Brain Stimulation 14 (2021) 241–249

Contents lists available at ScienceDirect

Brain Stimulation

journal homepage: http://www.journals.elsevier.com/brain-stimulation

Classification accuracy of TMS for the diagnosis of mild cognitive impairment

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ARTICLE INFO

Article history:
Received 5 August 2020
Received in revised form
17 November 2020
Accepted 8 January 2021
Available online 13 January 2021

Keywords:
Mild cognitive impairment
Alzheimer's disease
Frontotemporal dementia
Dementia with Lewy bodies
Transcranial magnetic stimulation
Diagnostic accuracy
Decision tree
Short interval intracortical inhibition
Intracortical facilitation
Short latency afferent inhibition

ABSTRACT

Objective: To evaluate the performance of a Random Forest (RF) classifier on Transcranial Magnetic Stimulation (TMS) measures in patients with Mild Cognitive Impairment (MCI).
Methods: We applied a RF classifier on TMS measures obtained from a multicenter cohort of patients with MCI, including MCI-Alzheimer's Disease (MCI-AD), MCI-frontotemporal dementia (MCI-FTD), MCI-dementia with Lewy bodies (MCI-DLB), and healthy controls (HC). All patients underwent TMS assessment at recruitment (index test), with application of reference clinical criteria, to predict different neurodegenerative disorders. The primary outcome measures were the classification accuracy, precision, recall and F1-score of TMS in differentiating each disorder.
Results: 160 participants were included, namely 64 patients diagnosed as MCI-AD, 28 as MCI-FTD, 14 as MCI-DLB, and 47 as healthy controls (HC). A series of 3 binary classifiers was employed, and the prediction model exhibited high classification accuracy (ranging from 0.72 to 0.86), high precision (0.72–0.90), high recall (0.75–0.98), and high F1-scores (0.78–0.92), in differentiating each neurodegenerative disorder. By computing a new classifier, trained and validated on the current cohort of MCI patients, classification indices showed even higher accuracy (ranging from 0.83 to 0.93), precision (0.87–0.89), recall (0.83–1.00), and F1-scores (0.85–0.94).
Conclusions: TMS may be considered a useful additional screening tool to be used in clinical practice in the prodromal stages of neurodegenerative dementias.

Introduction

Mild Cognitive Impairment (MCI) is a nosological entity useful to identify subjects at higher risk of developing dementia, suffering from cognitive complaints but with preserved daily living activities [1–3]. Initially developed to detect prodromal stages of Alzheimer's disease (AD) [4,5], the MCI term has been extended to subjects

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https://doi.org/10.1016/j.brs.2021.01.004

progressing to different dementing illnesses, such as dementia with Lewy Bodies (DLB) or frontotemporal dementia (FTD) [6–8].

While MCI due to AD has been carefully characterized and supportive diagnostic markers identified [4,5], the preclinical stages of DLB and FTD have only recently came out to a more careful description [6–8]. Reliable identification of etiological MCI subtypes would enable early intervention, would assist clinicians to anticipate treatment options and facilitate selection for trials of targeted therapies as these become available.

Currently, validated markers, divided into imaging modalities and CSF/plasma/serum fluid measures, are key on clinical grounds to accomplish the diagnosis of preclinical neurodegenerative dementias [9].

Amyloid Positron Emission Tomography (PET) imaging or cerebrospinal fluid (CSF) $A\beta_{1-42}$ and Tau dosages are undoubtfully useful for detecting the earliest stages of AD, and pattern of brain hypometabolism with ^{18}F-FDG-PET are of help in supporting differential diagnosis of preclinical neurodegenerative dementias [10]. Furthermore, dopamine transporter uptake in basal ganglia may guide in early DLB diagnosis [8]. However, the ideal marker, besides having high accuracy and reliability, should be non-invasive, simple to perform and inexpensive [9].

In this context, our group has recently developed an index using transcranial magnetic stimulation (TMS) measures of intracortical circuit excitability. TMS allows to non-invasively assess neurotransmitters imbalance and it has been demonstrated helpful to differentiate AD, FTD and DLB with high accuracy [11,12]. It has been widely demonstrated that AD and DLB are characterized by a deficit in short latency afferent inhibition (SAI) [13–19], a marker of cholinergic circuits [20], while FTD and DLB show a prominent change in short interval intracortical inhibition and facilitation (SICI-ICF) [21–25], which partially depend on GABAergic and glutamatergic circuits [20], respectively. The combined assessment of these TMS parameters has been proven helpful to identify neurodegenerative dementias since the earliest stages [26–30]. In a recent work, we have maximized the potential diagnostic performances of TMS measures in symptomatic AD and other neurodegenerative dementias by a machine learning approach [31]. We have trained and tested a Random Forest (RF) classifier, which resulted in high classification accuracy (ranging from 0.89 to 0.92) and high precision (0.86–0.92) in differentiating AD, DLB, and FTD [31]. Using the classification parameters obtained in the RF analysis, an automated and open-access R script was coded to allow the simple and straightforward entry of raw TMS measures, which are computed and elaborated, resulting in a diagnostic class for each diagnosis at the single subject level.

The present multicenter study, performed in a new cohort, was aimed to test the performances of the already computed machine learning approach, trained in patients with overt dementia, and to assess its classification accuracy in the preclinical stages of AD and other dementias. To this, we recruited a large sample of MCI subjects, grouped into MCI-Alzheimer's disease (MCI-AD), MCI-frontotemporal dementia (MCI-FTD), and MCI-dementia with Lewy bodies (MCI-DLB), according to clinical features and diagnostic markers, to whom we applied the RF classifier.

