MEDIZINISCHES LABOR BREMEN

# Detecting Alzheimer Amyloid Pathology by Plasma Biomarkers with Lumipulse™

Andreas Gerritzen[1], Sabine Gerritzen[1], Barbara Marcos Morgado[2], Hermann Esselmann[2], Niels Hansen[2] and Jens Wiltfang[2, 3]

[1]MVZ Medizinisches Labor Bremen GmbH, Haferwende 12, 28357 Bremen

andreas.gerritzen@mlhb.de; sabine.gerritzen@mlhb.de

[2]Universitätsmedizin Göttingen, Klinik für Psychiatrie und Psychologie, Von-Siebold-Straße 5, 37075 Göttingen

hermann.esselmann@med.uni-goettingen.de; barbara.marcosmorgado@med.uni-goettingen.de; niels.hansen@med.uni-goettingen.de; jens.wiltfang@med.uni-goettingen.de

[3]Deutsches Zentrum für Neurodegenerative Erkrankungen (DZNE), Von-Siebold-Straße 3a, 37075 Göttingen

## Abstract

New therapeutic regimens for Alzheimer's disease like monoclonal antibodies directed against ß-amyloid require reliable tools for early diagnosis, staging and monitoring of individual patients. While classical functional tests, MRT and PET scans and CSF based biomarker assays are well established and will keep necessary in the near future, plasma-based biomarkers appear to offer promising advantages in respect of easy accessibility and relatively low costs. We here describe the assessment of a plasma biomarker profile (ß-amyloid 1-40 and 1-42 including amyloid ratio, pTau181 and pTau217) analyzed with the Lumipulse™ CLIA assays (Fujirebio). In addition, the plasma protein dementia score (PPD-score) is introduced as a simple and easy to grasp tool for a combined interpretation of results.

In our approach to reach in-house CE conformity as LDT IVDR laboratory diagnostic tools for all four biomarkers, we characterized the analytical performance of the assays and established our own reference values. For this purpose, plasma specimens of three distinct cohorts (patients with and without evidence for brain amyloid pathology [biomarker proxy: CSF Abeta ratio Aß1-42/Aß1-40] and volunteer laboratory staff) were analyzed. Amyloid ratio, pTau217, pT217/aß42 ratio, AT$^{217}$-Term and PPD-score proved suitable in this setting as diagnostic tools with comparably high sensitivities and specificities. Aß42 and pT181 alone performed less satisfyingly.

## 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.

**Figure 1**
Lumipulse™ 1200 (Fujirebio)

## 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.

| | ß-amyloid 1-42 | ß-amyloid 1-40 | pTau 181 | pTau 217 |
|---|---|---|---|---|
| Intra-Assay | | | | |
| low | 3,9 | 1,8 | 4,5 | 2,8 |
| high | 1,5 | 2,1 | 3,2 | 3,8 |
| Inter-Assay | | | | |
| low | 3,9 | 2,1 | 5,4 | 3,8 |
| high | 1,7 | 2,2 | 3,5 | 3,9 |

**Table 1**
Intra- and Inter-Assay precision (% coefficient of variation) during repeated testing (n >= 20) of controls at two levels

## Discriminative Power of Assays

For verification of the plasma assays, we investigated three separate cohorts: 45 patients with CSF proven amyloid pathology (Alzheimer, AD), 48 patients without CSF amyloid pathology (non-AD) and 44 apparently healthy volunteers (laboratory staff, age 51 - 77 years). While all combined approaches with different ratios provided good discrimination of the cohorts, pTau217 seems to have the highest power as a single biomarker.

