Classification accuracy of blood-based and neurophysiological markers in the differential diagnosis of Alzheimer’s disease and frontotemporal lobar degeneration

Alberto Benussi1,2, Valentina Cantoni3, Jasmine Rivolta1, Silvana Archetti4, Anna Micheli5, Nicholas Ashton6,7,8,9, Henrik Zetterberg6,10,11,12,13, Kaj Blennow6,10 and Barbara Borroni1,2*

Abstract

Background: In the last decade, non-invasive blood-based and neurophysiological biomarkers have shown great potential for the discrimination of several neurodegenerative disorders. However, in the clinical workup of patients with cognitive impairment, it will be highly unlikely that any biomarker will achieve the highest potential predictive accuracy on its own, owing to the multifactorial nature of Alzheimer’s disease (AD) and frontotemporal lobar degeneration (FTLD).

Methods: In this retrospective study, performed on 202 participants, we analysed plasma neurofilament light (NfL), glial fibrillary acidic protein (GFAP), and tau phosphorylated at amino acid 181 (p-Tau181) concentrations, as well as amyloid β42 to 40 ratio (Aβ1–42/1–40) ratio, using the ultrasensitive single-molecule array (Simoa) technique, and neurophysiological measures obtained by transcranial magnetic stimulation (TMS), including short-interval intracortical inhibition (SICI), intracortical facilitation (ICF), long-interval intracortical inhibition (LICI), and short-latency afferent inhibition (SAI). We assessed the diagnostic accuracy of combinations of both plasma and neurophysiological biomarkers in the differential diagnosis between healthy ageing, AD, and FTLD.

Results: We observed significant differences in plasma NfL, GFAP, and p-Tau181 levels between the groups, but not for the Aβ1–42/Aβ1–40 ratio. For the evaluation of diagnostic accuracy, we adopted a two-step process which reflects the clinical judgement on clinical grounds. In the first step, the best single biomarker to classify “cases” vs “controls” was NfL (AUC 0.94, \( p < 0.001 \)), whilst in the second step, the best single biomarker to classify AD vs FTLD was SAI (AUC 0.96, \( p < 0.001 \)). The combination of multiple biomarkers significantly increased diagnostic accuracy. The best model for classifying “cases” vs “controls” included the predictors p-Tau181, GFAP, NfL, SICI, ICF, and SAI, resulting in an AUC of 0.99 (\( p < 0.001 \)). For the second step, classifying AD from FTD, the best model included the combination of Aβ1–42/Aβ1–40 ratio, p-Tau181, SICI, ICF, and SAI, resulting in an AUC of 0.98 (\( p < 0.001 \)).

Conclusions: The combined assessment of plasma and neurophysiological measures may greatly improve the differential diagnosis of AD and FTLD.

*Correspondence: bborroni@inwind.it

1 Neurology Unit, Department of Clinical and Experimental Sciences, University of Brescia, P.le Spedali Civili 1, 25123 Brescia, Italy

© The Author(s) 2022. Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/. The Creative Commons Public Domain Dedication waiver (http://creativecommons.org/publicdomain/zero/1.0/) applies to the data made available in this article, unless otherwise stated in a credit line to the data.
Background

Alzheimer’s disease (AD) and frontotemporal lobar degeneration (FTLD) are a major and increasing global health challenge, with cases estimated to reach 150 million worldwide in 2050 [1], due to the constant increase of elderly people as younger age mortality declines [2]. In this scenario, several priorities are being identified, particularly in view of the development of disease-specific and disease-modifying therapies that target distinct proteinopathies; the foremost concern is to promptly identify patients with a neurodegenerative disorder from healthy ageing with high diagnostic confidence and then discriminate neurodegenerative disorders from one another, to benefit patients with tailored therapies and prognostic counselling.

Currently, validated markers, divided into imaging modalities and cerebrospinal fluid (CSF) measures, are used on clinical grounds and have proven to be highly accurate in diagnosing dementia [3]. However, several drawbacks may limit the use of these markers; thus, they are considered only in selected cases. In particular, some are able to identify AD but are unhelpful in other forms of dementia (i.e. amyloid positron emission tomography [PET] imaging or CSF Aβ42 and tau concentrations), and others are not useful in early disease stages at the single subject level (i.e. brain magnetic resonance imaging [MRI]); moreover, the invasiveness of the procedure (i.e. CSF analysis) or the expensiveness (i.e. amyloid PET) may further limit their availability. Notably, the ideal marker, besides having high accuracy and reliability, should be non-invasive, simple to perform, and inexpensive [4].

In the last decade, non-invasive blood-based biomarkers have been extensively studied and refined, showing great potential in the identification of neurodegenerative disorders, even in the prodromal phases of disease [5–9]. Moreover, neurophysiological techniques, such as transcranial magnetic stimulation, have been shown to be non-invasive and accurate in the discrimination of different dementing conditions [10–12].

As each biomarker (blood-based or neurophysiological) has shown great potential for the individualized prediction of neurodegenerative conditions, in the clinical workup of patients with cognitive impairment, it will be highly unlikely that any biomarker will achieve the highest potential predictive accuracy on its own, owing to the multifactorial nature of AD and FTLD and its heterogeneous clinical presentations. Consequently, there is a necessity to identify a combination of measures that should be integrated to produce the most accurate, easily accessible, non-invasive, and cost-effective diagnostic algorithm for the classification of common neurodegenerative disorders.

The aim of the current study was to examine the diagnostic accuracy of blood-based and neurophysiological biomarkers, both taken individually and in combination with other biomarkers, in a two-step phase. In the first step, patients with neurodegenerative dementia, i.e. AD or FTLD, should be discriminated from healthy controls, whilst in the second step, AD should be identified from other conditions such as FTLD.

Materials and methods

Participants

This retrospective study included 202 participants from the Centre for Neurodegenerative Disorders, Department of Clinical and Experimental Sciences, University of Brescia, Brescia, Italy.

