Multipoint incremental motor unit number estimation (MUNE) as measure of disease progression in Amyotrophic Lateral Sclerosis

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By

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DECLARATION

I, Dr. Sujit Abajirao Jagtap, hereby declare that the projects in this book were undertaken by me under the supervision of the faculty, Department of Neurology, Sree Chitra Tirunal Institute for Medical Sciences and Technology.

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The candidate, Dr. Sujit Abajirao Jagtap, has carried out the minimum required project.

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I take this opportunity to express my sincere gratitude to Dr. A. Kuruvilla, Additional Professor of Neurology, SCTIMST, my guide for the study, for his expert guidance, constant review, kind help and keen interest at each and every step during the completion of the study.

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Introduction

Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disorder of undetermined etiology that primarily affects the motor neuron cell population. It is progressive and most patients eventually succumb to respiratory failure. The first detailed description in the literature was by Jean Martin Charcot in 1869, in which he discussed the clinical and pathological characteristics of "la sclerose laterale amyotrophique," a disorder that affected both upper and lower motor neurons. ¹ ALS is known by several other names including Charcot's disease, motor neuron disease, and in the United States, "Lou Gehrig disease" in remembrance of the famous "Iron Horse" of baseball who was diagnosed with ALS in the late 1930s. ²,³ The World Federation of Neurology Research Group on Neuromuscular Disorders has classified ALS as a disorder of motor neurons of undetermined cause, and several variants are recognized. Included in this group are primary lateral sclerosis (PLS), progressive bulbar palsy (PBP) and progressive muscular atrophy (PMA). It is important to recognize that ALS is a progressive dynamic disorder. Some cases present with the classic combination of upper motor neuron (UMN) and lower motor neuron (LMN) signs, but others may be UMN onset, LMN onset, or bulbar onset and only later develop signs of involvement of the other parts of the motor system. About 5-10 % of ALS is familial rather than sporadic.⁴ The most common inheritance pattern being autosomal dominant. Thus one comes across the terms, sporadic ALS (SALS) and familial ALS (FALS). A few other conditions have a phenotypical expression similar to that of ALS including Western Pacific ALS-parkinsonism-dementia complex (PDC) (or Guamanian ALS) and juvenile ALS. The incidence and prevalence rates for non-Western Pacific ALS are surprisingly uniform
throughout the world. The incidence is estimated at 1-3 per 100,000 and the prevalence varies from 6-8 per 100,000. Several epidemiological studies have suggested that the incidence of ALS may have increased in the past two decades and that this is a disease-specific finding rather than due to factors related to better national health care, economic prosperity, or case ascertainment. In sporadic spinal ALS, men are more often affected than women by a ratio of 1.2-1.6:1. However, several clinical papers have shown that there is a slight female predominance in the bulbar-onset variety and that there appears to be no consistent pattern of gender predominance in familial forms of the disease. ALS is reported to occur as early as in the second decade of life, but the most common onset is in the patient’s early sixties. It is notably rare in the very oldest segment of the general population, that is, those older than 85 years. This has yet to be explained. The mean disease duration from symptom onset to death is approximately 3 years, although some patients live for more than a decade, whereas others may succumb within a matter of a few months. Although no specific environmental factors have been linked with certainty to an increased risk of ALS, epidemiological research suggested increased mortality rates for ALS in electrical utility workers who were chronically exposed to electromagnetic fields. Population-based case control studies have also ascertained increased risk in those with a high dietary intake of glutamate and in smokers. A host of environmental trace elements have been evaluated as potential causative agents for ALS including selenium, aluminum, iron, manganese, copper, zinc, cadmium, and lead, but there is no convincing evidence that any one of these plays a major part in ALS pathogenesis.
Etiology:

Significant inroads have been made into understanding the pathogenesis of SALS and FALS. Several hypotheses have been put forward, including that of viral infection, activation of the immune system, exogenous toxins, and hormonal disturbances. However, there has been insufficient evidence to implicate any of these as the major cause of motor neuron degeneration in ALS. Perhaps the most significant breakthrough in understanding the cause of ALS (be it sporadic or familial) came in 1993, when Rosen et al. identified mutations in the gene encoding an enzyme called copper/zinc superoxide dismutase (SOD I) in patients with FALS.\(^{10}\) SOD I mutations, which can cause elevated intracellular levels of reactive oxygen species, are now identified in up to 20% of all patients with FALS. Most recently, mutations in a gene encoding a novel protein called ‘alsin’ have been identified in form of recessively inherited juvenile-onset ALS of North African origin. This protein shares structural homology to a guanine nucleotide exchange factor, which suggests a role in altered cell signaling.\(^{11}\) A significant body of basic and clinical research lends strong support to a new theory of ALS pathogenesis, which proposes selective motor neuron damage from a complex chain of injurious events involving excitotoxins, oxidative stress, neurofilament dysfunction, altered calcium homeostasis, mitochondrial dysfunction, enhanced motor neuron apoptosis, and proinflammatory cytokines.\(^{12}\) A number of ALS susceptibility genes have also been proposed, mutations of which are known to occur in small ALS populations or individual cases but which do not appear to account for the majority of SALS cases.
Pathogenesis of Sporadic Amyotrophic Lateral Sclerosis

Glutamate excitotoxicity and Free Radical Injury, Glutamate, which is the most abundant free amino acid in the CNS, is one of the major excitatory amino acid (LAA) neurotransmitters. Glutamate produces neuronal excitation and participates in many neuronal functions, including neuronal plasticity. In excess, however, it causes neurotoxicity. There are two types of glutamate receptors: (1) ionotropic and (2) metabotropic. The former is an integral, cation-specific particularly Ca⁺ ion channel type, which is further grouped into two major subtypes depending on receptor characteristics: the N-methyl-D aspartate (NMDA) receptors and the non-NMDA receptors (AMPA-kainate receptor). Metabotropic receptors are coupled to G proteins and cyclic guanosine monophosphate (cGMP), modulating the production of intracellular messengers and influencing ionotropic glutamate receptors. In ALS, motor neurons appear to receive the glutamate excitotoxic signal through non-NMDA receptors rather than NMDA receptors.

