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Blood biomarkers for Alzheimer's disease with the Lumipulse automated platform: Age-effect and clinical value interpretation

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ABSTRACT

Background: Advances in analytical methods have recently paved the way to Alzheimer's disease (AD) biomarkers testing in blood along with the more established CSF testing. To ensure a forthcoming application of this low-invasive diagnostic that might allow to recognize early onset of dementia, appropriate pathological cut-points need to be defined.

Methods: In this cross-sectional study we measured blood and CSF neurofilament light chain (NFL), phosphorylated tau (pTau 181), Amyloid-β1-42 (AB 1–42) and Amyloid-β1-40 (AB 1–40) on a fully automated chemiluminescent platform (Lumipulse, Fujirebio) in 80 cognitively impaired patients and 55 cognitively unimpaired subjects. Clinical cut points were calculated with receiver-operator characteristic (ROC) curve analysis and a head-to-head comparison of blood and CSF testing was performed.

Results: Blood NFL best discriminant thresholds to distinguish neurodegenerative diseases from controls varied age-dependently, being 19 and 33 pg/mL in subjects 50–65 years and > 65 years respectively. AD was best framed by AB 1–42/1–40 ratio < 0.079 and ptau181 > 1 pg/mL. Though a strong correlation for all biomarkers, only blood AB ratio was equal to CSF testing for AD diagnosis.

Conclusions: The specific context of use might be considered to define the cut-offs of blood biomarkers of neurodegenerative diseases. Future efforts towards reference materials for each AD blood biomarker will improve clinical cut-offs.

1. Introduction

Alzheimer's Disease (AD) is a neurodegenerative disease for which the in-vivo biological diagnosis is possible through imaging techniques (positron emission tomography, PET, with amyloid and tau tracers) and with cerebrospinal fluid (CSF) biomarkers that are currently approved as in-vitro diagnostic (IVD) tests for clinical use, i.e. Amyloid-β1-42 (AB 1–42), Amyloid-β1-40 (AB 1–40), phosphorylated tau at threonine 181 (pTau 181), and total tau [1]. As a breakthrough finding, neurofilament light chain (NFL) concentration has been proven to be elevated in heterogeneous diseases resulting in irreversible damages of the central or peripheral nervous system [2]. In the last few years, solid advances in analytical methods and innovative technologies have led to a progressive shift of biomarkers traditionally tested in CSF towards blood, thus facilitating early diagnosis and monitoring of neurodegenerative disorders in general and, in particular, AD, although these novel blood technologies are not yet reimbursed and widespread in the diagnostic laboratory services.

It is estimated that a long lasting disease course of AD is clinically silent [3], having the disease a long preclinical phase [4] that might potentially be examined with a low invasive diagnostic biomarker. Given the global social and economic impact of dementia and

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Table 1
Descriptive statistics. Mean values were compared by one-way ANOVA with Bonferroni's test for pairwise comparisons (* $p < 0.05$ with respect to CTRL; † $p < 0.05$ with respect to all the other groups). Median values were compared by Kruskal-Wallis test. CTRL = controls; FTD = frontotemporal dementia; PD = Parkinson disease; AD = Alzheimer's disease.