The cohort consisted of 127 patients meeting probable clinical criteria for a syndrome in the FTLD spectrum, namely 67 behavioural variant frontotemporal dementia (bvFTD), 44 primary progressive aphasia (PPA), 7 corticobasal syndrome (CBS) and 9 progressive supranuclear palsy (PSP) [13–16]. Moreover, 48 patients fulfilling clinical criteria for AD [17] and 27 healthy controls (HC), recruited among spouses or caregivers, were included as well.

Each participant underwent neurological evaluation, routine laboratory examination, and a neuropsychological and behavioural assessment. In all cases, the diagnosis was supported by brain structural imaging, whilst CSF concentrations of tau, p-Tau181, and Aβ1–42 were measured in a subset of cases (n = 142, 64.5%), as previously reported [18]. Furthermore, in familial cases (based on the presence of at least one dementia case among the first-degree relatives) and early-onset sporadic cases, genetic screening for GRN, C9orf72, and MAPT P301L mutations was performed; given the low frequency of MAPT mutations in Italy [19], we considered only the P301L mutation, and we sequenced the entire MAPT gene only in selected cases.

Each participant underwent blood collection for measurements of serum NfL, GFAP, p-Tau181, Aβ1–42, and Aβ1–40 biomarkers. Moreover, each included patient underwent transcranial magnetic stimulation (TMS) protocols that partially and indirectly reflect the activity of several neurotransmitters, including GABA_A by

**Keywords:** Frontotemporal dementia, Alzheimer’s disease, Serum, Biomarkers, Neurofilament light, GFAP, p-tau, Amyloid, Transcranial magnetic stimulation
short-interval intracortical inhibition (SICI), glutamate by intracortical facilitation (ICF), GABA by long-interval intracortical inhibition (LICI), and acetylcholine by short-latency afferent inhibition (SAI) [20–22].

Clinical evaluation
At baseline, patients underwent a standardized neuropsychological battery which included the Mini-Mental State Examination (MMSE), the short story recall test, the Rey complex figure (copy and recall), the phonemic and semantic fluencies, the token test, the clock-drawing test, and the trail-making test (part A and part B) [23]. Disease severity was assessed with the clinical dementia rating plus National Alzheimer’s Coordinating Center (NACC) behaviour and language domains (CDR plus NACC FTLD) global and sum of boxes, whilst the level of functional independence was assessed with the basic activities of daily living (BADL) and the instrumental activities of daily living (IADL) questionnaire. Furthermore, neuropsychiatric and behavioural disturbances were evaluated with the neuropsychiatric inventory (NPI) [24].

HCs underwent a brief standardized neuropsychological assessment (MMSE ≥ 27/30); psychiatric or other neurological illnesses were considered exclusion criteria.

Serum biomarkers
Plasma was collected by venipuncture, processed, and stored in aliquots at −80 °C according to the standardised procedures. Plasma NfL concentration was measured using a commercial single-molecule array (Simoa) NF-Light® assay (Quanterix, Billerica, MA) according to the manufacturer’s instructions [25]. Plasma p-Tau181 concentration was measured using an in-house Simoa assay developed at the University of Gothenburg [8]. In brief, the capture antibody (AT270, Invitrogen) which is specific for the threonine-181 phosphorylation site [26] was coupled to paramagnetic beads whilst the detector antibody (Tau12, BioLegend) was raised against the N-terminal epitope amino acid 6-QEFEVMEDHAGT-18 on human tau protein. Detailed analytical procedures and assay validation have been previously described [8]. Plasma GFAP, Aβ1–42, and Aβ1–40 concentrations were measured using commercial Simoa assays (Quanterix, Billerica, MA). All measurements were carried out using an HD-X analyser (Quanterix, Boston, MA) in one round of experiments, using one batch of reagents with operators blinded to clinical information.

Transcranial magnetic stimulation
A TMS figure-of-eight coil (each loop diameter 70 mm – D70² coil) connected to a monophasic Magstim Bistim² system (Magstim Company, Oxford, UK) was employed for all TMS paradigms, as previously reported [27]. Electromyographic (EMG) recordings were performed from the first dorsal interosseous muscle using 9 mm diameter, Ag–AgCl surface-cup electrodes. The active electrode was placed over the muscle belly and the reference electrode over the metacarpophalangeal joint of the index finger. Responses were amplified and filtered at 20 Hz and 2 kHz with a sampling rate of 5 kHz.

Resting motor threshold (RMT) was determined on the left motor cortex as the minimum intensity of the stimulator required to elicit motor evoked potentials (MEPs) with a 50-μV amplitude in 50% of 10 consecutive trails, recorded during full muscle relaxation.

SICI-ICF, LICI, and SAI were studied using a paired-pulse technique, employing a conditioning test design. For all paradigms, the test stimulus (TS) was adjusted to evoke a MEP of approximately 1-mV amplitude.

For SICI and ICF, the conditioning stimulus (CS) was adjusted at 70% of the RMT, employing multiple inter-stimulus intervals (ISIs), including 1, 2, and 3 ms for SICI and 7, 10, and 15 ms for ICF [28, 29]. LICI was investigated by implementing two supra-threshold stimuli, with the CS adjusted at 130% of the RMT, employing ISIs of 50, 100, and 150 ms [30]. SAI was evaluated employing a CS of single pulses (200 μs) of electrical stimulation delivered to the right median nerve at the wrist, using a bipolar electrode with the cathode positioned proximally, at an intensity sufficient to evoke a visible twitch of the thenar muscles [31]. Different ISIs were implemented (0, +4), which were fixed relative to the N20 component latency of the somatosensory evoked potential of the median nerve.

For each ISI and for each protocol, ten different paired CS-TS stimuli and fourteen control TS stimuli were delivered to all participants in a pseudo-randomized sequence, with an inter-trial interval of 5 secs (± 10%).

