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REVIEW ARTICLE

A review of electrophysiological studies of lower motor neuron involvement in amyotrophic lateral sclerosis

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Abstract

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease involving both the upper and lower motor neuron diseases. In this review, we studied and compared different articles regarding the electrodiagnostic criteria for diagnosis of lower motor neuron pathology in ALS. We reviewed the most recent articles and metaanalysis regarding various lower motor neuron electrodiagnostic methods for ALS and their sensitivities. We concluded that Awaji Shima criteria is by far the most sensitive criteria for diagnosis of ALS.

Keywords ALS · Lower motor neuron · Electrodiagnosis

Introduction

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease involving the upper and lower motor neurons. Electrophysiological investigations play a prominent role in the diagnosis of this disease. There are many years of experience with electrophysiological studies in ALS. Nevertheless, areas of controversy remain and attempts at advancing the electrophysiological diagnosis of ALS continue. This article aims to review the literature on electrophysiological studies in ALS. We will discuss the electrophysiological signs of lower motor neuron degeneration (i.e., denervation-reinnervation) and the evolution of the electrophysiological criteria for the diagnosis of this disease.

Method

We searched PubMed and Google Scholar for publications using the following keywords: ALS, amyotrophic lateral sclerosis, electromyography (EMG), nerve conduction studies, and electrophysiology. Studies with methodological errors such as poor definition of disease diagnosis, potential technical errors, and inadequate sample size were excluded. Search date was from the onset of indexing by these databases until December 2017. A second search was performed in October 2018, and data was updated. When the abstract and the text of a study were not accessible, the study was excluded. Studies were thoroughly reviewed for relevance. The text of all the studies was obtained using available online libraries.

Conventional nerve conduction studies

It has been long known that the motor amplitudes can be within normal limits early on and decrease as ALS progresses [1, 2]. The motor conduction velocity can be slightly decreased but generally remains less than 10 m/s which is below normal. In a study of three motor nerves in 61 patients with ALS, motor conduction velocity was below 80% of the normal values in one out of 149 (0.7%) measurements and distal latency was above 125% of the normal values in seven out of 163 (5%) measurements [3]. Ordinarily, one would not expect non-motor involvement in ALS [1]. However, near-nerve recording techniques of sensory nerves has demonstrated reduced minimum conduction velocity (i.e., the conduction velocity for the slowest response) and/or reduced peak-to-peak amplitude in more than half of the patients [4]. A prospective study of 35 patients with ALS showed decreased sural sensory amplitude or conduction velocity in 17% of patients [5]. In another study, distal sensory nerve conduction studies (NCS) were more often abnormal than conventional sensory NCS in ALS [6]. In this study, distal sensory NCS were obtained using a typical method for obtaining mixed nerve action potentials, with the stimulation intensity reduced to

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a level that muscle twitches were not observed. The absence of visible muscle twitch does not necessary indicate the absence of motor nerve stimulation. Therefore, this study may have measured mixed rather than purely sensory action potentials. Pathological studies have confirmed a non-length-dependent sensory and autonomic nerve fiber loss in patients with ALS [7]. Overall, while mildly reduced sensory and motor amplitudes and conduction velocities and mildly increased distal latencies can be seen in patients with ALS, significant abnormalities (compound muscle action potential (CMAP) latency > 150% or conduction velocity < 75% of normal), conduction block, and temporal dispersion are not consistent with ALS.

F waves are late responses elicited by the stimulation of a motor nerve and are thought to represent recurrent electrophysiological discharge arising in the anterior horn cells in response to the antidromic stimulation of these neurons. This phenomenon is unrelated to upper motor neuron signs in patients with ALS, indicating a purely lower motor neuron origin [8]. F wave latency is usually preserved or slightly increased in patients with ALS. In one study, only one (0.8%) of 125 patients measured F wave latencies in three nerves from 61 patients which increased to greater than 125% of normal limit [3]. F wave persistence can be decreased, and F wave amplitudes can be increased in patients with ALS [9]. The decreased number of motor units in patients with ALS results in the increased number of repeated F waves [10]. However, this phenomenon is not limited to the patients with ALS and merely reflects a decrease in the number of motor neurons capable of producing F waves [11]. Repeater F waves can be seen in up to 26% of healthy individuals [11]. Enlargement of the motor units due to collateral reinnervation may also contribute to this phenomenon. "Giant" F waves, defined as having an amplitude greater than 2 standard deviations above the average for healthy controls, can be seen in 16.6% of patients with ALS [12]. Overall, changes in F wave have high sensitivity for motor neuron loss but lack specificity.

Axonal reflex (A reflex) is commonly seen in patients with ALS, consistent with a denervation-reinnervation process [13]. Axonal reflex may be responsible for activation cramps seen in these patients. Small studies have shown that A reflex is relatively resistant to induced limb ischemia in patients with ALS as compared to healthy controls [14].

