A REVIEW OF CASES OF MOTOR NEURONE DISEASE SEEN AT GROOTE SCHUUR HOSPITAL FROM 2005 TO 2010

by

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Declaration

I, Amina Daude, hereby declare that the work on which this thesis is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university.

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Abstract

Background: Motor neurone disease (MND) is a rare progressive neurodegenerative disorder in which selective degeneration of the motor neurones of the brain and spinal cord occurs. Progressive weakness of limb, bulbar and respiratory muscles eventually results in death. Most descriptive and epidemiological studies of MND have been performed in the industrialized countries of Europe and North America. We know very little about the incidence or prevalence of MND in Africa in general and South Africa in particular. However, anecdotal evidence based on observations by clinicians in the neurology and geriatric medical clinics at Groote Schuur Hospital suggest that the condition is not uncommonly seen, even in younger patients. Furthermore, many cases appear to originate from the West Coast area of the Western Cape.

Aims: The proposed study aimed at describing the demographic and clinical characteristics of MND seen at Groote Schuur Hospital between 2005 and 2010. I hypothesized that disease duration, measured from age of onset of first symptoms to death, would be shorter in patients with bulbar-onset disease, in younger-onset disease, and in patients with higher CSF protein and blood creatine kinase levels at baseline. Furthermore, age of onset of the disease would be younger in familial compared with sporadic MND. I also hypothesized that smoking and certain occupational exposures might be risk factors for MND, that there would be a male preponderance of the disease, and that a disproportionate number of cases would come from the West Coast region.

Methods*:* This was a retrospective study. I reviewed the clinical notes of cases of motor neurone disease and collected data relevant to the aims and hypotheses described above. I applied the El Escorial diagnostic criteria for MND to check the validity of the diagnoses. Mortality data were obtained through the Burden of Diseases Research Unit at the South African Medical Research Council.

Results: Forty eight patients were identified who met El Escorial criteria for the diagnosis of probable or definite MND. The median age of onset of the disease was 54 (IQR 47-63) and the mean duration of the disease from earliest symptoms to death was 2 years (IQR 1-3). These did not differ significantly between bulbar and limb-onset disease sub-types. There was a male preponderance of the disease (60%) and the majority of patients (60%) were smokers. African patients tended to have a younger age of onset. Occupations involving potential exposure to chemicals were disproportionately represented in the MND patients

compared with the general population of the Western Cape. People from the West Coast region were not disproportionately represented in the patient population. Baseline CSF protein and serum creatine kinase levels were not associated with disease duration.

Conclusion: The characteristics of MND cases seen at Groote Schuur Hospital between 2005 and 2010 are similar to those described in the world literature. Smoking and chemical exposure may be risk factors for the disease. There was no evidence of clustering of cases. This study will serve as the basis for future larger prospective studies on MND prevalence and aetiology in South Africa.

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List of abbreviations

ALS	amyotrophic lateral sclerosis
ANG	angiogenin gene
BMAA	beta-methylamino-L-alanine
CPK	creatine phosphokinase
CSF	cerebrospinal fluid
EEDC	El Escorial diagnostic criteria
EMG	electromyography
ESR	erythrocyte sedimentation rate
FBC	full blood count
fMND	familial motor neurone disease
FTLD	fronto-temporal lobe dementia
FUS	fusen in sarcoma
GSH	Groote Schuur Hospital
Guam MND-PDC	Guam motor neurone disease – Parkinson's -dementia complex
HIV	human immunodeficiency virus
HREC	Human Research Ethics Committee
IFCN	International Federation of Clinical Neurophysiologists
LMN	lower motor neurone
MND	motor neurone disease
MRI	magnetic resonance imaging
MUNE	motor unit number estimation
MUP	motor unit potential
NCS	nerve conduction studies
OPTN	optineurone
PBP	progressive bulbar paralysis
PEG	percutaneous endoscopic gastrostomy
PLS	progressive lateral sclerosis
PMA	progressive muscular atrophy
SFEMG	single fibre electromyography

sMND	sporadic motor neurone disease
SOD1	superoxide dismutase gene 1
SSEP	somato-sensory evoked potentials
TARDB	transactive response DNA binding protein
TBH	Tygerberg Hospital
TMS	transcortical magnetic stimulation
UMN	upper motor neurone
VDRL	venereal diseases research laboratory
WCC	white cell count
WFN	World Federation of Neurology

