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ORIGINAL ARTICLE

Diagnostic accuracy of nerve excitability and compound muscle action potential scan derived biomarkers in amyotrophic lateral sclerosis

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Abstract

Background and purpose: The lack of reliable early biomarkers still causes substantial diagnostic delays in amyotrophic lateral sclerosis (ALS). The aim was to assess the diagnostic accuracy of a novel electrophysiological protocol in patients with suspected motor neuron disease (MND).

Methods: Consecutive patients with suspected MND were prospectively recruited at our tertiary referral centre for MND in Utrecht, The Netherlands. Procedures were performed in accordance with the Standards for Reporting of Diagnostic Accuracy. In addition to the standard diagnostic workup, an electrophysiological protocol of compound muscle action potential (CMAP) scans and nerve excitability tests was performed on patients' thenar muscles. The combined diagnostic yield of nerve excitability and CMAP scan based motor unit number estimation was compared to the Awaji and Gold Coast criteria and their added value was determined.

Results: In all, 153 ALS or progressive muscular atrophy patients, 63 disease controls and 43 healthy controls were included. Our electrophysiological protocol had high diagnostic accuracy (area under the curve [AUC] 0.85, 95% confidence interval [95% CI] 0.80–0.90), even in muscles with undetectable axon loss (AUC 0.78, 95% CI 0.70–0.85) and in bulbar-onset patients (AUC 0.85, 95% CI 0.73–0.95). Twenty-four of 33 (73%) ALS patients who could not be diagnosed during the same visit were correctly identified, as well as 8/13 (62%) ALS patients not meeting the Gold Coast criteria and 49/59 (83%) ALS patients not meeting the Awaji criteria during this first visit.

Conclusions: Our practical and non-invasive electrophysiological protocol may improve early diagnosis in clinically challenging patients with suspected ALS. Routine incorporation may boost early diagnosis, enhance patient selection and generate baseline measures for clinical trials.

KEYWORDS
ALS, Awaji criteria, CMAP scan, diagnosis, EMG, Gold Coast criteria, MND, nerve excitability

INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a devastating progressive neurodegenerative disorder affecting upper motor neurons (UMNs) and lower motor neurons (LMNs) [1]. Due to lack of reliable biomarkers and the heterogeneous presentation of the disease, diagnosing ALS involves a combination of clinical and electrodiagnostic assessments to establish UMN and LMN loss and an arduous process to exclude other potential conditions [2]. As a result, substantial diagnostic delays are introduced [3]. During this period, neurodegeneration can cause unsalvageable damage in other regions well before becoming clinically observable [4]. Early diagnosis is thus necessary to effectively administer potential treatments, such as riluzole, or to recruit patients for clinical trials [5]. Identifying biomarkers which provide additional evidence in the pre-symptomatic phase of ALS [6] could help steer the diagnostic process from an early stage.

Advanced non-invasive electrophysiological techniques, such as surface electromyography based motor unit number estimation (MUNE) methods and nerve excitability testing, are promising tools for detecting early disease-specific alterations in ALS. MUNE values derived from compound muscle action potential (CMAP) scans were shown to be a reliable biomarker of LMN loss [7, 8], as this technique overcomes the masking effects of re-innervation that often obscures motor unit loss [9, 10]. Nerve excitability tests are an additional useful tool for revealing biomarkers of LMN dysfunction, as phenomena attributed to hyperexcitable LMNs, such as multiplet discharges [11] and fasciculations [12], are ubiquitous in ALS. Several altered nerve excitability measures have been reported in patients with ALS [13–20], some of which were even found to precede axon loss [16, 18]. However, the accuracy of nerve excitability measures in differentiating ALS patients from clinically challenging disease controls, individually or in combination with MUNE, remains to be established [14].

The aim was therefore to evaluate the combined diagnostic accuracy of these electrophysiological techniques in a large prospective cohort of ALS patients and to compare the diagnostic yield to that of current diagnostic criteria for ALS.

METHODS

Study design

A prospective study was performed of a large cohort of consecutive suspected motor neuron disease (MND) patients, to provide class I evidence of the diagnostic yield of our electrophysiological protocol. All enrolled subjects underwent routine diagnostic tests and electrophysiological tests between 1 September 2020 and 10 May 2022. Our study protocol was approved by the Medical Ethics Committee of the University Medical Center Utrecht. All participants provided written informed consent and the study procedures were in accordance with the Declaration of Helsinki.

Patients and controls

Patients with suspected MND referred to our neuromuscular outpatient clinic of the University Medical Center Utrecht, a national tertiary referral centre, were screened. Exclusion criteria were any established cognitive or physical condition potentially hampering compliance with the study (e.g., severe frontotemporal dementia); coincidental active neuropathies; use of nerve excitability altering medication (such as riluzole); inability to tolerate electrical nerve stimulation; and absence of motor responses in the abductor pollicis brevis (APB). Patients who agreed to participate underwent a standardized electrophysiological carpal tunnel syndrome screening, to detect subclinical entrapment of the median nerve that could affect the results of our electrophysiological protocol. Patients were considered ineligible if any of these exclusion criteria were met or if participation was not possible for logistic reasons (e.g., unforeseen delays between clinical procedures). Age- and gender-matched healthy controls, without history of disorders affecting the median nerve, were recruited from a prospective population based register in The Netherlands [21], or were obtained from previous studies [22, 23], to derive reference values for the electrophysiological protocol.

