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Diagnostic performance of a fully automated chemiluminescent enzyme immunoassay for Alzheimer's disease diagnosis

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ABSTRACT

The variability of Alzheimer's disease (AD) cerebrospinal fluid (CSF) biomarkers (Aβ42, t-Tau and p-Tau) undermines their full-fledged introduction into routine diagnostics and clinical trials. The introduction of automatic systems can improve the diagnostic performance promoting standardization and reducing the impact of preanalytical and analytical factors.

Here we assessed the diagnostic performance of a fully automated chemiluminescent enzyme assay (LUMIPULSE) and compared it with that obtained by using the classical manual enzyme-linked immunosorbent assays (ELISAs). Patients were clinically diagnosed as AD (n = 42) and non-AD (n = 38). Clinical diagnosis was confirmed at follow-up. LUMIPULSE Aβ42 was reduced in AD (969.4 ± 329.6 pg/mL vs. 1625.9 ± 745.9 pg/mL, $p < 0.001$), whereas LUMIPULSE t-Tau was increased in AD (768.2 ± 281.0 pg/mL vs. 337.5 ± 159.1 pg/mL, $p < 0.001$) compared to non-AD patients. Both LUMIPULSE Aβ42 (AUC = 0.78, spec. = 0.74, sens. = 0.76) and t-Tau (AUC = 0.94, spec. = 0.93, sens. = 0.87) showed good accuracy in distinguish AD from non-AD and a high correlation with the manual ELISAs ($r = 0.87$, $p < 0.001$ and $r = 0.92$, $p < 0.001$, respectively). LUMIPULSE improves clinical accuracy in AD diagnosis, promoting the use of standardized values for CSF biomarkers with a good correlation with classical manual assays.

1. Introduction

Alzheimer's disease is the most common neurodegenerative disorder [1]. It is characterized by several intertwining pathological processes, including the extracellular deposition of amyloid plaques and the intracellular formation of neurofibrillary tangles and neuritic plaques [2]. These processes start years before the disease becomes clinically manifest [3] and are mirrored in CSF where levels of soluble Aβ peptide 1–42 (Aβ42) are decreased as consequence of the Aβ42 deposition into amyloid plaques in the brain parenchyma, whereas the levels of protein tau (t-Tau) and of its phosphorylated form at threonine 181 (p-Tau) are increased [4].

The most recent NIA-AA (National Institute on Aging and the Alzheimer's Association) 2018 criteria [5] strongly encourage the use of CSF biomarkers in clinical routine to support the diagnosis *in vivo* of AD. In fact, CSF biomarkers have proven both high sensitivity and specificity [6]. Furthermore, they are also helpful to predict the future development of dementia in patients with mild cognitive impairment (MCI) due to AD [7] showing a good performance in detecting early asymptomatic stage of the disease [8]. The European Medicines Agency indicated these biomarkers as a tool for patient enrolment in both clinical and pharmacological trials, in particular for testing disease modifying therapies [9,10]. Nevertheless, the main obstacle to their use in clinical routine depends on their variability. To date, the lack of standard protocols and certified reference materials as well as the intra- and inter-laboratory variability due to the manual procedures limit the establishment of universal discriminative cut-offs, which instead can be defined "center and CSF-collection specific" [11,12]. A recent paper provided some recommendations to reduce the impact of pre-analytical and analytical handling of CSF [13], but still remains the need for a consensus on how to collect and store CSF before analysis [14]. Several international initiatives have gone in this direction [14,15].

Abbreviations: Aβ42, soluble Aβ peptide 1–42; AD, Alzheimer's disease; AUC, area under the curve; CSF, cerebrospinal fluid; ELISA, enzyme-linked immunosorbent assay; MCI, mild cognitive impairment; MMSE, mini-mental state examination; NIA-AA, National Institute on Aging and the Alzheimer's Association; p-Tau, protein tau phosphorylated form at threonine 181; ROC, receiver operating characteristic; SMC, subjective memory complaint; t-Tau, total tau

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The most common technique for assessing CSF core AD biomarkers is the enzyme-linked immunosorbent assay (ELISA) [16]. This method is based on the 96-well plate immunoassay format; thus, most laboratories must await that a sufficient number of samples are collected before performing the analysis, leading to a delay on delivering test results. Recently, fully automated immunoassay platforms for assessing CSF AD core biomarkers have been developed to reduce variability, costs and waiting times [17,18]. These platforms seem to have a good concordance with classical manual ELISAs and visual amyloid positron emission tomographic assessment [19], but more studies are needed to assess their diagnostic performance in clinical routine.