Methods

Subjects

In this multicenter study, we considered subjects with an MCI diagnosis according to current clinical criteria, and age-matched healthy controls (HC).

MCI diagnosis required *i)* a cognitive complaint from the patient, a relative or from a physician for at least the previous 6 months, *ii)* objective deficits in one or more cognitive domains that are greater than would be expected for the patient's age and educational background, that do not represent lifelong patterns of lower cognitive function, and are not associated with acute medical or neurologic conditions, and *iii)* maintained independence in completing daily living activities [4].

At enrolment, each MCI subject underwent an extensive neuropsychological evaluation, according to standard procedures at each center and based on the expertise of each clinician, a brain MRI study and TMS protocols. MCI subjects were followed over time, and either conversion to dementia or stable cognitive function was recorded.

Each subject was further grouped according to clinical characteristics and biomarkers data into the label of either MCI-AD, MCI-FTD, MCI-DLB or HC.

MCI-AD was defined as amnestic-MCI along with at least one of the following features: *i)* positive amyloid markers (CSF or Positron Emission Tomography - amyloid PET imaging), *ii)* positive marker of neuronal injury with topographic specificity for dysfunction that occurs in AD (reduction of glucose metabolism in temporoparietal cortex at brain ^{18}F-FDG-PET) or *iii)* conversion to AD at follow-up [4,32]. A CSF AD-like profile was defined as $A\beta_{1-42} \leq 650$ pg/mL and Tau ≥ 400 pg/mL using a commercial ELISA assay [33], while PET amyloid imaging was acquired using 370 MBq (10 mCi) of ^{18}F-florbetapir or ^{18}F-flutemetamol, following the procedures provided by the ligand manufacturer [34].

MCI-FTD was defined as non-amnestic MCI along with at least one of the following features: *i)* pathogenetic mutation significative of FTD-related monogenic disorder, *ii)* positive marker of neuronal injury with topographic specificity for dysfunction that occurs in FTD (reduction of glucose metabolism in frontotemporal cortex at brain ^{18}F-FDG-PET), or *iii)* conversion to FTD at follow-up [35,36]. Pathogenetic mutations within *Granulin (GRN)* or *Microtubule Associated Protein Tau (MAPT)* or an expansion in *C9orf72* were considered.

MCI-DLB was defined by non-amnestic MCI with at least one core symptom (fluctuating cognition with variations in attention and alertness, recurrent visual hallucinations, REM behavior sleep disorder, one cardinal feature of parkinsonism), along with at least one of the following features: *i)* positive marker of neurodegeneration (single-photon emission computed tomography-[123I]FP-CIT Ioflupane I 123, SPECT DaTSCAN) or *ii)* conversion to DLB at follow-up [8].

HC was defined by at least one of the following features: *i)* no complaints of cognitive disturbances and unremarkable scores at a brief standardized neuropsychological assessment (MMSE $\geq$ 27/30), with no psychiatric or other neurological illnesses.

Exclusion criteria were as follows: *i*) history of head trauma, alcohol abuse, stroke or transient ischemic attack, epilepsy or medical causes of cognitive decline; *ii*) use of drugs that could affect TMS measures, *iii*) presence of a pacemaker or other cardiac devices, cochlear implants, or previous brain surgery, such as clipping of a cerebral aneurysm.

The study was performed in accordance with the Standard for Reporting of Diagnostic Accuracy (STARD) criteria, applying the reference and index test at recruitment (see Fig. 1). All subjects underwent an extensive clinical and instrumental work-up and the diagnosis was made by neurologists with expertise in neurodegenerative disorders (i.e., reference test). All subjects underwent TMS at recruitment, performed by examiners who had experience with neurophysiological techniques and who were masked to the results of the reference test (i.e., index test). Data analysis was performed by two separate statisticians.

Our primary research question was to determine the classification performance of MCI subtypes, considering the best