Chapter 1: Introduction

Motor neurone disease is a group of neurodegenerative disorders associated with selective degeneration of motor neurones in the brain and spinal cord. Jean-Martin Charcot first characterized the disease in 1869; it was then referred to as maladie de Charcot. The commonest form of motor neurone disease involves a combination of signs of upper and lower motor neurone degeneration. In the American literature the term amyotrophic lateral sclerosis or ALS is often used to describe this form of the disease. In the UK, the umbrella term motor neurone disease (MND) is more commonly used to describe all variants of the disease.

Studies of the incidence, prevalence and demographic characteristics of patients with MND have mostly been done in the industrialized countries. We know very little about the disease in Africa in general and southern Africa in particular. Our anecdotal evidence from the neurology and geriatric medicine clinics suggests that the disease is not uncommonly seen in younger patients.

This study aimed to describe the demographic and clinical characteristics of cases of MND seen at GSH between 2005 and 2010. I wished to determine whether a number of characteristics of MND, derived from studies in the developed world, are also seen locally. I also wished to test a number of hypotheses based on our clinical observations of the cases of MND seen in the Western Cape.

Chapter 2: Rationale, motivation, background to the study and a review of the literature

2.1 Introduction and history

Jean–Marie Charcot, a French neurologist, used the term "amyotrophic lateral sclerosis" for the first time in 1874 (Charcot, 1874). It was also known as "maladie de Charcot" in France. Studies conducted between 1865 and 1869 by Charcot and his colleague Joffroy described the clinico-pathological features of the disease (Charcot, 1869). In 1824 Charles Bell reported the motor functions of the anterior spinal nerve roots and the sensory functions of the posterior roots (Kazi, 2004). Aran in 1850 described cases of progressive muscular atrophy (PMA) (Aran, 1850). Five years later Cruveilhier correlated PMA with atrophy of the anterior horns of the spinal cord (Cruveilhier, 1853). In 1883 Dejerine related the disease of progressive bulbar palsy (PBP) to MND. In 1884 Kahler grouped the diseases of PMA, ALS and PBP together as "primary degenerations of the motor system" (Leigh, 1995). At this stage there were questions as to whether these syndromes, characterised by limb weakness, muscle atrophy and spasticity, were primarily muscle lesions or spinal cord diseases. Charcot was the first to establish the relationships between clinical presentations and autopsy findings. He used the disorder of motor neurone disease as the prototypic example of the use of his research method "méthode anatomoclinique" to correlate clinical signs detected in life with anatomical lesions seen at autopsy (Goetz, 2000; Sigerson, G 1881).

Figure 1 Jean Marie Charcot, (source: Kumar, 2011)

The term motor neurone disease (MND) was first used by Brain in 1933 (Turner, 2010). In the United States it is better known as amyotrophic lateral sclerosis (ALS) or Lou Gehrig disease, after the New York Yankees baseball player, Lou Gehrig, (Figure 2) who developed the disease in the late 1930s. In Africa and Australia MND is the most common term used.

Figure 2 Lou Gehrig, American baseball player (source: Universidad Francisco Marroquín 2008)

Figure 3 Joost Van Der Westhuizen, a South African rugby player, was diagnosed with MND in 2011 (source: Peacock, 2013)

However, practically, MND and ALS are used interchangeably to describe the spectrum of motor neurone disorders.

Throughout this text I shall use the term MND rather than ALS.

This chapter aims to provide the reader with a background understanding of motor neurone disease (MND) and its classification. It covers the definition of the disease, diagnostic criteria, clinical features, risk factors for its development and current management of the condition.

Most of this literature has been based on studies conducted in Europe and North America. Very little is known about MND in developing countries, in the African continent in general; and southern Africa in particular. The clinical forms of MND in developing countries may not be the same as those in the developed world.

2.2 Definitions

MND is a progressive and ultimately fatal neurodegenerative condition that causes upper and lower motor neurone loss at many levels in the nervous system.