The conditioned MEP amplitude, evoked after delivering a paired CS-TS stimulus, was expressed as a percentage of the average control MEP amplitude. The average values for SICI (1, 2, 3 ms ISI), ICF (7, 10, 15 ms ISI), LICI (50, 100, 150 ms ISI), and SAI (0, +4 ms ISI) were used for analysis.

Stimulation protocols were conducted in a randomized order. Audio-visual feedback was provided to ensure muscle relaxation during the entire experiment, and trials were discarded if EMG activity exceeded 100 μV in the 250 ms prior to TMS stimulus delivery. Less than 5% of trials were discarded for each protocol. All of the participants were capable of following instructions and reaching complete muscle relaxation; if, however, the data was corrupted by patient movement, the protocol was restarted, and the initial recording was rejected.
Statistical analysis
Continuous and categorical variables are reported as median (interquartile range) and n (%), respectively. Differences in clinical variables, biomarker concentrations, and neurophysiological measures between the groups were compared by Kruskal–Wallis H test, Mann–Whitney U test, χ² test, or Fisher’s exact test where appropriate.

A rank-based partial correlation was run to assess the relationship between serum biomarkers, clinical variables, and TMS measures, correcting for age.

To identify the most accurate combination of biomarkers, the initial model selection was performed using the R package MuMIn, which tests all different combinations of variables and then ranks the models according to the Akaike Information Criterion (AIC). AIC is a model performance metric which considers the trade-off between model fit and sparsity. Whilst R² and AUC explain how well a model performs on the observed data, AIC rather explains how well a model would perform on unseen data. In other words, the larger the difference in AIC values between the two models, the less likely it is that the model with the higher AIC value would provide better predictive performance on unseen data than the model with the lower AIC value. The model with the lowest AIC was selected as the model with the best trade-off between fit and complexity and then a stepwise removal of variables was performed as long as the ΔAIC was < 2 from the reference model identified (ΔAIC < 2). Thereafter, variables were removed further in a stepwise procedure to examine the added value of different variables and combinations of variable. Receiver operating characteristics (ROC) curve analyses were plotted, and the area under the curve (AUC) was selected as the model with the best trade-off between fit and complexity and then a stepwise removal of variables, and TMS measures, correcting for age.

To identify the most accurate combination of biomarkers, the initial model selection was performed using the R package MuMIn, which tests all different combinations of variables and then ranks the models according to the Akaike Information Criterion (AIC). AIC is a model performance metric which considers the trade-off between model fit and sparsity. Whilst R² and AUC explain how well a model performs on the observed data, AIC rather explains how well a model would perform on unseen data. In other words, the larger the difference in AIC values between the two models, the less likely it is that the model with the higher AIC value would provide better predictive performance on unseen data than the model with the lower AIC value. The model with the lowest AIC was selected as the model with the best trade-off between fit and complexity and then a stepwise removal of variables was performed as long as the ΔAIC was < 2 from the reference model identified (ΔAIC < 2). Thereafter, variables were removed further in a stepwise procedure to examine the added value of different variables and combinations of variable. Receiver operating characteristics (ROC) curve analyses were plotted, and the area under the curve (AUC) was selected as the model with the best trade-off between fit and complexity and then a stepwise removal of variables, and TMS measures, correcting for age.

Data availability
All study data, including raw and analysed data, and materials will be available from the corresponding author, B.B., upon reasonable request.

Results
Participant characteristics
A total of 202 patients were included in the present study, namely 127 FTLD, 48 AD patients, and 27 HC. Demographic and clinical characteristics, fluid biomarker levels, and neurophysiological measures are reported in Table 1. We observed significant differences in NfL, GFAP, and p-Tau181 levels between the groups but not for the Aβ1–42/Aβ1–40 ratio. NfL and GFAP levels were significantly increased in FTLD and AD, compared to HC (p = 0.005 and p < 0.001, respectively), whilst p-Tau181 levels were significantly increased in AD patients compared to FTLD and HC (both p < 0.001) (see Fig. 1). Regarding neurophysiological measures, AD showed decreased SAI compared to FTLD and HC (both p < 0.001), whilst FTLD showed reduced SICI, ICF, and LICI compared to AD and HC (both p < 0.001) (see Fig. 1).

Correlations between serum biomarkers, neuropsychological scores, and neurophysiological measures
Age-corrected biomarker concentrations correlated with several neuropsychological scores. In particular, we observed significant correlations between NfL and CDR plus NACC FTLD sum of boxes (r = 0.37, p < 0.001), NPI (r = 0.28, p < 0.001), and MMSE scores (r = −0.19, p = 0.013), but also with neurophysiological scores as SICI (r = 0.17, p = 0.025) and SAIs (r = −0.21, p = 0.006). GFAP also showed significant correlations with MMSE scores (r = −0.34, p < 0.001) and LICI (r = −0.16, p = 0.036). p-Tau181 values strongly correlated with the Aβ1–42/Aβ1–40 ratio (r = −0.30, p < 0.001), whilst NfL strongly correlated with GFAP levels (r = 0.29, p < 0.001).

Diagnostic accuracy of blood-based biomarkers and neurophysiological measures
For the evaluation of diagnostic accuracy, we adopted a two-step process which reflects a clinical judgement on clinical grounds. The first step allows us to classify each subject as “case” (i.e. patient with dementia) or “control”. If the subject falls into the “case” category, the next order of classification was considered, and the AD vs FTLD classifier was carried out.

As shown in Fig. 2A, the best single biomarker to classify “cases” vs “controls” was NfL (AUC 0.94 [95% CI 0.90–0.98], p < 0.001), followed by GFAP (AUC 0.86 [95% CI 0.80–0.92], p < 0.001). In the second step, as shown in Fig. 2B, the best single biomarker to classify AD vs FTLD was SAI (AUC 0.96 [95% CI 0.92–0.99], p < 0.001), followed by ICF (AUC 0.87 [95% CI 0.82–0.93], p < 0.001).