In this study we sought to evaluate the diagnostic performance of a fully-automated chemiluminescent platform (LUMIPULSE G600-II, Fujirebio Europe, Ghent, Belgium) in routine diagnostics of AD as compared to classical manual ELISAs.

2. Materials and methods

2.1. Cohort selection

In the period between 2014 and 2017, 80 patients were enrolled at the Center of Memory Disturbances of the University of Perugia. Patients underwent complete medical and neuropsychological examination, MRI scan for excluding other neurological conditions causing dementia and lumbar puncture (LP). The patients were clinically diagnosed as AD and non-AD according to previous NIA-AA 2011 criteria [23] and at least 1 year of clinical follow-up.

2.2. CSF collection

LP was performed according to international guidelines [22]. Following a standardized procedure, 10–12 mL of CSF were collected in sterile polypropylene tubes and centrifuged at room temperature for 10 min (2000 × g). Aliquots were frozen at −80 °C. CSF Aβ42 and t-Tau levels were measured by using LUMIPULSEG β-Amyloid 1–42 and LUMIPULSEG Total Tau assays (Fujirebio Europe, Gent, Belgium). All samples were analyzed together by using a single production lot number. LUMIPULSE CSF biomarkers concentrations were compared with CSF Aβ42 levels previously measured with the EUROIMMUN ELISA kit (EUROIMMUN AG, Lübeck, Germany) and with CSF t-Tau previously assessed by INNOTEST ELISA (Fujirebio Europe, Gent, Belgium). Different production lots numbers were used to generate data from both EUROIMMUN and INNOTEST manual ELISAs. Internal quality controls were assayed in each run.

2.3. Statistical analysis

Statistical analysis was performed with R statistical software. Descriptive statistics were reported. Correlation between CSF biomarkers was calculated by means of Spearman coefficients. Diagnostic accuracy was determined according to the results of ROC (receiver operating characteristic) analysis. Sensitivity and specificity were calculated at the point closest to the top left of the ROC curve. Comparisons of diagnostic accuracy between biomarkers were performed by means of DeLong test. A p-value < 0.05 was considered as significant in all the analysis.

3. Results

The patients were clinically diagnosed as AD (n = 42) and non-AD (n = 38) according to previous NIA-AA 2011 criteria [23] and with a minimal clinical follow-up of 1 year. Non-AD subjects included 23 MCI, 7 subjective memory complaints (SMC), 4 headache, 3 epilepsy and 1 psychiatric condition. Age, sex, mini-mental state examination (MMSE) and CSF biomarkers levels are reported in Table 1.

LUMIPULSE Aβ42 was significantly reduced in AD compared to non-AD (969.4 ± 329.6 pg/mL vs. 1625.9 ± 745.9 pg/mL, $p < 0.001$, Fig. 1A), providing good diagnostic accuracy (AUC = 0.78, spec. = 0.74, sens. = 0.76, Fig. 2A and Table 2). LUMIPULSE t-Tau was significantly increased in AD (768.2 ± 281.0 pg/mL) compared to non-AD patients (337.5 ± 159.1 pg/mL, $p < 0.001$, Fig. 1C). A similar trend was observed for Aβ42 and t-Tau assessed by ELISAs (Fig. 1B and D). ROC analysis showed an optimal diagnostic accuracy of t-Tau in distinguishing AD patients (AUC = 0.94, spec. = 0.93, sens. = 0.87). The LUMIPULSE Aβ1–42/t-Tau ratio provided an improvement of the diagnostic performance (AUC = 0.98, spec. = 0.93, sens. = 0.97) with respect to Aβ42 and t-Tau alone (Fig. 2). No significant difference was observed comparing the diagnostic accuracy between the levels of Aβ42, t-Tau and their ratio (Fig. 1, E and F) between LUMIPULSE and ELISAs (Table 2, Fig. 2).

Table 1
Demographics, MMSE and CSF profile of participants.

