



Discrimination of dementia with Lewy bodies from Alzheimer's disease via determination of the phospho-tau concentration in cerebrospinal fluid

De Vreese K¹, Blennow K², Andreasen N², Sindic C³, Ivanoiu A³, Hampel H⁴, Bürger K⁴, Parnetti L⁵, Lanari A⁵, Padovani A⁶, Di Luca M⁶, Bläser M¹, Pottel H¹, Vanmechelen E¹, Vanderstichele H¹, Hulstaert F¹

¹Innogenetics NV, Technologiepark 6, B-9052 Ghent, Belgium, E-mail: karen_de_vreese@innogenetics.com, ²Sahlgren's University Hospital, SE-431 80 Mölndal, Sweden, ³Cliniques Universitaires Saint-Luc, Avenue Mounier 53-59, B-1200 Brussels, Belgium, ⁴Ludwig Maximilian University, Nussbaumstrasse 7, D-80336 Munich, Germany, ⁵University of Perugia, Via Enrico dal Pozzo, I-06126 Perugia, Italy, ⁶Università degli Studi Brescia, Via Valsabbina 19, I-25124 Brescia, Italy

1. Introduction

Reliable diagnostic procedures are required for the development and later clinical use of therapies targeting specific pathologies associated with dementia. Determination of tau and β -amyloid₍₁₋₄₂₎ (A β 42) in cerebrospinal fluid (CSF) is already being used in certain clinical routine labs to differentiate early Alzheimer's disease (AD) from normal aging and psychiatric disorders¹.

A more specific marker for AD, phospho-tau_(181P), has been found to discriminate AD from dementia with Lewy bodies (DLB) with 80% correct classification, based on central lab testing². The present multicenter study was performed to confirm these earlier findings.

2. Objectives

- **Analytical performance:** repeatability/reproducibility of INNOTEST™ PHOSPHO-TAU_(181P) (Innogenetics NV, Belgium)
- **Clinical performance:** value of phospho-tau_(181P) for differentiation of AD versus DLB in the hands of users, alone and in combination with total tau and β -amyloid₍₁₋₄₂₎ as measured with INNOTEST™ hTAU Ag and INNOTEST™ β -AMYLOID₍₁₋₄₂₎ (Innogenetics NV, Belgium), respectively

3. Participating centers

| Site | Investigator | Diagnosis | | |
|---|----------------|-----------------|------------------|-----------------|
| | | AD ^a | DLB ^b | CS ^c |
| Sahlgren's University Hospital, Mölndal, Sweden | Prof. Blennow | 20 | 20 | 20 |
| Cliniques Universitaires Saint-Luc, Brussels, Belgium | Prof. Sindic | 20 | 10 | 14 |
| Ludwig Maximilian University, Munich, Germany | Prof. Hampel | 20 | 11 | 10 |
| University of Perugia, Perugia, Italy | Prof. Parnetti | 17 | 11 | 11 |
| Università degli Studi Brescia, Brescia, Italy | Prof. Padovani | 19 | 8 | 12 |
| Total | | 96 | 60 | 67 |

^a according to DSM-IV³ and NINCDS-ADRDA⁴

^b according to McKeith et al.⁵

^c CS = controls: apparently healthy, mechanical problems or depression without neurodegeneration

A number of samples were not included for statistical analysis because:

- they were not compliant with inclusion criteria (n = 10)
- the statistical analysis revealed a center-effect: phospho-tau_(181P) values were generally lower for one center (n = 32). Criteria for pooled analysis had prospectively been defined in the protocol. The cause of the center-effect is at present unknown and should be investigated further.

As a result, calculations with regard to cut-off value, sensitivity and specificity were performed on 72 AD, 52 DLB and 53 CS.

