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ORIGINAL COMMUNICATION

Analytical and clinical performances of the automated Lumipulse cerebrospinal fluid $A\beta_{42}$ and T-Tau assays for Alzheimer's disease diagnosis

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Abstract

Background Cerebrospinal fluid (CSF) biomarkers are increasingly used to diagnose Alzheimer's disease (AD). However, important methodological and technical remain regarding measurement variability between kit providers and users. We compared the Lumipulse fully automated assays with the manual INNOTEST assays (both from Fujirebio Europe NV, Gent, Belgium) on a clinically representative sample of patients and controls.

Methods CSF samples of 156 patients were used to quantify Amyloïd $A\beta_{1–42}$ peptide ($A\beta_{1–42}$) and Total-Tau (T-Tau) protein by chemiluminescent enzyme-immunoassay (Lumipulse). Patients were divided into several subgroups: Alzheimer (AD = 44), mild-cognitive impairment (MCI = 23), other dementias (OD = 36), non-dementing neurological conditions (ND = 11), and controls (CTRL = 42). Clinical cut-offs were determined by comparing AD and CTRL with ROC curves for the two markers and their related ratio (T-Tau/$A\beta_{1–42}$). Subgroups of 58 (for phosphorylated-Tau) and 115 samples (for $A\beta_{1–42}$ and T-Tau) were used to evaluate the concordance of this analyzer with the INNOTEST assays.

Results Lumipulse and INNOTEST assays showed good concordance for all markers, but systematic bias was observed justifying the need to redefine new clinical cut-offs. To discriminate AD from CTRL subjects, T-Tau/$A\beta_{1–42}$ ratio was the best biomarker, with a cut-off value of 1.12 (sensitivity 81.8% and specificity 92.9%). Similar clinical performances were observed for the Lumipulse and Innotests assays on the subsample of 115 subjects.

Conclusions Our results demonstrate that the Lumipulse $A\beta_{1–42}$ and T-Tau assays show good analytical and clinical performances in the context of patient evaluation referred to a memory clinic. Automated analyzers should be preferred for the measurement of CSF AD biomarkers to reduce inter- and intra-laboratory variability.

Keywords Cerebrospinal fluid · Alzheimer's disease · Chemiluminescent enzyme-immunoassay · Assay automation · Amyloïd peptide · Total-Tau

Introduction

Alzheimer's disease (AD) accounts for more than half of all dementias cases and its prevalence is known to increases exponentially with age [1]. Therefore, due to the aging population, its prevalence is expected to triple by 2050 [2].

Adrian Ivanoiu and Vincent van Pesch have contributed equally to this work as senior authors.

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It is also well demonstrated that the degenerative process begins many years before the first clinical manifestations [3]. Indeed, changes in cerebrospinal fluid (CSF) biomarkers can already be detected in prodromal AD, namely reduced amyloid β_{1-42} (Aβ42) peptide, increased Total-Tau (T-Tau), and Phosphorylated-Tau (P-Tau) levels [4]. These biomarkers have repeatedly demonstrated their added value in diagnosing AD patients and patients with mild-cognitive impairment (MCI) at risk for developing AD [5]. They have strengthened the link between the Alzheimer's clinical syndrome and the AD-related pathological process. The integration of this CSF signature as recommended by the International Working Group (IWG) illustrates the importance of CSF collection nowadays for diagnosing probable AD dementia [6]. However, while these biomarkers are now widely adopted, in both research and academic clinical settings, some important concerns are still to be addressed. One of the most important shortcomings of the methods currently used are related to the significant intra- and inter-lab variability in measured biomarker levels, particularly for Aβ42 [7, 8]. This variability is mainly explained by preanalytical (nature of sampling and storage tubes, delay between CSF collection and storage, etc) and analytical issues (various kit providers and users) [9]. While preanalytical issues can be resolved by implementing a rigorous collection and storage process, little has evolved for analytical problems, until recently [10]. In their recent review, Mattson and colleagues emphasized the importance of reducing the measurement variability of the CSF biomarkers and introduced fully automated assay systems [11]. Moreover, in an effort to further harmonize the different Aβ42 assays, the European Commission's Joint Research Centre (EC-JRC) in collaboration with the International Federation for Clinical Chemistry and Laboratory Medicine (IFCC) has released three certified reference materials (CRM) for Aβ42 [12]. Manufacturers are, therefore, now committed to commercialize re-calibrated Aβ42 assays.