We subsequently applied a data-driven model selection process to choose the best combination of biomarkers with the lowest AIC, removing as many variables as possible whilst maintaining a similar model performance defined as being within two AIC points of the lowest AIC model identified (ΔAIC < 2). Thereafter, variables were removed further in a stepwise procedure to examine
the performance of the more basic models (i.e. the most accurate model with exclusively neurophysiological or blood-based biomarkers, a model with only a single neurophysiological or blood-based biomarker, and a model with the combination of one neurophysiological and one blood-based biomarker).

The best model for classifying “cases” vs “controls” included the predictors p-Tau181, GFAP, NfL, SICI, ICF, and SAI, resulting in an AUC of 0.99 (95% CI 0.99–1.00), p < 0.001 (see Fig. 3). By selecting exclusively blood-based biomarkers (p-Tau181, GFAP, and NfL) or neurophysiological measures (SICI, ICF, and SAI), we obtained very similar results (AUC 0.96 [95% CI 0.93–0.98], p < 0.001 and AUC 0.96 [95% CI 0.93–1.00], p < 0.001, respectively). Even higher accuracy was reached by combining the best blood-based biomarker (NfL) (AUC 0.94 [95% CI 0.90–0.98], p < 0.001) with the best neurophysiological measure (SICI) (AUC 0.85 [95% CI 0.78–0.92], p < 0.001), resulting in an AUC of 0.96 (95% CI 0.94–0.99), p < 0.001 (see Fig. 3).

For the second step, classifying AD from FTD, the best model included the combination of Aβ1–42/Aβ1–40 ratio, or neurophysiological measures (SICI, ICF, and SAI), or serum biomarkers (NfL, GFAP, p-Tau181), resulting in an AUC of 0.99 (95% CI 0.99–1.00), p < 0.001 (see Fig. 3). By selecting exclusively blood-based biomarkers (p-Tau181, GFAP, and NfL) or neurophysiological measures (SICI, ICF, and SAI), we obtained very similar results (AUC 0.96 [95% CI 0.93–0.98], p < 0.001 and AUC 0.96 [95% CI 0.93–1.00], p < 0.001, respectively). Even higher accuracy was reached by combining the best blood-based biomarker (NfL) (AUC 0.94 [95% CI 0.90–0.98], p < 0.001) with the best neurophysiological measure (SICI) (AUC 0.85 [95% CI 0.78–0.92], p < 0.001), resulting in an AUC of 0.96 (95% CI 0.94–0.99), p < 0.001 (see Fig. 3).

For the second step, classifying AD from FTD, the best model included the combination of Aβ1–42/Aβ1–40 ratio,
Fig. 1 (See legend on previous page.)
p-Tau181, SICI, ICF, and SAI, resulting in an AUC of 0.98 (95% CI 0.96–1.00, \( p < 0.001 \) (see Fig. 4). Considering exclusively blood-based biomarkers, the best model included GFAP, p-Tau181, and NfL (AUC 0.82 [95% CI 0.75–0.88], \( p < 0.001 \)), whilst the best model with exclusively neurophysiological measures included SICI, ICF, and SAI (AUC 0.98 [95% CI 0.96–1.00], \( p < 0.001 \)). By combining the best blood-based biomarker (p-Tau181, AUC 0.70 [95% CI 0.61–0.78], \( p < 0.001 \)) with the best neurophysiological measure (SAI, AUC 0.96 [95% CI 0.93–0.99], \( p < 0.001 \)), we obtained an AUC of 0.96 (95% CI 0.93–0.99), \( p < 0.001 \) (see Fig. 4).

**Discussion**

In the last years, a giant step forward has been made in identifying new diagnostic markers in neurodegenerative dementias, ranging from imaging and neurophysiology to biological measures. However, to spread the use of these markers extensively, beyond research referral centres, they need to be easily accessible, inexpensive, not time-consuming, and procedurally easy to perform.

In this view, the recently proposed blood-based biomarkers hold the potential to be available in any centre [34], and TMS intracortical excitability measures may be considered an adjunctive screening tool to be performed during outpatient visits [35].

In the present work, we aimed at assessing the classification accuracy of these markers, taken individually or in combination, in a large sample of consecutive patients with AD or FTLD and healthy controls. We adopted an intuitive and straightforward step-by-step approach resembling clinical reasoning, and at first, we aimed at discriminating ongoing neurodegenerative dementia from healthy ageing, and then we sought to identify AD cases from other conditions such as FTLD. We considered the best parsimonious model fit, the best model fit with either blood-based or TMS markers, and the best model fit with a single marker.

Our results supported the usefulness of blood NfL dosage as the first screening tool to classify patients with neurodegenerative dementia as compared to healthy subjects (AUC = 0.94), with a further slight increase in accuracy when it was combined with other blood-based biomarkers, such as GFAP or pTau181 (AUC = 0.96) or with TMS assessment indirectly evaluating GABAergic neurotransmission, i.e. SICI (AUC = 0.96) (see Figs. 2 and 3). On the other hand, in this cohort, TMS intracortical excitability measures indirectly assessing cholinergic dysfunction, i.e. SAI, were found to be the best single marker to classify AD compared to FTLD patients (AUC = 0.96). In this case, minor further improvements in classification accuracy were obtained when considering all TMS measures, i.e. SICI and ICF (AUC = 0.98) or Ab1–42/Ab1–40 ratio and pTau181 markers (AUC = 0.98) (see Figs. 2 and 4).

Taken together, these findings confirm and extend previous data, corroborating the utility of non-invasive and cost-effective markers on clinical grounds in diagnosing patients with dementia and dementia subtypes, and suggest a specific use of these tools depending on the clinical question. Compared to well-established diagnostic markers, such as CSF or PET tracers [36], this approach had a robust performance and similar accuracy, when markers were considered individually and even more when assessed in combination.