The significance of glutamate excitotoxicity in neurodegeneration is strengthened by the observation that exogenous glutamate receptor agonists result in clinically observable neurotoxicity. An outbreak of food poisoning associated with contaminated muscles that clinically presented with chronic dementia and motor neuron disease was caused by domoic acid, another potent non-NMDA receptor agonist. In patients with ALS, a series of endogenous glutamate abnormalities have been demonstrated; for example, EAA is significantly increased in serum, plasma, and CSF. On the other hand, glutamate in CNS tissue and the glutamate-to-glutamine ratio are significantly decreased in ALS. When glutamate metabolism is studied by loading with oral
monosodium glutamate, plasma glutamate levels increase to a significantly greater
degree in patients with ALS than in healthy patients. These studies clearly support the
idea that glutamate excitotoxicity is involved in the pathogenesis of ALS, if not actually
the cause. Glutamate is normally released from presynaptic axon terminals into the
synaptic cleft where it binds to its receptors causing signal transduction to occur. After
signal transduction, interstitial glutamate must be reabsorbed into its main reservoir, the
surrounding astrocytic glial cells. This absorption process involves specific transporter
proteins known as GLT (glutamate transporter) or EAAT (excitatory amino acid
transporter) proteins, which have been sub classified according to their distribution
within cells of the CNS. Among these, the astrocytic glutamate transporter, termed
GLT1 or EAAT2, is markedly reduced in the motor cortex and anterior horn cells of
patients with ALS, which supports earlier evidence that interstitial or extracellular
(including CSF and plasma) glutamate is increased in ALS. Rothstein et al. found
intriguing abnormalities in the DNA encoding GLT1 in more than 60% of patients with
ALS (predominantly the sporadic form). However, subsequent research suggests that
GLT1 does not appear to be a candidate gene for FALS or SALS. Impaired glutamate
transport reduces clearance of glutamate from the synaptic cleft, which may leave
excessive amounts of free excitatory neurotransmitter to repeatedly stimulate the
glutamate receptor and thus allow calcium ions enter the neuron. Excess calcium ions
are usually buffered by intracellular calcium-buffering proteins, such as parvalbumin or
calbindin, and by mitochondria that may also function as an extra calcium reservoir. Low
levels of parvalbumin, calbindin, and altered mitochondrial function have been detected
in ALS models. When calcium ion levels exceed this reduced buffering capacity, they
may catalyze activity in specific destructive enzymes that are not activated under normal conditions including xanthine oxidase, phospholipase, and nitric oxide synthase. These enzymes produce free radicals, including reactive oxygen and nitrogen species, which cause harmful nitration of tyrosine residues on key neuronal proteins and ultimately may also cause apoptosis. It has recently been proposed that regional differences in the levels of activity of buffering systems and in glutamate receptor subtype expression may explain the selective vulnerability of certain motor neuron pools within the CNS.

Immunological and Inflammatory abnormalities.

Several pieces of evidence implicate an immune process in the pathogenesis of ALS. Immune complexes have been identified in gut and renal tissue from patients with ALS. Furthermore, up to 10% of patients with ALS may have a monoclonal gammopathy and fewer than 5% have low-level titers of anti-GM1 antibody. Moreover, serum antibodies to L-type voltage-gated calcium channels have been found in some patients with ALS but not in others. Activated spinal cord microglial cells, elevated inflammatory cytokine levels, and most recently, marked increased expression of cyclooxygenase-2 have also been found in ALS tissue samples.\textsuperscript{14,15} However, all available immunotherapies, including cyclophosphamide, IVIG, plasmapheresis, corticosteroids, and total lymphoid irradiation, have failed to alter the course of ALS.\textsuperscript{16} Although this might indicate that immune mechanisms are not of primary importance in the pathogenesis of ALS, there is hope that cell-targeted immune therapy and anti-inflammatory therapy may be useful.
Neurofilament Dysfunction.

Abundant neurofilaments are present in the cytoskeleton of motor axons where they are vital for bi-directional axonal transport. Abnormal axonal spheroids, consisting of neurofilament-derived material, have been identified in tissue from patients with ALS. Subsequent research shows that abnormally slow axonal transport (referred to as "axonal strangulation") may be important in ALS, perhaps as a result of oxidative stress-induced neurofilament injury. However, it is possible that increased levels of neurofilament may actually represent a protective reaction of the cell body to harmful calcium levels or to other substances. Mutations in the genes for neurofilament subunits appear to confer increased risk for the later development of SALS.

The neurofilament heavy chain is thought to be important in the correct spacing of neurofilaments from each other and thus in the regulation of axonal diameter. In rare cases of SALS (and very rarely FALS), mutations have been found in the heavy-chain gene segment that encodes an amino acid repeat motif. Overexpression of another intermediate motor neuron-specific protein called peripherin may lead to accumulation of toxic intraneuronal aggregates as has been demonstrated in patients with SALS and in mice with SOD1 mutations. In fact, selective motor neuron toxicity in the setting of peripherin overexpression appears to occur in mice that lack light subunits, which implies that the light subunit may somehow prevent a harmful interaction between peripheral and other neurofilament subunits. Furthermore, proinflammatory cytokines appear to increase the duration of peripheral overexpression at sites of neuronal injury.
Susceptibility Genes for Sporadic Amyotrophic Lateral Sclerosis

The survival motor neuron (SMN) proteins are encoded by inversely homologous genes located on chromosome 5q. In one study, no deletions in the SMN1 gene were found in SALS ($n = 177$) or FALS ($n = 66$), but a pure adult-onset LMN disorder associated with homozygous deletion of the SMN2 gene was described in five cases.\textsuperscript{19} A French study of 167 patients with ALS revealed that the SMN1 gene copy number was abnormal in 16\% of cases compared with only 4\% of controls, which suggests that the SMN1 gene may be a susceptibility factor for ALS.\textsuperscript{20} Other rare mutations have been identified in patients with ALS, including in the APEX nuclease gene, cytochrome oxidase c subunit gene, the copper chaperone of SOD 1 gene, and the leukemia inhibitory factor gene. As with the genes for GLT1/EAAT2, neurofilament heavy chains, SMN protein, and the apolipoprotein H4 genotype, there is insufficient evidence to implicate these mutations in the direct pathogenesis of all ALS, but they may act as genetically determined susceptibility factors.
Review of literature

It is widely agreed that when the clinical symptoms of ALS first appear, the biological disease must have been developing for some time and is well into its course. Electrophysiological investigations in patients in the early stages of the disease suggest that an extensive remodeling of motor units takes place by continuous denervation and reinnervation process before affected individuals can recognize muscle weakness. A study in patients with acute poliomyelitis estimated that as many as 50% of the motor neurons are lost before muscle weakness is detected. Therefore an important preclinical asymptomatic stage likely precedes progressive muscle weakness in ALS. Muscle weakness in ALS usually begins in a focal area, first spreading to contiguous muscles in the same region before involvement of another region.

The first presentation may appear very similar to a focal mononeuropathy; this is sometimes called the pseudo-neuritic presentation, more commonly, however, limb weakness appears to occur in muscles derived from more than one peripheral nerve and/or nerve root distribution; this is called a monomelic presentation. Onset of muscle weakness is more common in the upper than the lower extremities (classic, spinal ALS), but in approximately 25% of patients, weakness begins in bulbar-innervated muscles (bulbar-onset ALS). On rare occasions (1-2% of patients), the weakness starts in the respiratory muscles (dyspnea onset). Some patients present with weakness that is restricted to one side of the body (Mills' hemiplegic variant) and up to 10% of patients appear with bilateral upper extremity wasting, which is known as the "flail arm" or flail person in the barrel variant. Symptoms of muscle weakness vary, depending on which motor function is impaired. For example, when weakness begins in the hand and
fingers, patients report difficulty in turning a key, buttoning, opening a bottle cap, or turning a door knob. When weakness begins in the lower leg, foot drop may be the first symptom or the patient may complain of instability of gait, falling, or fatigue when walking. When bulbar muscles are affected, the first symptoms may be slurred speech, hoarseness, or an inability to sing or shout, which may be soon followed by progressive dysphagia. Indeed, patients with bulbar onset ALS often initially consult ear, nose, and throat specialists and not only experience progressive impairment in bulbar function but also excessive drooling (sialorrhea) and weight loss.