Upper motor neurones, by definition, are neurones that originate in the motor cortex of the brain and terminate within the brainstem (corticobulbar tracts) or spinal cord (corticospinal tracts). They form part of the pyramidal motor system. Lower motor neurones are nerve cells that originate in the anterior horn of grey matter of the spinal cord or the brainstem cranial nerve nuclei. They end at the neuro-muscular junction and innervate the somatic limb muscles as well as the muscles of the lower face, tongue and oro-phaynx (Figure 4).

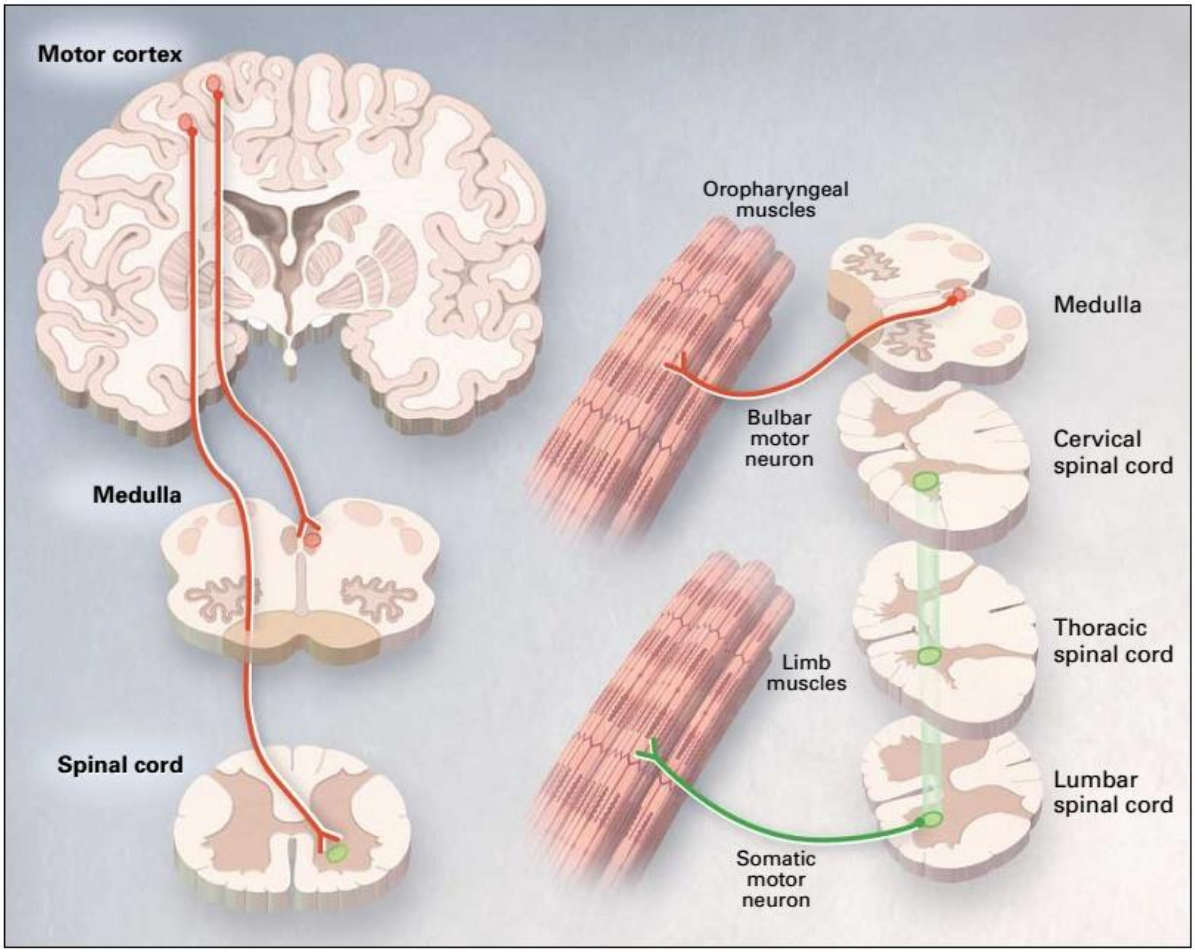

Figure 4 UMN and LMN (source: Rowland, 2001)

Damage to upper motor neurones results in muscle weakness, spasticity, and increased deep tendon reflexes (see table 1). Lower motor neurone lesions causes muscle atrophy (amyotrophy), fasciculations, weakness, and reduced or absent tendon reflexes (Table 1 and Figure 5).