There has been great progress in validating blood-based biomarkers for individualized prediction of neurodegenerative diseases, and it has been widely and consistently demonstrated that serum/plasma NFL and GFAP are markers of neurodegeneration and astrogliosis, respectively [36, 37], even though not sufficiently helpful in discriminating between AD and FTLD [38, 39]. Conversely, plasma Ap1–42/Ap1–40 ratio or pTau181 are able to identify AD with high accuracy as compared to other neurodegenerative disorders [6, 40]. We indeed reported a twofold increase of plasma pTau181 in AD as compared to FTLD and HC, but we failed to demonstrate significant differences in Ab42/Ab40 ratio between the groups (see Table 1 or Fig. 1), possibly due to the methodological differences in sampling procedures [40, 41] or insufficient robustness (too low fold change between cases with and without brain amyloidosis) of this biomarker [42].

Along with blood-based markers, TMS intracortical connectivity measures, which rely on the biological bases of diseases and their associated specific transmitter impairment, are able to identify a now well-established cholinergic deficit in AD and a significant impairment in GABA and glutamatergic circuits in FTLD [43–46]. Indeed, SAI, a marker of sensorimotor integration, has been shown to partially reflect the activity of cholinergic circuits [47]. Furthermore, SICI is considered to reflect short-lasting postsynaptic

(See figure on next page.)

**Fig. 2** ROC curve analysis. ROC curves for differentiating A "cases" vs "controls" and B AD vs FTLD patients. ROC, receiver operating characteristic; AUC, area under the curve; HC, healthy controls; AD, Alzheimer’s disease; FTLD, frontotemporal lobar degeneration; NFL, neurofilament light; GFAP, glial fibrillary acidic protein; SICI, average short-interval intracortical inhibition (1, 2, 3 ms); ICF, average intracortical facilitation (7, 10, 15 ms); LICI, average long-interval intracortical inhibition (50, 100, 150 ms); SAI, average short-latency afferent inhibition (0, 4–4 ms) expressed as the ratio of the unconditioned motor evoked potential (MEP)
Fig. 2 (See legend on previous page.)

A

Patients vs HC

B

AD vs FTD

Legend:

- pTau
- NFL
- GFAP
- Aβ1-42/1-40
- mean SICI
- mean ICF
- mean SAI
- mean LICI

| Biomarker   | AUC            |
|-------------|----------------|
| pTau 1:1    | 0.69 (0.61-0.77)** |
| NFL         | 0.94 (0.90-0.98)*** |
| GFAP        | 0.86 (0.80-0.92)*** |
| Aβ1-42/1-40 | 0.58 (0.49-0.68)   |
| mean SICI   | 0.85 (0.78-0.92)*** |
| mean ICF    | 0.82 (0.74-0.90)*** |
| mean SAI    | 0.70 (0.61-0.79)** |
| mean LICI   | 0.65 (0.54-0.76)*  |

| Biomarker   | AUC            |
|-------------|----------------|
| pTau 1:1    | 0.70 (0.61-0.80)*** |
| NFL         | 0.68 (0.59-0.76)*** |
| GFAP        | 0.66 (0.56-0.75)** |
| Aβ1-42/1-40 | 0.56 (0.46-0.66)   |
| mean SICI   | 0.86 (0.78-0.93)*** |
| mean ICF    | 0.87 (0.82-0.93)*** |
| mean SAI    | 0.96 (0.92-0.99)*** |
| mean LICI   | 0.70 (0.62-0.79)*** |
inhibition mediated through the GABA_A receptors at the level of local interneurons, whilst ICF is thought to represent a net facilitation most likely mediated by glutamatergic NMDA receptors [20, 28, 31].

**Limitations**

We acknowledge that this study entails some limitations. First, the generalizability of these findings needs to be further demonstrated in future studies. Second, other
recently proposed blood biomarkers may be considered in the near future, and the model may be further refined also considering different assays on the market. Third, we did not have neuropathological confirmation of the present case series; however, each subject underwent comprehensive clinical and neuropsychological evaluation along with structural and functional imaging assessment. Finally, we did not compare the performance of blood-based and neurophysiological markers with more validated determinations in CSF or with imaging measures.

Conclusions
Despite these limitations, the implementation of this model may be proposed as the first screening tool in subjects with the suspected cognitive decline with cost benefits, especially in primary care centres. These findings support the use of blood-based biomarkers and TMS intracortical excitability measures to identify patients who may undergo secondary CSF or imaging testing. Future studies should evaluate their performances in the prodromal stages of dementia.

Acknowledgements
We thank our participant volunteers and their families for their participation.

Authors’ contributions
Conception and design of the study: AB and BB. Acquisition and analysis of data: AB, VC, JR, SA, AM, NA, HZ, KB, and BB. Drafting of the manuscript and figures: AB and BB. Revising the manuscript for intellectual content: AB, VC, JR, SA, AM, NA, HZ, KB, and BB. The authors read and approved the final manuscript.

Funding
AB was partially supported by the Airalzh-AGYR2020 and by Fondazione Cariplo, grant n° 2021–1516; HZ is a Wallenberg Scholar supported by grants from the Swedish Research Council (#2018–02532); the European Research Council (#681712 and #10103962); the Swedish State Support for Clinical Research (#ALFGBG-71320); the Alzheimer Drug Discovery Foundation (ADDF), USA (#201809–201662); the AD Strategic Fund and the Alzheimer’s Association (#ADSF-21–831375–C, #ADSF-21–831381–C and #ADSF-21–831377–C); the Olay Thon Foundation; the Erling-Persson Family Foundation; Stiftelsen för Gamla Tjänarinnor; Hjärnfonden, Sweden (#FO2019-0228); the European Union’s Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 860197 (MIRAIIDE); the European Union Joint Programme – Neurodegenerative Disease Research (JPND2021-00694); and the UK Dementia Research Institute at UCL (UKDRI-1003). KB was supported by the Swedish Research Council (#2017–00915); the Alzheimer Drug Discovery Foundation (ADDF), USA (#RDAPB18-2016165); the Swedish Alzheimer Foundation (#AF–742881); Hjärnfonden, Sweden (#FO2017-0243); the Swedish state under the agreement between the Swedish government and the County Councils; the ALF-agreement (ALFFGBG-715986); the European Union Joint Program for Neurodegenerative Disorders (JPND2019-466–236); the National Institute of Health (NIH), USA (grant #1R01AG063938-01); and the Alzheimer’s Association 2021 Zenith Award (ZEN-21–848495). BB was supported by the RF-2018–12366655 grant (Italian Ministry of Health).