Sympathetic skin response

Sympathetic skin response (SSR) is rarely used in routine practice. This test measures the changes in skin potential generated by sweat in response to various stimuli. Recent pathological studies have shown involvement of peripheral autonomic nerve fibers in patients with ALS [7]. A study of 25 patients with ALS showed a significant increase in SSR latency in these patients as compared to 22 age-matched healthy individuals and six patients with muscular dystrophy. In addition, SSR was absent in at least one limb in 40% of ALS patients [15]. They did not report whether these findings were correlated with disease duration. This finding was not reproduced in two other studies. In a study of 31 patients with ALS compared to 48 age-matched healthy controls, palmar SSR was normal in all patients and plantar SSR was abnormal in only seven (22.6%) of the patients [16]. Patients with abnormal SSR had more advanced disease. In a study of 16 patients with ALS compared to 12 age-matched controls, plantar SSR was absent in only three patients [17]. These patients had subtle signs of sympathetic nervous system involvement on other tests of this system. While an abnormal SSR suggesting an involvement of the autonomic nervous system can be seen in patients with advanced ALS, it is likely of little clinical value.

Needle EMG

Qualitative and quantitative EMGs are sensitive tools for the detection of acute and chronic lower motor involvement in patients with ALS. Proximal and distal limb muscles are routinely examined and appear to confer a high sensitivity for the detection of both acute and chronic denervation. In a study of 51 patients with ALS, the examination of the tibialis anterior showed fibrillation potentials and/or positive sharp waves in at least one site in 37 (72.5%) patients and in at least two sites in 32 (63%) patients. Clinically, normal muscles showed fibrillation potentials and/or positive sharp waves in 22 (67%) of the patients. Increased amplitude of motor unit potentials was seen in 46 muscles (90%), but increased duration was only seen in 23 muscles (45%) [18]. Another study examined a proximal muscle and a distal muscle in each of the upper and lower limbs of 36 ALS patients. Fibrillation potentials were noted in at least one muscle in 100% of the examined upper limbs and in 97% of the examined lower limbs. Chronic motor unit changes were seen in at least one muscle in each of the limbs in all patients [19]. In a retrospective study of 135 ALS subjects, a total of four muscles (two from the cervical segment and two from the lumbosacral segment) was examined. The muscles tested showed fibrillation potentials in 40–77% of times. A combination of fibrillation potentials and chronic motor changes was found in only 25% of muscles (19 = 85).

Paraspinal muscles have a slightly lower sensitivity for the detection of fibrillation potentials than distal muscles in the same myotome. The examination of the thoracic paraspinal muscles, however, may increase the sensitivity and specificity of EMG examination for the diagnosis of ALS. In one study of 36 patients, fibrillation potentials were found in 94% of examined thoracic paraspinal muscles, as compared to 70% and 69% of cervical and lumbar paraspinal muscles, respectively [18]. In a prospective case-control study of 55 patients with ALS compared to 54 patients with other neuromuscular disorders mimicking ALS, thoracic paraspinal needle examination was found to be useful for distinguishing between the two groups [20]. This study defined abnormal examination as

fibrillation potentials and positive sharp waves but not necessarily motor unit remodeling. Abnormal thoracic paraspinal needle examination was found in 67% of the patients with ALS if only one or two thoracic segments were sampled and in 74% of these patients if four segments were sampled. In comparison, only 19% of the patients with other neuromuscular disorders had an abnormal thoracic paraspinal needle examination. When two muscles in each of the upper and lower limbs, two cranial or bulbar muscles, and three thoracic paraspinal muscles were tested, the limb and thoracic paraspinal muscles showed equally high sensitivity of 90% and 84%, respectively. Moderate sensitivity and high specificity of thoracic paraspinal muscle EMG were reproduced in another retrospective cohort study of 64 patients examined for the question of ALS [21]. This study showed a sensitivity of 0.74 and a specificity of 0.93 for fibrillation potentials and positive sharp wave in these muscles [21]. To sum up, EMG examination of thoracic paraspinal muscles is useful for confirming the diagnosis of ALS, but only moderately useful for ruling out this diagnosis.

The utility of cranial and bulbar muscles in diagnosis of ALS has been a subject of several studies with somewhat variable results (Table 1). In contrast to the limb and paraspinal muscles, cranial and bulbar muscles showed low sensitivity of 23% for the EMG detection of acute denervation [20]. In a study of five cranial and bulbar muscles in 21 ALS patients, tongue had the highest sensitivity for fibrillations (eight out of 21 examinations, 38%) followed by mentalis (three out of 11 examinations, 27%), frontalis (five out of 20 examinations, 25%), and masseter (four out of 21 examinations, 19%). None of the six examined temporalis muscles showed fibrillation potentials in this study. On the other hand, chronic motor unit changes were most common in masseter (71%), followed by tongue (67%) and mentalis (64%). Overall, the highest chance of observing both fibrillations and motor unit changes was noted in tongue (29%). When only patients with bulbar-onset ALS were included, the combination of denervation and reinnervation was seen in 50% of examined tongues [29]. Cappellari and colleagues [19] similarly found that the sensitivity of bulbar muscle examination for the detection of fibrillation potentials is low in patients with limb-onset ALS (0% in masseter, 7.4% in facial, and 18.5% in tongue) and low to moderate in patients with bulbar-onset ALS (0% in masseter, 22.2% in facial, and 77.7% in tongue). The sensitivity for the detection of chronic motor unit remodeling was moderate in patients with limb-onset ALS (37% in masseter, 25.9% in facial, and 40.7% in tongue) and bulbar-onset ALS (44.4% in masseter, 33.3% in facial, and 66.7% in tongue) [29]. Another study of ten patients with limb-onset ALS and eight patients with bulbar-onset ALS showed fibrillation potentials in tongue in 50% and 62.5%, respectively [22]. The same group examined the frontalis, the masseter, and the sternocleidomastoid muscles in ALS patients with (five patients) and without (nine patients) bulbar signs and symptoms [23]. They found similar fibrillation potential occurrence of 60% in all three muscles in patients with bulbar involvement. Unlike the other studies, a high number of patients without bulbar involvement also showed fibrillation potentials (60% in masseter and the sternocleidomastoid muscles and 33% in the frontalis muscle). In this study, quantitative motor unit action potential analysis showed neurogenic motor units in all patients with bulbar involvement and 66% of patients without bulbar involvement [23]. In a small study, sternocleidomastoid and tongue had the same sensitivity (50%) in nine patients with bulbar-onset ALS but the former had higher sensitivity (60% vs. 20%) in 15 patients with limb-onset ALS for the detection of abnormal spontaneous activity, recruitment, or motor unit