Routine diagnostic tests

At the initial presentation for diagnostic workup in our hospital, all patients underwent the relevant routine investigations including (1) thorough medical history, extensive standardized neurological examination to identify UMN and LMN involvement in any of the four body regions (bulbar, cervical, thoracic, lumbosacral); (2) an elaborate electrodiagnostic protocol (standardized electromyographic examination of muscles of all four body regions) to detect LMN involvement; (3) appropriate laboratory testing (for further details see Table S1). If considered appropriate, additional ancillary tests (i.e., imaging, cognitive screening, nerve conduction studies, DNA testing) were performed. Final diagnosis was established by a panel of experienced neurologists (including HSG and LHvdB) and reviewed after a minimal follow-up period of 6 months. In keeping with the recent consensus criteria for ALS, cases of progressive muscular atrophy (PMA) were grouped with ALS patients [24]. All suspected ALS patients who eventually received other final diagnoses were considered disease controls. This final grouping was taken as the clinical reference standard.

The following patient characteristics were recorded: disease duration (from symptom onset to visit), diagnostic delay (from symptom onset to final diagnosis), follow-up duration (from study visit to final diagnosis), region of symptom onset (bulbar, thoracic, upper limb or lower limb), the revised ALS Functional Rating Scale (ALSFRS-R) [25] score during the visit and the rate of functional decline (dF = [48 − ALSFRS-R]/disease duration). In addition, one rater (DS) assessed muscle strength of the APB, sampled in the electrodiagnostic study protocol described below, using the Medical Research Council score. Experienced physical burden during the

study procedures was recorded using one combined ordinal visual analogue score [26] (Figure S1), ranging from 0 to 10 (no burden to worst pain imaginable).

Electrophysiological protocol

All recordings were performed by an experienced examiner (DS) and were integrated in the first diagnostic workup at the hospital. Electrical stimuli were supplied to the median nerve at the wrist and the evoked CMAP responses were recorded from the APB. Additionally, a standardized warming protocol was implemented, as described previously [27], to maintain the examined arm at 37°C. The complete protocol took approximately 50 min [23, 28].

Nerve excitability testing

Standardized nerve excitability tests were conducted using the QTRAC software (Institute of Neurology, London, UK). Four measures were selected for analysis that had previously been shown to consistently detect early excitability differences between ALS patients and healthy controls [29], including (1) strength–duration time constant (SDTC), a biomarker associated with persistent Na^+ channel currents [20]; (2) the average threshold change after a 40% depolarizing current of 10 and 20 ms (TEd10–20); (3) the average threshold change after a 40% depolarizing current of 90 and 100 ms (TEd90–100); (4) the peak threshold reduction during the recovery cycle between an interval of 2 to 200 ms (superexcitability). These four standard measures (SDTC, TEd10–20, TEd90–100 and superexcitability) can be directly obtained from the tests in standard recording protocol for nerve excitability (TRONDNF, described in detail elsewhere [14, 30]).

Compound muscle action potential scan

Detailed stimulus–response curves were recorded, termed CMAP scans, in which all motor units innervating a muscle are gradually recruited [10]. During these scans, the stimulus current was reduced in steps of 0.2% (2 Hz, stimulus duration 0.1 ms) from supramaximal until no further responses were elicited. These recordings were used to derive MUNE [9] using the MScanFit tool (version 2) in the QTRAC software, as described in detail previously [9, 31].

Diagnostic accuracy of the electrophysiological protocol

The primary study outcome was the individual and combined diagnostic accuracy of MUNE and the four nerve excitability measures in the full cohort. The combined accuracy of our electrophysiological protocol was also assessed in clinical subgroups, including patients with axon loss present or absent in the thenar muscles, defined by MUNE values below the lower fifth percentile of the values observed in healthy controls (MUNE <50); patients with disease onset in the bulbar or spinal regions; and patients who could be diagnosed on the day of study visit or patients who required follow-up before a final diagnosis could be established.

The secondary study outcome was the diagnostic accuracy of our combined electrophysiological protocol compared to current diagnostic criteria for ALS. The Awaji criteria (AC) and the more recent Gold Coast criteria (GCC) were examined. The latter do not require UMN dysfunction if LMN dysfunction is present in at least two regions [2]. The AC, in contrast, require the presence of UMN dysfunction [2]. Therefore, PMA patients were omitted from analysis of the AC. To establish the additional value of our electrophysiological measures, the protocol's diagnostic yield was examined in the diagnostic categories of the AC and GCC during the first routine workup.

