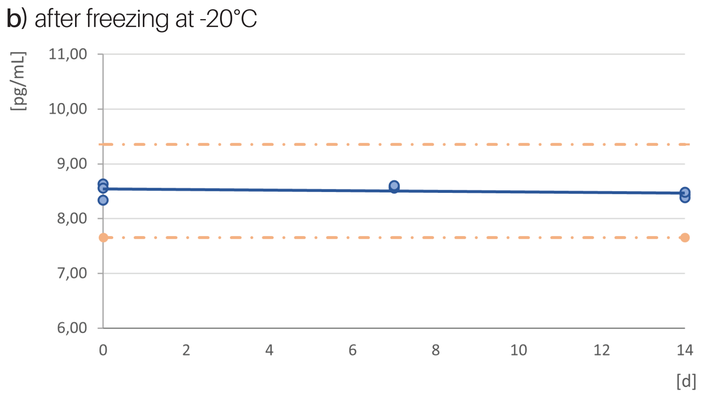
The widely used EDTA plasma proved a suitable specimen for dementia associated proteins assays. While plasma should be separated from the cellular parts of the blood specimen soon after puncture, stability of the relevant markers is expected then at least for 48 hours (maybe up to three days) at 4°C. For longer storage we recommend freezing of plasma aliquots at -20°C, which guarantees prolonged stability but renders the logistic road of specimens from patient to laboratory analysis more demanding.

Fig. 2: Stability of dementia associated biomarkers at the example of phospho-Tau 217: consecutive values (triplicates) in plasma pools spiked with a small amount of CSF

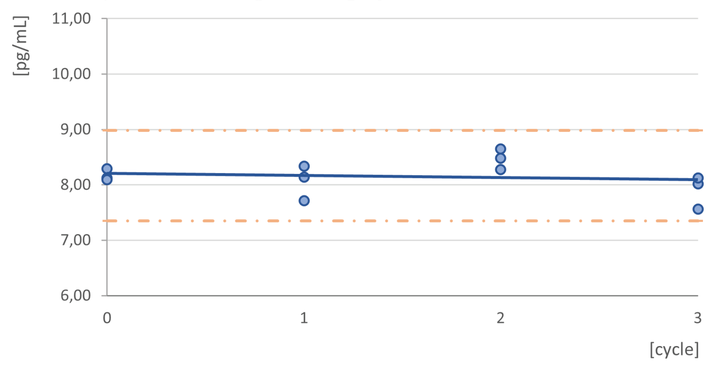
a) after storage at 2 - 8°C



b) after freezing at -20°C



c) after repeated freezing/thawing cycles



Intra- and Inter-Assay Precision

Unlike the usual 15 - 20 (up to 30) % deviations in manually performed ELISA assays, the fully automated Lumipulse™ enhanced chemiluminescence method provides much better precision criteria. This is crucial for its intended use in plasma based Alzheimer diagnostics as the detectable levels of proteins are low.

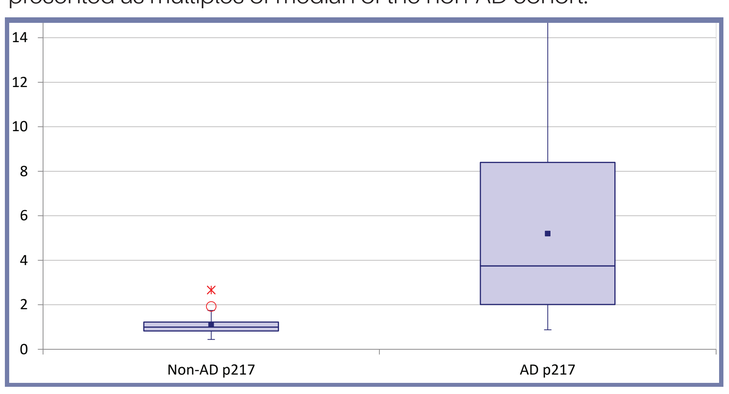
Tab. 1: Intra- and Inter-Assay precision in VK % during repeated testing (n >= 20) of controls at two levels

	ß-amyloid 1-42	ß-amyloid 1-40	phospho-Tau 181	phospho-Tau 217
Intra-Assay VK [%]				
low	3,9	1,8	4,5	2,8
high	1,5	2,1	3,2	3,8
Inter-Assay VK [%]				
low	3,9	2,1	5,4	3,8
high	1,7	2,2	3,5	3,9

Discriminative Power of Assays

For verification of the plasma assays we investigated two separate cohorts: 45 patients with CSF proven amyloid pathology (Alzheimer, AD) and 48 neurological patients without CSF amyloid pathology (Non-AD). While all approaches provided good discrimination of the cohorts, pTau217 seems to have the highest power.

Fig. 3: Discrimination of the two cohorts shown by the example of pTau217 as the most powerful single biomarker. All values are presented as multiples of median of the non-AD cohort.



Tab. 2: Distribution of pTau217 results in the two cohorts below (-), within 5% plus/minus (+/-) and above (+) the cutoff value range

	-	+/-	+
non-AD (48)	37	4	7
AD (45)	3	2	40

Combined interpretation of results

Future blood based dementia diagnostics will call for an integrated interpretation of the core dementia biomarkers. In analogy to the Erlangen score - well established for CSF based dementia diagnostics – we propose a simple and easy to grasp algorithm for future routine laboratory diagnostics: the Plasma Protein Dementia Score (PPD-score). This employs the ß-amyloid quotient (Aß42 x 10/Aß40), pTau181 and pTau217. Normal results score at zero, equivocals count 1, and pathologic results sum up with 2 points. The PPD-score needs to be validated in larger and independent cohorts most importantly including other dementias to judge its differential diagnostic performance. Moreover, the grey zone to determine equivocal results needs to be defined, and the possible added value of additional biomarkers like the ratio pTau217/pTau181 be verified. Within the present set of data the PPD-score will be compared to other composite scores recently suggested like the AT^{217}-Term (Aß1-40/Aß1-42 x pTau217) or the pTau217/pTau181 ratio, which is promising for severity staging and monitoring of treatment efficacy.

Tab. 3: Combined interpretation of the four assays at one glimpse with Plasma-Protein-Dementia-Score (PPD-score): cumulative numbers of individuals with 0 – 4 PPD-points in the two cohorts

PPD-points	0	1	2	3	4	Av.
non-AD (48)	25	11	8	3	1	0,83
AD (45)	0	1	3	19	22	3,37
AD risk	**not significant**		**improbable**	**probable**		

Conclusion

The newly established assays for blood plasma based Alzheimer diagnostics on the Fujirebio Lumipulse™ platform are very promising tools for the early and easy to access determination of candidate patients who will profit most from therapeutic measures. All assays proved robust and suitable for routine laboratory use. While phospho-Tau 217 seems to be the single biomarker with the highest discrimination power (supporting recent findings from other investigators) composite scores like the PPD-score or the AT^{217}-Term may offer added diagnostic value regarding differential diagnostics and the identification of early preclinical cases like subjective cognitive decline due to Alzheimer´s Diseases (SCD-AD).

Scientific Literature

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