In light of these recent developments, the first aim of the present study was to compare the analytical performances of the recently developed fully automated chemiluminescent enzyme-immunoassay (CLEIA) Lumipulse assays (Fujirebio Europe NV, Gent, Belgium) with the standard manual Aβ42, T-Tau, and Phosphorylated-Tau (P-Tau) INNOTEST Enzyme-linked immunosorbent assays (ELISA). Our second aim was to evaluate the clinical performances of the Lumipulse analyzer for Aβ42 and T-Tau quantification, and their related ratio (T-Tau/Aβ42), to diagnose AD patients, by comparing them to cohorts of patients suffering from other neurological diseases with or without dementia and control subjects. In particular, we aimed to establish cut-offs useful for the clinician using the new Lumipulse analyzer by choosing a control group without central nervous system conditions susceptible to alter the levels of the AD biomarkers and a group of well-characterized probable AD patients.

Study subjects and methods

Participants

156 patients were included in this retrospective study. Patients underwent lumbar puncture at the Cliniques Universitaires Saint-Luc between 2012 and 2018. Each participant also underwent a thorough clinical and paraclinical evaluation, including medical history, neurological examination, and neuroimaging [Fluorodeoxyglucose (FDG)-PET scan and/or magnetic resonance imaging]. Finally, each demented patient underwent a standard neuropsychological testing. Based on these data, a senior neurologist (AI) with advanced experience in neurodegenerative disorders classified each patient into five distinct subgroups: Alzheimer (AD) ($n=44$), mild-cognitive impairment (MCI) ($n=23$), other dementias (OD) ($n=36$), non-dementing neurological conditions (ND) ($n=11$), and controls (CTRL) ($n=42$). AD cases were "Probable AD dementia" according to the 2011 criteria of the National Institute of Neurological and Communicative Disorders and Stroke-Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) [13]. Each OD patient fulfilled the DSM-IV criteria for dementia and the specific diagnostic criteria of the disorders considered (e.g., fronto-temporal dementia, Lewy body dementia, etc.). ND patients were subjects without dementia but with neurological conditions potentially affecting the CSF biomarkers (i.e., bacterial meningitis, multiple sclerosis, and stroke). CTRL patients were strictly selected and neither had cognitive impairment nor central nervous system disorders, based on clinical and imaging data. Detailed clinical diagnoses are listed in Supplementary Table 1. The patients included signed an internal regulatory document, stating that residual samples used for diagnostic procedures can be used for retrospective academic studies, without any additional informed consent (ethics committee approval 2007/10SEP/233).

Sample collection and preparation

CSF samples were collected as part of the routine diagnostic procedure at the L3/L4 or L4/L5 interspace in polypropylene tubes (Sarstedt, ref 60.541.004) and centrifuged at 2000*g* during 10 min at controlled room temperature. Aliquots of 500 µL were then prepared in small polypropylene tubes (Thermo Fisher Scientific, ref 3431-11) and frozen at − 80 °C until analysis. The centrifugation and freezing processes were performed within an hour after CSF drawing, according to the published guidelines [14].

Imprecision and linearity of the Lumipulse assay

Imprecision was evaluated by analyzing low- and high-quality control samples 10 times during the same analytical run (repeatability) or 30 times over a period of 15 days (reproducibility). To do so, mean, standard deviation (SD) and coefficient of variation (CV) were calculated. For linearity assessment, a CSF sample with a high T-Tau concentration was serially diluted with using the manufacturer's diluent. Linearity was assessed by weighted linear regression and mean recovery (+SD) was calculated.

Concordance between methods

The comparison study for Aβ42 and T-Tau was based on 115 randomly selected samples from the cohort described above. Since the P-Tau Lumipulse assay became available only at the end of our investigations, a brief comparison study on 58 samples was performed. Following thawing, samples were analyzed successively on the Lumipulse and on the INNOTEST assays. The latter were performed by the same laboratory technician, as usually done for the routine workflow. Passing–Bablok regression analysis was used to determine proportional bias (slope) and constant bias (intercept). A Bland–Altman study was also carried out to calculate the mean bias and relative bias distribution.