Table 1 Summary of the data on the sensitivity of cranial and bulbar muscles for the detection of acute denervation potentials (fibrillations, positive sharp waves, and fasciculation potentials)

Reference	Temporalis	Masseter	Facial	SCM	Upper trapezius	Mentalis	Tongue
Kuncl et al. [21]	0/6 (0%)	2/16 (13%)	2/8 (25%)	NR	NR	NR	6/22 (27%)
Preston et al. [22]	NR	4/21 (19%)	5/20 (25%)	NR	NR	3/11 (27%)	8/21 (38%)
Finsterer et al. [23]	NR	NR	NR	NR	NR	NR	10/18 (56%)
Finsterer et al. [24]	NR	12/14 (86%)	7/14 (50%)	13/13 (100%)	NR	NR	NR
Cappellari et al. [19]	NR	0/36 (0%)	4/30 (13%)	NR	NR	NR	12/36 (33%)
Li et al. [25]	NR	NR	NR	3/12 (25%)	6/13 (46%)	NR	4/6 (67%)
Cho et al. [26]	NR	NR	NR	11/17 (65%)	15/17 (88%)	NR	9/17 (53%)
Sonoo et al. [27]	NR	NR	NR	18/104 (17%)	65/104 (63%)	NR	9/104 (9%)
Xu et al. [28]	NR	NR	NR	41/100 (41%)	49/100 (49%)	NR	NR
Pooled data	ID	18/87 (21%)	18/72 (25%)	86/246 (35%)	135/234 (58%)	ID	58/224 (26%)
Confidence interval	ID	14% to 30%	16% to 36%	29% to 41%	51% to 64%	ID	21% to 32%

The data is pooled for all patients with and without bulbar symptoms and presented as the number of abnormal muscles/total examined muscles (percentage)

SCM sternocleidomastoid, *NR* not reported, *ID* inadequate data

morphology [24]. In this study, the upper trapezius and sternocleidomastoid muscles had comparable sensitivities [24]. These findings were confirmed in another study indicating a higher rate of abnormal EMG findings in the upper trapezius and sternocleidomastoid muscles as compared to tongue in patients with no bulbar symptoms, but a higher rate of abnormal EMG examinations in tongue compared to the other two muscles in patients with bulbar symptoms [25]. It has been argued that the upper trapezius and sternocleidomastoid muscles represent an upper cervical region rather than a bulbar region, explaining the higher incidence of abnormal EMG examination in these muscles in patients with limb-onset ALS. This concern was addressed in a study comparing 104 patients with ALS and 32 patients with cervical spondylosis and upper limb weakness. This study found spontaneous activity in none and chronic motor unit changes in 6% and 19% of the sternocleidomastoid and upper trapezius muscles, respectively, in patients with cervical spondylosis. In patients with ALS, spontaneous activities were seen in 17% and 63% and chronic motor unit changes were seen in 60% and 64% of the sternocleidomastoid and upper trapezius muscles, respectively [26]. A study of 80 patients with cervical spondylosis of unspecified level and muscle weakness showed no spontaneous activity in the upper trapezius muscle. EMG examination was done in 43 of these patients after cervical spine surgery and showed spontaneous activities (defined as fibrillation potentials, positive sharp waves, or fasciculation) in five patients. In this study, the sensitivity of the upper trapezius was 77.8%, 55.6%, and 10.7% and the sensitivity of the sternocleidomastoid muscle was 66.7%, 42.2%, and 14.3% for the detection of spontaneous activities in ALS patients with bulbar (27 patients), upper limb (45 patients), and lower limb (28 patients) onset, respectively. Overall, the examination of the tongue, facial, and masseter muscles bears a higher yield in patients with bulbar symptoms while examination of the upper trapezius and sternocleidomastoid muscles is more likely to provide evidence for denervation in patients without bulbar symptoms. Table 1 summarizes this data and provides the pooled sensitivity of cranial and bulbar muscles for denervation potentials [27].