Pseudobulbar palsy may present with inappropriate or forced crying or laughter, which is often a source of great emotional distress for patients. Excessive forced yawning may also be a manifestation of pseudobulbar palsy. In the rare patient who presents with progressive respiratory muscle weakness, the first port of call may be to a pulmonologist or even to the intensive care unit; the diagnosis of ALS is then made when the patient cannot be weaned from the ventilator. Head-drop (or droop) may be a feature in ALS and is caused by weakness of cervical and thoracic paraspinal muscles. Fasciculation’s are not commonly the presenting feature of ALS, but they develop in almost all patients soon after onset. In fact, absence of fasciculation’s should prompt one to seriously reconsider the diagnosis. In some patients, waves of fasciculation’s, called Lambert’s waves, are seen spreading across the chest or back. Muscle cramps are one of most common symptoms in patients with ALS and often precede other symptoms by many. Although cramps are common in healthy individuals and most commonly occur in calf muscles, in ALS they can occur in unusual muscles such as in the thigh, abdomen, back, upper extremity, hand, neck, jaw, and even the tongue.
Other signs and symptoms include exertional fatigue that mimics myasthenia. As dysphagia worsens, reduced caloric intake worsens fatigue and accelerates muscle weakness. Aspiration of liquids, secretions, and food becomes a risk. Patients may complain that they produce copious amounts of abnormally thick oral secretions, which may drool excessively from the mouth. This sialorrhea is made worse as perioral muscles weaken and/or head-drop develops. Weight loss is often rapidly progressive; indeed it has been suggested that this does not simply reflect poor caloric intake but represents a form of ALS cachexia. Marked loss of muscle bulk exposes joints and associated connective tissues to abnormal mechanical stresses that can lead to joint contractures, joint deformities, painful pericapsulitis, and bursitis. Sleep disturbances, in the form of increased awakenings from increased hypopneas and hypoxia, have been shown to be common in ALS and contribute to daytime sleepiness, morning headaches, and fatigue, as respiratory difficulty worsens, patients may be unable to lie supine because of worsening diaphragmatic weakness and thus compensate by using multiple pillows. In more advanced stages, patients are unable to be in bed at all. Other manifestations of ventilatory failure include dyspnea on exertion and eventually dyspnea at rest. As the disease advances, motor function is progressively impaired and activities of daily living (e.g., self-hygiene, bathing, dressing, toileting, and walking, feeding, and verbal communication) become difficult. Accordingly, a patient's quality of life starts to progressively deteriorate. It may be difficult to distinguish daytime fatigue, broken sleep, affect lability, and sighing from depression, but it is vitally important to be aware of the latter. Depression is a common and underdiagnosed problem in ALS, which not only negatively affects quality of life but also shortens survival.
Atypical Features of ALS

There are certain clinical features that are unusual if not absent in ALS including sensory loss, dementia, extrapyramidal dysfunction, eye movement abnormalities, autonomic disturbances, and abnormal sphincter control. When patients have these signs, the diagnosis of ALS should not be made until all possible alternative diseases are excluded. Although the sensory system is characteristically spared, some patients do report vague sensory symptoms such as numbness or aching and there is electrophysiological evidence that ascending afferent pathways may be involved despite the absence of objective sensory loss on physical examination.

Overt dementia is estimated to occur in approximately 5% of non-Western Pacific ALS where it may even be the presenting feature. It is usually of the frontotemporal dementia (FTD) variety, and most commonly presents with word-finding difficulties, deficits in visual perception, and abnormal confrontation naming. Patients may exhibit poor judgment and other deficits in executive processing. There is some evidence that this form of dementia or cognitive impairment is much more common not only in bulbar-onset ALS but also in all subtypes of ALS. One needs to be cautious that language disturbances (especially anomia) may be masked by dysarthria.

A prospective neuropsychological study of cognition in ALS identified deficits in up to a third of patients and a subsequent study reported an incidence of FTD in almost 50% in patients with bulbar-onset ALS. Of 36 cases meeting criteria for FTD, 5 (14%) also met criteria for definite ALS. Dementia in ALS is pathologically distinct from other dementing illnesses; the most reliable pathological marker of cognitive impairment in SALS is superficial linear spongiosis in neocortical, entorhinal, and cingulate tissue.
The motor neurons of Onufrowicz in the sacral cord are essentially spared in ALS, and thus patients generally do not complain of significant problems with sphincter control (although some may report mild urgency of micturition). Similarly, eye movements are typically normal in ALS; it takes detailed quantitative testing to be able to identify abnormal vertical ocular saccades. Approximately 5% of patients with ALS exhibit signs of extrapyramidal tract dysfunction, usually in the form of retropulsions during attempted ambulation. Autonomic symptoms do not come to the attention of patients with ALS, although there is electrophysiological evidence of abnormal sweat production and cardiac denervation in the early stages of disease in sonic panciiiis

**Natural History of the Disease**

It has been estimated that up to 40% of anterior horn cell motor neurons are lost before the clinical detection of motor abnormalities; this suggests that a prolonged preclinical phase may be part of ALS. However, once the clinical palsies evident, there appears to be a generally linear decline in motor function over time. There is a characteristic pattern of spread of disease. When onset is in one upper extremity, spread is often first to the contralateral side, then the ipsilateral lower extremity, the contralateral lower extremity, and finally the bulbar region. Onset in the lower extremity often follows a similar pattern, yet again with final involvement of the bulbar region. ²³ Bulbar-onset ALS tends to spread to the distal upper extremities first, with spread to thoracic myotomes, and then the lower extremities. Overall, the pattern suggests that rostral-caudal involvement is faster than caudal-rostral spread. During the course of the disease, transient improvement, plateaus, or sudden worsening can occur, but spontaneous improvement is exceedingly rare.
**Prognosis**

Based on several epidemiological studies the median duration of ALS ranges from 23-52 months and the mean duration from 27-43 months. About 25% of patients survive 5 years and 8-16% of patients survive beyond 10 years. A number of factors influence the prognosis of ALS including the age at onset, clinical type, and duration from onset to the time of diagnosis. However, it must be emphasized that there is a wide range of rates of progression in each category of patient; the previous rate of progression in a particular patient is a better indicator of prognosis than any other feature. In general, the younger the patient, or the longer the duration between onset and diagnosis, the better the prognosis. A worse prognosis is found in those whose rate of progression is rapid within the first 6 months of diagnosis. Several clinical subtypes harbor a better prognosis; these include PLS, PMA, pseudobulbar (rather than bulbar) palsy, the pseudo-neuritic presentation, and the flail-arm variant. Those who survive beyond 46 months and those who are psychologically well adjusted or not depressed have a better prognosis. Those who have low-amplitude CMAPs in the setting of normal sensory potentials (the generalized low motor-normal sensory pattern) as revealed by nerve conduction studies appear to have a poor prognosis. Dyspnea-onset ALS has a shorter survival. Low serum chloride levels are associated with a short-term survival without ventilatory support because they reflect accumulation of bicarbonate due to respiratory failure. Data on bulbar-onset ALS vary, but mean survival ranges between 12 and 26 months. Malnutrition is an independent risk factor for poor outcome.
Laboratory Studies

In some instances a diagnosis of definite ALS can be reached based on the history and clinical examination alone. However, often the diagnosis is not so obvious and further investigations are necessary. Because there is no single test that can make a diagnosis of ALS, all of these investigations are performed to exclude other disorders that may clinically mimic ALS and its variants. All such testing is an extension of a thorough history and physical examination and includes blood tests, the EDX, and neuroimaging. There is no single blood test that may objectively diagnose SALS.