Quantification of certified reference materials (CRM)

Three Aβ1-42 CRM samples (ERM®-DA480/IFCC, ERM®-DA481/IFCC and ERM®-DA482/IFCC) were quantified, on both assays, in the same analytical run as clinical samples. Bias, expressed as relative percentages of the target values, were calculated. Of note, CRM for Total-Tau were not yet available at the time of our study.

Statistical analysis

Medcalc software version 14.8.1 was used to perform the Bland–Altman and Passing–Bablok analysis. GraphPad Prism 7 software was used to perform other statistical analysis. To test for differences between subgroups, non-parametrical Kruskal–Wallis followed by Dunn's multiple comparison tests were performed for the AD biomarkers. One-way ANOVA and Chi-square test were applied for the age and gender variables, respectively. ROC curves and derived Youden's index were used to determine optimal cut-offs able to discriminate AD from control or non-AD patients. Two-tailed p values (<0.05) were considered as statistically significant.

Results

Analytical evaluation of the Lumipulse assays

Performances of the Lumipulse assay

Repeatability and reproducibility gave satisfactory results. Calculated CVs for measures of low and high Aβ42, T-Tau and P-Tau control samples were all below 5% and lower than the manufacturer's values (Supplementary Table 2 and 3). Sustained linearity for T-Tau over a tested range of 11–1496 pg/mL was observed. The following equation of linearity was obtained: $y=1.0031x-0.7891$ with $r^2=0.999$ and a mean recovery (SD) of 95.17% (7.98%) was obtained.

Concordance between the Innotest and Lumipulse assays

Passing–Bablok regression indicated good agreement between the two assays. For both Aβ42, P-Tau, and T-Tau, there was a significant systematic bias observed across all measured ranges. Mean Aβ42 Lumipulse measured values were lower than those obtained by the Innotest assay [systematic difference (Y intercept) of − 100 pg/mL (CI_{95}: − 131; − 64)], but this bias was constant across all the measured range (slope: 1.04; CI_{95}: 0.97–1.11). The observed correlation coefficient (R) was 0.94 (CI_{95}: 0.91–0.96). As showed on the Bland–Altman plot, the relative bias seems, indeed, higher at low values with a mean bias of 14.9% on the measured range (Fig. 1). On the other hand, mean T-Tau values were higher on the Lumipulse analyzer [constant bias (Y intercept) of + 33 pg/mL (CI_{95}: 21–49)]. However, as illustrated in the Bland and Altman plot, this bias seems negative at low values and positive at high values (slope 0.88; CI_{95}: 0.88–0.91), leading to a very low mean bias of − 1.2% when calculated on the total range values (Fig. 1). Observed correlation coefficient (R) was 0.98 (CI_{95}: 0.97–0.98). Finally, for P-Tau, a constant bias of − 12.3 pg/ml was observed across all the measured range (slope 0.88; CI_{95}: 0.77–1.07) with a correlation coefficient of 0.93 (CI_{95}: 0.88–0.96) and a mean bias of 16.7%. For all assays evaluated, more than 95% of measured values were comprised between ± 1.96 SD. (Fig. 1) Demographic characteristics and CSF biomarker levels of the patients included in the correlation study are detailed in Supplementary Table 4.

Quantification of Aβ42 certified reference materials

Quantification of the ERM-DA480/IFCC material gave the same result on both platforms (428 pg/mL). These values were slightly below the target (450 pg/mL; bias of − 4.9%) but comprised in the uncertainty range (380–520 pg/mL).