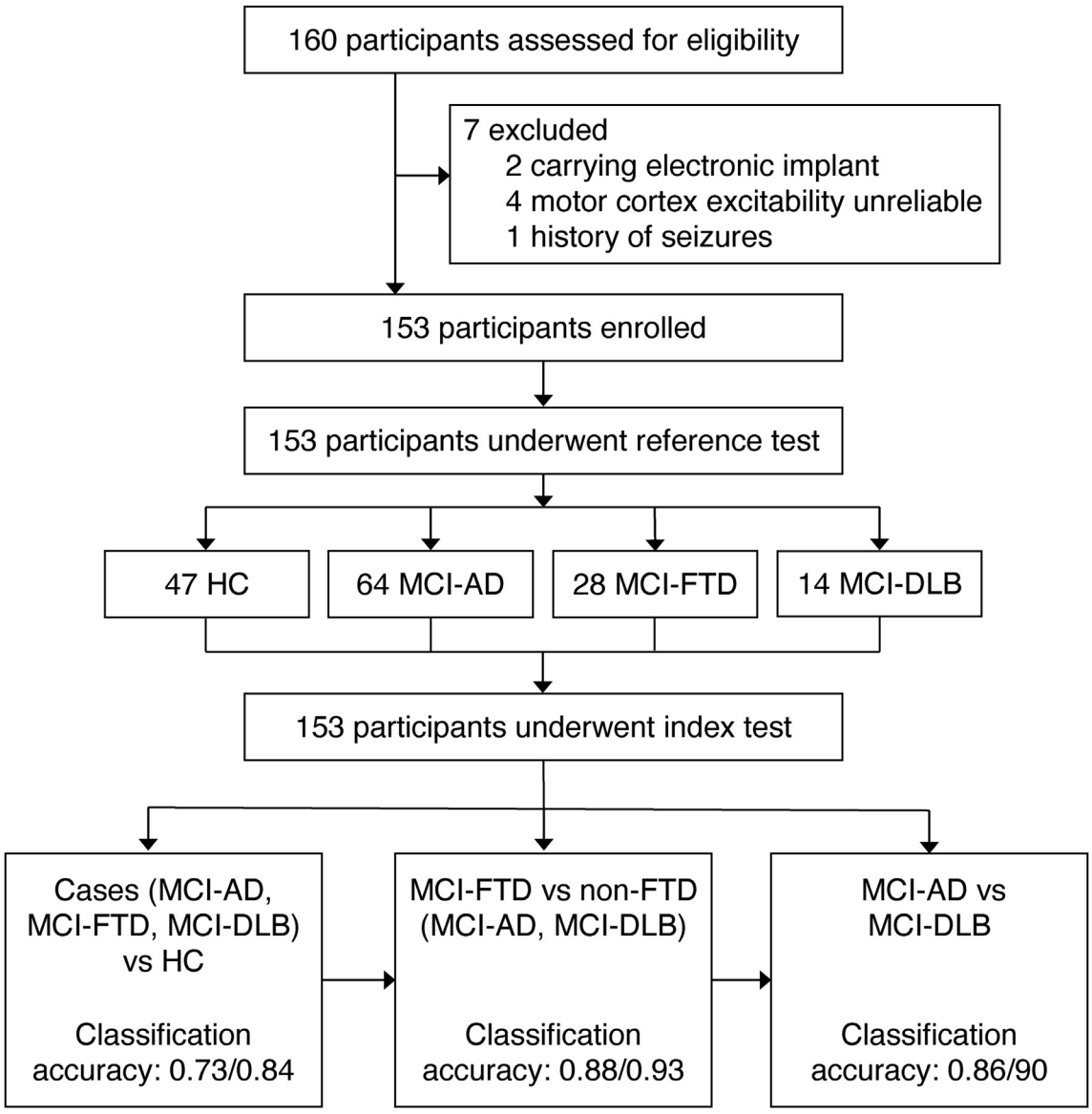

Fig. 1. Flow diagram of the study.
After the index test, results were sorted on the basis of the reference standard. Classification accuracy is reported by: applying the already published algorithm [31]/new algorithm trained on the present cohort of patients.
MCI = mild cognitive impairment; MCI-AD = MCI-Alzheimer's disease; MCI-FTD = MCI-frontotemporal dementia; MCI-DLB = MCI-dementia with Lewy bodies; HC = healthy controls.

combination of TMS indicators as previously computed by Random Forrest models [31].

Full written informed consent was obtained from all participants according to the Declaration of Helsinki. The study protocol was approved by the local ethics committees of the participating centers.

Transcranial magnetic stimulation parameters

The four centers applied comparable TMS protocols. A TMS figure-of-eight coil (each loop diameter 70–90 mm) connected to a monophasic Magstim Bistim or Bistim^2 system (Magstim Company, Oxford, UK) was employed for all TMS paradigms. Electromyographic (EMG) recordings were performed from the first dorsal interosseous (FDI) muscles 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.

To locate the precise representation of the target muscle on the contralateral primary motor cortex, the TMS coil was positioned approximately 4 cm laterally and 2 cm anteriorly to Cz, tangentially on the scalp with the coil handle pointed 45° posteriorly and laterally to the sagittal plane. The "hot spot" was defined as the point in which magnetic stimulation resulted in the maximum motor evoked potential (MEP) amplitude with the minimum stimulator intensity. To obtain this, stimulator intensity was increased from 35% of the maximal stimulator output (MSO) in 5% steps until MEPs with an approximately 0.5–1 mV amplitude could be recorded. The coil was then moved in 0.5 cm steps medially, laterally, posteriorly and anteriorly while evoking 3 MEPs at each site. This was performed until the site in which the largest MEPs

could be located, which was marked with a felt tip pen on the scalp to ensure constant placement of the coil throughout the experiment.

RMT was defined as the minimal stimulus intensity needed to produce MEPs with an amplitude of at least 50 μV in 5 out of 10 consecutive trials during complete muscle relaxation, which was controlled by visually checking the absence of EMG activity at high-gain amplification. The active motor threshold (AMT) was defined as the minimal stimulus intensity needed to produce MEPs with an amplitude of at least 200 μV in 5 out of 10 consecutive trials and it was determined during a slight tonic contraction of the target muscle at approximately 20% of the maximal muscle strength. MT was determined according to the relative frequency method, in which we started at a stimulus intensity of 35% MSO with the coil placed over the motor "hot spot", and stimulus intensity was gradually increased in steps of 5% MSO until TMS consistently evoked MEPs with peak-to-peak amplitudes of >50 μV in each trial for RMT. Thereafter, stimulus intensity was gradually lowered in steps of 1% MSO until there were less than 5 positive responses out of 10 trials. All centers recorded MT and RMT, while only certain centers recorded AMT, according to individual preferences at each center for determining the conditioning stimulus intensity for SICI and ICF.

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

For SICI and ICF, the conditioning stimulus (CS) was adjusted at 70% of the RMT or 5% of below AMT (based on individual preferences at each center), employing multiple interstimulus intervals (ISIs), including 1, 2, 3, 5 ms for SICI and 7, 10, 15 ms for ICF [37,38].

Long interval intracortical inhibition (LICI), which predominantly reflects $GABA_B$ergic transmission, was elicited by applying two suprathreshold stimuli at long ISIs (50, 100, 150 ms), with the CS set at 130% of the RMT preceding the TS [39].