However, there are several blood tests that are usually performed for the evaluation of patients with suspected ALS. The list includes serum CK concentration, blood count, chemistry panel (including calcium, phosphate, and magnesium), Venereal Disease Research Laboratories test results, GM1 autoantibody titers, sedimentation rate, serum protein immunofixation or Immunoelectrophoresis, thyroid function studies including thyroid-stimulating hormone, and vitamin B12 levels. The CK concentration may be modestly elevated, particularly early in the disease. Patients older than 50 years and smokers of any age should have a chest radiograph taken. If any lesion is identified, an anti-Hu antibody level should be determined. Certain patients may have clinical features that suggest a disorder of the neuromuscular junction and may therefore benefit from testing for antibodies against the acetylcholine receptor or voltage-gated calcium channel. If there is biochemical evidence of adrenal insufficiency, it is important to do long-chain fatty acid [VLCFA] assay to investigate for possible adrenomyeloneuropathy. Young-onset ALS with atypical clinical features should prompt
the physician to obtain a Hex-A assay. If there is a positive family history, it is important to counsel the patient in preparation for SOD1 mutation analysis.

There are no specific features on muscle biopsy to distinguish ALS from other neurogenic disorders and this test should be reserved for cases that are more suggestive of a myopathy. The EDX examination is an invaluable tool in the investigation of ALS and its variants. It serves as an adjunct to the clinical examination and is particularly useful in determining the presence or extent of LMN disease. Again, none of the EDX findings is ALS specific, but they can strongly support the diagnosis. Furthermore, this investigation may be repeated at intervals to more objectively monitor disease progression. Sensory nerve conduction study results are characteristically normal, unless the patient happens to have a coincidental mononeuropathy or polyneuropathy. Motor nerve conduction study results may be normal, although the conduction velocity and CMAP amplitude may be diminished in keeping with the extent of motor axon loss. There should be no evidence of conduction slowing or block, which would suggest a primarily demyelinating disorder. Severe motor axon loss may give rise to the "generalized low motor-normal sensory" EDX pattern, which may portend a poorer prognosis. The needle electrode examination characteristically reveals a combination of acute (positive sharp waves and fibrillation potentials) and chronic (neurogenic firing pattern with evidence of increased amplitude and duration, polyphasic motor unit potentials) changes in a widespread distribution that is not in keeping with any single root or peripheral nerve distribution. Fasciculation potentials are usually identified; their absence should prompt an investigation for another disorder. Other common findings include moment-to-moment amplitude variation that indicates impaired
motor unit stability and repetitive discharges known as doublets. Mention should be made of a special EDX finding, the split-hand phenomenon; in some patients, EDX reveals severe changes in muscles of the lateral hand (thenar eminence) but relative sparing of the medial hand (hypothenar eminence). EDX changes should be observed in a certain topographical distribution and ideally should be carried out in at least three of the four regions of the neuraxis (bulbar, cervical, thoracic, and lumbosacral).

**Motor Unit Number Estimates (MUNEs)**

Determination of MUNEs is a quantitative method of assessing loss of AHCs but it is less commonly used than is MUP recruitment. The number of motor units in a muscle is estimated in MUNEs by (1) measuring the size of the CMAP evoked by supramaximal nerve stimulation and (2) dividing the supramaximal CMAP by the average size of single motor unit potentials (S-MUPs). Estimates of the size of S-MUPs can be made by measuring “all” or “none” responses at threshold stimulation, from the size of F waves, from spike-triggered surface averages, or from measurements of CMAP variance. Motor unit number estimates can be most reliably used in neurogenic processes, where the reliability increases as the disease progresses.

The method is most readily performed in distal muscles that lend themselves to surface stimulation and recording techniques but requires 5 to 10 min per nerve for full assessment. Reproducibility is now comparable with that for CMAP. Motor unit number estimates have shown that reinnervation by collateral sprouting can prevent reduction in strength and CMAP amplitude with loss of up to half the motor units in a slowly progressive ALS. The loss of motor units measured by MUNEs in individual muscles is rapid over a few months and more gradual for the remaining motor units. In
ALS, some of the S-MUPs seen on MUNEs are much larger than others, indicating much more collateral sprouting and increase in size. Motor unit number estimates quantitation is the most reliable method to measure the loss of motor neurons in clinical trials.32,34

Other Studies with Limited Application in ALS

Some uncommon electrophysiological studies can provide insight into clinical phenomena. Comparison of macro-EMG and twitch forces has shown that the late deterioration of strength in patients with ALS results from a decline in force of surviving motor units as well as from loss of motor neurons and corticospinal degeneration.35

There is a growing interest in methods to monitor disease progression in amyotrophic lateral sclerosis (ALS). A reliable and sensitive method is relevant, for example, as an outcome measure in therapeutic trials. Besides clinical methods to monitor disease progression, such as the ALS functional rating scale (ALSFRS) and the Medical Research Council (MRC) scale, quantitative methods that are more directly related to the underlying disease process are of interest. Motor unit number estimation (MUNE) techniques38, are all based on surface electromyography (sEMG) measurements. These methods are non-invasive and, in contrast to the MRC scale and the compound muscle action potential (CMAP), are not influenced by the compensatory reinnervation process following denervation due to lower motor neuron degeneration.

The number of axons innervating a muscle or group of muscles is a critical piece of information in identifying and characterizing a neurogenic disease. Techniques to estimate motor unit number (MUNE) measure the number of functioning motor units in a muscle. Traditional methods include the measurement of amplitude on nerve
conduction studies (NCS) and of motor unit potential (MUP) recruitment on needle
electromyography (EMG). Both methods provide useful information in clinical diagnostic
EMG, but do not provide numerical measures that can be reliably compared with either
normal values or changes over time. Motor unit number estimates (MUNE) attempt to
rapidly and reliably measure the actual number of axons that innervate a muscle. In
many cases such a measure of the loss of axons in a neuromuscular disease would be
more valuable than the standard NCS measures of amplitude, latency and conduction
velocity or the standard EMG measures of fibrillation, MUP recruitment and MUP
measurements. Nonetheless the clinical importance of determining the number of motor
axons in a nerve innervating a muscle has spurred many electromyographers and
neurophysiologists to search for a clinically useful MUNE method over the past 30
years.