Availability of data and materials
All study data, including raw and analysed data, and materials will be available from the corresponding author, B.B., upon reasonable request.

Declarations

Ethics approval and consent to participate
Full written informed consent was obtained from all subjects according to the Declaration of Helsinki. The Brescia Ethics Committee approved the study protocol.

Consent for publication
Not applicable.

Competing interests
HZ has served at scientific advisory boards and/or as a consultant for Abbvie, Alector, Annexon, Artery Therapeutics, AZTherapies, CogRx, Denali, Eisai, Neuragen, Novo Nordisk, Pinteon Therapeutics, Red Abbey Labs, Passage Bio, Roche, Samumed, Siemens Healthineers, Triplett Therapeutics, and Wave; has given lectures in symposia sponsored by Cellectricon, Fujirebio, Alzeccure, Biogen, and Roche; and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program (outside submitted work). KB has served as a consultant at advisory boards or at data monitoring committees for Abcam, Axon, BioArctic, Biogen, JOMDD/Shimadzu, Julius Clinical, Lilly, MagQu, Novartis, Roche Diagnostics, and Siemens Healthineers and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program. BB has served at scientific boards for Denali, Wave, Alector, and Aviadbio.

Author details
1 Neurology Unit, Department of Clinical and Experimental Sciences, University of Brescia, P.le Spedali Civili 1, 25123 Brescia, Italy. 2 Neurology Unit, ASST Spedali Civili Brescia, Brescia, Italy. 3 Department of Molecular and Translational Medicine, University of Brescia, Brescia, Italy. 4 Biotechnology Laboratory and Department of Diagnostics, Civic Hospital of Brescia, Brescia, Italy. 5 Casa Di Cura San Francesco, Bergamo, Italy. 6 Institute of Neuroscience and Physiology, Sahlgrenska Academy at University of Gothenburg, Mölndal, Sweden. 7 Wallenberg Centre for Molecular and Translational Medicine, University of Gothenburg, Mölndal, Sweden. 8 King’s College London, Institute of Psychiatry, Psychology & Neuroscience, Maurice Wohl Clinical Neuroscience Institute, London, UK. 9 NIH Biomedical Research Centre for Mental Health & Biomedical Research Unit for Dementia at South London & Maudsley NHS Foundation, London, UK. 10 Clinical Neurochemistry Laboratory, Sahlgrenska University Hospital, Mölndal, Sweden. 11 UK Dementia Research Institute at UCL, London, UK. 12 Department of Neurodegenerative Disease, UCL Institute of Neurology, London, UK. 13 Hong Kong Center for Neurodegenerative Diseases, Clear Water Bay, Hong Kong, China.

Received: 12 April 2022   Accepted: 22 September 2022
Published online: 13 October 2022

References
1. Nichols E, Vos T. The estimation of the global prevalence of dementia from 1990–2019 and forecasted prevalence through 2050: an analysis for the Global Burden of Disease (GBD) study 2019. Alzheimer’s Assoc Int Conf. The Author(s). Published by Elsevier Ltd. This is an Open Access article under the CC BY 4.0 license; 2021;2667:1–21. Available from: https://alzconfex.com/alz/2021/meetingapp.cgi/Paper/51496
2. Livingston G, Huntley J, Sommerlad A, Ames D, Ballard C, Banerjee S, et al. Dementia prevention, intervention, and care: 2020 report of the Lancet Commission. Lancet. 2020;396:413–46.
3. Frisoni GB, Boccardi M, Barkhof F, Blennow K, Cappa S, Chiotti K, et al. Strategic roadmap for an early diagnosis of Alzheimer’s disease based on biomarkers. Lancet Neurol. Elsevier Ltd. 2017;16:661–76. Available from: http://linkinghub.elsevier.com/retrieve/pii/S147444221730159X
4. The Ronald and Nancy Reagan Research Institute of the Alzheimer’s Association and National Institute on Aging Working Group. Consensus report of the working group on “Molecular and biochemical markers of Alzheimer’s disease.” Neurobiol Aging. Elsevier; 1998;19:109–16. Available
The heritability of frontotemporal lobar degeneration: validation of plasma phospho-tau181 as a biomarker for Alzheimer’s disease: development and validation of a prediction model using data from four prospective cohorts. Lancet Neurol. 2020;19(5):422–33 (in press).

10. Benussi A, Grassi M, Palluzzi F, Koch G, Di Lazzaro V, Nardone R, et al. Classification accuracy of transcranial magnetic stimulation for the diagnosis of neurodegenerative dementias. Ann Neurol. 2020;87:394–404. Available from: (http://www.ncbi.nlm.nih.gov/pubmed/31925823).

11. Benussi A, Grassi M, Palluzzi F, Cantiello V, Cotelli MS, Premi E, et al. Classification accuracy of TMS for the diagnosis of mild cognitive impairment. Brain Stimul. 2021;14:241–9. Available from: (https://linkinghub.elsevier.com/retrieve/pii/S1935861X21001005X).

12. Benussi A, Dell’Era V, Cantiello V, Ferracci C, Caratossolo S, Rizzoli L, et al. Discrimination of atypical parkinsonians with transcranial magnetic stimulation. Brain Stimul. 2018;11:366–73. Available from: http://linkinghub.elsevier.com/retrieve/pii/S1935861X17309695 Elsevier Ltd.

13. Armstrong MJ, Litvan I, Lang AE, Bak TH, Bhatia KP, Borroni B, et al. Criteria for the diagnosis of corticobasal degeneration. Neurology. 2013;80:496–503. Available from: http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=23359374&retmode=ref&cmd=prlinks From the University of Maryland (M.J.A., S.G.R., W.J.W.), Baltimore; University of California San Diego (I.L.), San Diego; Morton and Gloria Shulman Movement Disorders Center and the Edmond J. Safra Program in Parkinson’s Disease (A.E.L.), Toronto Western.