SAI was evaluated employing a CS consisting of a single pulse (200 μs) of electrical stimulation at 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 [40]. Different ISIs were implemented ($^{-}$4, 0, $^{+}$4, $^{+}$8 ms), which were fixed relative to the peak latency of the N20 component of the somatosensory evoked potential of the median nerve.

For each ISI and for each protocol (SICI-ICF, LICI and SAI), from 5 to 10 (depending on each center) different paired CS-TS and control TS were delivered in all participants in a pseudo randomized sequence, with an inter-trial interval of 5 s (±10%). Protocols were also performed in a random order, with an average of 5 min elapsing between the end and the beginning of the subsequent protocol.

The conditioned MEP amplitude, evoked after delivering a paired CS-TS, was expressed as percentage of the average control MEP amplitude. Audio-visual feedback was provided to ensure muscle relaxation during the entire experiment and trials were discarded if EMG activity exceeded 100 μV 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.

The operators who administered TMS were blinded to the subjects' status; standardized TMS procedures were employed for all participants and stimuli were delivered in a randomized sequence, thus reducing possible biases in TMS recordings.

For the purpose of the present study we considered as potential indicators each of the following parameters: SICI and ICF (at 1, 2, 3, 5, 7, 10, 15 ms ISIs), LICI (at 50, 100, 150 ms ISIs), and SAI (at −4, 0, +4, +8 ms ISIs). For every patient, considering that protocols were performed with slightly different parameters between each center (i.e., not every ISI was performed in all centers), machine learning algorithms were applied (see next section) to infer average values and trends of each measure for each paired-pulse protocol.

Statistical analysis

TMS raw measures were compared with three two-way mixed ANCOVA (for SICI-ICF, LICI and SAI) with GROUP as between-subjects factor and ISI as within-subjects factor, including age at TMS and center as covariates. If a significant main effect was observed, group differences were evaluated with *post hoc* tests (Bonferroni correction for multiple comparisons). Mauchly's test was used to check for sphericity violation, applying Greenhouse-Geisser epsilon determinations. We reported F statistics with (degrees of freedom for the two-way interaction term, degrees of freedom of the error term), with the Greenhouse-Geisser correction.

We evaluated significant associations between TMS measures and CSF biomarkers using Pearson's partial correlation, corrected for age, in patients with MCI-AD.

The previously computed RF classifier, obtained in ~650 patients with symptomatic neurodegenerative dementias (not in the MCI phase) and healthy controls [31], was tested in this new cohort of MCI subjects without dementia. Briefly, in the previous study, TMS intracortical connectivity measures, namely SICI-ICF, SAI and LICI, were considered. For each TMS protocol, regression analysis to capture both average values (i.e., intercept or zero-order parameters) and trends (i.e., regressor coefficients) at different ISIs were performed, and regression parameters estimating mean ISIs and curve trends at different ISIs were considered. Finally, RF classifier [41] was carried out with regression parameters used as predictors for hierarchical binary classification, after covariate adjustment by age at TMS, sex and center. To avoid classifier over-performance on a specific dataset, and consequent loss of classification generality and reproducibility, we performed a K-fold cross-validation analysis (for full details see **Supplementary Materials**).

Moreover, we computed a new RF classifier, trained and validated on the current cohort of MCI patients, considering the same parameters and comparable approach. As in the previous RF classifier, we considered the following indices of classification performance [42]: *i)* classification accuracy, i.e., the ratio of correctly predicted (positive or negative) observations to the total observations; *ii)* precision, i.e., the ratio of correctly predicted positive observations to the total predicted positive observations (precision estimates classifier's ability to predict really positive observations when the test is positive); *iii)* recall, i.e., the ratio of correctly predicted positive observations to the total true positive observations (recall estimates the amount of true positive observations that were correctly classified as positive); and *iv)* F1-score, i.e., the harmonic average of precision and recall.

Descriptive analyses were carried out using SPSS software (SPSS 21.0. Armonk, NY). RF classifier and evaluation of classification performance were carried out in R-4.0.0, using *RandomForest* package [43], with ntree = 1000 = number of trees to grow and mtry = sqrt(9) = number of variables randomly sampled as candidates at each split; *reprtree* package [44] for selection of the most representative tree; *CMA* package [45] for performance evaluation with K-fold cross-validation.

Results

Participants

One hundred sixty participants were assessed for eligibility, and seven were excluded because they could not undergo TMS testing (4%), because carrying electronic implants (n = 2), they had a positive history of seizures (n = 1), or because 1 mV MEPs could not be obtained by using stimulator intensities <85% of the maximum stimulator output (n = 4) (see Fig. 1).

Thus, one hundred fifty-three subjects were considered in the present analysis. Demographic characteristics of the diagnostic groups are reported in Table 1. We included sixty-four participants with MCI-AD, twenty-eight with MCI-FTD, fourteen with MCI-DLB, and forty-seven HC.

Sixty-one out of one hundred six (57.7%) MCI subjects had at least one amyloid marker (PET amyloid or CSF $A\beta_{1\text{-}42}$) and eighty-seven (82.1%) had at least one marker of neuronal injury (CSF Tau or ^{18}F-FDG-PET or SPECT-DaTSCAN). Eight out of twenty-eight MCI-FTD (28.6%) had an inherited monogenic disorder (4 *GRN*, 3 *C9orf72* and 1 *MAPT* mutations). Forty-three (40.6%) MCI subjects had at least 18-month follow-up, with an average of 23.1 ± 12.7 months.