		CTRL	FTD	PD	AD	Statistical analysis
	n.	55	24	9	47	
gender (F/M)		33/22	11/13	4/5	26/21	Chi-square = 1.774, p = 0.0621
age (years)	range	15–81	51–78	57–76	59–81	
	mean ± SD	46 ± 15	65 ± 8*	68 ± 7*	70 ± 6*	F = 43.93, p < 0.0001
	median (25 %-75 %)	43 (36–55)	65 (58–70)	70 (65–72)	71 (67–74)	p < 0.0001
NFL pg/mL	n.	54	23	9	44	
	range	4–32.6	12.5–84.6	15.9–117.3	9.9–191.8	
	mean ± SD	11.1 ± 6.5	37.3 ± 25.2*	39.1 ± 31.1*	31.4 ± 27.4*	F = 13.06, p < 0.0001
	median (25 %-75 %)	9.2 (7–13.2)	26.3 (17–49.9)	26.1 (24–43)	25.6 (19.7–34.3)	p < 0.0001
pTau181 pg/mL	n.	37	12	9	47	
	range	0.3–5.4	0.6–2.2	0.6–1.7	0.6–5.3	
	mean ± SD	0.9 ± 0.9	1.2 ± 0.5	1.3 ± 0.4	2.5 ± 1.1†	F = 20.66p < 0.0001
	median (25 %-75 %)	0.6 (0.5–1)	1.2 (0.8–1.5)	1.2 (1–1.5)	2.3 (1.8–2.9)	p < 0.0001
AB 1–42 pg/mL	n.	37	12	9	47	
	range	8–30.1	17.9–30.2	11.2–29	8.1–29.3	
	mean ± SD	21.8 ± 4.4	23.2 ± 3.4	23.3 ± 5.6	18.7 ± 4.3†	F = 6.66, p = 0.0004
	median (25 %-75 %)	22.2 (20.2–23.8)	22.5 (20.9–24.9)	25.3 (20.6–25.8)	19 (16.6–21.6)	p = 0.0001
AB1-40 pg/mL	n.	37	12	9	47	
	range	77.7–292.3	208.5–359.4	144.2–423	116.4–436.1	
	mean ± SD	223.1 ± 41.9	280.7 ± 44.7*	289.5 ± 77.2*	260.9 ± 62.4*	F = 6.16, p = 0.0007
	median (25 %-75 %)	225.2 (213.6–243.6)	279.4 (252.2–305.4)	299.4 (241.2–322.7)	264.9 (232.3–294.9)	p = 0.0001
1–42/1–40	n.	37	12	9	47	
	range	0.072–0.113	0.074–0.106	0.059–0.095	0.059–0.092	
	mean ± SD	0.098 ± 0.011	0.083 ± 0.009*	0.081 ± 0.010*	0.072 ± 0.007†	F = 59.24p < 0.0001
	median (25 %-75 %)	0.101 (0.095–0.107)	0.082 (0.078–0.084)	0.082 (0.078–0.086)	0.072 (0.067–0.074)	p < 0.0001

particularly AD, with an estimated number of patients of about 131 million worldwide by 2050 [5], the advantage of a blood testing to recognize early onset of dementia or individuals who will develop symptoms in the near future or even to accurately diagnosis a specific type of dementia is undoubted, although its feasibility is yet to be proven [1].

Since its definition [6] and the subsequent support by the National Institute on Aging–Alzheimer's Association (NIA-AA) [7], the A/T(N) classification has been widely used, albeit formally in research context only, to estimate the three main pathological processes that are involved in AD, i.e. brain deposition of amyloid and phosphorylated-tau dependent neurodegeneration thus encompassing both imaging-based and fluid-based biomarkers. The biological definition of a patient with suspected "AD continuum" [7] will probably enable the appropriate administration of upcoming pharmacological treatments and facilitate the inclusion in clinical trials, therefore shifting the application of A/T(N) as standard of care would also improve the clinical management of these patients. Moreover, the need for a structured workflow for AD in vivo staging based on biomarkers has been recently described [3], also promoting the further use of blood biomarker in particular as routine clinical laboratory tests [8]. In fact, although no such blood-based test has received IVD certification yet, several studies have addressed pre-analytical and analytical issues [9,10], and standard operating procedures for sample handling have been recently published by the Standardization of Alzheimer's Blood Biomarkers (SABB) working group [11]. The implementation of a laboratory test for clinical diagnostic use with appropriate reference ranges or threshold levels is essential, but in the field of blood biomarkers for AD, in spite of several published works, a shared consensus is still lacking, although it would facilitate the administration of the forthcoming anti-amyloid immunotherapies [1].

In the present study, first, we aim to identify age-related clinical cut-offs levels for blood biomarkers in neurodegenerative disorders including AD, detected with the chemiluminescent immunoassay (CLIA) automated platform Lumipulse. Specifically, we will focus on NFL, pTau181, AB 1–42 and AB 1–40. Next, we plan to compare blood biomarkers to CSF biomarkers to differentiate patients with neurodegenerative diseases from cognitively unimpaired subjects.

2. Methods

2.1. Subjects enrolled

We retrospectively included blood and CSF diagnostic leftovers of 80 subjects who underwent clinical examination at the Regional Brain Aging Center and at the Neurology Clinic of the University-Hospital of Padova (Study protocol CE:3950/AO/2016, informed consent was obtained from each subject): 47 patients with AD at different disease staging including mild cognitive impairment, 24 patients with frontotemporal dementia (FTD) and 9 patients with Parkinson disease or atypical parkinsonism (PD), diagnosed according to published criteria [7,12–17]. An additional cohort of 26 patients with newly diagnosed Multiple Sclerosis [18] was included for the verification of NFL ranges in CSF (MS group); these patients were in stable condition after at least one month from steroid therapy and not treated with disease-modifying drugs. As a cognitively unimpaired control group (CTRL) 55 subjects were considered: 30 were employees who underwent regular occupational health monitoring, 25 were patients who underwent neurological and cognitive examination due to a non-neurodegenerative disorder (i.e. migraine, psychiatric disorders).