14. Hoglinger GU, Respondek G, Stamelou M, Kurz C, Josephs KA, Lang AE, et al. Clinical diagnosis of progressive supranuclear palsy: the Movement Disorder Society Criteria. Mov Disord. 2017;32:853–64. Available from: https://doi.org/10.1002/mds.26987 Wiley Subscription Services, Inc., A Wiley Company.

15. Pascozsky K, Hodges J, Knopman DS, Kramer JH, Neuhaus J, et al. Sensitivity of revised diagnostic criteria for the behavioural variant of frontotemporal dementia. Brain. 2011;134:2456–77. Available from: (http://www.ncbi.nlm.nih.gov/pubmed/2105220? via%3Dihub cited 2019 Apr 30)

16. Gorno-Tempini ML, Hillis AF, Weintraub S, Kertesz A, Mendez MF, Cappa SF, et al. Classification of primary progressive aphasia and its variants. Neurology. 2011;76:1006–14. Available from: http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=21325651&retmode=ref&cmd=prlinks Memory and Aging Center, Department of Neurology, UCSF, 350 Parnassus Avenue, Suite 905, San Francisco, CA 94143–1207, USA. marilu@memory.ucsf.edu

17. McKhann GM, Knopman DS, Chertkow H, Hyman BT, Jack CR, Kawas CH, et al. The diagnosis of dementia due to Alzheimer’s disease: recommendations from the National Institute on Aging-Alzheimer’s Association workgroups on diagnostic guidelines for Alzheimer’s disease. Alzheimers Dement. 2011;7:263–9.

18. Borroni B, Benussi A, Cosseddu M,archetti S, Padovani A. Cerebrospinal fluid tau levels predict prognosis in non-inherited frontotemporal dementia. Neurology. 2014;83:1347–9. Available from: (http://www.ncbi.nlm.nih.gov/pubmed/24029600).

19. Fostinelli S, Ciani M, Zanardini R, Zanetti O, Binetti G, Ghidoni R, et al. The heritability of frontotemporal lobar degeneration: validation of pedigree classification criteria in a Northern Italy cohort. J Alzheimers Dis. 2018;61:753–60. Available from: (http://www.ncbi.nlm.nih.gov/pubmed/29268689).

20. Ziemann U, Reis J, Schwenkreis P, Rosanova M, Strassel A, Badawy R, et al. TMS and drugs revisited. Clin Neurophysiol. 2015;126:1847–88. Available from: https://doi.org/10.1016/j.clinph.2014.08.028.Department of Neurology & Stroke, and Hertie Institute for Clinical Brain Research, Eberhard-Karls-University Tübingen, Tübingen, Germany. Electronic address: ulf.ziemann@uni-tuebingen.de. International Federation of Clinical Neurophysiology.

21. Di Lazzaro V, Bella R, Benussi A, Bologna M, Borroni B, Capone F, et al. Diagnostic contribution and therapeutic perspectives of transcranial magnetic stimulation in dementia. Clin Neurophysiol. 2021;132:2568–607. Available from: https://doi.org/10.1016/j.clinph.2021.05.035 International Federation of Clinical Neurophysiology.

22. Benussi A, Cantiello V, Borroni B. The role of transcranial magnetic stimulation in the diagnosis of dementia. Nova Science Publishers, Inc, 2021. Available from: https://novapublishers.com/shop/horizons-in-neurosciences-research-volume-44/.

23. Giunta M, Libri I, Premi E, Brattini C, Paghera B, Archetti S, et al. Clinical and radiological features of posterior cortical atrophy (PCA) in a GRN mutation carrier: a case report. Eur J Neurol. 2020;17. Available from: (http://www.ncbi.nlm.nih.gov/pubmed/34782010).

24. Benussi A, Ashton NJ, Karkar T, Abbesi A, Saraceno C, Ghidoni R, et al. Prodromal frontotemporal dementia: clinical features and predictors of progression. Alzheimers Res Ther. 2021;13:188. Available from: (http://www.ncbi.nlm.nih.gov/pubmed/36026432 Acta Neuropathologica Communications.

25. Goedert M, Jakes R, Crowther RA, Cohen P, Vanmechelen E, Vandermeeren M, et al. Epitope mapping of monoclonal antibodies to the paired helical filaments of Alzheimer’s disease: Identification of phosphorylation sites in tau protein. Biochim J. 1994;301:871–7.

26. Benussi A, Premi E, Gazzina S, Cantiello V, Cotelli MS, Giunta M, et al. Neurotransmitter imbalance dysregulates brain dynamic fluidity in frontotemporal degeneration. Neurobiol Aging. 2020;94:33. Available from: https://doi.org/10.1016/j.neurobiolaging.2020.05.017 Elsevier Inc.

27. Kuirai T, Caramia MD, Roithl JC, Day BL, Thompson PD, Ferbert A, et al. Corticothalamic inhibition in human motor cortex. J Physiol. 1993;471:501–19. Available from: https://doi.org/10.1113/jphysiol.1993.sp019912 UCL Institute of Neurology, London, United Kingdom.

28. Ziemann U, Roithl JC, Ridding MC. Interaction between intra-cortical inhibition and facilitation in human motor cortex. J Physiol. 1996;496:873–81. Available from: https://doi.org/10.1113/j Physiol.1996.sp021734.

29. Valls-Solé J, Pascual-Leone A, Wassermann EM, Hallett M. Human motor evoked responses to paired transcranial magnetic stimuli. Electroencephalogr Clin Neurophysiol. 1992;85:355–64. Available from: http://linkinghub.elsevier.com/retrieve/pii/S016855979290048G Human Cortical Physiology Unit, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, MD 20892.