Fasciculations are frequently observed in patients with ALS. They are not affected by sleep, suggesting a lower motor neuron origin for this phenomenon [28]. Collision studies have suggested multiple anatomical origins within the lower motor neuron pathway for these abnormal potentials [30]. In some instances, fasciculations are not abolished by spinal or nerve blockade but are abolished by neuromuscular junction blockade, suggesting a distal nerve origin [31]. On the other hand, the observation of synchronous fasciculations in muscles supplied by different nerves but the same spinal level suggests that some fasciculations originate at the spinal root level [32]. Other possible origins of fasciculations include proximal axonal sprouting, with a mechanism similar to that of axonal reflex [13].

The concern with using fasciculations as an electrophysiological sign for denervation in patients with ALS relates to the sensitivity and specificity of this abnormal potential [33]. Fasciculations occur at lower frequencies than fibrillations and positive sharp waves. In a study of 51 patients with ALS, fasciculation potentials were found in 26 (51%) muscles while fibrillation potentials and positive sharp waves were found in 37 (72.5%) muscles [17]. In bulbar muscles, fibrillation potentials were found twice as often as fasciculation potentials [23, 29]. In contrast to all other reports, de Carvalho [34] found a significantly higher prevalence of fasciculation potentials as compared to fibrillation potentials or positive sharp waves in 60 patients with ALS. Because fasciculation has a lower firing rate, the difference in recording duration may explain the difference in these observations. Fasciculations can be seen in the absence of fibrillation potentials and positive sharp waves. Hence, it is plausible that using either of these spontaneous activities as a sign of active denervation would increase the overall sensitivity of needle examination, albeit moderately.

Fasciculations can be seen in more benign conditions, raising concerns about their specificity. A small study suggested that diffuse fasciculations (six out of eight muscles tested in four limbs) rather than localized fasciculations are seen in patients with ALS. The control group in this study was small (two patients) and included patients with localized neuromuscular disorders, hence not allowing an estimation of the sensitivity or specificity of this finding [33]. Another small study suggested that variation in the motor unit origins of two consecutive fasciculation potentials distinguishes patients with ALS from patients with other neurogenic disorders [34]. Another study showed complex fasciculations, defined as consisting of two more components that also occur independently, in 14 of 17 ALS patients but only one of six patients with Kennedy's disease [35]. Contrary to these observations, a comparison of fasciculation potentials recorded from 63 muscles in 28 patients with ALS and 21 muscles in 11 patients with benign fasciculation syndrome showed that the complexity of waveform, amplitude, area, duration, the presence of double fasciculations, and discharge interval highly overlap between the two and cannot distinguish benign and malignant fasciculations [36]. In ALS, benign fasciculation syndrome, and spinal muscle atrophy alike, the generation of fasciculation potentials remains active and unchanged with time [37]. Therefore, the clinical context and the association of chronic motor unit changes are probably the most reliable mode to differentiate between these conditions.

Other types of abnormal spontaneous activity are rarely seen in ALS. Generalized myokymia has been occasionally reported in patients with ALS including patients with confirmed diagnosis on autopsy [38, 39]. In one of these patients, proximal nerve block did not abolish the myokymic discharges, suggesting a distal origin of this abnormal

electrophysiological activity [38]. A retrospective study of 96 ALS patients found myokymic discharges in 5.2% of patients and 0.81% of all examined muscles [40]. Hence, finding this type of abnormal spontaneous activity should not raise any question about the diagnosis.

Single-fiber EMG

Increased jitter and blocking on single-fiber EMG are non-specific findings seen with both the denervation and reinnervation of the muscle and with the disorders of the neuromuscular junction. Effort has been made to identify characteristics that may distinguish the increased jitter and blocking of the above two conditions. A bimodal rather than unimodal distribution of jitter, known as the long "flip-flop" phenomenon, may signify denervation-reinnervation [41]. Variability in the amplitude and shape of the motor unit potentials, quantified as "jiggle," is increased in patients with ALS. This phenomenon can distinguish motor unit changes in patients with ALS from chronic denervation-reinnervation potentials in patients with poliomyelitis [42]. A study suggested that the phenomenon of blockade of two spikes within a complex motor unit potential, known as *paired blocking*, may be more specific to a neurogenic phenomenon such as ALS [41]. In this study, the blocked spike pair typically appeared late in the motor unit complex, suggesting that they arise from non-myelinated, newly formed nerve sprouts. However, neither of these findings have been reproduced and the sensitivity and specificity of these phenomena have not been established. These studies have limited utility due to their complex and time-consuming nature.

Increased fiber density represents reinnervation and may inversely correlate with the rate of progression in patients with ALS [17, 42]. In comparison to other conditions involving the lower motor neurons, however, fiber density is only moderately increased in ALS due to the rapid rate of denervation [42]. This finding may suggest that collateral reinnervation is less efficient in ALS [17]. While not of diagnostic value, the impaired collateral reinnervation has important therapeutic significance. There are ongoing efforts to improve the outcome of ALS via enhancing collateral reinnervation. It is not clear if estimating collateral reinnervation using fiber density can allow prognostication of the rate of progression in ALS.

Motor unit number estimation

Compensatory mechanisms increase the individual motor unit twitch tension and preserve the total muscle twitch tension until fewer 10% of motor units remain [43]. Hence, the change in the muscle strength lags in time behind the change in the number of motor units. Motor unit number estimation (MUNE) aims to address this problem. While this concept may confer a diagnostic value, it is chiefly useful as a surrogate marker in ALS clinical trials.