Statistical analysis

Patient characteristics were compared using *t* tests or Mann–Whitney *U* tests for non-normally distributed data. Categorical data were compared with chi-squared tests. Missing data due to test-specific noise or movement artefacts were infrequent ($n=6$) and were imputed to the cohort median. First, a set of logistic regression models was used to assess the diagnostic accuracy of MUNE and the individual nerve excitability measures. Secondly, the accuracy of a model was examined with all nerve excitability measures. Lastly, MUNE and all nerve excitability measures were combined into a final model. Differences in model performance were established using the likelihood ratio χ^2 test. Diagnostic accuracy of the electrophysiological measures was quantified using the area under the receiver operator characteristic curve (AUC), sensitivity, specificity, and positive and negative predictive value. No separate cut-offs were obtained for MUNE and the excitability measures, but rather the linear predictions from the final multivariable model were used as electrophysiological risk scores. The cut-off for this electrophysiological risk score was determined arbitrarily by maximizing the summed sensitivity and specificity. Diagnostic measures were denoted as mean plus 95% confidence intervals. *p* values <0.05 were considered significant. Statistical analyses were performed in R (R Core Team, 2020, R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Baseline characteristics

In all, 344 patients were recruited and screened, of whom 228 (66%) were eligible for inclusion. A summarizing flowchart of recruitment is shown in Figure 1. Twelve (5%) recordings were omitted, predominantly those of patients whose final diagnosis had not been

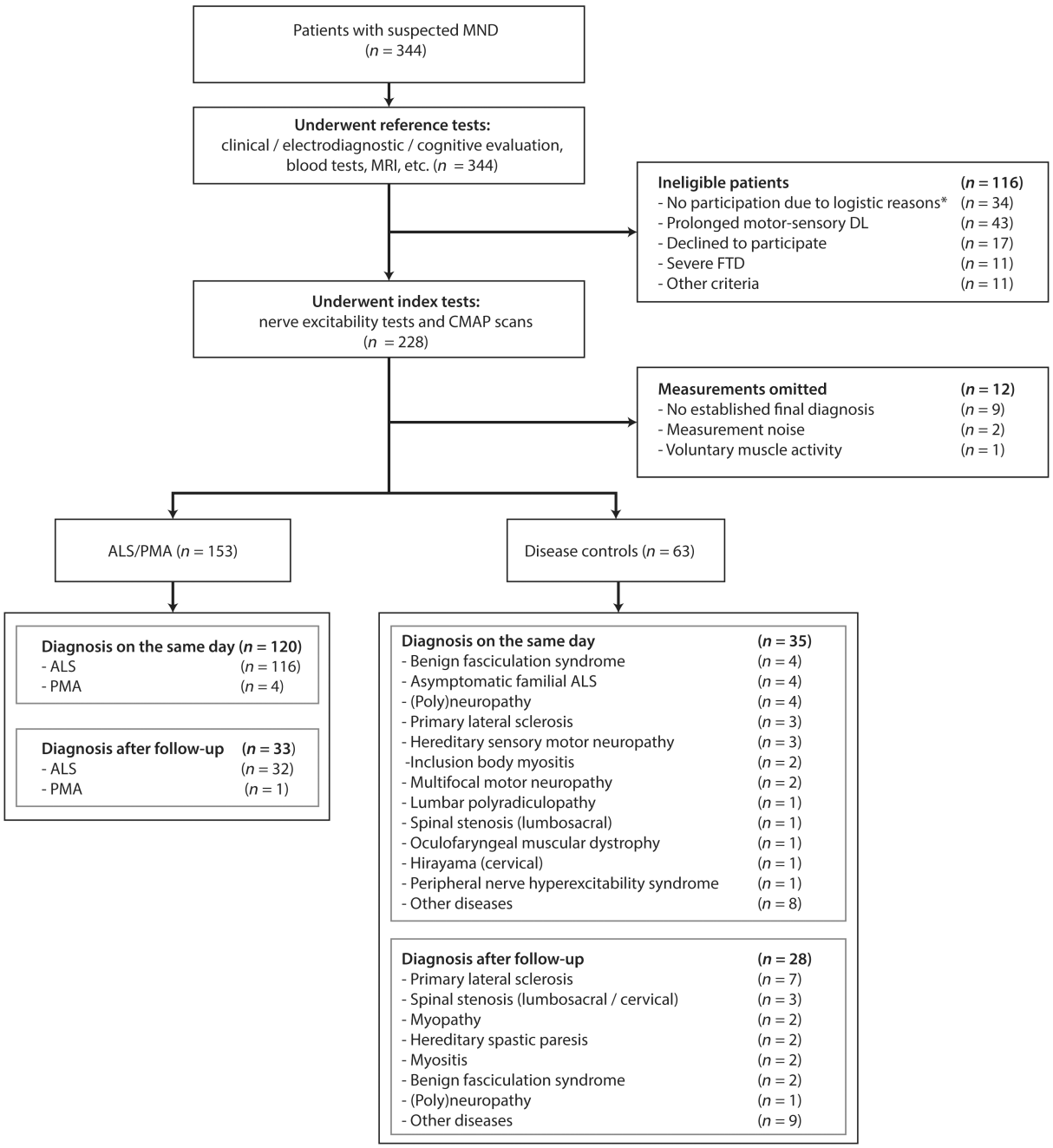

FIGURE 1 Flowchart of the study. *Logistic reasons such as unforeseen delays during reference tests. ALS, amyotrophic lateral sclerosis; CMAP, compound muscle action potential; DL, distal latency; FTD, frontotemporal dementia; MND, motor neuron disease; MRI, magnetic resonance imaging; PMA, progressive muscular atrophy.

established at the time of writing. Of the final study population of 216 patients, 153 (67%) patients received the final diagnosis of ALS (PMA, $n=5$). The remaining 63 (33%) patients were considered to be disease controls. Lastly, 43 healthy controls (56% male) were recruited with a mean (SD) age of 64 (8) years. The baseline characteristics of the ALS patients, disease controls and the ineligible ALS patients are summarized in Table 1. No difference in age and gender was observed between ALS patients and disease controls. Median disease duration was shorter in the ALS group, who had more functional impairment and faster rates of functional decline than disease controls. Experienced physical burden during measurements was low, as indicated by the visual analogue scores (median [interquartile range] = 2 [1–4]).