The many MUNE methods differ primarily in how the size of the surface recorded
single motor unit potentials (SMUP) is determined. In each case the size of the
compound muscle action potential (CMAP) is divided by the size of the SMUP to
determine the number of motor units in the muscle. The major methods that have been
developed include: (1) measurement of the all- or-none steps in the CMAP with
incremental stimulation; (2) F-wave measurements; (3) multiple point stimulation of
individual motor axons at different points along the nerve; (4) spike-triggered averaging
of the SMUP corresponding to the firing of motor unit potentials on needle EMG; and (5)
Poisson statistical analysis of the variation in CMAP size with repeated stimulation.
Most MUNE techniques are based on the ratio of the maximal CMAP divided by an
average surface motor unit action potential (SMUP). MUNE appears to be a more
sensitive marker of disease progression in ALS as compared to clinical measures.\textsuperscript{38} High-density surface MUNE (HD-MUNE) is a recently developed technique that combines high-density surface EMG with elements of two other MUNE techniques: the increment counting technique (ICT) and the adapted multiple point stimulation (aMPS).\textsuperscript{40} In ICT nerve stimulation intensity is increased step-wise, starting at a sub-threshold level. Every incremental step that leads to a discrete increase in CMAP amplitude is considered as the added contribution of one single motor unit. Dividing the latest CMAP response by the number of incremental steps will provide average MUP amplitude. However, several motor axons with similar stimulation thresholds can have a probability of firing at a certain stimulation intensity, which leads to a variable CMAP amplitude (‘alternation’) on repetitive stimuli.\textsuperscript{41}

Statistical MUNE was employed in a multicenter trial of creatine in ALS, and was shown to be reliable, reproducible, and to decline with disease progression. However, motor unit amplitude stayed constant over 7 months, a finding believed to reflect an artifact of the method. The statistical method was revised to reflect more accurately the presence of larger motor units and employed in a 12-month study of Celecoxib in ALS. MUNE declined by 49\% in 12 months; however, motor unit amplitude again stayed constant over the same period. Statistical MUNE estimates motor unit number based on the variability of response to a repeated stimulus of constant strength, with an underlying assumption that this variability is due solely to the number of motor units responding in an intermittent manner. Based on studies showing that single motor units in ALS display excessive amplitude variability when stimulated repeatedly, response variability in ALS patients is in large part due to single unit changes. Thus the statistical
Kevin J. Felice studied 21 amyotrophic lateral sclerosis (ALS) patients, aged 36–76 years (mean: 58 years), at baseline and months 4, 8, and 12: thenar motor unit number estimate (MUNE) using multiple point stimulation, mean thenar surface recorded motor unit action potential negative-peak area, thenar compound muscle action potential amplitude, isometric hand grip strength, total Medical Research Council (MRC) manual muscle testing score, Appel ALS rating scale, and forced vital capacity (FVC). The absolute mean rate of change per month was significantly greater (P< 0.01) for MUNE values than for MRC and FVC values in the 21 ALS patients. In a subset of patients (n= 6) with slowly progressive disease, the absolute mean rate of change per month was significantly greater (P< 0.01) for MUNE values than for all other test values. In addition, MUNE values were the most sensitive index for documenting changes in disease progression over time .

Eric C. Yuen et al studied fiber density, compound muscle action potential (CMAP) amplitude, and motor unit number estimate (MUNE) of the abductor digiti minimi and grip strength longitudinally to determine the effects of ALS on these measurements and to evaluate which of these tests may be more sensitive in evaluating progression of ALS and possibly predicting survival. Ten patients were examined at months 0, 3, and 6. A significant decrease in MUNE and increase in fiber density were observed at months 3 and 6 (p < 0.02) compared with baseline (month 0). Mean CMAP and grip strength declined, but not significantly. The decrease in MUNE over 6 months was significantly greater than that of CMAP and grip strength (p < 0.025). The
significant changes in MUNE and fiber density over time suggest that they are more sensitive in measuring the rate of progression of ALS. To evaluate further the utility of these tests, these patients were arbitrarily divided into equal groups based on length of survival. MUNE declined significantly in the group with shorter survival ($p < 0.01$). Conversely, fiber density increased significantly in patients with longer survival ($p < 0.01$). With similar statistical analysis there were no significant differences in decline of CMAP or grip strength in either subgroup over 6 months. The study suggested that MUNE and fiber density are more sensitive than CMAP and grip strength in detecting progression of ALS. The greater increase in fiber density identifies a group of patients with ALS who will have longer survival, and that a greater decline in MUNE identifies a group with a worse prognosis.44

In study by Schefner et al fifty healthy subjects were evaluated twice and 71 subjects with ALS were studied repeatedly for up to 500 days. Side and nerve studied was based on clinical examination findings. Nerves were stimulated at 3 specified locations and 3 increments were obtained at each location. Average single motor unit action potential (SMUP) amplitude was calculated by adding the amplitude of the third increment at each location and dividing by 9; SMUP was divided into maximum CMAP amplitude to determine the MUNE. Test-retest variability was 9% in normal subjects. Average MUNE for normal subjects was 225 (± 87), and was 41.9 (± 39) among subjects with ALS at baseline. Subjects with ALS showed clear decrements over time, with an overage rate of decline of approximately 9% per month. SMUP amplitude increased with time in a fashion consistent with the known pathophysiology of ALS. Multipoint incremental MUNE has a number of attributes that make it attractive as an
outcome measure in ALS and other diseases characterized by motor unit loss. It has repeatability and rates of decline that favorably compare to other previously described methods.45

Timothy J. Doherty et al used Multiple point stimulation (MPS) as a method of estimating the numbers of motor units in the median innervated thenar muscles of young and older control subjects. Stimulation at multiple sites along the course of the median nerve was employed to collect a sample of the lowest threshold, all-or-nothing surface recorded motor unit action potentials (S-MUAPs). The average, negative peak area, and peak-to-peak amplitude of the sample of S-MUAPs was determined and divided into the corresponding value for the maximal compound muscle action potential to derive the motor unit estimate (MUE). In 37 trials from 17 younger subjects (20-40 years), the mean MUE was 288 ± 95 SD based on negative peak area and, in 33 trials from 20 older subjects, mean values were 139 ± 68. In 23 young and older subjects, MPS was performed on at least two occasions and the MUEs were found to be highly correlated (r = 0.88). 31

In study by Kevin J. Felice, thenar motor unit number estimate (MUNE) reproducibility was assessed in 20 patients with ALS and 16 normal subjects using the multiple point stimulation (MPS) technique. The MUNE was calculated by dividing the thenar compound muscle action potential negative-peak (n-p) area by the mean n-p area of 10 lowest threshold, all-or-nothing, surface-recorded motor unit action potentials. Two trials (test-retest) were performed by the same examiner either on separate days or on the same day with new electrode placements. The mean test MUNE was 43.4 (SD: 35.9, range: 6-145) for ALS patients and 219.4 (SD: 80.8, range:
122-368) for normal subjects. Test-retest MUNE differences were not significant for ALS patients or normal subjects. The test-retest correlation coefficient ($r$) was 0.99 for ALS patients and 0.85 for normal subjects. The mean difference between test-retest values was 10% for ALS patients and 17% for normal subjects. Test-retest reproducibility of the thenar MUNE using the MPS technique is high in both ALS patients and normal subjects. The reliability of the MPS technique in estimating motor unit numbers makes it a useful outcome measure in following the course of patients with progressive lower motor neuron disease, especially those enrolled in experimental drug trials. 32
Aims and objectives

To study demographic profile, clinical features in patients with ALS

To study motor unit number estimation (MUNE) at baseline and at 6 month

To compare MUNE, ALS functional rating scale and MRC sum score at baseline and at 6 month for progression of disease and to know which the better predictor of progression.
Materials and methods

The patients with ALS as defined by the modified El Escorial Criteria were included in study. Patients were excluded if they had another disease that could impact assessment of peripheral motor neuron loss due to ALS. Modified ALS functional rating scale\textsuperscript{36} as well as the Medical Research Council (MRC) sum score\textsuperscript{37} was calculated at baseline and then at 6 month.