30. Tokimura H, Di Lazzaro V, Tokimura Y, Oliviero A, Proﬃce P, Inosa A, et al. Short latency inhibition of human hand motor cortex by somatosensory input from the hand. J Physiol. 2000;523 Pt 2:503–13. Available from: (http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=11593099&etmode=ref&cmd=prlinks from the National Institute on Aging-Alzheimer’s Association workgroups on diagnostic guidelines for Alzheimer’s disease. Alzheimers Dement. 2011;7:263–9.

31. Tokimura H, Di Lazzaro V, Tokimura Y, Oliviero A, Proﬃce P, Inosa A, et al. Short latency inhibition of human hand motor cortex by somatosensory input from the hand. J Physiol. 2000;523 Pt 2:503–13. Available from: (http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=11593099&etmode=ref&cmd=prlinks from the National Institute on Aging-Alzheimer’s Association workgroups on diagnostic guidelines for Alzheimer’s disease. Alzheimers Dement. 2011;7:263–9.

32. Tokimura H, Di Lazzaro V, Tokimura Y, Oliviero A, Proﬃce P, Inosa A, et al. Short latency inhibition of human hand motor cortex by somatosensory input from the hand. J Physiol. 2000;523 Pt 2:503–13. Available from: (http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=11593099&etmode=ref&cmd=prlinks from the National Institute on Aging-Alzheimer’s Association workgroups on diagnostic guidelines for Alzheimer’s disease. Alzheimers Dement. 2011;7:263–9.

33. Burnham KP, Anderson DR. Multimodel inference: understanding AIC and BIC. Sociological Methodology. 2004;34:261–304.

34. Treushjisen CE, Verberk IMW, Thijssen EH, vanEGAARDEN L, HANNON O, ZETTERBERG H, et al. Blood-based biomarkers for Alzheimer’s disease: towards clinical implementation. Lancet Neurol. 2022;21:66–77. Available from: https://doi.org/10.1016/S1474-4422(21)00361-6 Elsevier Ltd.
35. Benussi A, Alberici A, Ferrari C, Cantoni V, Dell’Era V, Turrone R, et al. The impact of transcranial magnetic stimulation on diagnostic confidence in patients with Alzheimer disease. Alzheimers Res Ther. 2018;10:94 Available from: http://www.ncbi.nlm.nih.gov/pubmed/30279956A, http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC6145195.

36. Mattsson N, Insel PS, Landau S, Jagust W, Donohue M, Shaw LM, et al. Diagnostic accuracy of CSF Ab42 and florbetapir PET for Alzheimer’s disease. Ann Clin Tranl Neurol. 2014;1:534–43.

37. Khalil M, Teunissen CE, Otto M, Piehl F, Sormani MP, Gattinger T, et al. Neurofilaments as biomarkers in neurological disorders. Nat Rev Neurol. 2018;14:577–89. Available from: https://doi.org/10.1038/s41582-018-0058-z Springer US

38. Choulilas J, Thomas A, Malpetti M, Donaghy P, Kane J, Mak E, et al. Differential levels of plasma biomarkers of neurodegeneration in Lewy body dementia, Alzheimer’s disease, frontotemporal dementia and progressive supranuclear palsy. J Neurol Neurosurg Psychiatry. 2022;93(6):651–8 (jnpn-2021-327788).

39. Zhu N, Santos-Santos M, Illán-Gala I, Montal V, Estellés T, Barroeta I, et al. Plasma glial fibrillary acidic protein and neurofilament light chain for the diagnostic and prognostic evaluation of frontotemporal dementia. Transl Neurodegener. 2021;10:1–12.

40. Li Y, Schindler SE, Bollinger JG, Oxod V, Mawuenyega KG, Weiner MW, et al. Validation of plasma amyloid-β 42/40 for detecting Alzheimer disease amyloid plaques. Neurology. 2022;98:6688–99.

41. Panneve J, Shaw LM, Korecka M, Waligorska T, Teunissen CE, Stoops E, et al. The global Alzheimer’s Association round robin study on plasma amyloid β methods. Alzheimers Dement Diagnosis Assess Dis Monit. 2021;13:1–7.

42. Benedet AL, Brum WS, Hansson O, Karikari TK, Zimmer ER, Zetterberg H, et al. The accuracy and robustness of plasma biomarker models for amyloid PET positivity. Alzheimers Res Ther. 2022;14:26. Available from: https://doi.org/10.1186/s13195-021-00942-0 BioMed Central.

43. Borroni B, Benussi A. Recent advances in understanding frontotemporal degeneration. F1000Research. 2019;8:2098. Available from: (https://f1000research.com/articles/8-1279/v1).

44. Benussi A, Dell’Era V, Cantoni V, Cotelli MS, Cosceddu M, Spallazz M, et al. TMS for staging and predicting functional decline in frontotemporal dementia. Brain Stimul. 2020;13:386–92. Available from: (http://www.ncbi.nlm.nih.gov/pubmed/31787557).

45. Benussi A, Dell’Era V, Cantoni V, Cotelli MS, Cosceddu M, Spallazz M, et al. Neurophysiological correlates of positive and negative symptoms in frontotemporal dementia. Arighi A, editor. J Alzheimers Dis. 2020;73:1133–42. Available from: https://doi.org/10.3233/JAD-190986

46. Benussi A, Alberici A, Buratti E, Ghidoni R, Gardoni F, Dl Luca M, et al. Toward a glutamate hypothesis of frontotemporal dementia. Front Neurosci. 2019;13:304. Available from: https://doi.org/10.3389/fnins.2019.00304/full

47. Di Lazzaro V, Pilato F, Dileone M, Saturno E, Oliviaero A, Marra C, et al. In vivo cholinergic circuit evaluation in frontotemporal and Alzheimer dementias. Neurology. 2006;66:1111–3. Available from: https://doi.org/10.1212/01.wnl.0000204183.26231.23 Institute of Neurology, Università Cattolica, Rome, Italy. vdlazzaro@rm.unicatt.it: AAN Enterprises

Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.