Several methods of MUNE have been introduced. These methods share the basic paradigm of estimating the surface motor unit potential (SMUP) and calculating the motor unit number by dividing the estimated SMUP into the CMAP. It is the method for the estimation of the SMUP that distinguishes these methods from one another. As such, all of these methods are equally subject to the sources of error in measuring CMAP, namely electrode placement over the motor point and volume-conducted electrical activity of the co-stimulated muscle [44]. They also assume that all motor units in the evaluated muscle are activated and are recorded by CMAP. Clearly, this assumption is not always true. Smaller SMUPs are not recorded, causing a systematic overestimation of SMUP and underestimation of MUNE [45]. In addition, each of these methods is subject to its unique sources of error in the estimation of SMUP and the assumption that the estimated SMUP is representative of all motor units in the muscle.

The earliest method of MUNE estimates SMUP using an incremental increase in the stimulation intensity and observing all or none changes in the recorded surface muscle action potential [43]. This method assumes an ability to finely grade electrical stimulation to evoke an all-or-none single motor axon and a sensitivity to record the corresponding all-or-none SMUP from the muscle in a stepwise fashion. It also assumes that the recorded SMUPs are representative of the average SMUP in the studied muscle. A decline over time in incremental MUNE in patients with ALS has been well established [46, 47]. In 96 patients with ALS, incremental MUNE correlated with isometric strength in the abductor digiti minimi ($r = 0.63$) and showed one of the highest rate of decline per month over a 12-month follow-up period [47]. Serious questions were raised over the validity of this method when it showed a spurious decrease in the motor unit number in patients with Duchenne muscular dystrophy [48]. This low estimation of the motor unit numbers has been attributed to several reasons. The proposed explanations include the phenomenon of alternation, i.e., the activation of a variable number of axons with the same stimulus intensity and duration, and a selection bias due to activation of large rather than small axons at low stimulus intensities [49]. It is also possible that the smaller, perhaps distant, motor units in patients with muscular dystrophy were not identifiable, thus inflating the recorded SMUP size. This method was most useful for distal muscles, where an accessible motor nerve and a lack of interference from the neighboring muscle(s) allow for finely graded stimulation of the nerve.

Further MUNE techniques aimed to improve the estimation of SMUP and, by extension, the motor unit number. Multiple point stimulation (MPS) MUNE is intended to eliminate the phenomenon of alternation and to improve the ability to evoke an all-or-none single motor axon [50]. This method has excellent test-retest reliability in the abductor pollicis brevis and abductor digiti minimi muscles with the correlation

coefficient of 0.8 to 0.9 [50, 51]. MPS MUNE in the thenar muscle was compared to SMUP, CMAP, isometric hand grip, manual muscle testing, Appel ALS rating scale, and forced vital capacity in a 12-month longitudinal study of 21 ALS patients [52]. In this study, MPS MUNE had the highest rate of decline of ~ 5% per month. The finding that MPS MUNE has a higher rate of change as compared to other ALS outcome measures has been reproduced [51, 53, 54]. In two independent studies, thenar MPS MUNE declined by an average of 9% per month [51, 53]. These studies suggested that MPS MUNE is the most sensitive measure of change in patients with ALS.

The spike-triggered averaging method of MUNE (STA-MUNE) uses simultaneous needle and surface EMG recordings to identify and record voluntary SMUPs time locked to motor unit potentials [54]. The main advantage of this method is the ability to estimate SMUP in proximal muscles where finely graded stimulation of the nerve is not possible. Potential sources of error in the estimation of SMUP using this method include spurious trigger potentials, a shift in trigger potential in shape, amplitude, or temporal relation to the surface potential, and the distance between the trigger potential and the surface electrode [55]. In a cross-sectional study of 31 patients with ALS, STA-MUNE showed moderate to good reliability with the test-retest correlation coefficient of 0.54, which increased to 0.65 in muscles with motor unit loss [56]. The correlation between STA-MUNE values and isometric muscle strength was modest. This finding may partly relate to the fact that decreased muscle strength in patients with ALS is related to both upper and lower motor neuron loss. In comparison to CMAP and maximal voluntary contraction, STA-MUNE showed a greater difference between patients with ALS and healthy controls, suggesting that this parameter is more sensitive for the detection of motor unit loss [57].

Statistical MUNE uses hundreds of CMAPs evoked via a submaximal stimulus and calculates the Poisson variance for the set of recordings [58]. Assuming that the recorded set follows the Poisson distribution, the variance will be equal to SMUP. Note that a Poisson variance is different from a statistical variance. This method assumes all SMUPs have similar amplitudes and there is no failure in neuromuscular transmission that could account for the variability of CMAP amplitudes. A 3-year longitudinal study of 19 asymptomatic carriers of SOD1 mutation showed a decrease in statistical MUNE in two patients, who became symptomatic several months later [59]. Statistical MUNE was used in two major clinical trials. While the estimated motor unit number did decrease over time in both treated and untreated patients, SMUP remained unchanged over several months [60, 61]. This finding raised doubt about the validity of statistical MUNE, and as a result, this method has fallen out favor despite attempts to improve the validity of techniques by altering the recording parameters [62]. The loss of validity of this technique over time is likely an inherent error due to the increased variability in motor unit size as the disease advances (cf. assumptions above). Changes in axonal excitability in patients with ALS may also contribute to the loss of validity of this technique in these patients [63]. Most recently, statistical MUNE has been improved upon using software that allows analysis of a larger sample of recordings [64].

