**COMPARISON OF TWO COMMERCIAL ENZYME-LINKED IMMUNOSORBENT ASSAYS FOR CEREBROSPINAL FLUID MEASUREMENT OF AMYLOID β1-42 AND TOTAL TAU**

**Abstract**

Amyloid β1-42 (Aβ1-42), total tau (t-tau), and phosphorylated tau (p-tau) are the main cerebrospinal fluid (CSF) biomarkers for early diagnosis of Alzheimer’s disease (AD). Detection of AD is critically important in view of the growing number of potential new drugs that may influence the course of the disease in its early phases. However, cut-off levels for these CSF biomarkers have not yet been established. Variability in absolute concentrations of AD biomarkers is high among studies and significant differences were noticed even within the same datasets. Variability in biomarkers levels in these assays may be due to many aspects of operating procedures. Standardization of pre-analytical and analytical procedures in collection, treatment, and storage of CSF samples is crucial because differences in sample handling can drastically influence results. Multicenter studies showed that usage of ELISA kits from different manufacturers also affects outcome. So far only very few studies tested the efficiency of ELISA kits produced by different vendors. In this study, the performance of Innogenetics (Gent, Belgium) and Invitrogen (Camarillo, CA, USA) ELISA kits for t-tau and Aβ1-42 was tested. Passing-Bablok analysis showed significant differences between Invitrogen and Innogenetics ELISA methods, making it impossible to use them interchangeably.

**Keywords**

• Alzheimer’s disease • Amyloid Aβ1-42 • Biomarkers • Cerebrospinal fluid • ELISA • Standardization • Tau proteins

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1. Introduction

Alzheimer’s disease (AD) is the major primary cause of dementia. Ferri and collaborators estimated that 24 million people suffered from dementia in 2005, with this number reaching 81 million by 2040 [1]. Clinical diagnosis of AD, which is still based on symptomatology, is accurate in only 63 to 90% of dementia cases [2]. A growing number of potential treatments for AD are in different phases of preclinical and clinical research and thus much effort is dedicated to identify reliable biomarkers to enable an accurate diagnosis of AD.

Three main cerebrospinal fluid (CSF) biomarkers of AD, amyloid β1-42 (Aβ1-42), total tau (t-tau), and phosphorylated forms of tau (p-tau) reflect two major neuropathological hallmarks of AD - neurofibrillary tangles and senile plaques [3]. These CSF biomarkers are altered in early stages of AD, even before the occurrence of the first dementia symptoms, and permit to differentiate patients with prodromal AD (i.e. those with mild cognitive impairment, MCI) who often progress to AD, from healthy controls [4,5]. CSF biomarkers are also used for differentiation of AD from other primary causes of dementia, such as vascular dementia, frontotemporal dementia (FTD), and dementia with Lewy bodies [6-10]. Reduction of Aβ1-42 in CSF of AD patients is explained by Aβ1-42 aggregation into senile plaques, increase of t-tau reflects neuronal degeneration, while elevation of p-tau is a consequence of neurofibrillary degeneration and consequent tangles formation in the brain [11-13]. Although numerous studies in which diagnostic accuracy of CSF biomarkers was analyzed have been published, an ideal biomarker (with specificity and sensitivity over 85%) could not yet be defined.

High variability in concentrations of CSF biomarkers is observed among different centers and laboratories [14-16]. Causes of variations could be either due to pre-analytical and analytical factors or differences in ELISA kits from various manufacturers. Pre-analytical procedures refer to selection of research participants, CSF sampling and treatment, sample storage (temperature, tube type), and freeze/thaw cycles [7,17]. Analytical factors
that influence results include differences in laboratory procedures among different centers [16]. Variability among ELISA kits from different manufacturers is due to differences in production processes of reagents (e.g., usage of different materials for reagents, preparation of standards, antibody purification, and plate coating). Lot-to-lot variability among assays of the same kit is also an issue. Post-analytical procedures such as curve-fitting type, curve-fitting software, and number of samples analyzed (usually singlets or duplicates) can also affect outcome [16].

There are insufficient data on comparability of ELISA kits developed by different vendors. This study compares the performance of Innogenetics (Gent, Belgium) and Invitrogen (Camarillo, CA, USA) ELISA kits for t-tau and Aβ42. Analyses were performed in the Laboratory for Developmental Neuropathology (LDN), Croatian Institute for Brain Research, University of Zagreb Medical School, Zagreb, Croatia, and in the Laboratory for Neurobiochemistry (LNB), Department of Laboratory Diagnostics, University Hospital Centre, Zagreb, Croatia.