2.2. Laboratory testing

NFL, pTau181, AB 1–42 and AB 1–40 were tested in 105 K2-EDTA plasma samples and 85 CSF samples; in a subset of 30 subjects only serum sample was available, and only NFL was tested. Analyses were carried out at Laboratory Medicine of University-Hospital of Padova on Lumipulse G1200 (Fujirebio, Japan). CSF samples included 47 AD, 17 cognitively unimpaired, 12 with FTD and 9 with PD, who underwent lumbar puncture as a standard of care for suspected cognitive decline. Diagnostic leftovers of plasma, serum and CSF samples were aliquoted in polypropylene tubes and stored at – 80° until analysis, that was performed after the samples were thawed at room temperature for at least 30 min, then vortexed for 10 s and centrifuged at 2000 g for 5 min.

Table 2
Logistic regression analysis. The presence or absence of neurodegenerative diseases was the dependent variable. Gender, age, NFL, pTau181 and AB 1–42/1–40 ratio were predictors. OR = Odds Ratio; SE = Standard Error; CI = Confidence Intervals.

	OR	SE	Z	p value	95 % CI	
Gender	3.637	3.862	1.22	0.224	0.454	29.150
Age	1.112	0.0678	1.74	0.082	0.987	1.253
NFL	1.126	0.0812	1.65	0.098	0.978	1.297
pTau181	0.546	0.240	−1.38	0.169	0.230	1.293
AB 1–42/1–40 ratio	$6.16e^{-67}$	$3.09e^{-65}$	−3.04	0.002	$1.4e^{-109}$	$2.67e^{-24}$
Constant	141.114	694.697	1.01	0.315	0.009	2188

2.3. Statistical analysis

Biomarkers concentrations were summarized as range, mean, standard deviation (SD), median and interquartile range. Normality distribution was tested with Shapiro Wilk W test. Distributions between groups were compared with Kruskal-Wallis test, Bonferroni test and Mann-Whitney test as appropriate. Correlation between age and biomarkers concentrations for each group was tested in appropriated logistic regression models and with Spearman's rank test. Clinical cut points were calculated with receiver-operator characteristic (ROC) curve analysis and Youden's index cut-off with confidence intervals 95 %. Correlation between biomarkers concentrations in blood and CSF was calculated with Spearman's rank test. Analyses were performed on Stata SE v. 13.0 and GraphPad Prism v. 5.01.

3. Results

3.1. Blood biomarkers descriptive analysis

The concentration levels of the blood biomarkers in patients and controls are reported in Table 1 along with age and gender distributions.

Logistic regression analysis was then performed considering the presence or absence of neurodegenerative diseases as dependent variable and, as predictors, age, gender, NFL, pTau181 and AB 1–42/1–40 ratio. A significant independent association between neurodegenerative diagnosis and AB 1–42/1–40 ratio was found and a minor, although not significant, effect was observed for age and NFL (Table 2).

Each blood biomarker was then individually evaluated to verify its diagnostic capacity considering the potential effect of age.

3.1.1. NFL

NFL levels correlated with age when considering patients and controls overall (Spearman's r = 0.752, p < 0.0001). The correlation between age and NFL levels was confirmed in controls (r = 0.721p < 0.0001) and in AD (r = 0.413p = 0.005). Since none of the patients was younger than 50 years, while part of controls belonged to this age category, age effect was re-evaluated after controls were subdivided into two groups: those younger and those older than 50 years. In controls aged less than 50 years (young controls) no significant correlation was found between NFL levels and age (r = 0.169p = 0.324), while this correlation was confirmed in those older than 50 years (r = 0.684p = 0.002). Since NFL in young controls (n. 36, mean 7.7 pg/mL, SD 1.7) had a normal distribution (Shapiro Wilk W test p = 0.649) and did not correlate with age, we defined the reference range in this cohort by mean ± 2SD, this resulting in: 4–11 pg/mL.

To define the best NFL threshold to distinguish patients with a neurodegenerative disease (AD and not-AD) from controls, ROC curves were performed considering subjects older than 50 years old altogether and after subdividing them into two further age-classes, 50–65 and > 65 years. The age of 65 years was chosen to define age-classes because this is considered the cut-off age to define young-onset versus late-onset dementia [19].