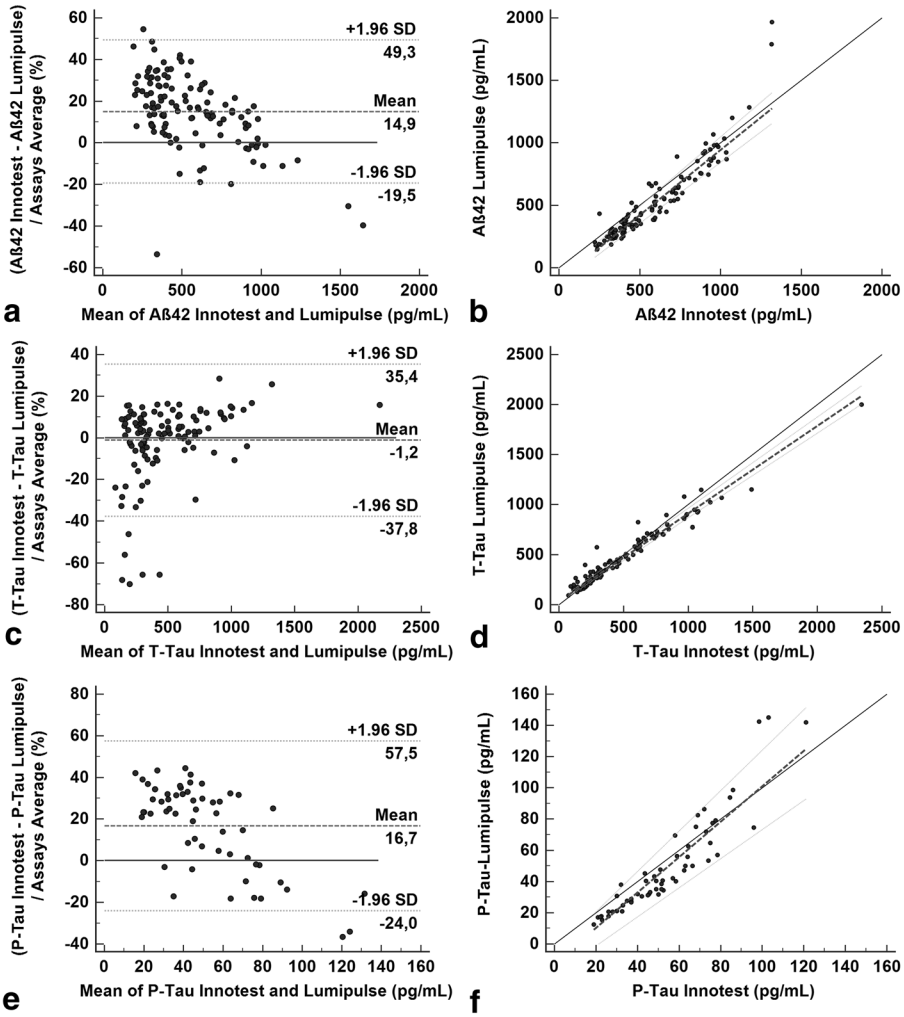

Fig. 1 Difference plot (**a**, **c** and **e**) and Passing–Bablok analysis (**b**, **d** and **f**) for the amyloïd β1–42 peptide (Aβ42), the total-Tau (T-tau) and Phosphorylated-Tau (P-Tau) Lumipulse assays compared with the gold standard Innotest assays ($n = 115$ samples for the first two markers and $n = 58$ for P-Tau). For difference plots analysis, grey dashed lines represent the mean bias. In the Passing–Bablok graphs, grey dashed lines represent the slope observed, while the continuous dark line represents a slope of 1.0

ERM-DA481/IFCC and ERM-DA482/IFCC quantification on the Lumipulse assay showed also satisfactory results comprised in the uncertainty range with bias of − 5.9% and − 12.5%, respectively. Values obtained with these two CRM's were, however, outside the uncertainty range for the Innotest assay (bias of − 16.9% and − 21.6%) (Table 1).