Motor unit number estimation and nerve excitability measures of ALS patients

Amyotrophic lateral sclerosis patients had lower MUNE values compared to disease controls ($p<0.001$). The strength–duration test,

TABLE 1 Summary baseline characteristics.

Characteristic	ALS, $N=153$	Disease controls, $N=63$	p value[a]	Ineligible ALS, $N=75$	p value[b]
Age, years	64 (9)	62 (12)	0.3	70 (9)	<0.001
Sex, male/female	95/58 (62%/38%)	36/27 (57%/43%)	0.6	42/33 (55%/45%)	0.5
Disease duration, months	10 (6–17)	15 (8–53)	0.003	9 (5–14)	0.2
Diagnostic delay, months	10 (7–17)	19 (10–55)	<0.001	9 (5–15)	0.2
Follow-up duration, months[c]	3.5 (1.1–6.2)	5.8 (2.4–8.6)	0.036	0.68 (0.23–3.49)	0.012
Region of symptom onset[d]			<0.001		0.5
Bulbar	42 (27%)	10 (16%)		26 (35%)	
Thoracic/respiratory	0 (0%)	2 (3%)		0 (0%)	
Cervical	60 (39%)	10 (16%)		25 (33%)	
Lumbosacral	51 (33%)	39 (62%)		24 (32%)	
ALSFRS-R	41 (38–44)	43 (41–46)	0.003	38 (33–42)	<0.001
dF	0.6 (0.4–1.1)	0.1 (0.1–0.5)	<0.001	1.02 (0.54–1.67)	<0.001
MRC score, 5/4/≤3	92/51/10 (60%/33%/7%)	52/10/1 (83%/16%/2%)	0.008	–	–

Note: Data presented as mean (SD), median (IQR) or *N* (%).

Abbreviations: ALS, amyotrophic lateral sclerosis; ALSFRS-R, Amyotrophic Lateral Sclerosis Functional Rating Score (revised); dF, (48 − ALSFRS-R)/disease duration; IQR, interquartile range; MRC, Medical Research Council.

[a]ALS versus disease controls.

[b]ALS versus ineligible ALS.

[c]Only for patients requiring follow-up to receive a final diagnosis (ALS $n=33$, disease controls $n=28$ and ineligible ALS $n=16$).

[d]$n=2$ disease controls with unclear region of symptom onset omitted.

threshold electrotonus and recovery cycle, from which the excitability measures were derived, are shown in Figure 2 and the measures are summarized in Table 2. ALS patients had a larger range of SDTC values compared to disease controls, as can be observed in Figure 2a,b. An additional variable was defined to address this nonlinear association of SDTC with ALS, defined as $SDTC_{nonlinear}=abs(SDTC - SDTC_{norm})$, $SDTC_{norm}$ being the mean obtained from our healthy controls. As such, this variable describes the absolute deviation of a patient's SDTC from the average in healthy controls. TEd10–20 and TEd90–100 were strongly correlated ($R=0.46$, $p<0.001$) and merged into a modified S2 accommodation (S2m, difference between TEd10–20 and TEd90–100). This S2m was markedly reduced in ALS patients ($p<0.001$, Figure 2c,d). Lastly, ALS patients exhibited higher superexcitability ($p<0.001$) than disease controls (Figure 2e,f).

Diagnostic accuracy of the measures in the protocol

Motor unit number estimation had the highest individual diagnostic accuracy of all the measures (AUC = 0.79, 95% confidence interval [95% CI] 0.72–0.85). Although the nerve excitability measures had lower AUCs individually, when combined their diagnostic accuracy was markedly improved (AUC = 0.77, 95% CI 0.70–0.83), even matching the performance of the model with MUNE ($p=0.39$, Figure 3a). Our final model contained all measures, that is, MUNE, S2m, superexcitability, SDTC and $SDTC_{nonlinear}$. This model had the highest accuracy of all (AUC = 0.85, 95% CI 0.80–0.90) and significantly outperformed the model with only MUNE ($p<0.001$).

The coefficients from the final multivariable model in Table 2 were used to obtain individual electrophysiological risk scores, as follows: risk score = −0.033*MUNE − 0.188*S2m + 0.083*superexcitability + 7.096*SDTC + 8.153*$SDTC_{nonlinear}$. In this linear predictor, reductions in MUNE and S2m or increases in superexcitability and SDTC increase the risk score, indicating a higher probability of ALS.

Our electrophysiological risk scores had high diagnostic accuracy, yielding a sensitivity of 82% (95% CI 76%–87%) and specificity of 75% (95% CI 63%–85%) at a cut-off of 0.548 (Table 3, Figure 3b). Of note, 34/63 disease controls received diagnoses of non-peripheral nerve disorders. To ensure that these cases did not positively bias our results, this analysis was repeated using only disease controls with peripheral nerve disorders and the ALS patients. The results were comparable to those in the full cohort, yielding a sensitivity of 82% (95% CI 75%–88%) and specificity of 76% (95% CI 59%–91%).