4. Assay principle

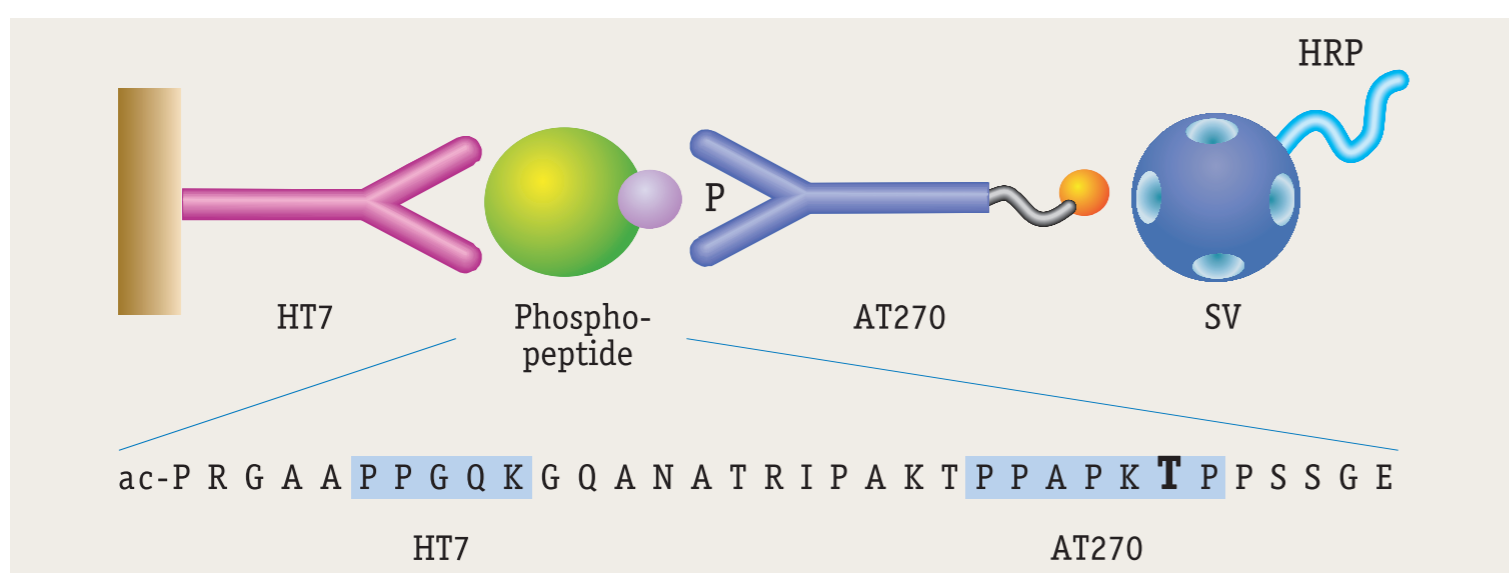


Figure 1. Assay principle of INNOTEST™ PHOSPHO-TAU_(181P)

5. Study results

Analytical performance

Three artificial CSF samples, spiked with a known concentration of phospho-tau_(181P), were tested. The samples were selected in the normal working concentration region of the assay.