Table 3 reports the results of ROC curves with the best identified cut-points to distinguish neurodegenerative diseases or AD from controls considering the two age cohorts above defined. Although no statistically significant difference was found between the AUC, NFL performed better in patients aged 50–65 years compared to those older than 65 years, with optimal cut-points being 19 pg/mL and 33 pg/mL, respectively.

3.1.2. pTau181

pTau181 levels were also age-correlated when considering all subjects (Spearman's r = 0.608p < 0.0001), while when considering the different groups this correlation was confirmed in controls only (Spearman's r = 0.484p = 0.002) but not in AD patients (r = 0.017p = 0.911).

As described for NFL, ROC curves were performed considering the previously defined age classes (Table 4). No significant difference between the AUCs was found. The best cut-point in subjects < 65 years was 1 pg/mL for neurodegenerative diseases considered overall and for AD considered singly. The cut-point value should be almost doubled in subjects > 65 years, although in this age-class pTau181 showed an unsatisfactory discriminant power (AUC ranging from 0.490 to 0.611). Because of the low consistence of age-correlation of pTau181, ROC curves were also calculated for the entire cohort, showing excellent performances in diagnosing AD, also with 1 pg/mL cut-off.

3.1.3. AB 1–42/1–40 ratio

Since the individual production of beta amyloid protein has a high variability between subjects, the most useful biomarker for amyloid pathology is considered 1–42/1–40 ratio, which was analyzed as above to identify a cut-point related to age.

Age was correlated with 1–42/1–40 ratio considering all cases (r = -0.6251p < 0.0001), controls (r = -0.5311p = 0.0007), but not AD (r = 0.1359p = 0.363).

By ROC curves analyses (Table 5) AB 1–42/1–40 ratio in the 50–65 years age class was highly discriminant between patients with neurodegenerative diseases and controls. The AUC was significantly higher with respect to that obtained considering the whole cohort of > 50 years subjects (z statistics = 2.20p = 0.028), and also considering the > 65 years cohort (z statistics = 3.59p = 0.0003). Considering AD diagnosis,

Table 3
ROC curve analyses with best cut-offs for blood NFL in different contexts of use. CTRL vs. any neurodegenerative diseases comprised AD + FTD + PD; CTRL vs. AD diagnosis; CTRL = controls; AD = Alzheimer's disease; FTD = frontotemporal dementia; PD = Parkinson disease; SE = Standard Error; CI = Confidence Intervals.

Blood NFL	N.	AUC	SE	95 % CI		Cut-point pg/mL	Sensitivity	Specificity
≥ 50 years								
CTRL vs. Neurodegenerative diseases	94	0.777	0.060	0.658	0.895	19	0.8	0.72
CTRL vs. AD	62	0.777	0.067	0.646	0.908	19	0.82	0.72
50–65 years								
CTRL vs. Neurodegenerative diseases	34	0.854	0.064	0.729	0.980	19	0.71	1
CTRL vs. AD	21	0.836	0.101	0.638	1.000	19	0.73	1
>65 years								
CTRL vs. Neurodegenerative diseases	60	0.655	0.092	0.474	0.836	33	0.35	1
CTRL vs. AD	41	0.657	0.100	0.462	0.852	33	0.33	1

Table 4
ROC curve analyses with best cut-offs for blood pTau181 in different contexts of use. CTRL vs. any neurodegenerative diseases comprised AD + FTD + PD; CTRL vs. AD diagnosis; CTRL = controls; AD = Alzheimer's disease; FTD = frontotemporal dementia; PD = Parkinson disease; SE = Standard Error; CI = Confidence Intervals.

pTau181	N.	AUC	SE	95 % CI		Cut-point pg/mL	Sensitivity	Specificity
≥ 50 years								
CTRL vs. Neurodegenerative diseases	79	0.692	0.107	0.482	0.901	1.0	0.85	0.55
CTRL vs. AD	58	0.774	0.106	0.566	0.981	1.8	0.77	0.82
50–65 years								
CTRL vs. Neurodegenerative diseases	25	0.829	0.126	0.583	1.000	0.9	0.94	0.71
CTRL vs. AD	18	0.883	0.118	0.651	1.000	1.0	1	0.86
>65 years								
CTRL vs. Neurodegenerative diseases	54	0.490	0.172	0.154	0.826	1.8	0.6	0.75
CTRL vs. AD	40	0.611	0.209	0.200	1.000	1.8	0.78	0.75
All ages								
CTRL vs. Neurodegenerative diseases	105	0.869	0.041	0.788	0.949	1.0	0.9	0.76
CTRL vs. AD	84	0.915	0.037	0.843	0.987	1.0	0.96	0.78

Table 5
ROC curve analyses with best cut-offs for blood AB 1–42/1–40 ratio in different contexts of use. CTRL vs. any neurodegenerative diseases comprised AD + FTD + PD; CTRL vs. AD diagnosis; CTRL = controls; AD = Alzheimer's disease; FTD = frontotemporal dementia; PD = Parkinson disease; SE = Standard Error; CI = Confidence Intervals.