Diagnostic sensitivity in clinical subgroups

The diagnostic sensitivity of the risk scores from the electrophysiological protocol was examined in subgroups of patients based on clinical characteristics. The corresponding group sizes, sensitivities and specificities are presented in Table 3. Diagnostic accuracy was marginally higher in patients with axon loss in the thenar muscles (present vs. absent, AUC = 0.82 [95% CI 0.71–0.91] vs. AUC = 0.78 [95% CI 0.70–0.85], Figure 4a). Comparable diagnostic accuracy was achieved in patients with symptom onset in either the spinal (AUC = 0.86, 95% CI 0.80–0.91) or bulbar region (AUC = 0.85, 95% CI 0.73–0.95) (Figure 4b). Our electrophysiological protocol produced

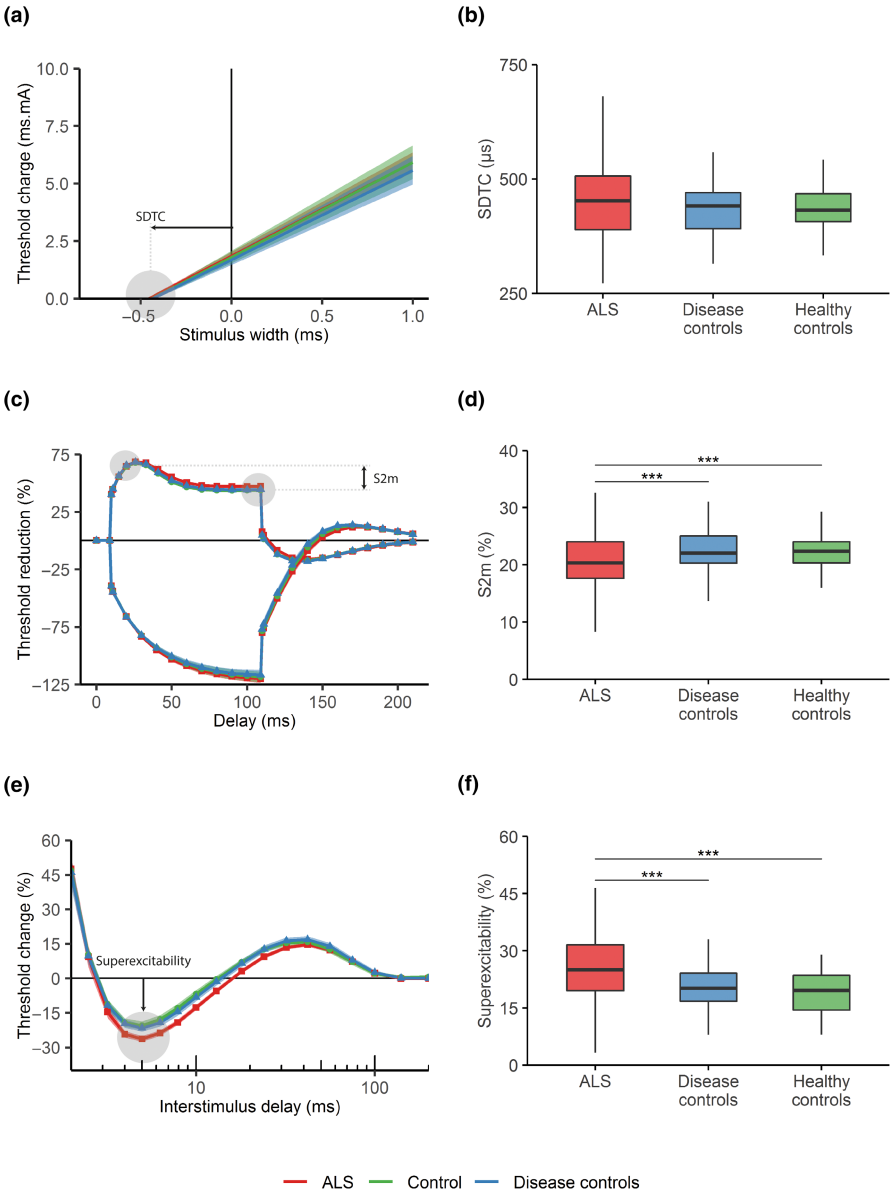

FIGURE 2 Averaged nerve excitability recordings per group, with (a) the strength–duration test and the resulting strength–duration time constant in (b); (c) the threshold electrotonus test and the resulting modified S2 accommodation (S2m) in (d); (e) the recovery cycle and the resulting superexcitability in (f). Shaded areas in the recordings indicate 95% confidence interval of each measurement point. ALS, amyotrophic lateral sclerosis; SDTC, strength–duration time constant. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$.

higher risk scores in patients who received their diagnosis on the same day, that is, less diagnostically challenging patients, than in patients who required follow-up after the study visit to establish a final diagnosis (AUC = 0.90, 95% CI 0.84–0.95; AUC = 0.74, 95% CI 0.60–0.85; Figure 4c). Nevertheless, our electrophysiological protocol allowed the correct classification of 73% of the ALS patients (24/33) who could not be diagnosed on the same day according to our clinical reference standard.