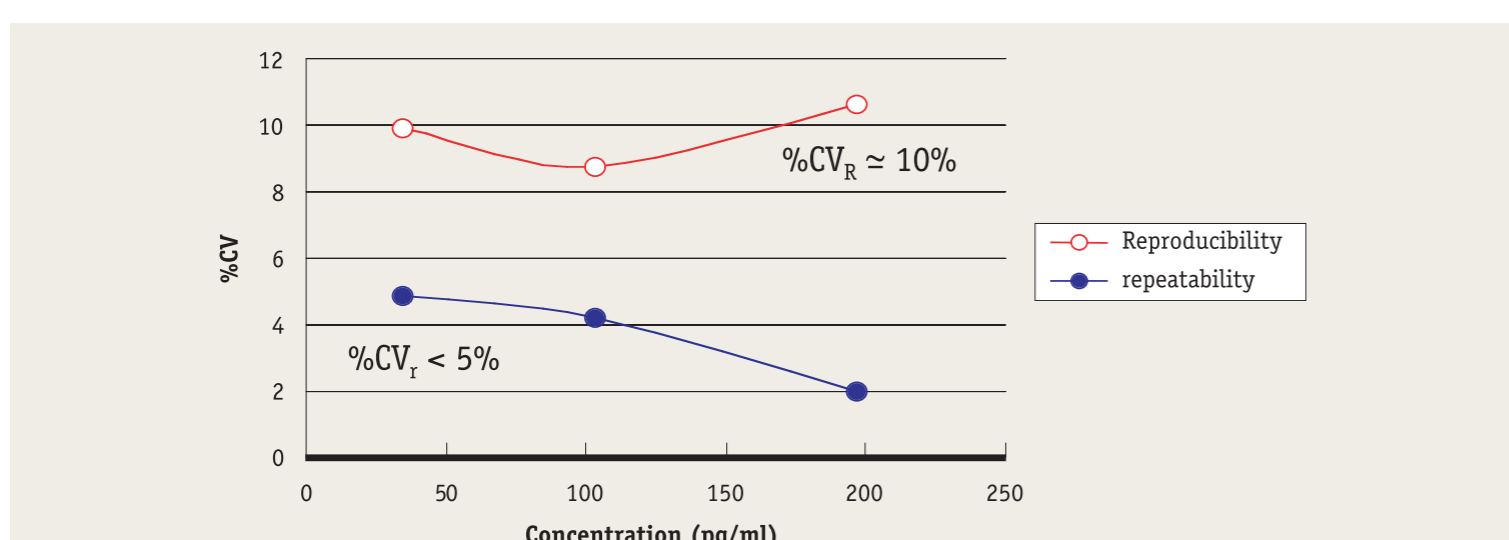


Figure 2. %CV for repeatability (r) and reproducibility (R).

The FDA guidance for industry states that the %CV should not exceed 15%, except for the lower limit of quantitation where it should not exceed 20%.