AB 1–42/1–40 ratio	N.	AUC	SE	95 % CI		Cut-point pg/mL	Sensitivity	Specificity
≥ 50 years								
CTRL vs. Neurodegenerative diseases	79	0.848	0.069	0.713	0.984	0.096	0.99	0.64
CTRL vs. AD	58	0.919	0.051	0.819	1.000	0.077	0.85	0.91
50–65 years								
CTRL vs. Neurodegenerative diseases	25	1.000	0.000	1.000	1.000	0.096	1	1
CTRL vs. AD	18	1.000	0.000	1.000	1.000	0.087	1	1
>65 years								
CTRL vs. Neurodegenerative diseases	54	0.612	0.108	0.400	0.820	0.077	0.66	0.75
CTRL vs. AD	40	0.750	0.116	0.522	0.978	0.077	0.83	0.75
All ages								
CTRL vs. Neurodegenerative diseases	105	0.926	0.027	0.872	0.979	0.094	0.97	0.76
CTRL vs. AD	84	0.966	0.018	0.931	1.000	0.079	0.89	0.95

Table 6
Descriptive statistics. Mean values were compared by one-way ANOVA with Bonferroni's test for pairwise comparisons: * $p < 0.05$ with respect to CTRL. Median values were compared by Kruskal-Wallis test. CTRL = controls; FTD = frontotemporal dementia; PD = Parkinson disease; AD = Alzheimer's disease; MS = Multiple sclerosis.

		CTRL	FTD	PD	AD	MS	Statistical analysis
	n.	17	12	9	47	26	
gender (F/M)		12/5	5/7	4/5	26/21	18/8	Chi-square = 5.0381, p = 0.411
age (years)	range	15–74	57–78	57–76	59–81	23–67	
	mean ± SD	46 ± 18.5	67 ± 7*	68 ± 6.5*	70 ± 6*	36 ± 9*	F = 43.77, p < 0.0001
	median (25 %-75 %)	43 (36–58)	67 (62–73)	70 (65–72)	71 (67–74)	33.5 (30–42)	p < 0.0001
CSF NFL pg/mL	n.	17	11	8	40	26	
	range	85–1623	697–4999	797–3746	467–3947	126–4017	
	mean ± SD	375 ± 380	1646 ± 1272*	1577 ± 1027*	1349 ± 743*	1290 ± 1128*	F = 5.1, p = 0.0003
	median (25 %-75 %)	259 (165–414)	1157 (836–2395)	1162.5 (870–2003)	1219.5 (749–1802)	945.5 (413–1925)	p < 0.0001

Table7
ROC curve analyses with best cut-offs for NFL in CSF in different contexts of use. CTRL vs. any neurodegenerative diseases comprised AD + FTD + PD + MS; CTRL vs. AD diagnosis; CTRL vs. MS diagnosis. CTRL = controls; AD = Alzheimer's disease; FTD = frontotemporal dementia; PD = Parkinson disease; MS = Multiple sclerosis; SE = Standard Error; CI = Confidence Intervals.

CSF NFL	N.	AUC	SE	95 % CI		Cut-point pg/mL	Sensitivity	Specificity
≥ 50 years								
CTRL vs. Neurodegenerative diseases	66	0.780	0.138	0.509	1	578	0.92	0.60
CTRL vs. AD	45	0.775	0.135	0.511	1	888	0.70	0.80
>65 years								
CTRL vs. Neurodegenerative diseases	47	0.773	0.165	0.450	1	901	0.81	0.75
CTRL vs. AD	34	0.775	0.158	0.465	1	919.5	0.80	0.75
All ages								
CTRL vs. Neurodegenerative diseases	102	0.904	0.046	0.814	0.995	447.5	0.89	0.82
CTRL vs. AD	57	0.934	0.048	0.846	1	450	1	0.82
CTRL vs. MS	43	0.833	0.063	0.708	0.957	378	0.85	0.71