Diagnostic accuracy of the electrophysiological protocol and consensus criteria

Sensitivity and specificity of the AC were 60% (95% CI 52%–68%) and 95% (95% CI 89%–100%), respectively, when considering probable or definite ALS as a positive test result. When also considering possible ALS as a positive test result, the sensitivity of the AC increased to 90% (95% CI 85%–95%) with a decreased specificity of 70% (95% CI 59%–81%). Note that PMA patients were omitted from the ALS group for the analysis of the AC. In contrast, the GCC had higher sensitivity (92%, 95% CI 87%–95%), albeit with lower specificity (78%, 95% CI 66%–88%).

The number of ALS patients and disease controls in the diagnostic categories of AC and GCC, as well as the corresponding sensitivities and specificities from the electrophysiological protocol, are presented in Table 3. Our electrophysiological protocol correctly identified 73/89 (82%) of the ALS patients with probable or definite ALS according to the AC. Also, 49/59 (83%) of the ALS patients who could not be diagnosed by the AC at the time of study participation (possible ALS or not meeting the criteria) were correctly identified.

TABLE 2 Summary of nerve excitability and MUNE scan measures in the study cohort and the corresponding univariable and multivariable diagnostic predictors.

Measures	ALS[a] mean (95% CI)	Disease controls mean (95% CI)	Healthy controls mean (95% CI)	Univariable coefficient ± SE	Multivariable coefficient ± SE
MUNE (–)	49 (44–54)***	85 (76–93)	85 (76–93)	−0.032 ± 0.0053***	−0.033 ± 0.006***
S2m (%)	19.9 (19.0–20.8)***	22.7 (21.8–23.7)	22.5 (21.6–23.5)	−0.108 ± 0.034**	−0.188 ± 0.053***
SDTC (ms)[b]	0.45 (0.44–0.47)	0.43 (0.42–45)	0.44 (0.42–0.45)	2.428 ± 2.278	7.096 ± 2.893*
$SDTC_{nonlinear}$ (ms)[b]	0.07 (0.06–0.08)**	0.05 (0.04–0.06)	0.04 (0.03–0.05)	9.142 ± 3.833*	8.153 ± 4.634
Superexcitability (%)	25.0 (24.0–27.0)***	21 (19–22)	19 (17–21)	0.103 ± 0.026***	0.083 ± 0.028**

Abbreviations: ALS, amyotrophic lateral sclerosis; CI, confidence interval; S2m, modified S2 accommodation; MUNE, motor unit number estimate; SDTC, strength–duration time constant; $SDTC_{nonlinear}$, abs(SDTC − 0.44).

[a]Asterisks indicate significance level with respect to disease controls.

[b]SDTC was modelled with an additional term due to a nonlinear association with diagnosis.

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

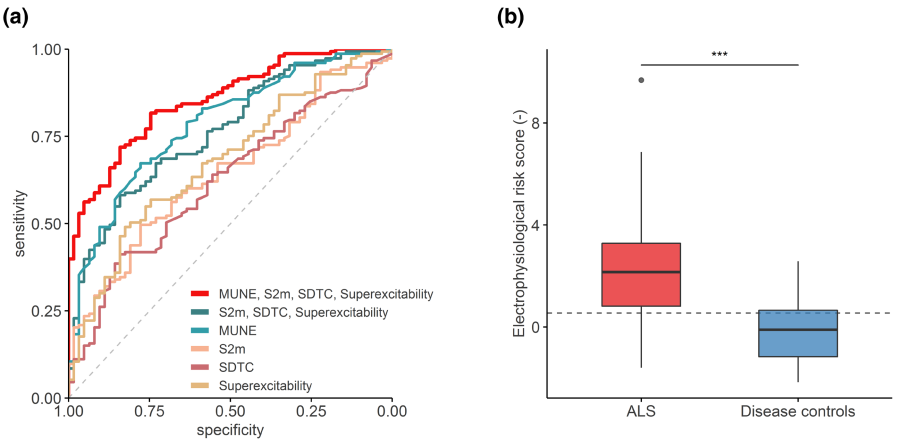

FIGURE 3 (a) Receiver operator characteristic curves of the individual measures or combined measures. (b) Resulting electrophysiological risk scores from the model containing MUNE, S2m, SDTC (and its nonlinear term) and superexcitability. The dotted line represents the cut-off at which maximum specificity and sensitivity was obtained. MUNE, motor unit number estimates; S2m, modified S2 accommodation; SDTC, strength–duration time constant (modelled with an additional nonlinear term). ***$p < 0.001$.

Furthermore, 117/140 (84%) of the ALS patients correctly diagnosed by the GCC were identified, and it was also possible to identify an additional 8/13 (62%) of ALS patients who did not meet the GCC at the time of study participation. Overall, these findings illustrate that the combined electrophysiological protocol with MUNE and nerve excitability could help diagnose approximately 60%–80% of the ALS patients who could otherwise not be diagnosed at the time of their first diagnostic workup with the AC, GCC and our clinical reference standard.