The Amyotrophic Lateral Sclerosis Functional Rating Scale (ALSFRS) is an instrument for evaluating the functional status of patients with Amyotrophic Lateral Sclerosis. It can be used to monitor functional change in a patient over time. It measures (1) speech, (2) salivation, (3) swallowing, (4) handwriting, (5) cutting food and handling utensils (with or without gastrostomy), (6) dressing and hygiene, (7) turning in bed and adjusting bed clothes, (8) walking, (9) climbing stairs, (10) breathing. One weakness of the ALSFRS as originally designed was that it granted disproportionate weighting to limb and bulbar, as compared to respiratory, dysfunction. A revised version of the ALSFRS incorporates additional assessments of dyspnea, orthopnea, and the need for ventilatory support.

The MRC sum score is a summation of the MRC grades (range, 0–5) given in full numbers of the following muscle pairs: upper arm abductors, elbow flexors, wrist extensors, hip flexors, knee extensors, and foot dorsal flexors.\textsuperscript{7} The MRC sum score ranges from 0 (“total paralysis”) to 60 (“normal strength”).

This study was approved by the Institutional Review Board.
Motor unit number estimation (MUNE) method

Median nerve of the right or left hand was studied. Recording electrodes were placed on the median nerve innervated abductor pollicis brevis (APB) muscle, using the standard belly-tendon method. There were 3 stimulus locations; for the median nerve, stimulus locations were 2 cm proximal to the wrist crease, 4 cm proximal to the first stimulation site, and in the cubital fossa. Filter settings were 2 Hz–10 KHz. For each stimulation site, optimum stimulus location was determined using a submaximal stimulus and moving the stimulator to evoke the greatest response. The location was marked, and stimulating electrodes applied; self-adhesive circular motor electrodes were employed. For the most distal site, a maximal response was obtained. Amplifier settings were then changed to 200µV/division, and stimulus control increased to the maximum allowable; gradation in at least tenths of milliamps was necessary.

A standard 3-site motor conduction program was used, with traces set to superimpose. Subthreshold stimuli were applied at a rate of approximately 1/second, with stimulus intensity slowly increased until an all-or-nothing initial response was obtained. Baseline to peak amplitude was measured. For both initial and subsequent incremental responses, the minimum negative peak amplitude considered to be acceptable for recording was 25 µV. Tracings with an initial positive component were measured from baseline to negative peak as well, disregarding the positive portion of the response. The initial response was recorded on trace 1, after which stimulus intensity was increased until a clearly defined incremental response (of more than 25 µV incremental amplitude) was obtained. This response was recorded on trace 2, and a
second increment obtained with further slight increase in stimulus intensity. The final
potential was recorded on trace 3. The negative peak amplitude of the third response
was recorded. Stimulation at the second and third location was identical to the first and
second.

**Calculation of MUNE and single motor unit action potential amplitude (SMUAP):**

The amplitude of the third response at each site was summed, and then divided
by 9 to yield the average single motor unit action potential (SMUP) amplitude. This
amplitude was divided into the maximum compound motor unit action potential (CMAP)
amplitude to yield the MUNE. For evaluation of rate of decline, change from baseline
was evaluated over time.

**Time intervals and nerve selection:**

The goal was to study subjects at baseline and at 6 month interval. At the first
visit, the upper extremities were evaluated clinically. For patients with clinically
detectable weakness in both upper extremities, the stronger of the 2 hands was chosen.
If there was weakness only in one extremity, that extremity was studied. For that hand,
motor and sensory nerve conduction studies of the median nerve were performed using
standard techniques, to rule out the presence of median neuropathy at the wrist. If a
median neuropathy was detected sensory and motor studies of the ulnar nerve were
performed. If a significant ulnar neuropathy at the elbow or wrist was detected, or the
CMAP amplitude was less than 5 mV, the other hand was studied in similar fashion.
The underlying goal was to choose a nerve/muscle not affected by focal neuropathy and
with a CMAP amplitude in the low normal range. If all nerves studied had CMAPs
reduced in amplitude, the nerve with the largest motor response was chosen for study.
Results:

MUNE was done in 23 healthy control (11 male & 12 female, age), mean age was 48 years (range 31-70). The mean MUNE was 62.60 (SD 17.45, range 37-94). MUNE values of controls had no correlation with age (Pearson's r=0.183; p=0.34) or sex (Unpaired t test, p=0.15).

Of the 29 patients studied, 19 were male and 10 female. Age of onset was 24.5 to 78.9 year (mean 51.5). Duration of symptom was 1 to 60 month (mean 13 month). There were 17 patients with spinal onset and 12 bulbar onset. Mean duration of symptom in spinal onset was 18 month (range 3-60 month) while in bulbar onset 6 month (range 1-18). Age of onset in spinal onset group was 24.5 to 61.6 year (mean 45.0). Age of onset in bulbar onset group was 45 to 78.9 year (mean 58.6). Three patients had anarthria and 5 underwent PEG.

Three patients had definite, 11 Probable, 7 Lab supported probable and 8 had Possible ALS as per modified El Escorial criteria.

The mean MUNE in patients at entry in the study was 21.80 (SD 19.46, range 4-73). At the entry in the study mean MUNE in male was 18.4 (SD 15.52) & 28.96 (SD 24.70) in females without any statistical significance.
Table 1: MUNE in Control and ALS patient

<table>
<thead>
<tr>
<th>MUNE</th>
<th>Control</th>
<th>Patient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>62.60</td>
<td>21.80</td>
</tr>
<tr>
<td>Std. Deviation</td>
<td>17.45</td>
<td>19.46</td>
</tr>
<tr>
<td>Range</td>
<td>37-94</td>
<td>4-73</td>
</tr>
</tbody>
</table>

In spinal onset group MUNE was 15.9 (SD 14.60) & 30.16 (SD 22.89) in bulbar onset group without any statistical significance.