Motor unit number index (MUNIX) is a less technically demanding method that uses amplitude and power of CMAP and surface interference potentials to calculate an index that reflects the number of motor units [65]. There is good correlation ($n = 20$; $R = 0.83$; $P < 0.01$) between MUNIX and multiple point stimulation MUNE [66]. MUNIX has a good test-retest reliability of ~ 0.7 [64]. The inter- and intra-rater reliabilities of MUNIX have been confirmed in healthy individuals and ALS patients in independent studies as well as in larger studies from the same group [67–69]. In a 1-year longitudinal study of four healthy subjects and 13 patients with ALS, hypothenar MUNIX showed good reliability, which decreased in ALS patients as compared to healthy subjects even when CMAP amplitude was normal, and decreased over time by greater than 5% per month [67]. A large longitudinal study confirmed these findings and showed lower MUNIX in six muscles in seven patients with ALS compared to eight healthy controls and a larger drop in MUNIX over a 15-month follow-up compared to CMAP, slow vital capacity, and the revised ALS functional rating scale [70]. Grimaldi et al. [71] showed that the global MUNIX sum score, defined as the summing MUNIX score of four muscles (ADM + trapezius + APB + TA), has a correlation with patient's clinical symptoms ($P = 0.01$). Another study by Neuwirth and colleagues [72] showed that MUNIX may detect motor neuron loss in early stages of ALS before the patient has apparent weakness. In this study, MUNIX was measured in muscles without weakness every 3 months. An average monthly decline of 5% in MUNIX was observed. The same rate of decline is MUNIX continued after the onset of muscle weakness. This rate of decline was greater than the rate of decline in the ALS functional rating scale and CMAP, suggesting that MUNIX may be more sensitive for monitoring of disease progression in ALS.

Although decreased MUNE has been considered as supportive evidence for lower motor neuron degeneration in ALS criteria [73], it has mainly appealed to investigators as an outcome measure. In patients with ALS, neither cross-sectional values nor longitudinal changes correlate between MUNE with CMAP amplitude, fiber density, motor unit amplitude, and maximal isometric strength [49, 56]. The rate of change in MUNE in patients with ALS is higher than that in other outcome measures, suggesting a potential for higher sensitivity as an outcome measure [52, 74, 75]. Averaging MUNIX measured from multiple muscles may decrease the variability and improve the sensitivity of this test for detecting change over time [76].

Electrophysiological criteria for ALS

The two main functions of electrophysiological studies in the diagnosis of ALS are to provide evidence for lower motor neuron degeneration and to rule out ALS mimics. Despite years of research in the electrophysiological markers of upper motor neuron degeneration, these studies have not found a role in ALS criteria. Therefore, the electrophysiological criteria established for ALS merely concern lower motor neuron loss.

The Lambert criteria were the earliest electrophysiological criteria for ALS [76]. These criteria required evidence of active denervation (fibrillation and fasciculation potentials) as well as reinnervation (increased motor unit potential amplitude and duration and reduced recruitment) in either upper and lower limbs or one of the upper or lower limbs and bulbar muscles. In addition, these criteria required normal sensory conduction studies and normal motor conduction velocity (or greater than 70% of normal when CMAP is significantly reduced). These stringent criteria had relatively low sensitivity. In one study of 133 patients with diagnosis of ALS confirmed on prospective follow-up and repeat EMG studies, 37% did not meet the Lambert electrophysiological criteria at presentation [2].

The El Escorial World Federation of Neurology criteria [71] required features of LMN degeneration in at least two muscles of different root or spinal nerve innervations and different cranial or peripheral nerve innervations in at least three (for a definite diagnosis) or two (for a probable diagnosis) of the four bulbar, cervical, thoracic, and lumbosacral regions. The thoracic paraspinal muscles were introduced as a separate region for the first time in this set of criteria. These criteria employed electrophysiological findings as definite or supportive evidence of lower motor neuron degeneration. In addition, several electrophysiological features were listed as compatible or inconsistent with lower motor neuron degeneration. Reduced recruitment, fibrillation potentials, and large motor units were required to be present at the same time for a definite evidence of lower motor neuron degeneration. Two regions with electrophysiological findings supportive of lower motor neuron degeneration could be substituted for one region with definite electrophysiological findings of lower motor neuron degeneration. These criteria were criticized for low sensitivity and delayed diagnosis [77]. For example, a study showed pathological evidence of ALS in five out of eight patients who did not meet a diagnosis of probable or definite ALS based on these criteria at the time of death [78]. A retrospective study of 73 patients with ALS suggested a sensitivity of 0.57 if two muscles in two of the four regions are required for the diagnosis of ALS.