TMS measures of intracortical excitability

TMS measures, i.e., SICI-ICF, SAI and LICI in the different diagnostic groups are reported in Fig. 2. We observed a significant interaction at the two-way mixed ANCOVA for SICI-ICF [$F(12.5, 392.3) = 14.0$, $p < 0.001$, partial $\eta^2 = 0.31$, $\varepsilon = 0.70$] and SAI [$F(7.1, 220.0) = 2.9$, $p = 0.002$, partial $\eta^2 = 0.09$, $\varepsilon = 0.79$]. For LICI we did not observed a significant ISI × DIAGNOSIS interaction [$F(4.6, 85.4) = 0.9$, $p = 0.494$, partial $\eta^2 = 0.05$, $\varepsilon = 0.76$] but only an simple main effect for ISI [$F(1.5, 85.4) = 4.0$, $p = 0.031$, partial $\eta^2 = 0.07$, $\varepsilon = 0.76$]. *Post-hoc* differences, corrected for multiple comparisons, between groups and at each ISI, are reported in Fig. 2. Briefly, in comparison to healthy controls, SICI-ICF resulted significantly impaired in both MCI-FTD and MCI-DLB, while SAI was significantly impaired in MCI-AD and MCI-DLB.

We did not observe significant differences in SICI-ICF measures obtained with different conditioning stimulus intensities (70% RMT or AMT-5%), which were distinctly adopted between centers, $F(1, 144) = 1.46$, $p = 0.229$, $\eta^2 = 0.01$, nor in patients with and without a biomarker supported diagnosis, SICI-ICF: $F(4.1, 584.8) = 1.0$, $p = 0.407$, $\eta^2 = 0.01$, $\varepsilon = 0.69$; SAI: $F(2.4, 338.9) = 1.0$, $p = 0.233$, $\eta^2 = 0.01$, $\varepsilon = 0.79$; LICI: $F(1.6, 234.1) = 1.0$, $p = 0.30$, $\eta^2 = 0.70$, $\varepsilon = 0.81$. We did not observe a significant effect of CENTER in the different measures: SICI-ICF $F(1, 147) = 2.5$, $p = 0.320$, $\eta^2 = 0.03$; SAI $F(1, 147) = 2.0$, $p = 0.490$, $\eta^2 = 0.02$; LICI $F(1, 147) = 2.7$, $p = 0.210$, $\eta^2 = 0.04$.

In MCI-AD, we did not observe a significant association between CSF measures ($A\beta_{1\text{-}42}$, Tau, pTau) and TMS measures (average SICI, ICF, LICI or SAI) (all $p > 0.05$).

Classification performance

As reported in Fig. 3, a hierarchical series of subsequent 3 binary (two-groups) and independent classifiers were employed: *i)* MCI vs HC; *ii)* MCI-FTD vs MCI-non-FTD (MCI-AD or MCI-DLB), *iii)* MCI-AD vs MCI-DLB. The specific order of classification resulted in the greatest accuracy, i.e., fewer classification errors.

The first two-groups classification allowed us to classify each subject as "case" (i.e., MCI) or "control"; if the subject fell into the "MCI" category, the next order of classification was considered, and the MCI-FTD vs MCI-non-FTD classifier was carried out; once again, if the patient fell into the "MCI-non-FTD" category, the third classifier allowed us to classify the patient into MCI-AD vs MCI-DLB.

Classification indexes of the previously computed RF classifier [31], and of RF run on the present sample with 5-fold-cross validation, adjusted for age, sex and center, are reported in Table 2, after removing outliers with Brier score >1 [46].

By applying the previously published RF classifier, trained on a cohort of 547 patients with overt dementia (not in the MCI phase) out of ~ 650 participants, the prediction model exhibited overall

Table 1
Demographic and clinical characteristics of included subjects.

Variable	MCI-AD	MCI-FTD	MCI-DLB	HC
Subjects (n)	64	28	14	47
Age, years	70.9 ± 6.0§	64.0 ± 9.3$^{\dagger *\ddagger}$	72.4 ± 6.4§	69.7 ± 7.0§
Gender, F%	45.3	46.4	28.6	55.3
Education, years	9.9 ± 4.0	9.9 ± 4.2	10.0 ± 43.8	9.7 ± 4.0
MMSE scores	25.6 ± 1.3$^{\S *}$	27.2 ± 2.0†	26.7 ± 1.8*	28.2 ± 1.2†
CSF $A\beta_{1\text{-}42}$, pg/mL (n)	504.8 ± 149.1 (27)$^{\S\ddagger *}$	922.5 ± 190.2 (12)†	809.9 ± 246.8 (8)†	954.6 ± 301.4 (8)†
CSF Tau, pg/mL (n)	724.7 ± 465.4 (27)$^{\S\ddagger *}$	356.2 ± 149.6 (12)†	350.6 ± 143.6 (8)†	286.1 ± 124.8 (8)†
CSF pTau, pg/mL (n)	144.8 ± 236.9 (27)	51.4 ± 11.5 (12)	58.1 ± 20.6 (8)	52.0 ± 12.5 (8)
Amyloid PET, pos/neg	15/0	0/5	1/3	0/3
^{18}F-FDG-PET, pos/neg	26/0	4/0	–	–
SPECT-DaTSCAN, n pos	–	–	11	–
Monogenic disease, n pos	–	8	–	–
TMS parameters				
RMT (% MSO)	49.0 ± 8.5§	44.3 ± 8.7†‡	43.7 ± 2.7§	47.9 ± 8.8
SICI	0.39 ± 0.15§‡	0.54 ± 0.23$^{\dagger *}$	0.65 ± 0.30$^{\dagger *}$	0.33 ± 0.12§‡
ICF	1.40 ± 0.44§‡	0.89 ± 0.19$^{\dagger *}$	1.05 ± 0.30†	1.27 ± 0.26§
SAI	0.74 ± 0.18$^{\S *}$	0.49 ± 0.10†‡	0.76 ± 0.27$^{\S *}$	0.46 ± 0.15†‡
LICI	0.30 ± 0.18§	0.63 ± 0.44$^{\dagger\ddagger *}$	0.26 ± 0.25§	0.27 ± 0.22§