2. Materials and methods

2.1. Pre-analytical procedures

All patients with suspected dementia were recruited from the University Hospital Centre, Zagreb, underwent complete blood tests including electrolytes, albumin, thyroid function, levels of vitamin B12, VDLR test for syphilis, Mini-Mental State Examination (MMSE), and neurological examination [19]. After exclusion of patients with secondary causes of dementia, selected 90 patients, upon signing the informed consent, underwent lumbar puncture. Out of these, 55 patients fulfilled NINCDS-ADRDA criteria for probable AD, 33 patients suffered from MCI, while 2 patients fulfilled criteria for FTD and corticobasal degeneration (CBD) [20,21]. Additionally, eight healthy control subjects (HC) with no evidence of dementia, or neurologic and psychiatric symptoms, were included. CSF was taken in the L3/L4 or L4/L5 intervertebral spaces, always between 9 a.m. and 11 a.m., and collected in polypropylene tubes. Leukocyte and erythrocyte cell counts, lactate, glucose, total protein concentration, Treponema Pallidum Hemagglutination Assay (TPHA), and IgG index were also determined in native CSF. At LNB, CSF samples were centrifuged for 10 minutes at 4,000 g, dispensed into 150 µl aliquots and stored at -80°C. At LDN all pre-analytical procedures were exactly the same except for centrifugation (10 minutes at 2,000 g).

2.2. Analytical procedures

CSF t-tau levels were determined on 58 CSF samples of 36 AD patients, 19 patients with MCI and 3 control subjects by using ELISA kits. Among these 36 AD patients, Aβ42 concentrations were determined in CSF samples from 32 patients, plus one AD patient that didn’t have determined levels of t-tau. Four AD patients didn’t have determined Aβ42 levels. Concentration of Aβ42 was not measured in samples of either MCI patients or HC. Invitrogen ELISA kits were used at LDN (Tau /Total/ Human ELISA Kit, Aβ1-42 Human ELISA Kit), while at LNB analyses were performed using Innogenetics ELISA kits (Innotest hTau-Ag, Innotest β-amyloid (1-42)). In both laboratories, the analyses were done on CSF samples of the same patients, only using different ELISA kits. Additionally, t-tau levels were determined on 39 CSF samples of 18 AD patients, 14 patients with MCI, 2 patients with FTD and CBD, and 5 HC using Innogenetics ELISA kits (Innotest hTau-Ag, Innotest β-amyloid (1-42)). In both laboratories, ELISA analyses were performed according to the manufacturers’ protocols in both laboratories. At LNB washing was performed manually, while t-tau and Aβ42 concentrations were calculated on plate reader using curve-fitting software and 4-parameter algorithm. At LDN plates were washed in an automatic washer. Protein concentrations were determined using the same algorithm in GraphPad Prism 5.0 demo version software (San Diego, CA, USA).

2.3. Statistical analysis

T-tau levels among AD, MCI, and HC were compared using a Kruskal-Wallis test, followed by the Mann-Whitney U test for pairwise comparisons. Concentrations of Aβ42 and t-tau obtained by Innogenetics and Invitrogen ELISA kits were compared with the Passing-Bablok method [22]. Levels of proteins measured by both methods in each group (AD, MCI, or HC) were compared using Wilcoxon matched pairs test. Statistical analyses were performed with SPSS 19.0.1 (SPSS Inc., Chicago, IL, USA) and MedCalc 12.4.0.0 (Mariakerke, Belgium). P values less than 0.05 were considered statistically significant.

3. Results

Demographic data of all patient groups including HC are presented in Table 1. T-tau and Aβ42 CSF concentrations obtained by both Innogenetics and Invitrogen ELISA kits are summarized in Table 2 (See Figure 1, and Table 3) and Figure 2, respectively. Protein levels are expressed as means, medians, and percentile ranges (P25-P75).

There was a significant difference in t-tau levels measured by Innogenetics ELISA among AD, MCI, and HC groups (χ² = 9.625, df = 2, p = 0.008). T-tau levels were significantly higher in AD patients than in either MCI patients (U = 215.5, Z = -2.239, p = 0.025) or HC (U = 9, Z = -2.372, p = 0.012), but did not differ significantly among MCI and HC (p > 0.05). There was also a significant difference in t-tau levels measured by the Invitrogen kit among all groups (χ² = 9.1, df = 2, p = 0.011). The difference was again significant between AD and MCI group (U = 209.5, Z = -2.342, p = 0.019), or AD and HC (U = 12.5, Z = -2.187, p = 0.022), while differences in t-tau levels did not reach significance between MCI and HC groups (p > 0.05).

| Table 1. Demographic data of groups included in this study with MMSE scores. AD – Alzheimer’s disease, MCI – Mild cognitive impairment, HC – healthy control, MMSE – Mini-Mental State Examination. |
|---------|---------|---------|---------|
| Group   | Age     | Gender | MMSE    |
|         | Mean ± SD (Range) | Women vs. Men | Mean ± SD (Range) |
| AD (n = 55) | 73.3 ± 6.5 | 29 vs. 26 | 19.5 ± 4.7 |
| MCI (n = 33) | 67.1 ± 11.3 | 20 vs. 13 | 24.9 ± 3.1 |
| HC (n = 8) | 58.8 ± 19.7 | 5 vs. 3 | 27.8 ± 2.4 |