	AD	Non-AD	P-value
N	42	38	
Gender (Male)	16 (39.0%)	16 (42.1%)	0.822
Age	74.1 ± 5.9	71.7 ± 7.6	0.119
MMSE	21.1 ± 5.7	27.1 ± 1.9	< 0.001
CSF Aβ42 LUMIPULSE	969.4 ± 329.6	1625.9 ± 745.9	< 0.001
CSF Aβ42 ELISA	522.9 ± 211.4	889.4 ± 333.3	< 0.001
CSF t-Tau LUMIPULSE	768.2 ± 281.0	337.5 ± 159.1	< 0.001
CSF t-Tau ELISA	786.6 ± 436.8	313.3 ± 162.4	< 0.001

LUMIPULSE Aβ42 and t-Tau were significantly different in AD patients also when age was considered as potential confounder ($p < 0.001$). The same holds true for ELISA Aβ42 and t-Tau ($p < 0.001$).

When compared with the classical manual ELISAs, both LUMIPULSE Aβ42 and LUMIPULSE t-Tau showed a good correlation with the EUROIMMUN and the INNOTEST assays ($r = 0.87$, $p < 0.001$; and $r = 0.92$, $p < 0.001$, respectively. Fig. 3). It is worth to note that, despite the correlation observed between LUMIPULSE and EUROIMMUN, the mean values of Aβ42 measured by LUMIPULSE were higher with respect to those assessed by ELISA.

4. Discussion

Our results support the use of LUMIPULSE G600-II for measuring CSF Aβ42 and t-Tau levels in routine diagnostics of AD. LUMIPULSE was accurate as manual assays. The higher levels of Aβ42 measured by LUMIPULSE compared to manual assays, make crucial the investigation of appropriate cut-offs in larger cohorts. The discrepancy between the Aβ42 levels measured by using the LUMIPULSE and EUROIMMUN kits might be due to differences in the technology as well as in the antibody pairs utilized for detecting the Aβ42 peptide. Furthermore, even if both systems use recombinant Aβ42 as the calibrator, each vendor produces and assigns the calibrator values individually, thus making the standardization of the results difficult.

Automation gives the opportunity to measure CSF biomarkers in any single sample, without waiting for the collection of several cases as classically done in manual ELISA setting. This will allow to reduce the variability due to pre-analytical factors. These aspects are of crucial importance in view of incorporating biomarker assays in the clinical routine, offering a much faster and easier management in diagnosis. The forthcoming accessibility of Aβ40 and p-Tau will be essential for using this platform in routine diagnostics.

5. Conclusions

Our results support the use of LUMIPULSE G600-II for measuring CSF Aβ42 and t-Tau levels in routine diagnostic of AD.