Demographic and clinical characteristics, and neurophysiological parameters are expressed as mean ± standard deviation; SICI, ICF, LICI and SAI are represented as ratio of mean conditioned and unconditioned (i.e., control) motor evoked potential (MEP) amplitude.
MCI = mild cognitive impairment; MCI-AD = MCI-Alzheimer's disease; MCI-FTD = MCI-frontotemporal dementia; MCI-DLB = MCI-dementia with Lewy bodies; HC = healthy controls; n = number; F = female; MMSE = Mini-Mental State Examination; CSF = cerebrospinal fluid; Amyloid PET = amyloid positron emission tomography; ^{18}F-FDG-PET = fluorodeoxyglucose positron emission tomography; SPECT-DaTSCAN = single-photon emission computed tomography-DaTSCAN; TMS = transcranial magnetic stimulation; RTM = resting motor threshold; SICI = mean short interval intracortical inhibition (1, 2, 3 ms); ICF = mean intracortical facilitation (7, 10, 15 ms); SAI = mean short latency afferent inhibition (0, +4 ms); LICI = mean long interval intracortical inhibition (50, 100, 150 ms); MSO = maximum stimulator output.
*$p < 0.05$ vs HC; $^{\dagger}p < 0.05$ vs MCI-AD, $^{\ddagger}p < 0.05$ vs MCI-DLB; $^{\S}p < 0.05$ vs MCI-FTD using one-way ANOVA or chi-square tests, as appropriate (*post hoc* tests with Bonferroni correction for multiple comparisons, only after a significant interaction).

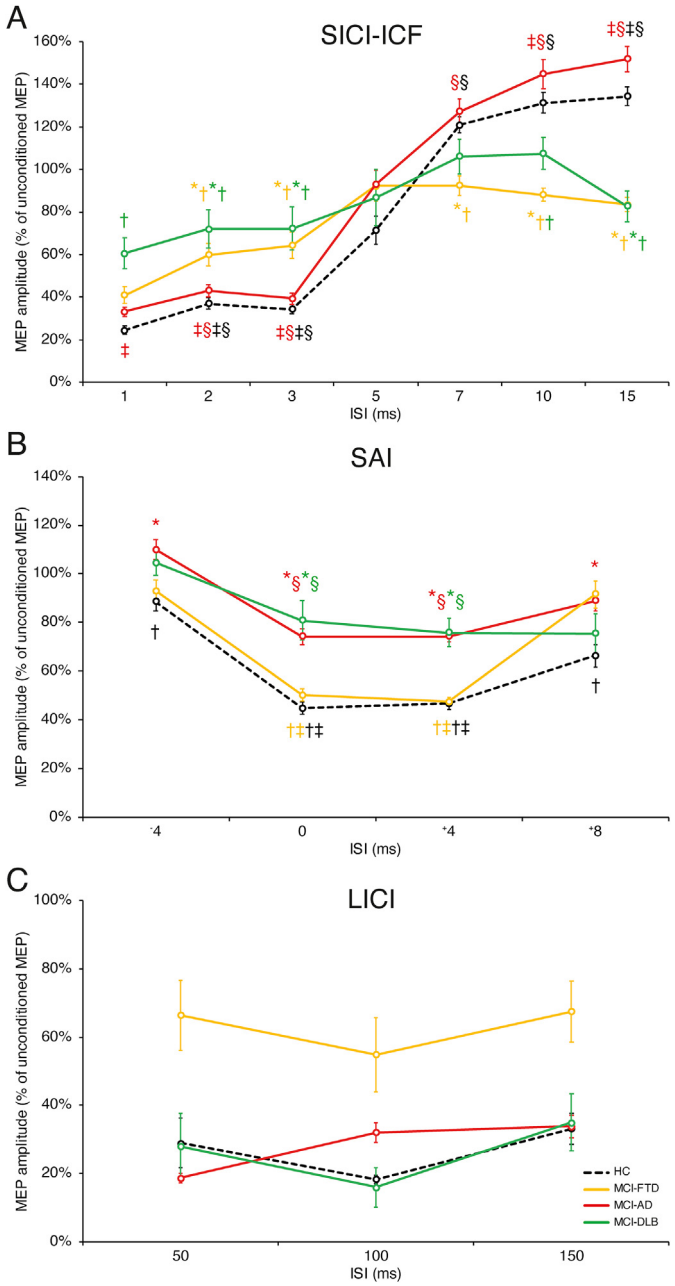

Fig. 2. TMS connectivity parameters according to diagnostic groups.
(A) SICI at ISI of 1, 2, 3, and 5 ms and ICF at ISI of 7, 10, and 15 ms, (B) SAI at ISI of $^{-}$4, 0, $^{+}$4, and 8 ms, relative to the N20 peak latency, and (C) LICI at ISI of 50, 100, and 150 ms in subjects with MCI-AD, MCI-FTD, MCI-DLB and in HC. Data are presented as a ratio to the unconditioned motor evoked potential amplitude; error bars represent standard errors. MCI = mild cognitive impairment; MCI-AD = MCI-Alzheimer's disease; MCI-FTD = MCI-frontotemporal dementia; MCI-DLB = MCI-dementia with Lewy bodies; HC = healthy controls; ICF = intracortical facilitation; ISI = interstimulus interval; LICI = long-interval intracortical inhibition; MEP = motor evoked potential; SAI = short-latency afferent inhibition; SICI = short-interval intracortical inhibition. *$p < 0.05$ vs HC; $^{\dagger}p < 0.05$ vs MCI-AD, $^{\ddagger}p < 0.05$ vs MCI-DLB; $^{\S}p < 0.05$ vs MCI-FTD using one-way ANOVA (*post hoc* tests with Bonferroni correction for multiple comparisons).