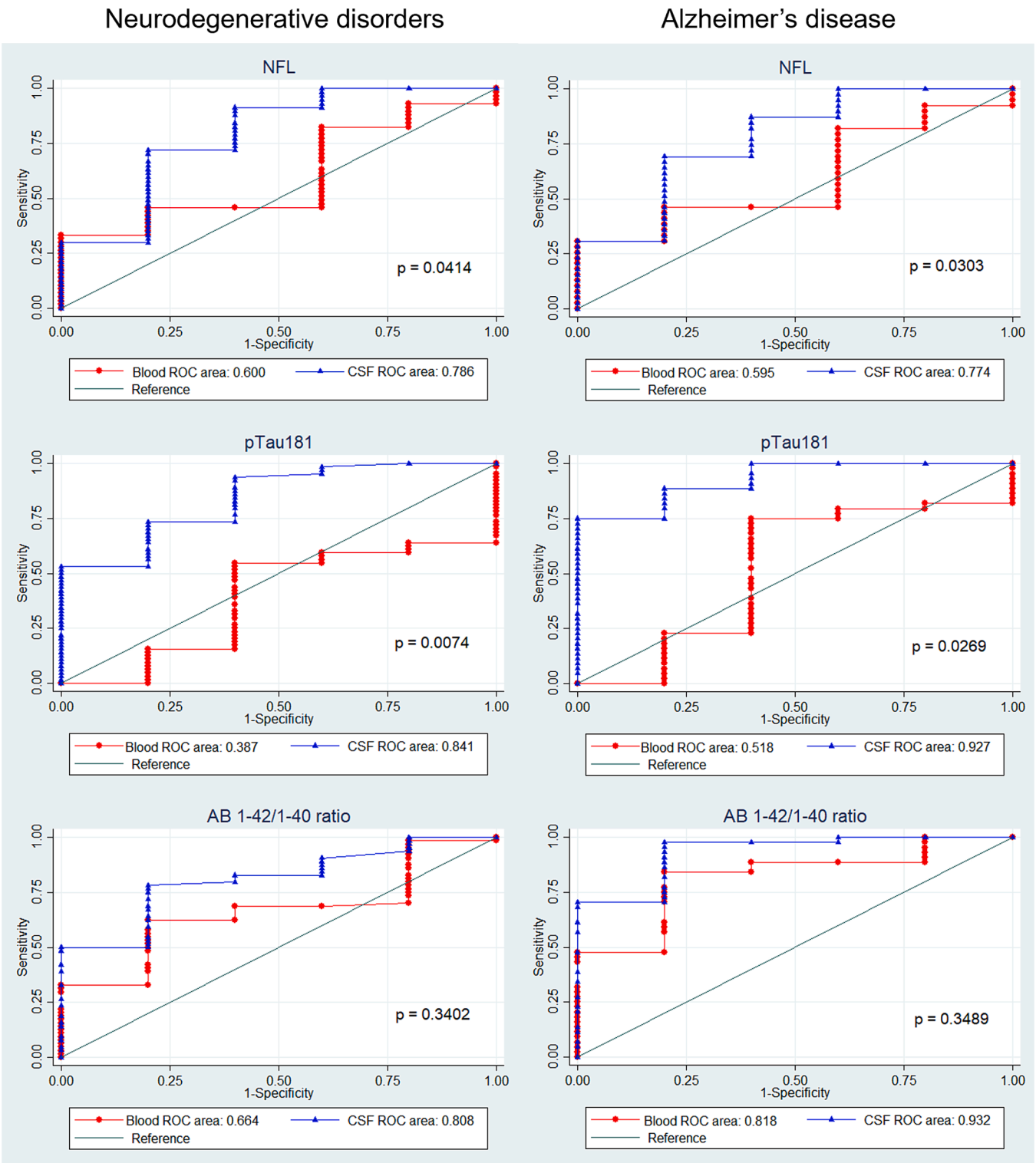

Fig. 1. Head-to-head comparison of blood and CSF ROC curves for neurofilament light chain (NFL), phosphorylated Tau (pTau181) and Amyloid-β1-42/1–40 (AB 1–42/1–40) ratio in distinguishing neurodegenerative disorders (left panels) and Alzheimer' disease (right panels) from controls in patients > 50 years old.

AB 1–42/1–40 ratio was confirmed to be highly discriminant in all age classes, with no significant difference between ROC curves. The best cut-off for AD considering all ages was 0.079, while that for neurodegenerative diseases was 0.094.

3.2. *CSF NFL descriptive analysis*

NFL concentrations, age and gender distribution for patients tested on CSF is reported in Table 6.