Clinical performance

- **Discrimination of AD versus DLB using phospho-tau_(181P)**

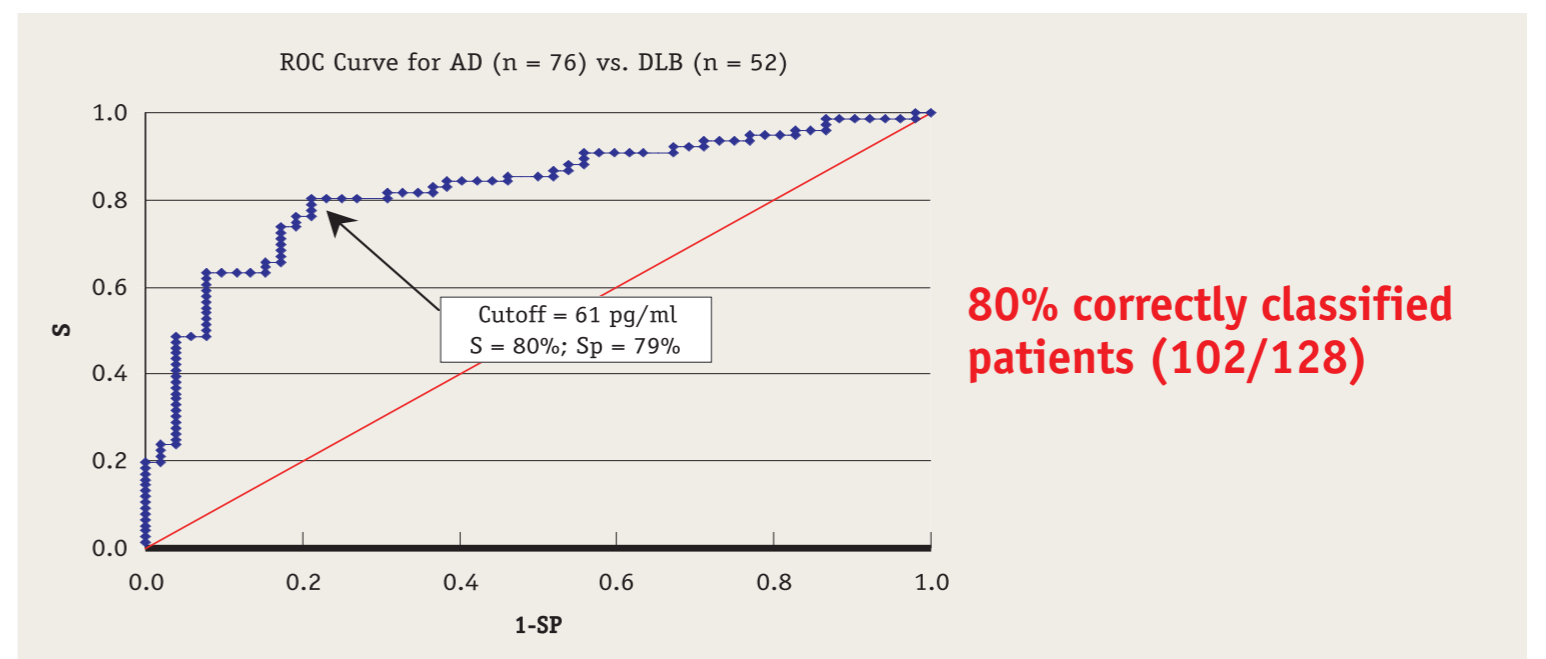


Figure 3. ROC curve for AD versus DLB

- **Classification of AD patients, DLB patients and CS in the correct group, based on a combination of biomarkers**