At 6 month MUNE was 8.46 (SD 14.03) & 24 (SD 15.37) in spinal and bulbar onset group, respectively. Limb onset patients have 74.02% of baseline value while bulbar onset patients have only 24.74% MUNE at 6 month follow up when compared to baseline value, Unpaired t test, p=0.001

Table 2: Baseline and 6 month follow up MUNE in spinal and bulbar onset group

<table>
<thead>
<tr>
<th>MUNE</th>
<th>Spinal onset</th>
<th>Bulbar onset</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>15.90</td>
<td>30.16</td>
</tr>
<tr>
<td>6 month follow up</td>
<td>8.46</td>
<td>24.00</td>
</tr>
<tr>
<td>% decline</td>
<td>25.97</td>
<td>75.26</td>
</tr>
</tbody>
</table>
Figure 1: CMAP and SMUAP in healthy control and patient at baseline and 6 month
Mean ALS FR score was 37.12(SD 6.4) at study entry and 32 (SD7.9) at 6 month follow up which showed statistically significant decline (p<0.001).

Mean MRC sum score was 50.86(SD 11.72) at study entry and 44.73 (SD 14.64) at 6 month follow up which showed statistically significant decline (p<0.001).

Of the MUNE, ALS-FR and MRC sum score, MUNE has highest sensitivity for progression of the disease although ALS FR and MRC sum score was also sensitive for progression of the disease.

Table 3: Comparison between MUNE, ALSFRS and MRC sum score at baseline and 6 month

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>Paired t test</th>
</tr>
</thead>
<tbody>
<tr>
<td>MUNE</td>
<td>26.89</td>
<td>20.42</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>MUNE 6 month</td>
<td>15.86</td>
<td>16.37</td>
<td></td>
</tr>
<tr>
<td>ALS FRS</td>
<td>37.18</td>
<td>6.48</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ALS FRS 6 month</td>
<td>32.00</td>
<td>7.94</td>
<td></td>
</tr>
<tr>
<td>MRC sum score</td>
<td>50.86</td>
<td>11.72</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>MRC6 month Follow up</td>
<td>44.73</td>
<td>14.64</td>
<td></td>
</tr>
</tbody>
</table>
Figure 2: Graph showing Comparison between MUNE, ALSFRS and MRC sum score at baseline and 6 month
Eleven patients expired during follow up with in 3 month to 12 month of first visit. The mean MUNE in these patient was 9.3 (range 4-26) and CMAP amplitude of 3.02 (range 0.5-7.8).

Kaplan Meier Survival curve with MUNE value of below and above 5, was significantly associated with death with mean survival time of 7.5 month, P value (Log rank test) = 0.002

**Table 4: Number of patients who expired with MUNE value below and above 5**

<table>
<thead>
<tr>
<th>MUNE</th>
<th>Total number</th>
<th>Number of Deaths</th>
<th>Mean survival in months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Above 5</td>
<td>22</td>
<td>5</td>
<td>10.545</td>
</tr>
<tr>
<td>Below 5</td>
<td>7</td>
<td>6</td>
<td>7.571</td>
</tr>
<tr>
<td>Total</td>
<td>29</td>
<td>11</td>
<td>9.828</td>
</tr>
</tbody>
</table>
Figure 3: Kaplan Meier Survival curve with MUNE value of below and above 5
ROC curve analysis was done for prediction of death during follow up showed highest area under curve for MUNE suggesting higher sensitivity for MUNE over ALS- FRS and MRC sum score.

**Table 5: ROC curve analysis was done for prediction of death during follow up**

<table>
<thead>
<tr>
<th>Variable(s)</th>
<th>Area</th>
<th>Asymptotic Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>MUNE</td>
<td>.843</td>
<td>.002</td>
</tr>
<tr>
<td>ALS FR</td>
<td>.725</td>
<td>.045</td>
</tr>
<tr>
<td>MRC score</td>
<td>.720</td>
<td>.051</td>
</tr>
</tbody>
</table>
Figure 4: ROC curve analysis for prediction of death during follow up

Diagonal segments are produced by ties.
Discussion

This study shows that multipoint incremental MUNE value declines faster than other commonly employed outcome measures used in ALS trials. Using the multipoint method, % change of MUNE was found to be greater than change in MRC sum score or the revised ALS functional rating scale over a 6 month period, approaching 60% on average. A similar decline in multipoint MUNE in natural history study of patients with ALS was found in the study by Mitsumoto H et al. A study by Shefner, et al also showed average 60% decline in MUNE at one year. A study employing an entirely different technique, the incremental method, by Dantes M, et al identified virtually the same rate of decline.

The protocol performing this method of motor unit estimation is simple can be performed on any EMG machine and in a uniform fashion. The amplitude was chosen as the attribute measured rather than area, which requires more judgments on the part of the evaluator with respect to cursor placement. Using baseline to peak amplitude also eliminates the need to make judgments as to whether waveforms with prominent positive dips should be excluded, as the only decision point is whether a given waveform is more than 25 µV greater than its predecessor. We recognize that some units can change the overall waveform area without affecting amplitude; however, our data suggest that reliable data can be obtained using strict amplitude criteria. Another criterion that was strictly followed for both normal subjects and subjects with ALS was to not include any units with negative peak amplitude of less than 25 µV; this criterion was applied both to initial waveforms and subsequent increments. While we cannot eliminate
the possibility that normal muscle does in fact contain units smaller than 25 µV, prior studies with a variety of techniques suggest that such units are rare.\textsuperscript{38,49}

The ALSFRS-R is commonly used as the primary outcome in recent ALS trials. The rates of decline of MUNE, ALSFRS-R and MRC Sum score are shown in table 5. Multipoint incremental MUNE compares favorably to both CMAP amplitude and ALSFRS-R, when expressed as% change from baseline.\textsuperscript{49}

There are several attractive practical aspects to this form of MUNE worth highlighting. First, it is relatively easy to perform, even in patients with a large number of motor unit potentials, and able to complete a measurement session within about 10 minutes. Second, specialized equipment is not necessary to perform the measurements. Finally, it is also well-tolerated by patients; in addition to being rapidly performed, it requires relatively low stimulus intensities. This method could also be applied to muscles of the foot, although stimulus intensities required for nerve stimulation at some stimulus locations may make the procedure somewhat more uncomfortable. However, the data presented here suggest that limiting investigation to the upper extremities still yields data that compare well to other outcome measures that evaluate more global deficits.

As with all MUNE methods, this technique is vulnerable to bias. First, sampling is limited to units near electrical threshold; this potentially could bias the sample toward larger units. Second, using amplitude as the measure of interest may lead to errors in estimation when summation of units is not linear. It is also possible that the same unit may be sampled at different locations, acting to further reduce the sample on which MUNE is estimated. Neither this method nor any other MUNE method has been
specifically validated against an objective assessment of motor unit number; indeed, it is hard to conceive of such a study being performed in humans. Despite this, MUNE using a variety of techniques has been shown to predict meaningful clinical outcomes including survival\(^3\).\(^3\)\(^2\),\(^4\)\(^2\),\(^4\)\(^4\),\(^5\)\(^0\),\(^5\)\(^1\) Thus, MUNE should be considered a surrogate marker of disease progression in ALS rather than a quantitative estimate of an underlying biological process.