NFL concentrations were age-correlated when considering the entire cohort (Spearman's r = 0.408, p < 0.0001), which was confirmed only in two groups: CTRL (r = 0.882, p < 0.0001) and AD (r = 0.423p =

0.006). Moreover, NFL and age were not correlated in the entire cohort for subjects younger than 50 years (r = 0.068, p = 0.693, n = 36).

ROC curves were performed considering the entire cohort and after subdividing subjects by age in the subgroups older than 50 years and older than 65 years. CSF NFL allowed distinguishing the presence of any neurodegenerative disorder from not affected subjects with similar performances considering both age subgroups (AUC = 0.780 and AUC = 0.773 for subjects older than 50 and 65 years respectively), being the best cut-points 578 pg/mL and 901 pg/mL. Threshold levels very close to the latter (888 and 920 pg/mL) allowed distinguishing AD from controls in the two age classes of subjects with more than 50 and more than 65 years (Table 7).

3.3. Blood vs. CSF biomarkers

Blood and CSF NFL levels were highly correlated (Spearman's r = 0.8484, p < 0.0001, n. obs = 74). There was also a significant correlation between blood and CSF pTau181 levels (r = 0.5907, p < 0.0001, n. obs = 71) and 1–42/1–40 ratio (r = 0.566, p < 0.0001, n. obs = 71), although AB 1–42 and AB 1–40 taken singly were not (p = 0.7107 and p = 0.6505 respectively).

For the > 50 years subjects for whom both blood and CSF samples were available, the head-to-head comparison of ROC curves for NFL, pTau181 and ratio 1–42/1–40 in blood versus CSF highlighted substantial better performances of CSF NFL and pTau181 measurement in the context of neurodegenerative disorders (Fig. 1 panel A) and in the context of AD diagnosis (panel B), while for blood 1–42/1–40 ratio the AUC was not significantly different from CSF both for a neurodegenerative diagnosis (AUC = 0.664 vs. 0.808, p = 0.3402) and AD (AUC = 0.818 vs. 0.932, p = 0.3489).

4. Discussion

This work aimed to verify age-related clinical cut-offs for blood biomarkers of AD; several works have previously evaluated age-related refence ranges, i.e. normal ranges, particularly for NFL [20,21], nevertheless, given the high between-subject biological variation (BV) [22], the index of individuality (II) [23] and the strong association with age for NFL, concentrations thresholds to recognize specific pathological processes seem to be more useful in clinical practice.

In regards to the index of individuality (II) for the blood AD biomarkers, it is worth mentioning that different publications found it to be < 0.6 for NFL [22,23] due to the relatively low within-subject BV (CVI) and the high between-subject BV (CVG), while for pTau181 and 1–42/1–40 ratio the II of 0.69 and 0.68 respectively was recently published, thus suggesting that a measure of fold changing rather than a reference range seem to have stronger clinical significance [24]. As a matter of fact, the use of a low invasive matrix as blood will imply that several patients will undergo routinely follow-up with serial measurements, therefore reporting the appropriate cut-off for each clinical context should also be accompanied by the calculation of reference change value (RCV) along with the confidence intervals that might help the clinician to understand whether the observed variation is of significant magnitude [25].

The ability of blood NFL alone to discriminate patients with any neurodegenerative disorder or AD from cognitively unimpaired subjects was overall satisfactory (AUC 0.777 for both settings) when considering subjects older than 50 y, showing however a better performance among subjects aged 50–65 years and a limited usefulness in older individuals. This might be due to a neurodegenerative process that date back to many years before, with a low-grade chronic NFL release in the biofluids. Consequently, as time goes by, NFL thresholds increase, moving from 11 pg/mL to 19 pg/mL and to 33 pg/mL for the age-classes < 50, 50–65 and > 65 years, "red-flags" for a neuropathologic mechanism.

Blood pTau181 performed overall differently depending on the age-class and the exact context, having a good performance when defining AD of all ages versus cognitively unimpaired patients with a sensitivity of 96 % at 1 pg/mL, but also when defining AD versus any other condition including FTD and PD, though with a higher cut-point of 1.6 pg/mL that increased the specificity from 78 % to 88 % (data not shown). PTau181 concentrations measured also with Lumipulse technology were indeed slightly higher in a recently published work [26], where cognitively unimpaired A-/T- subjects had a mean value of 1.59 pg/mL, though with a standard deviation (SD) of 1.5 pg/mL. Similarly, the mean level of blood pTau181 in A+/T + patients was higher [26] (2.92 vs. 2.5 pg/mL in our study), although, given that the AD continuum group of our cohort encompasses both MCI and overt AD, we cannot exclude an underestimation effect due to a lower number of patients with advanced tauopathy in our cohort.