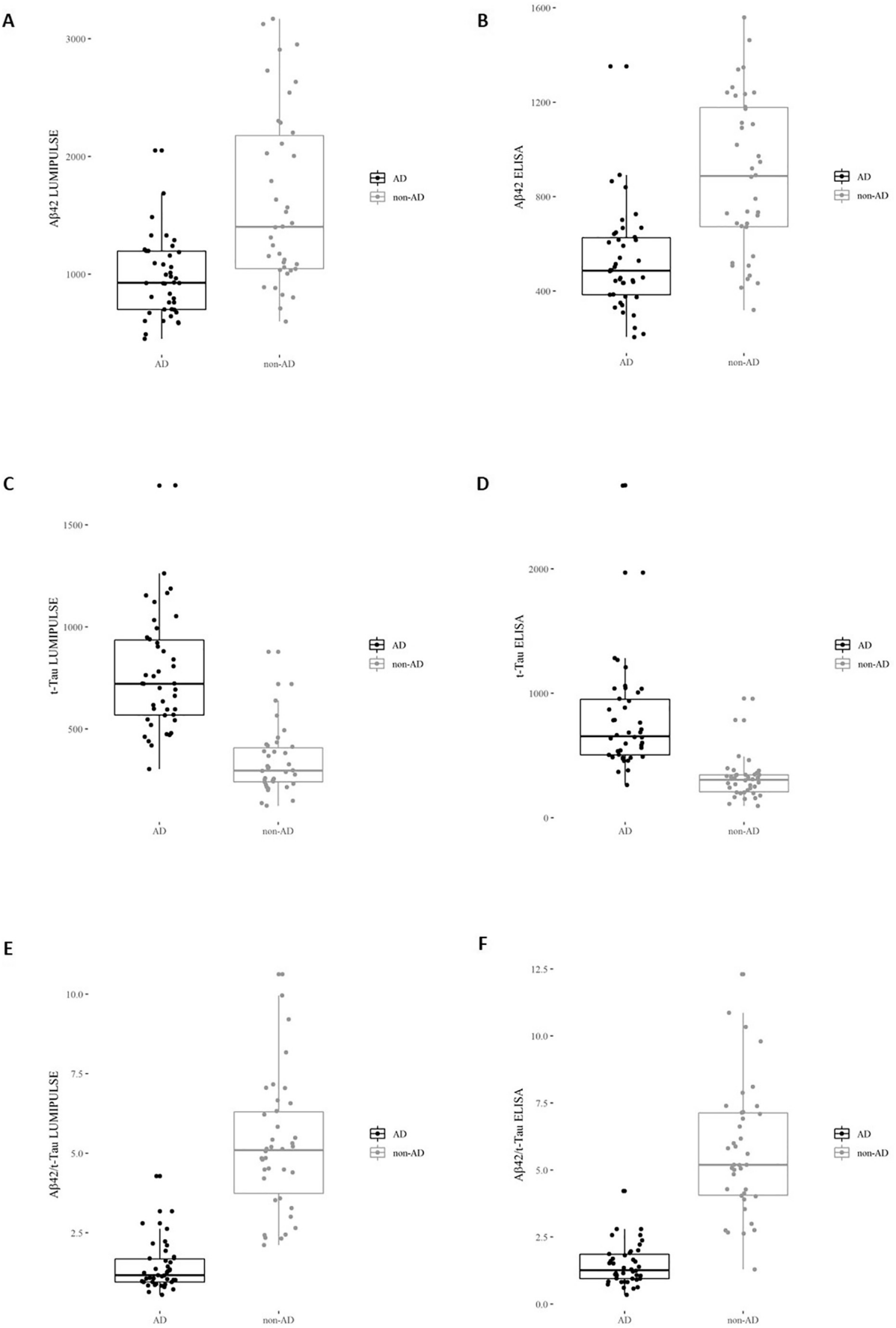

Fig. 1. Boxplots reporting the distribution of CSF biomarkers Aβ42 and t-Tau and their ratio within diagnostic groups. Minimum, maximum, median and inter-quartile ranges are shown.