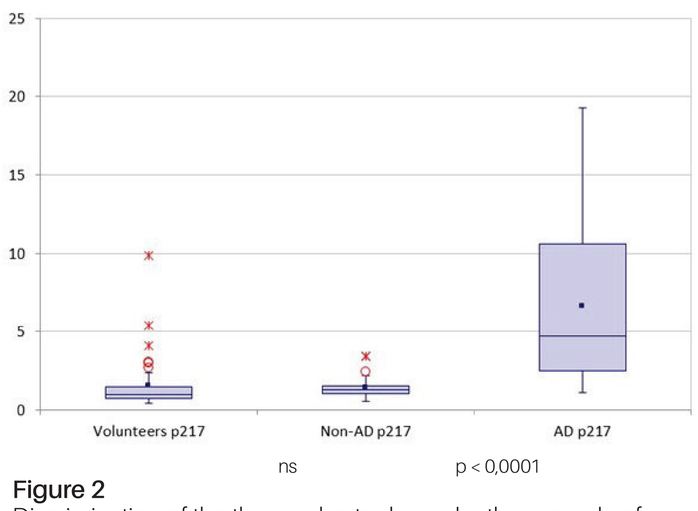


**Figure 2**
Discrimination of the three cohorts shown by the example of pTau217 as the most powerful single biomarker. All values are presented as multiples of median of the volunteers' cohort.

## Results: single biomarkers, ratios and score

Phospho-Tau 217 (pTau217) proved the single plasma biomarker with the highest power to detect amyloid pathology in the setting of our three cohorts. Aß42 and pTau181 were not that satisfying as single biomarkers for this purpose. The best performance, however, was granted by different calculated ratios and scores combining the results of more than one single biomarker.

| | Aß42 | Amyloid ratio* | pTau181 | pTau217 | pT217/aß42 | AT$^{217}$-Term** | PPD-score° |
|---|---|---|---|---|---|---|---|
| cutoff | 24,2 | 0,81 | 1,19 | 0,126 | 0,0057 | 0,1589 | 2,5 |
| Sensitivity % | 75,6 | 93,3 | 68,9 | 91,1 | 95,6 | 93,3 | 91,1 |
| Specificity % | | | | | | | |
| vs. Non-AD | 70,8 | 89,6 | 77,1 | 85,4 | 91,7 | 85,4 | 91,7 |
| vs. Volunteers | 72,7 | 93,2 | 72,7 | 79,5 | 81,8 | 84,1 | 93,2 |

**Table 2**
Sensitivities and specificities of single biomarkers and calculated ratios for the detection of amyloid pathology in the three cohorts
* Amyloid ratio: aß42 x 10/aß40
** AT$^{217}$-Term: aß40/aß42 x pTau217
° PPD-Score: Plasma Protein Dementia Score

## Combined interpretation of results: PPD-score

In analogy to the well-established Erlangen score for CSF Alzheimer biomarker diagnostics, we propose a simple and easy to grasp algorithm for the combined interpretation of plasma-based results (Plasma Protein Dementia Score, PPD-score). This employs the ß-amyloid ratio (Aß42 x 10/Aß40), phospho-tau 181 (pTau181) and phospho-Tau 217 (pTau217). Normal results score at zero, equivocals count 1, and pathologic results sum up with 2 points. with "and/or" counting in the pTau fraction. Thus, 0 – 4 points are possible results in the PPD score.

| PPD-points | 0 | 1 | 2 | 3 | 4 | Av. |
|---|---|---|---|---|---|---|
| | | | | | | |
| Volunteers (44) | 27 | 2 | 12 | 2 | 1 | 0,82 |
| 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 | | |

**Table 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 and average point sum in the three cohorts

## Conclusion

The newly established assays for plasma-based Alzheimer diagnostics on the Fujirebio Lumipulse™ platform are very promising tools for the detection of amyloid pathology. They might turn out helpful for the determination of candidate patients who will profit most from therapeutic measures, especially with amyloid antibodies like Lecanemab and Donanemab. The assays performed robustly and appeared suitable for routine laboratory use. While phospho-Tau 217 proved to be the single biomarker with the highest discrimination power (supporting 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). Further experiences from larger cohorts will add evidence if the plasma biomarkers gather a position as autochthone diagnostic tools, i.e. for first line screening followed by CSF based biomarkers and neuro-imaging methods.