The revised El Escorial criteria, sometimes referred to as *Airlie House criteria*, aimed to improve its sensitivity and allow for an earlier diagnosis [79]. These criteria introduced a "Clinically Probable ALS - Laboratory-supported" diagnostic category. This category allows for an upgrade in diagnosis when upper motor neuron signs are only present in one region but electrophysiological findings are indicative of lower motor neuron degeneration in two regions and, together with MRI, exclude other causes. Lower motor neuron findings need to be rostral to the upper motor neuron findings. In contrast to its predecessor, either of fibrillation potentials or positive sharp waves and either of large motor unit potentials, reduced interference pattern or unstable motor unit potentials constitute sufficient evidence for lower motor neuron degeneration. Quantitative EMG methods are also acceptable evidence for lower motor neuron degeneration. However, fasciculation potentials (or absence thereof) do not provide adequate diagnostic evidence for (or against) lower motor neuron degeneration. These criteria require one rather than two abnormal muscles in the bulbar and thoracic paraspinal regions, as compared to two in the original El Escorial criteria. Other aspects of electrophysiological utility in the diagnosis of ALS remained unchanged in the revised El Escorial criteria.

Retrospective evidence supports that the use of one abnormal muscle in the bulbar and thoracic paraspinal regions and two abnormal muscles in the cervical and lumbar regions increases the overall sensitivity without a large impact on the specificity of these criteria over the original El Escorial criteria [21]. However, the sensitivity remains low. A new diagnostic criterion, the Awaji-Shima criteria, was proposed to further improve the sensitivity of ALS diagnosis [80]. These criteria assign the same significance as clinical features of lower motor neuron changes to neurogenic EMG changes and thereby upgrade the laboratory-supported probable ALS of the revised El Escorial criteria to probable ALS. According to these criteria, neurogenic fasciculation (i.e., those with complex morphology or instability and those associated with chronic neurogenic changes) possesses the same significance as fibrillation potentials and positive sharp waves. The potential for unstable motor unit as a sign of chronic denervation and the possible role of TMS and MRI to support upper motor neuron involvement are acknowledged, but these tests are not considered adequate for the diagnosis of ALS in these criteria.

The Awaji-Shima criteria were used mainly because of the concern for the specificity of fasciculation potentials. The proponents of the Awaji-Shima criteria argue that while fasciculation potentials in isolation do occur in otherwise healthy individuals, complex fasciculations at the presence of chronic neurogenic EMG features do not. Several studies have attempted to address the difference between the two criteria. The main difficulty with settling this controversy is the wide range of sensitivity and specificity calculated for the two criteria as outlined below. The calculated sensitivity and specificity in various studies range from 19 to 100%. Based on the

various studies outline below, we have calculated the *inter-observer variability*.

There are several studies that indicate the Awaji-Shima criteria increase the sensitivity for the diagnosis of ALS without a significant decrease in the specificity. A study of 55 patients with referral question and final diagnosis of ALS based on follow-up clinical progression showed a statistically significant increase on diagnostic sensitivity to 95% using the Awaji-Shima criteria as compared to 53% using the revised El Escorial criteria for definite ALS [81]. For patients with bulbar-onset ALS, the increased sensitivity was more dramatic (from 38 to 87%). This study did not examine the specificity of the two criteria. In 51 patients with clinically suspected ALS, the Awaji-Shima electrophysiological diagnostic criteria were met in 94.3% but the revised El Escorial electrophysiological diagnostic criteria were met in only 40% at the initial visits, indicating a statistically significant difference [82]. In 35 of these patients with extended follow-up, the interval between the disease onset and the time of meeting the electrophysiological criteria was about 6 months shorter with the Awaji-Shima criteria than with the revised El Escorial criteria. In another retrospective study of 46 ALS patients, all with bulbar-onset ALS probable or certain after follow-up, the Awaji criteria were more sensitive in early diagnosis of bulbar-onset ALS as compared to the revised El Escorial criteria. The diagnostic sensitivity was 19.5% for the El Escorial criteria, 28.2% for the revised El Escorial criteria, and 49.98% for the Awaji criteria [20]. However, all the three criteria remained of low diagnostic sensitivity in this study. A retrospective study of 205 patients with clinically suspected ALS showed a sensitivity of 60.7% for the Awaji-Shima criteria and a significant sensitivity higher than 28% for the revised El Escorial criteria, for the diagnosis of probable or definite ALS [83]. The specificity was 95.9% for both criteria. A prospective study of 200 referrals for the question of ALS showed a sensitivity of 66% for laboratory-supported probable ALS or higher on the revised El Escorial criteria and 85% for probable or definite ALS on the Awaji-Shima criteria [84]. This difference was statistically significant. The specificity of the Awaji-Shima criteria was reported to be 100% in this study. Another large study showed a 20% increase in diagnostic yield with the Awaji-Shima criteria [85]. This increased yield was solely related to the inclusion of EMG finding as equivalent to clinical features. A prospective study by Li and colleagues [86] showed that the Awaji criteria exhibited greater diagnostic sensitivity of 78% as compared to 36% for the revised El Escorial criteria for probable or definite ALS in a Chinese ALS population. Overall, several studies indicate an improvement to diagnostic sensitivity without a loss of specificity for the Awaji-Shima criteria.