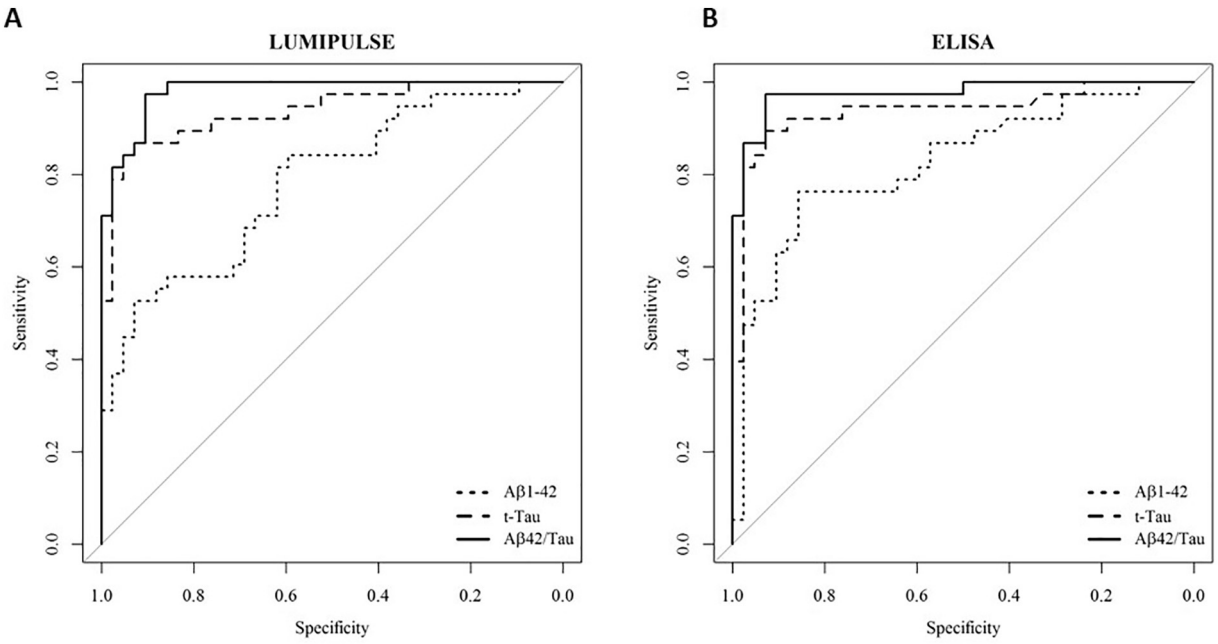

Fig. 2. ROC curves of CSF biomarkers. No significant difference was observed comparing the diagnostic accuracy between the levels of biomarkers between LUMIPULSE and ELISAs.

Table 2
AUC values, Sensitivity and Specificity of LUMIPULSE and ELISA assays.

	AUC (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
CSF Aβ42 LUMIPULSE	0.78 (0.68–0.88)	0.76 (0.53–0.92)	0.74 (0.55–0.95)
CSF Aβ42 ELISA	0.83 (0.73–0.92)	0.76 (0.63–0.89)	0.86 (0.73–0.95)
CSF t-Tau LUMIPULSE	0.94 (0.89–0.99)	0.87 (0.76–0.95)	0.93 (0.83–1.00)
CSF t-Tau ELISA	0.94 (0.88–1.00)	0.92 (0.82–0.97)	0.93 (0.83–1.00)
CSF Aβ42/t-Tau LUMIPULSE	0.98 (0.88–1.00)	0.97 (0.87–1.00)	0.93 (0.83–1.00)
CSF Aβ42/t-Tau ELISA	0.98 (0.94–1.00)	0.97 (0.89–1.00)	0.95 (0.86–1.00)

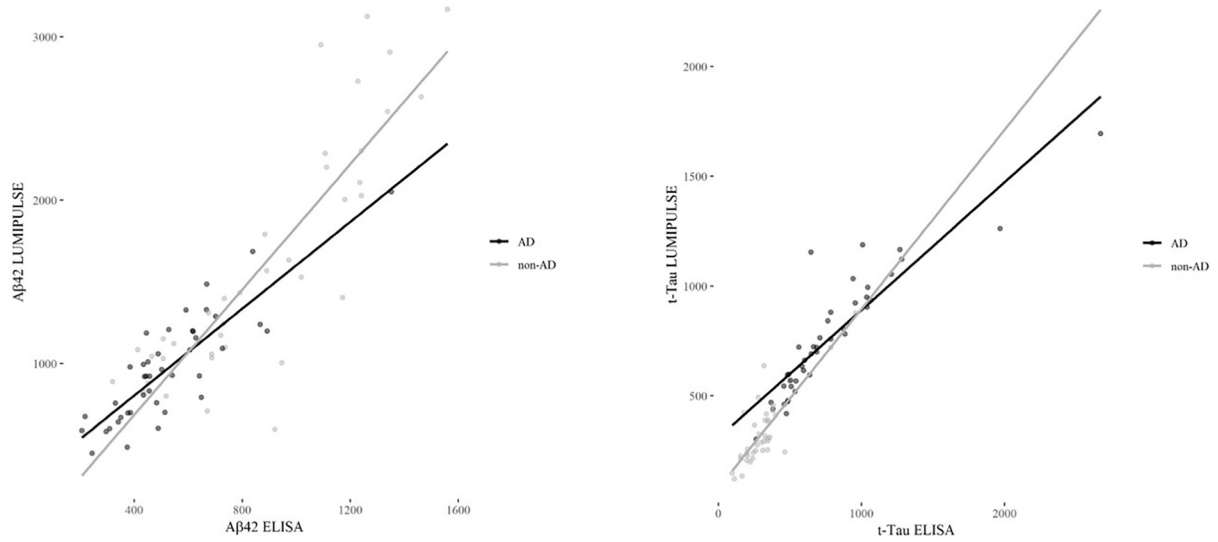

Fig. 3. Correlation analysis between classical manual ELISA and LUMIPULSE. Left: Aβ42 LUMIPULSE vs. Aβ42 ELISA (r = 0.87, 95% CI = 0.82 to 0.93). Right: t-Tau LUMIPULSE vs. t-Tau ELISA (r = 0.91, 95% CI = 0.87 to 0.95).

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