high accuracy (ranging from 0.72 to 0.86), high precision (0.72–0.90), high recall (0.75–0.98), and high F1-scores (0.78–0.92) (see Table 2).

By computing a new classifier, both trained and validated on the current cohort of MCI patients, classification indices with 5-fold-cross validation adjusted for age, sex and center, showed even higher accuracy (ranging from 0.83 to 0.93), precision (0.87–0.89), recall (0.83–1.00), and F1-scores (0.85–0.94) (see Table 2).

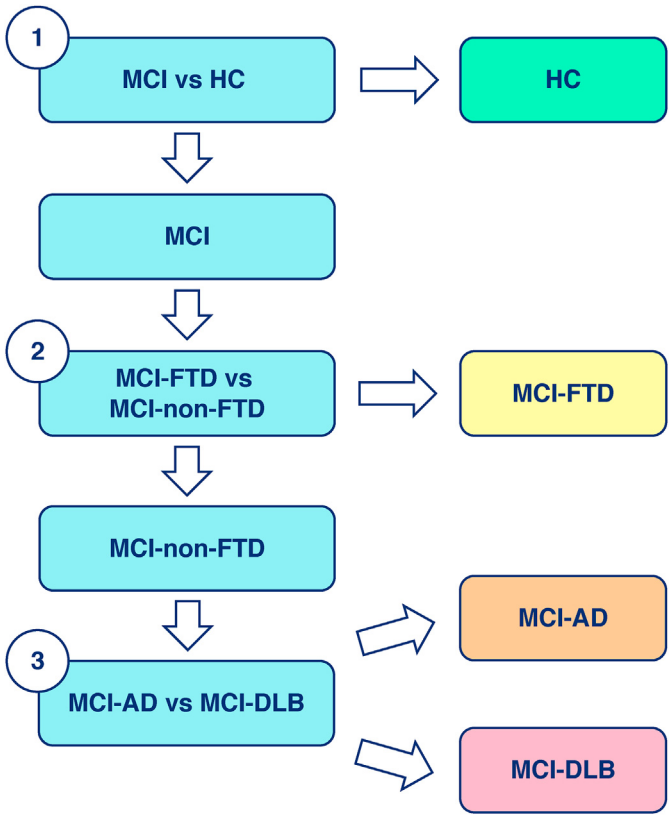

Fig. 3. Series and order of employed binary classifiers.
1) MCI vs HC; 2) MCI-FTD vs MCI-non-FTD (MCI-AD or MCI-DLB), 3) MCI-AD vs MCI-DLB.

Discussion

In the present work we applied previously validated RF algorithms obtained from a very large multicenter cohort of patients with dementia (>650 patients) [31] to a new multicenter cohort of MCI patients with a biomarker supported diagnosis or long-term follow-up.

We observed high levels of classification accuracy, precision and recall, by applying an intuitive and straightforward step-by-step approach: in the first step healthy controls are identified and excluded, in the second step MCI-FTD is recognized from neurodegenerative disorders that affect the central cholinergic system (i.e., MCI-AD and MCI-DLB), while in the third step MCI-AD is differentiated from MCI-DLB. The number of necessary steps to perform a correct diagnosis depends on the diagnostic output of the previous classifier (1 for HC, 2 for MCI-FTD and 3 for MCI-AD and MCI-DLB), resulting in a diagnostic class for each diagnosis at the single subject level.

This was achieved by applying a previously published algorithm carried out considering patients with dementia, which is freely available online (https://github.com/fernandoPalluzzi/tmsClassifier), and by retraining and validating the algorithm on the present cohort of MCI patients, obtaining even higher performances.

The diagnostic assessment of patients with MCI is still complex and significantly varies between centers. It frequently requires different techniques (i.e., MRI imaging, amyloid PET, ^{18}F-FDG-PET,

Table 2
Classification accuracy, precision, recall and F1-score of the two-group classifiers (5-fold cross-validation). Random Forest performance was evaluated with *a*) the classification trees of the published RF classifier [31] and *b)* training new classification trees, after removing outliers with Brier score >1 (8, 5, 5 for 1st, 2nd and 3rd RF classifier, respectively). Both *a)* and *b*) use as features the MCI-TMS regression parameters adjusted by center, age and sex as covariates.

Two-group classifier	*First classifier* MCI vs HC	*Second classifier* MCI-FTD vs MCI-non-FTD	*Third classifier* MCI-AD vs MCI-DLB
a) Previously published classifier [31]			
Accuracy	0.73	0.88	0.86
Precision	0.72	0.81	0.90
Recall	0.98	0.75	0.93
F1-score	0.83	0.78	0.92
b) Present sample classifier (adjusted)			
Accuracy	0.84	0.93	0.90
Precision	0.86	0.87	0.90
Recall	0.93	0.84	1.00
F1-score	0.90	0.85	0.94

MCI = mild cognitive impairment; MCI-AD = MCI-Alzheimer's disease; MCI-FTD = MCI-frontotemporal dementia; MCI-DLB = MCI-dementia with Lewy bodies; RF = Random Forrest.