DISCUSSION

In this study, the diagnostic accuracy of two novel electrophysiological techniques was examined: CMAP scan based MUNE and nerve excitability testing, in patients with a suspected MND. It was shown that their integration into a combined electrophysiological protocol produces early and distinctive electrophysiological evidence of LMN dysfunction in patients with ALS. Importantly, this study indicates that these features may serve as diagnostic markers that distinguish patients with ALS from clinically representative disease controls with high accuracy. Of further relevance, the electrophysiological protocol is feasible during routine diagnostic visits and introduces minimal additional burden for the patients.

Diagnosing ALS requires evidence of UMN and LMN dysfunction [32, 33], but in the absence of reliable biomarkers substantial diagnostic delays are not uncommon [3]. Altered nerve excitability in ALS patients has previously been recognized as an early feature of the LMNs, mostly attributed to a pattern of altered Na^+ and K^+ channel conductance [13–18, 34]. It was found that our measures of nerve excitability, when combined, have diagnostic value comparable to MUNE. This finding is in line with the notion above that ALS is characterized by a pattern of changes in multiple parameters of nerve excitability. The risk scores obtained from all the measures in the electrophysiological protocol demonstrated superior performance over MUNE or nerve excitability alone in classifying ALS patients. Importantly, several previous studies showed that nerve excitability changes were present in ALS patients, even

TABLE 3 Summary of the diagnostic accuracy of the risk scores from the electrophysiological protocol in the full cohort, clinical subgroups and diagnostic categories of the Awaji and Gold Coast criteria.

Diagnostic approach	*N*, ALS/DC	Sensitivity, % (95% CI)	Specificity, % (95% CI)	PPV, % (95% CI)	NPV, % (95% CI)
Electrophysiological protocol	153/53	82 (76–87)	75 (63–85)	89 (83–94)	63 (51–94)
Axon loss					
Absent	61/52	57 (56–68)	85 (74–94)	81 (69–92)	63 (46–68)
Present	92/11	98 (95–100)	27 (0–57)	92 (86–97)	60 (0–100)
Onset region					
Spinal	111/51	84 (77–90)	73 (60–85)	87 (81–93)	67 (55–80)
Bulbar	42/10	76 (62–88)	80 (50–100)	94 (85–100)	44 (21–69)
Final diagnosis					
At study visit	120/35	84 (77–90)	83 (69–93)	94 (90–98)	60 (47–74)
Required follow-up	33/28	73 (57–87)	64 (46–82)	71 (54–85)	67 (50–83)
Awaji[a]					
Probable/definite	89/3	82 (74–90)	0 (0–0)	96 (91–99)	0 (0–0)
Possible/NMC	59/60	83 (73–92)	78 (67–88)	79 (68–88)	82 (71–92)
Gold Coast					
ALS[b]	140/14	84 (78–90)	50 (24–78)	94 (90–98)	23 (9–39)
NMC	13/49	62 (33–88)	82 (70–92)	47 (24–69)	89 (79–98)

Note: Unless otherwise stated, data represent all patients ($n = 216$).

Abbreviations: ALS, amyotrophic lateral sclerosis; CI, confidence interval; DC, disease controls; NMC, not meeting criteria; NPV, negative predictive value; PPV, positive predictive value.

[a]Patients with progressive muscular atrophy ($n = 5$) omitted from the ALS group.

[b]According to the criteria.

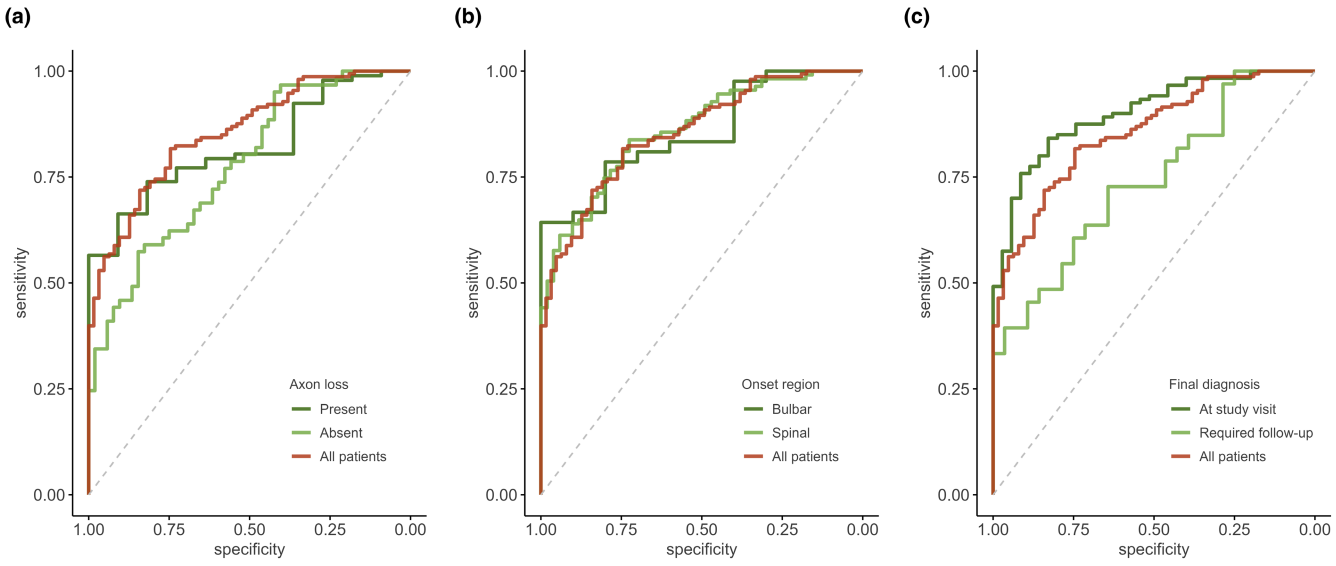

FIGURE 4 Receiver operator characteristic curves indicating diagnostic accuracy of the electrophysiological protocol in all patients and clinical subgroups of (a) patients with axon loss present or absent in the examined muscles; (b) patients with onset in the bulbar or spinal regions; (c) patients who received a final diagnosis on the day of study visit or patients who required follow-up to receive a final diagnosis.