- Discrimination AD versus CS

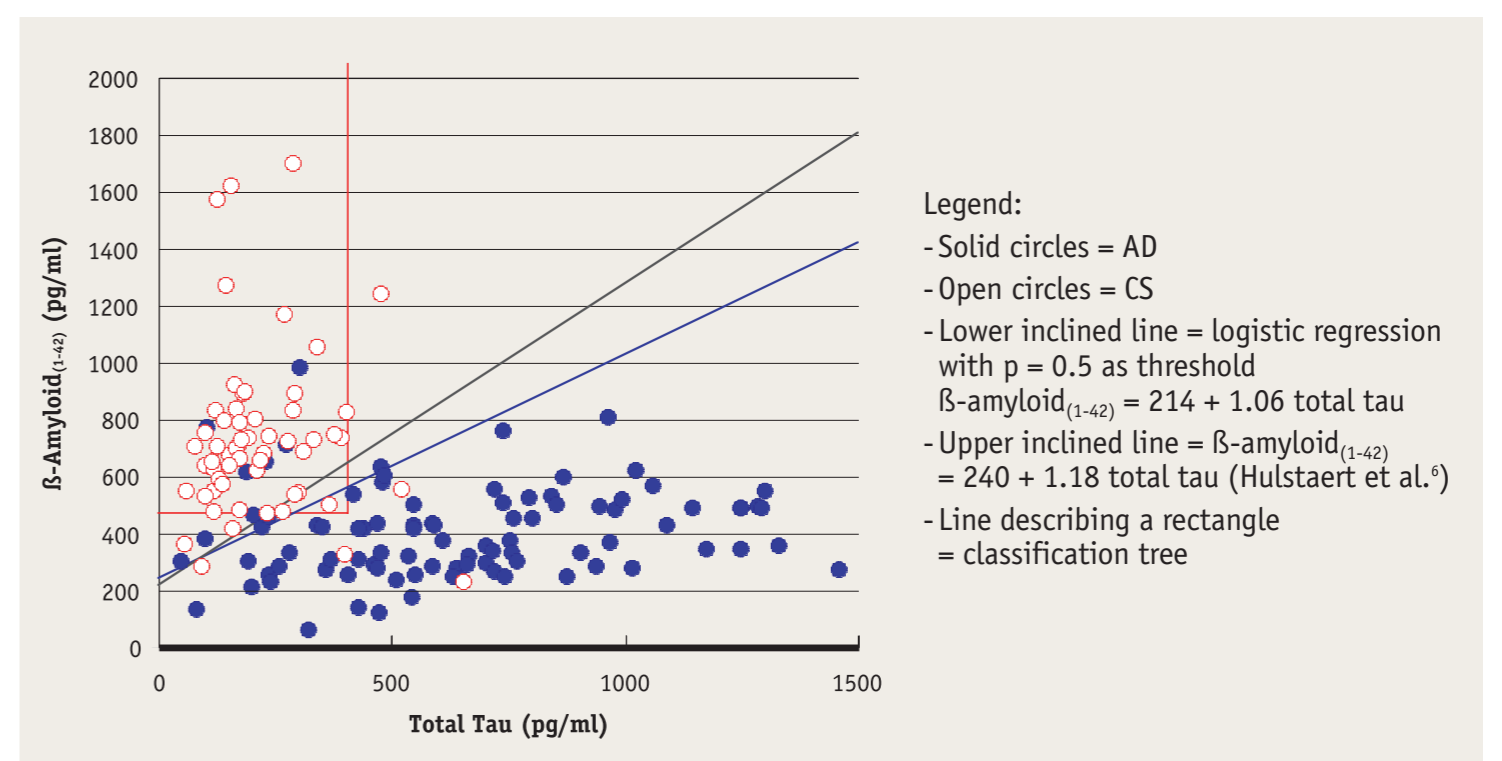


Figure 4. Scatter plot for total tau and β -amyloid₍₁₋₄₂₎ for discriminating AD from CS

The discrimination line as reported by Hulstaert et al.⁶ is very robust and gives in the current study a sensitivity of 92.3% and a specificity of 83.1%.

The combination of total tau and β -amyloid₍₁₋₄₂₎ results in an overall accuracy of 90%. Phospho-tau_(181P) does not seem to give additional value in this specific discrimination.

- Discrimination AD versus DLB

Phospho-tau_(181P) is the most important variable among the different biomarkers to discriminate between AD and DLB. Overall accuracy is 80%.

6. Conclusion

- INNOTEST™ PHOSPHO-TAU_(181P) is a robust assay. The %CV repeatability is below 5% and the %CV reproducibility is around 10%, which is well below the guidelines set by the FDA.
- Phospho-tau_(181P) is significantly increased in the AD group compared to the DLB group (p < 0.001) and the CS group (p < 0.001).
- The overall accuracy of phospho-tau_(181P) to discriminate AD from DLB is 80%, confirming the results of the previous internal study². This accuracy has been observed using a cut-off of 61 pg/ml, for which 'sensitivity + specificity' is maximal. Optimization for sensitivity or specificity, based on clinical setting, will impact on the cut-off value.
- The center-effect observed in this multicenter study implies that the values provided should be used as a guidance only. Each laboratory using these markers in a clinical setting should establish local reference ranges.
- Validation of the previously reported discrimination line⁶ on the independent data set of this study proves the robustness of this model for discrimination of AD from CS.
- Combination of CSF biomarkers can aid in (differential) diagnosis, e.g. total tau + β -amyloid₍₁₋₄₂₎ for discrimination of AD from CS, and phospho-tau_(181P) for differentiation of AD and DLB.

References

1. Andreasen N et al., Archives in Neurology 2001;58: 373-379.
2. Vanmechelen E et al., Alzheimer's Disease: Advances in Etiology and Therapeutics. Edited by K. Iqbal, S.S. Sisodia & B. Winblad. 2001 John Wiley & Sons, Ltd.
3. APA (American Psychiatric Association): Diagnostic and statistical manual of mental disorders, Fourth Edition. Washington, DC, American Psychiatric Association, 1994.
4. McKhann G et al., Neurology 1984; 34: 939-944.
5. McKeith IG et al., Neurology 1996; 47: 1113-1124.
6. Hulstaert F et al., Neurology 1999; 52: 1555-1562.

Presented at

18th International Conference of Alzheimer's Disease International - October 23-26, 2002 - Barcelona, Spain