In contrast to these studies, others have found no difference in the diagnostic yield of the Awaji-Shima and the revised El Escorial criteria. In a retrospective study implementation of the Awaji criteria for 135 patients with ALS and 25 with progressive muscular dystrophy primarily diagnosed with the El Escorial criteria [87], diagnostic sensitivity increased only in 5.9% of subjects. They found no statistical difference in the number of patients diagnosed with clinically possible, clinically probable, and definite ALS using the Awaji or El Escorial criteria. A similar study of 213 patients with suspected ALS showed a sensitivity of 70% for laboratory-supported probable or higher ALS with the revised El Escorial criteria and 71% for probable and definite ALS with the Awaji criteria [86]. Specificity was 100% for both criteria. A retrospective study of 113 patients with ALS showed a sensitivity of 61% by the revised El Escorial criteria and 71% by the Awaji-Shima criteria for probable and definite diagnosis of ALS [88]. This study did not calculate statistical significance for the difference in sensitivity. Using their reported data, we calculated a non-significant P value of 0.16 for this difference. This study also does not explicitly discuss how the laboratory-supported probable category on the revised El Escorial criteria was treated, and does not report the specificity for the two criteria. The non-significant increase in diagnostic sensitivity in this study came exclusively from the patients with bulbar-onset ALS. A small retrospective study of 46 patients with ALS showed a sensitivity of 85% for laboratory-supported probable or higher on the revised El Escorial criteria and a similar sensitivity of 87% for probable or higher on the Awaji-Shima criteria. Specificity was not reported [89]. Another retrospective study of 139 patients found a statistically non-significantly higher sensitivity of 43% with the revised El Escorial criteria versus 37% with the Awaji-Shima criteria for probable (including laboratory-supported) and definite ALS [90]. Specificity was not reported. Overall, a number of studies have not found any significant difference between the sensitivity and specificity of the two criteria.

Several meta-analyses have attempted to address the controversy. A meta-analysis aimed to summarize the findings on the sensitivity and specificity of these two criteria [91]. This study included all of the above studies except for the most recent large, negative study [90], not available at the time of the meta-analysis. This study indicated a significantly higher pooled sensitivity of 81.1% for the Awaji criteria as compared to 62.2% for the revised El Escorial criteria. The specificity was similar (98%) for both criteria. In a subgroup analysis, the difference in sensitivity was greater for patients with bulbar-onset ALS (82.9% and 46.1%) as compared to patients with limb-onset ALS (69.4% and 63.8%) for the Awaji-Shima criteria and the revised El Escorial criteria. We pooled the most recent study [91] with other studies pooled in this recent study, but we excluded two studies that used probable and definite but not laboratory-supported probable on the revised El Escorial criteria [81, 85]. Based on this pooled data of 967 patients, the sensitivity of the Awaji-Shima criteria and the revised El

Table 2 Comparison of the sensitivity of the Awaji-Shima and revised El Escorial criteria in a recent meta-analysis

Reference	Awaji-Shima criteria (%)	Revised El Escorial criteria (%)
Geevasinga et al. [94]	70	58
Jung and Bae [92]	70	59
Higashihara et al. [93]	81	62.2

Escorial criteria was calculated as 69% (95% CI, 66% to 72%) and 54% (95% CI, 51% to 58%), respectively. This difference is less dramatic compared to the difference reported on the previous meta-analysis but remains statistically significant ($P < 0.0001$, two-tailed chi-square test). Another recent meta-analysis by Jang and Bae [92] concluded that the Awaji criteria were more sensitive than the revised El Escorial criteria. The calculated sensitivities were 70% (95% CI, 67% to 73%) versus 59% (95% CI, 56% to 62%), respectively [93]. An in-depth look at this meta-analysis showed that the Awaji-Shima criteria increased the diagnostic sensitivity of clinically definite or clinically probable ALS. Using the Awaji-Shima criteria, 11% of patients classified as clinically possible by the revised El Escorial criteria were upgraded to clinically probable or clinically definite ALS. In the sub-analysis that was limited to the clinically probable–laboratory-supported category of the revised El Escorial criteria, the Awaji criteria resulted in downgrading of 20% of the patients in this category to clinically possible. This finding is curious as the Awaji-Shima criteria were designed to upgrade and not to downgrade the clinically probable–laboratory-supported category. Therefore, a downgrade may suggest a previous classification error when applying the revised El Escorial criteria. In another meta-analysis by Geevasinga et al. [94] showed the Awaji criteria to be 12% more sensitive in the diagnosis of ALS (70% vs. 58%). In this study, ALS patients included clinically definite and probable ALS. In this study, the increase was more evident in bulbar-onset disease [92]. Overall, the meta-analysis studies point to a higher sensitivity for the Awaji-Shima criteria for the diagnosis of probable and definite ALS. A large part of this increase is related to upgrading laboratory-supported probable ALS to probable ALS in these criteria (Table 2). A smaller part of the increase is related to giving fasciculation potentials an equivalent status as fibrillation potentials and positive sharp waves. The effect is likely more pronounced in patients with bulbar-onset ALS.

Conclusion

Electrophysiological studies play an important role in the diagnosis and as an outcome measure in patients with ALS. The utility of these studies in establishing the presence of lower motor neuron degeneration, and the diagnosis of ALS is well recognized. Nevertheless, this utility continues to evolve. Further research is required to advance the role of electrophysiological studies in establishing the presence of upper motor neuron degeneration and in monitoring progression.

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