Clinical performance evaluation of the Lumipulse assays

Demographic characteristics and cerebrospinal fluid biomarker levels

Demographic characteristics and CSF biomarker levels of the different patient cohorts are detailed in Table 2. There were no statistical gender differences between the five subgroups. According to one-way ANOVA testing, age was significantly different among subgroups ($p = 0.02$). However,

Table 1 Measurement accuracy of Amyloïd $\beta_{1–42}$ certified reference materials (CRM) from the European Reference Materials (ERM) department

Certified values for Amyloid $\beta_{1–42}$ peptide CRM (± uncertainties)	Innotest assay (pg/mL)—bias (%)	Lumipulse assay (pg/mL)—bias (%)
ERM-DA480/IFCC 450 ± 70 pg/mL	428 (− 4.9)	428 (− 4.9)
ERM-DA481/IFCC 720 ± 110 pg/mL	**599 (− 16.9)**	677 (− 5.9)
ERM-DA482/IFCC 1220 ± 180 pg/mL	**956 (− 21.6)**	1068 (− 12.5)

Values in bold are outside the certified range

Table 2 Demographics of the study population and results of the Lumipulse assays for the amyloïd β1–42 peptide (Aβ42) and total-Tau protein (T-tau)

	AD ($n=44$)	MCI ($n=23$)	OD ($n=36$)	ND ($n=11$)	CTRL ($n=42$)	p value
Age (years) + SD	69.7 + − 9.0	67.4 + − 9.0	69.9 + − 7.7	64 + − 10.2	64.7 + − 8.2	0.02
% of women	47.7	52.2	58.3	54.5	52.4	0.92
Median Aβ42 (pg/mL) [interquartile range]	344[d,e] [299–434]	468 [340–758]	380[e] [249–595]	720[a] [379–917]	611[a,c] [468–870]	< 0.0001
Median T-Tau (pg/mL) [interquartile range]	653[b,c,e] [427–896]	455[a] [247–556]	328[a,d] [234–455]	704[c,e] [493–823]	276[a,d] [206–351]	< 0.0001
Median T-Tau/Aβ42 [interquartile range]	1.87[b,c,e] [1.32–2.47]	0.83[a] [0.40–1.58]	0.94[a] [0.50–1.26]	1.07 [0.52–1.64]	0.39[a] [0.32–0.55]	< 0.0001

Values displayed are median with interquartile range shown between square brackets. Statistically significant differences between subgroups using the Kruskal–Wallis test, followed by Dunn's multiple comparison tests are indicated as follows: [a]for significantly different from AD; [b] for significantly different from MCI; [c] significantly different from OD; [d] significantly different from ND; [e]: significantly different from CTRL

SD standard deviation, *AD* Alzheimer's disease, *MCI* mild-cognitive impairment, *OD* other dementias, *ND* non-demented, *CTRL* control patients

this difference did not survive adjustment for multiple comparisons using the Holm–Sidak's test.

The results for Lumipulse CSF $A\beta_{42}$, T-Tau, and T-Tau/Aβ42 for the five subgroups of patients are displayed in Table 2 and Fig. 2, together with the statistical analysis. AD patients showed, as expected, the most important differences in Aβ42, T-Tau, and T-Tau/Aβ42 levels in comparison to CTRL subjects, all statistically significant. OD patients showed significantly lower Aβ42 but not T-Tau levels than CTRLS. In that subgroup, Aβ42 was not statistically different from that of the AD group. MCI patients showed intermediate results for all markers but not statistically different from CTRLs. Finally, in comparison to CTRLs, ND patients showed comparable values for Aβ42, elevated T-Tau levels, and, therefore, an intermediate T-Tau/Aβ42 ratio (Table 2 and Fig. 2).

Diagnostic accuracy and ROC curve analyses

AD diagnostic accuracy of the Lumipulse was assessed by means of ROC analysis (using clinical diagnosis as gold standard). To discriminate AD from CTRL subjects, T-Tau was the best single biomarker at a cut-off value of 381 pg/mL [sensitivity: 86.4% (72.6–94.8%); specificity: 83.4% (68.6–93.0%), area under the curve (AUC): 0.90 (0.83–0.96)]. Aβ42 had a lower discriminating power ($p=0.049$) at an optimal cut-off value of 437 pg/mL [sensitivity: 77.3% (62.2–88.5%); specificity: 81.0% (65.9–91.4%); AUC: 0.79 (0.68–0.89)]. When dividing T-Tau by Aβ42 values, an optimal cut-off of 1.12 was found. This ratio significantly outperformed Aβ42 performances ($p=0.0008$) but not T-Tau ($p=0.601$) [sensitivity: 81.8% (67.3–91.8%); specificity: 92.9% (80.5–98.5%), AUC: 0.91 (0.84–0.98)], even if a slightly higher AUC could be observed (0.91 vs 0.90 for T-Tau) (Fig. 3). When applying the same methodology to discriminate AD from non-AD patients (OD, ND and CTRL), Aβ42 had again a lower discriminating performance [AUC: 0.68 (0.58–0.77)] than T-Tau [AUC: 0.83 (0.75–0.90)] ($p=0.009$). Again, the T-Tau/Aβ42 ratio improved significantly the discrimination between the two subgroups [AUC: 0.84 (0.77–0.92)] as compared to Aβ42 ($p=0.015$) but not for T-Tau ($p=0.72$) (Fig. 3b).