SPECT DaTSCAN, lumbar puncture), which may not be available in all centers and may be invasive or expensive, particularly if multiple techniques may be required to perform an accurate diagnosis.

The early identification of MCI in the context of the prodromal phase of a neurodegenerative disorder may bring several advantages: access to a range of evidence-based early interventions, symptomatic treatment on cognitive functioning and non-cognitive symptoms, and disease modification, considering the now increasing evidence that disease-modifying treatments for most neurodegenerative disorders must be administered early in the disease course [47,48]. Moreover, disease staging and monitoring the response to treatments will increase the demand for accurate and easily repeatable measures. An early diagnosis also provides the opportunity to prepare financial and end-of-life plans while cognitive impairment remains mild.

As compared to other biological markers, TMS measures are possibly able to selectively identify most of the spectrum of neurodegenerative dementias, such as AD, FTD, DLB and distinguish them from healthy ageing. This may rely on the biological bases of diseases and their associated specific neurotransmitter impairment. Several studies have identified a now well-established cholinergic deficit in AD and DLB [49] while in FTD a significant impairment in GABA and glutamatergic circuits has been observed [50].

The most common TMS protocols herein adopted have been shown to reflect several intracortical circuits, which in turn partially and indirectly reflect the activity of several neurotransmitters. Indeed, SICI and LICI are considered to reflect short-lasting postsynaptic inhibition mediated through the $GABA_A$ and $GABA_B$ receptors at the level of local interneurons [37,38], while ICF is thought to represent a net facilitation most likely mediated by glutamatergic NMDA receptors [20,38]. Moreover, SAI, a marker of sensorimotor integration, has been shown to partially reflect the activity of cholinergic circuits.

As expected, in this study we observed a significant impairment of SICI-ICF in MCI-FTD and MCI-DLB, while SAI was impaired in both DLB and AD patients. Regarding LICI, we observed a non-significant impairment only in MCI-FTD. The combination of these measures has been shown to be accurate in differentiating these neurodegenerative diseases from one another.

The strength of the present work relies on the relatively large sample size of patients with MCI, with a multicenter enrolment and the machine-learning approach to data analysis to obtain highest possible values of diagnostic accuracy. Moreover, the validation of a previously published algorithm on a new cohort of subjects with prodromal neurodegenerative dementia further strengthens our observations.

Compared to well-established diagnostic markers, as CSF or amyloid PET imaging, which have accuracies ranging from 0.92 to 0.94 [51], TMS measures performed only slightly worse. Indeed, previous studies have also shown that TMS, in addition to routine clinical assessment in patients with dementia or MCI, has a significant effect on diagnostic accuracy and confidence, comparable to well-established biomarkers of amyloidosis [26,52].

We acknowledge that this study entails some limitations: first, we cannot exclude that healthy controls included in the present study are biomarker positive in a preclinical phase of disease, since the majority did not undergo any biological marker assessment; second, not all patients had a biomarker supported diagnosis, however all of those without a biomarker converted to a specific dementia at follow-up; third, if MCI-AD [4] and, most recently, MCI-DLB [8] have been carefully defined, MCI-FTD needs to be further characterized; fourth, it is still not clear which is the best biomarker to consider in the diagnosis of MCI (i.e., CSF or amyloid PET imaging in MCI-AD) and thus which should be used as the reference test.

Despite these limitations, the addition of TMS measures to the routine diagnostic assessment could allow for an earlier diagnosis, when combined with clinical and conventional methods of diagnosis. These findings support for the use of TMS intracortical excitability measures to be translated from the experimental setting to the clinical practice, even in the prodromal phases of neurodegenerative dementias.

Search terms

Mild Cognitive Impairment; Alzheimer's disease; frontotemporal dementia; dementia with Lewy bodies; transcranial magnetic stimulation; diagnostic accuracy; decision tree; short interval intracortical inhibition; intracortical facilitation; short latency afferent inhibition.

CRediT authorship contribution statement

Alberto Benussi: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Roles, Writing - original draft, Writing - review & editing. **Mario Grassi:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Roles, Writing - original draft, Writing - review & editing. **Fernando Palluzzi:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Roles, Writing - original draft, Writing - review & editing. **Valentina Cantoni:** Investigation, Writing -

review & editing. **Maria Sofia Cotelli:** Investigation, Writing - review & editing. **Enrico Premi:** Investigation, Writing - review & editing. **Francesco Di Lorenzo:** Investigation, Writing - review & editing. **Maria Concetta Pellicciari:** Investigation, Writing - review & editing. **Federico Ranieri:** Investigation, Writing - review & editing. **Gabriella Musumeci:** Investigation, Writing - review & editing. **Camillo Marra:** Investigation, Writing - review & editing. **Paolo Manganotti:** Investigation, Writing - review & editing. **Raffaele Nardone:** Investigation, Writing - review & editing. **Vincenzo Di Lazzaro:** Investigation, Writing - review & editing. **Giacomo Koch:** Investigation, Writing - review & editing. **Barbara Borroni:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Supervision, Roles, Writing - original draft, Writing - review & editing.

Declaration of competing interest

Nothing to report.

Acknowledgements

This study is supported by the Airalzh-AGYR2020 grant issued to A.B. We thank the patients and their families for participating in this research. We also thank the many physicians, genetic counselors, and families for referring patients.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.brs.2021.01.004.

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