before axon loss could be detected [16, 18]. Corroborating these findings, the risk scores from our combined electrophysiological protocol had high accuracy in patients without axon loss. The cut-off for axon loss that was used to establish this subgroup was determined in healthy controls, as a single observation of MUNE within the range observed in healthy controls cannot be considered evidence of motor unit loss. Consequently, our findings indicate that nerve excitability changes with discriminative potential are present in the LMNs of ALS patients, even before traditional evidence of neurodegeneration can be established. The potential

of the electrophysiological protocol with MUNE and excitability tests was further highlighted by the early and correct classification of the majority of ALS patients who only received their diagnosis during later follow-up visits.

Nerve excitability predictors were selected that could capture early pathophysiological features of ALS, based on the results of a large meta-analysis of previous studies [29]. Consequently, reproducibility of our findings is likely with minimal bias. Despite our more conservative approach, combined MUNE and nerve excitability measures yielded excellent diagnostic characteristics in our full study cohort and subgroups. Interestingly, most exclusions occurred due to electrophysiological evidence of (bilateral) median nerve entrapment with substantially higher prevalence than the established Dutch population average [35]. Other causes for exclusions were in line with expectations, mainly logistic or substantial cognitive deficits.

Our established sensitivities and specificities of the AC were in line with those of a previous study [36]. The sensitivity of the GCC was also in line with that of previous studies [37, 38], albeit with lower specificity. This reduction in specificity probably originated from the lack of ancillary information available at the first diagnostic visit, which was used as a benchmark for our electrophysiological protocol. Additionally, the role of our institute as tertiary referral centre may have reduced the specificity, as diagnostically challenging mimics are more likely to be referred than less challenging mimics. The risk scores from our electrophysiological protocol had higher sensitivity than the AC, at the cost of lower specificity. The GCC had higher sensitivity and comparable specificity to our electrophysiological protocol. Still, the high diagnostic accuracy of our electrophysiological protocol performed in just one muscle during the first visit indicates that combined loss of LMNs (MUNE) and excitability changes are highly characteristic of ALS. Despite the differences in overall performance, a substantial percentage (60%–80%) of ALS patients who did not meet the GCC or AC at the time of participation was correctly identified. These findings indicate that these novel electrodiagnostic techniques may complement conventional electrodiagnostic testing in the early stages of the diagnostic process.

Our study has several limitations. Recordings were only derived from a single muscle–nerve combination, which could explain the absence of axon loss in the majority of disease controls and a large proportion of the ALS patients. Inclusion of additional nerves could further improve detection rates, assessing more widespread abnormalities by using a combination of multiple nerves and body regions. Testing multiple nerve–muscle combinations with nerve excitability and CMAP scan based MUNE is feasible [39–41]. The application of nerve excitability measures is inherently restricted to LMNs. Combination with cortical excitability tests could provide a unique opportunity, thereby yielding complementary biomarkers for UMN involvement [42, 43].

A current key objective in ALS research is to improve clinical trial outcomes, mainly by reducing diagnostic delay and by making patient stratification more accurate [44]. Nerve excitability measures are considered sensitive biomarkers for evaluating effects of novel treatment strategies [28, 45] with prognostic potential [46, 47]. Our study shows that a combination of MUNE and nerve excitability tests also provides directly applicable measures with added diagnostic value. Routine incorporation of the presented electrophysiological protocol into clinical practice may boost early diagnosis, enhance patient selection and generate baseline measures for clinical trials.

AUTHOR CONTRIBUTIONS

DJL Stikvoort García: conceptualization of study design, acquisition and analysis of data, drafting and revision of manuscript. HS Goedee: conceptualization of study design, acquisition and analysis of data, drafting and revision of manuscript, obtained funding. BTHM Sleutjes and LH van den Berg: conceptualization of study design, analysis of data, drafting and revision of manuscript, obtained funding. LJ van Schelven: provided technical expertise and support, drafting and revision of manuscript.

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CONFLICT OF INTEREST STATEMENT

None of the authors have potential conflicts of interest to disclose of relevance to this manuscript. D.J.L. Stikvoort García reports no disclosures; B.T.H.M Sleutjes has received research grants from ALS Foundation Netherlands; L.J. van Schelven reports no disclosures; H.S. Goedee has received research speaker fees from Shire/Takeda paid to the institution and grants from Prinses Beatrix Spierfonds; L.H. van den Berg serves on the scientific advisory boards for Ferrer, Amylyx, Biogen, Cytokinetics, Sanofi, Corcept and has received research grants from the Netherlands ALS Foundation Netherlands.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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