**Conclusion:**

Multipoint incremental MUNE is a valuable tool for outcome measurement in patients with ALS and can be also extended to other diseases characterized by loss of motor unit. It can be rapidly performed in any EMG machine and is reproducible
Summary

Improved outcome measures are necessary to reduce sample size and increase power in amyotrophic lateral sclerosis (ALS) clinical trials. Motor unit number estimation (MUNE) is a potentially attractive tool. We studied multipoint incremental MUNE and the revised Amyotrophic Lateral sclerosis functional rating scale (ALSFRS-R) in natural history study of subjects with ALS.

Twenty three healthy subjects were evaluated baseline and 29 subjects with ALS were studied at base line and after 180 days. The mean MUNE was 62.60 (SD 17.45, range 37-94) and 21.80 (SD 19.46, range 4-73) in healthy controls and patients at entry in the study. In spinal onset group MUNE was 15.9 (SD 14.60) & 30.16 (SD 22.89) in bulbar onset group without any statistical significance. At 6 month MUNE was 8.46 (SD 14.03) & 24 (SD 15.37) in spinal and bulbar onset group, respectively. Limb onset patients have 74.02% of baseline value while bulbar onset patients have only 24.74% MUNE at 6 month follow up when compared to baseline value, Unpaired t test, p=0.001. Mean ALS FR score was 37.12(SD 6.4) at study entry and 32 (SD7.9) at 6 month follow up which showed statistically significant decline (p<0.001). Mean MRC sum score was 50.86(SD 11.72) at study entry and 44.73 (SD 14.64) at 6 month follow up which showed statistically significant decline (p<0.001). Of the MUNE, ALS-FRS and MRC sum score, MUNE has highest sensitivity for progression of the disease although ALS FR and MRC sum score was also sensitive for progression of the disease. Eleven patients expired during follow up with in 3 month to 12month of first visit. The mean MUNE value in these patient was 9.3 (range 4-26). Kaplan Meier Survival curve with MUNE value of below and above 5, was significantly associated with death with mean
survival time of 7.5 month, P value (Log rank test) = 0.002. ROC curve analysis was done for prediction of death during follow up which showed highest area under curve for MUNE suggesting highest sensitivity of MUNE over ALS- FR and MRC sum score.

Multipoint incremental MUNE is a valuable tool for outcome measure in ALS and other diseases characterized by motor unit loss. It can be rapidly performed on any EMG machine and has repeatability.
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Revised El Escorial Research Diagnostic Criteria for ALS (Brooks et al., 2000)

The diagnosis of ALS requires:

1) Evidence of LMN degeneration by clinical, electrophysiological or neuropathological examination;

2) Evidence of UMN degeneration by clinical examination, and

3) Progressive spread of symptoms or signs within a region or to other regions, as determined by history or examination,

Together with the absence of:

[1] Electrophysiological and pathological evidence of other disease that might explain the signs of LMN and/or UMN degeneration, and

[2] Neuroimaging evidence of other disease processes that might explain the observed clinical and electrophysiological signs

Categories of clinical diagnostic certainty on clinical criteria alone

**Definite ALS** - UMN signs and LMN signs in 3 regions

**Probable ALS** - UMN signs and LMN signs in 2 regions with at least some UMN signs rostral to LMN signs

**Probable ALS – Laboratory supported** - UMN signs in 1 or more regions and LMN signs defined by EMG in at least 2 regions

**Possible ALS** - UMN signs and LMN signs in 1 region (together), or UMN signs in 2 or more regions, UMN and LMN signs in 2 regions with no UMN signs rostral to LMN signs

**UMN signs:** clonus, Babinski sign, absent abdominal skin reflexes, hypertonia, loss of dexterity.

**LMN signs:** atrophy, weakness. If only fasciculation: search with EMG for active denervation.

**Regions reflect neuronal pools:** bulbar, cervical, thoracic and lumbosacral
ANNEXURE –II

ALS Functional Rating Scale-Revised

1. SPEECH
No change value = 4
Noticeable speech disturbance value = 3
Asked often to repeat words or phrases value = 2
Alternative communication methods value = 1
Unable to communicate verbally value = 0
Q1. Score =

2. SALIVATION
No change value = 4
Slight excess saliva, nighttime drooling value = 3
Moderately excessive saliva, minimal drooling value = 2
Marked excess of saliva, some drooling value = 1
Marked drooling, requires constant tissue value = 0
Q2. Score =

3. SWALLOWING
No change value = 4
Occasional choking episodes value = 3
Modified the consistency of foods value = 2
Supplemental tube feedings value = 1
NPO (do not eat anything by mouth) value = 0
Q3. Score =

4. HANDWRITING
No change value = 4
Slow or sloppy, all words legible value = 3
Not all words legible value = 2
Able to hold pen, unable to write value = 1
Unable to hold pen value = 0
Q4. Score =

5a. CUTTING FOOD AND HANDLING UTENSILS
(patients without gastrostomy)
No change value = 4
Somewhat slow and clumsy, needs no help value = 3
Sometimes needs help value = 2
Foods cut by someone else value = 1
Needs to be fed value = 0
Q5a. Score =

5b. CUTTING FOOD AND HANDLING UTENSILS
(patients with gastrostomy)
Uses PEG without assistance or difficulty value = 4
Somewhat slow and clumsy, needs no help value = 3
Requires assistance with closures and fasteners value = 2
Provides minimal assistance to caregiver value = 1
Unable to perform any manipulations value = 0
Q5b. Score =

6. DRESSING AND HYGIENE
No change value = 4
Performs without assistance with increased effort or decreased efficiency value = 3
Intermittent assistance or different methods value = 2
Requires daily assistance value = 1
Completely dependent value = 0
Q6. Score =

7. TURNING IN BED AND ADJUSTING BEDCLOTHES
No change value = 4
Slower or more clumsy, without assistance value = 3
Can turn alone or adjust bed clothes value = 2
Can initiate but requires assistance value = 1
Helpless in bed value = 0
Q7. Score =

8. WALKING
No change value = 4
Change in walking, no assistance or devices value = 3
Requires assistance to walk value = 2
Can move legs or stand up, unable to walk from room to room value = 1
Cannot walk or move legs value = 0
Q8. Score =

9. CLIMBING STAIRS
No change value = 4
Unsteady and/or more fatigued value = 3
Requires assistance to walk value = 2
Can only walk up one step at a time value = 1
Cannot climb stairs value = 0
Q9. Score =

10. DYSPNEA
No change value = 4
Occurs only with walking value = 3
Occurs at rest, either sitting or lying value = 2
Significant shortness of breath considering mechanical support value = 1
Q10. Score =

11. ORTHOPNEA
No change value = 4
Occasional shortness of breath, does not routinely use more than two pillows value = 3
Requires more than 2 pillows to sleep value = 2
Can only sleep sitting up value = 1
Q11. Score =

12. RESPIRATORY INSUFFICIENCY
No respiratory support value = 4
Intermittent use of BiPAP® value = 3
Continuous use of BiPAP® at night value = 2
Invasive mechanical ventilation value = 0
Q12. Score =

Total Score = / 48