According to the cut-offs derived from the comparison of AD patients versus CTRLs, 43.5%, 52.2%, and 39.1% of MCI patients showed abnormal Aβ42, T-Tau, and T-Tau/Aβ42 ratio levels, respectively. These proportions were, respectively, 66.7%, 38.9%, and 30.6% in the OD subgroup. Finally, ND patients presented abnormal Aβ42 levels in 25% of cases, whereas 81.8% had elevated T-Tau values. Conversely, only 36.4% had an abnormal T-Tau/Aβ42 ratio. Of note, the lowest prevalence of cases with a CSF AD signature in the main groups of interest for a differential diagnosis with AD (MCI and OD) were for the T-Tau/Aβ42 ratio, showing that it is also a good measure for this purpose.

Clinical performance comparison between INNOTEST and Lumipulse assays

When analyzing the data issued from 115 samples (27 AD and 88 non-AD) measured on both assays, Aβ42 showed a slightly higher AUC with the INNOTEST assay [0.75; (0.66–0.83)] than the Lumipulse assay [0.71; (0.61–0.79)], but this difference did not reach statistical significance ($p=0.055$). T-Tau showed similar performances both on INNOTEST [AUC: 0.86 (0.78–0.92)] and Lumipulse assay [AUC: 0.86 (0.79–0.92)]. Again, T-Tau/Aβ42 showed slightly higher clinical performances with INNOTEST assays [AUC: 0.90 (0.83–0.95)] versus Lumipulse assays [AUC: 0.88 (0.80–0.93)], although not statistically significant ($p=0.38$).