The 1–42/1–40 ratio in blood had the best performances in defining AD patients, with an AUC of 0.966 for AD versus cognitively unimpaired and a specificity of 95 % at the best cut-off of 0.079. This finding is in accordance with the aforementioned paper by Bellomo et al. [26] that in a classification model for predicting A+/T + profile identified a Youden best cutoff of 0.080. Moreover, in our current work the cut-off of 0.094 had 97 % sensitivity in the identification of any neurodegenerative disorder, that is rather higher than the mean value of cognitively unimpaired of 0.088 (SD = 0.011) found in the previous work [26].

Although the overall good performances of blood, the head-to-head comparison with CSF biomarkers in our study encourages blood analyses of NFL and 1–42/1–40 ratio, while more cautious evaluation should be reserved to pTau181, that should be studied in a higher number of subjects. In the overall context of neurodegenerative disorders, and specifically in the AD setting, blood measurements of NFL but mainly of 1–42/1–40 ratio might support AD diagnosis versus cognitively unimpaired controls, although they cannot substitute CSF testing. This is true also for analytical considerations, as desirable performance specifications of laboratory methods should encounter the low CVI that has already been reported [22]. Indeed, it has been reported that plasma 1–42/1–40 ratio has a lower fold change than CSF when comparing subjects with and without amyloid pathology [27], thus resulting more affected by an analytical bias. Blood phosphorylated tau proteins at different isoforms have instead shown more promising results, with concentrations that change between subjects affected and not-affected by amyloid deposition similarly as CSF [27]. Moreover, plasma pTau217 has been proven clinically equivalent to CSF testing in revealing amyloid pathology at PET and even superior to CSF testing for tau pathology [28] and extensive clinical validation of its quantification also on CLIA technology is currently under consideration.

As limitations of our study we should include the limited number of cases included, and the lack of data on renal function and body mass index (BMI) for the entire cohort: although adjustment for these variables was not proven to be clinically relevant [29], it has been reported that these might impact the concentration of at least NFL [30].

It is extremely important to circumscribe the extension of subjects that might potentially be included in this diagnostic, as the general population will not probably be the ultimate field of application, due to the general costs and the burden that this would rest on the health systems worldwide. Nevertheless, while for more invasive diagnostics as CSF biomarkers and PET "The value of knowing", as previously stated [31], at the moment is cautiously to be guaranteed to patients with objectively measured cognitive impairment, blood biomarkers have the potential to be assigned to the "preventive" or pre-symptomatic setting, that might also encompass screening of individuals with subjective cognitive complaints. Yet for the pre-symptomatic setting, it has been revealed that CSF biomarkers levels in subject that will develop AD change even 18 years before diagnosis [32], compelling further studies on the transferability of this finding in the blood matrix, also giving the recognition of possible modifiable risk factors for dementia [33,34].

As already pointed out, blood biomarkers step forward to guarantee equality in the access to diagnosis in developing countries, or in specific remote areas with limited access to more complex health resources [35].

Table 8
Proposed Lumipulse clinical cut-offs for blood biomarkers.

Blood Biomarker	Age-class	Clinical cut-off	Best context of use
NFL	< 50 years	> 11 pg/mL	Neurodegenerative process
NFL	50–65 years	> 19 pg/mL	Neurodegenerative process
NFL	> 65 years	> 33 pg/mL	Neurodegenerative process
pTau181	All ages	> 1 pg/mL	AD
AB 1–42/1–40 ratio	All ages	< 0.094	Neurodegenerative process
AB 1–42/1–40 ratio	All ages	< 0.079	AD

Identification of relevant clinical thresholds for biomarkers is crucial for their implementation in the clinical practice, however it is necessary to highlight that this goal will be achieved only when reference materials (RM) and reference measurement procedures (RMP) will be established for each blood biomarker, effort that is currently ongoing [35]. RM and RMP will eventually ensure standardization in the commercially available analytical methods and the harmonization between clinical laboratories that will also allow the feasibility of international guidelines for diagnosis, treatment and monitoring of these patients.

4.1. Conclusion

Finally, based on the current work the proposed clinical cut-offs for the three biomarkers in blood for specific context of use are summarized in Table 8.

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Declaration of Competing Interest

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Data availability

Data will be made available on request.

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