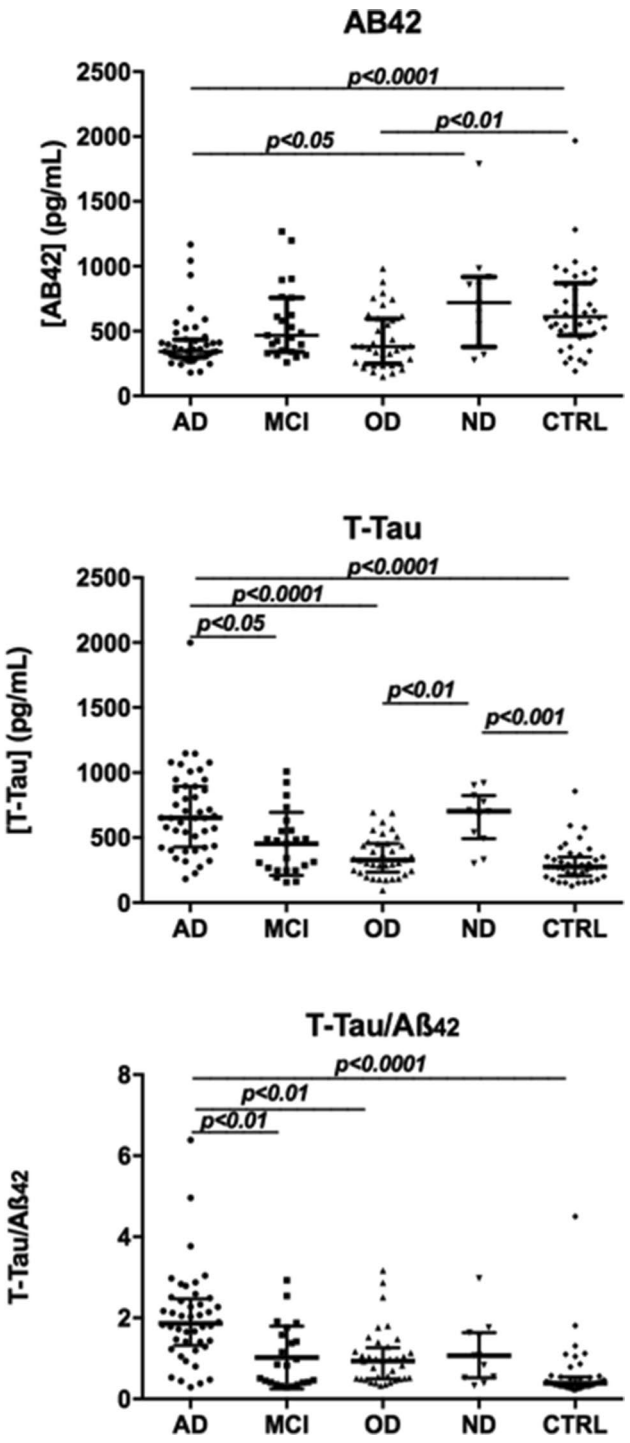

Fig. 2 Scatter plots of CSF amyloïd β1–42 peptide (Aβ42) and total-Tau protein (T-tau) measured with the Lumipulse in several disease categories. Median with interquartile range is indicated in all subgroups. Kruskal–Wallis test followed by Dunn's multiple comparison tests were performed and significant *p* values shown. *AD* Alzheimer disease, *MCI* mild-cognitive impairment, *OD* other dementias, *ND* non-demented, *CTRL* control patients

Discussion

Since the development of the INNOTEST assays in the early 2000s, few novel measurement technologies have emerged in the field of CSF-based AD diagnosis [15]. However, more recently, several CE-approved automated platforms have been developed, which are expected to play a key role in the standardization process of AD biomarkers in the near future. Indeed, it is now well established that there is measurement variability linked to kit users, both within and between laboratories [7, 9]. These platforms have the advantage to standardize the process of multiple incubation times, reaction temperature, and also pipetted volumes.

In our study, the Lumipulse showed excellent analytical performance both in- and between-runs. As expected, a significant systematic difference was observed for both Aβ42, T-Tau, and P-Tau, underlying the need to redefine new clinical cut-offs when switching from manual ELISA to an automated platform. Recently, Lumipulse target values for the Aβ42 calibrators were re-adjusted using the CRM samples. To evaluate this, we measured three Aβ42 CRM on the Lumipulse assay which all gave results comprised within the certified ranges. On the other hand, measured values on the manual ELISA Innotest assays were out of certified range for the middle and high CRM materials.

In this study, we examined the ability of Aβ42 and T-Tau biomarkers to discriminate clinically defined AD from non-AD or CTRL patients. As expected, median levels of Aβ42 were lower, whereas median levels of T-Tau were higher in the AD group compared to both other groups. According to our results, the best discriminating marker was the T-Tau/Aβ42 ratio, as reported by several others teams [16–18]. The use of this ratio seems to outperform the use of a single biomarker for patients having conditions potentially affecting T-Tau and Aβ42 levels.

The proportion of MCI patients presenting a pathological T-Tau/Aβ42 ratio in our study was 39.1%; this is in line with previously published studies having assessed the proportion of MCI patients who will progress to defined Alzheimer's disease [19, 20]. However, due to the limited cohort, the mixed types of presentations (amnestic and nonamnestic MCI), and the absence of longitudinal follow-up, no firm conclusions can be drawn from these preliminary MCI analyses.

Numerous studies have evaluated the performance of AD biomarkers to discriminate AD patients from non-AD patients. However, these studies are characterized by a high variability in the selection criteria of the non-AD group. Some studies included both non-demented and demented patients, while others restrain the selection to

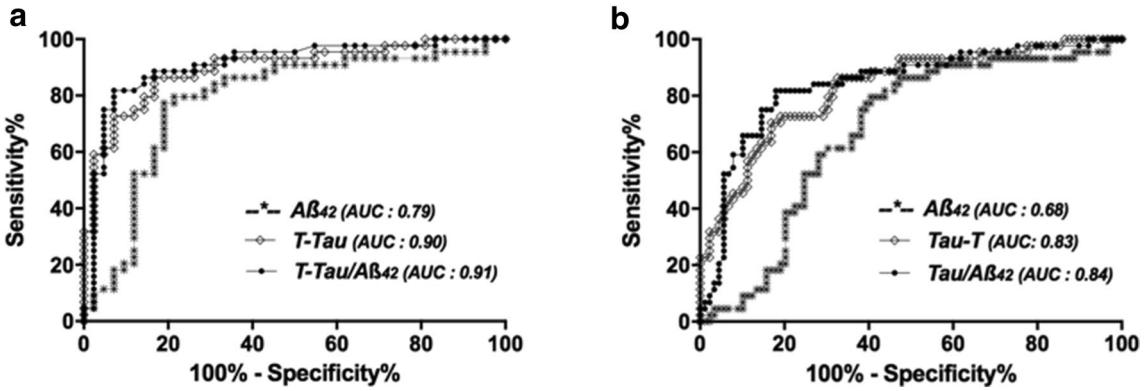

Fig. 3 ROC curves analysis of CSF biomarkers on Lumipulse for Alzheimer 's disease (AD) diagnosis. Receiver-operating characteristic (ROC) curves obtained for amyloïd β1–42 peptide (Aβ42) and total-Tau protein (T-tau) and T-Tau/Aβ42 ratio for discriminating AD vs. CTRL patients (**a**) or AD vs. non-AD (**b**) (including other dementias, non-demented, and CTRL patients). *AUC* area under the curve

patients showing no clinical evidence of dementia [17, 21–24]. This selection issue is, in our opinion, not sufficiently addressed in the existing guidelines. Indeed, selection bias probably contributes to the inter-laboratory cut-off variability. With a carefully selected control group, our results clearly illustrate that the ROC AUCs for AD diagnosis are higher when the non-AD group is composed solely of non-demented CTRL patients than with a non-AD group including also OD and ND patients. It is well known that a significant proportion of patients considered as suffering from non-AD dementia also show some AD pathology either upon imagery, PET, CSF analysis, or at post-mortem examination, indicating the common prevalence of mixed dementias [25–27]. Moreover, some non-AD dementias, like tauopathies, also show elevated CSF T-Tau levels, therefore, reducing the discriminating power of this marker [28]. Finally, due to the occurrence of atypical presentations, diagnostic mistakes (a clinical presentation of non-AD dementia having, in fact, AD pathology) are also possible. The ND group included conditions which, although not accompanied by dementia, could have altered the levels of biomarkers, in particular for T-Tau, by widespread neuronal destruction.

Our study has a major limitation, since we were not able to determine the diagnostic performances of the P-Tau$_{181}$ protein. Indeed, this assay was not CE/IVD-approved at the time of our investigation. Only a brief analytical evaluation was performed, once the kit was available. Nevertheless, Aβ42 and T-Tau outperformed this marker in several previously published studies and few AD profiles are characterized by isolated high P-Tau values [21, 27]. Moreover, P-Tau181 was initially introduced to allow easier differential diagnosis between AD and other neurodegenerative dementias, but this indication is still debated [5, 27].

In conclusion, our work demonstrates the optimal analytical and clinical performance of the new Aβ42 and T-Tau Lumipulse assays in a single-center investigation. We here described clinical cut-offs able to discriminate AD patients from CTRL patients with these recently approved assays. Thanks to the better operational standardization and Aβ42 CRM-adjusted calibrators' release, it is expected that these automated platforms will enhance intra- and inter-laboratory reproducibility. Further efforts are needed to continue the preanalytical standardization of processes across laboratories and to develop CRM for the T-Tau protein.

Compliance with ethical standards

Conflicts of interest The authors declare that they have no conflict of interest related to this work.

Ethical standards The study protocol was in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration. Residual samples used for diagnostic procedures can be used for retrospective academic studies, without any additional informed consent.

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