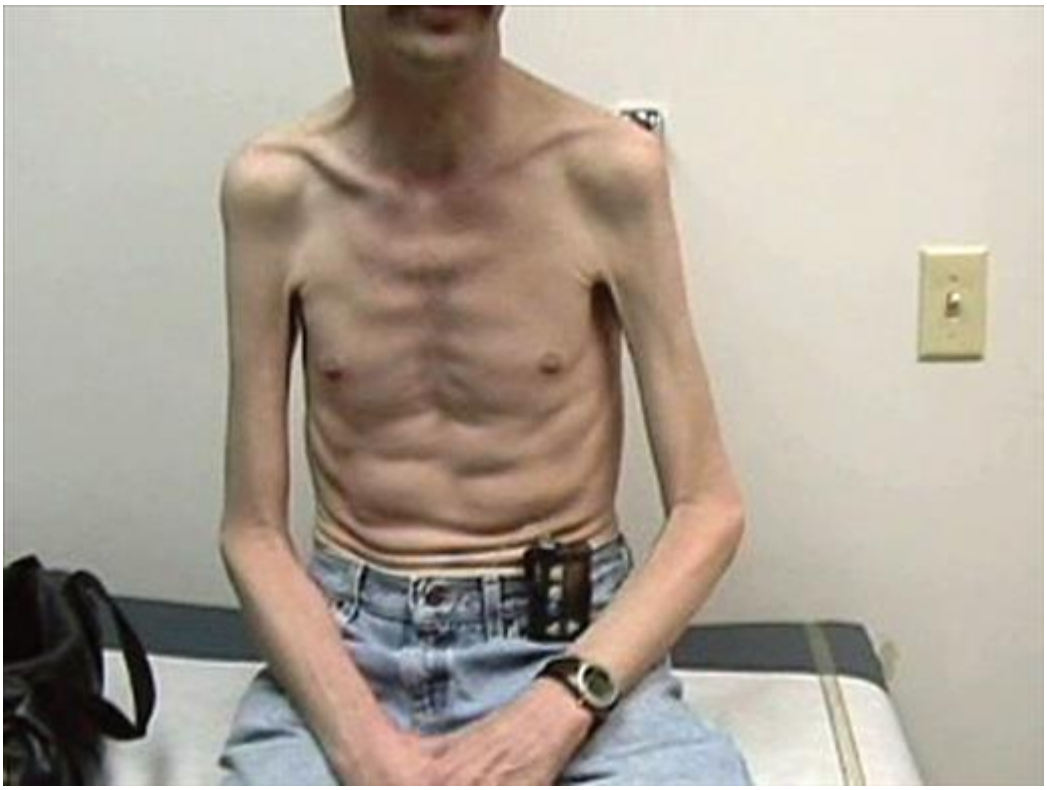

Figure 5 Muscle atrophy in the upper limbs (source: Berger, 2005)

In MND, the bulbar muscles involved in speech, chewing and swallowing are affected, as well as the muscles of the limbs, neck and trunk. The latter involves the muscles of respiration.

Table 1: Features of upper and lower motor neurone dysfunction

LMN dysfunction symptoms and signs	**UMN dysfunction symptoms and signs**
flaccid paralysis-weakness	spastic paralysis-weakness
muscle atrophy	hypertonia/spasticity
fasciculations	hyperreflexia
cramps	extensor plantar responses
fatigue	Hoffman's sign
hypotonia	
hyporreflexia/arreflexia	

The term “amyotrophy” is derived from Greek. “A” means “no” or “negative”. “Myo” refers to muscle and “trophic” means “nourishment”. Hence “no muscle nourishment” or wasting away. “Lateral” refers to the area of the corticospinal tracts in the spinal cord; and “sclerosis” means scarring or hardening (Wikipedia, 2014).

In summary**:** ALS or MND is a disease in which the upper and lower motor neurones of the nervous system degenerate. The cause of this process, as we shall see, remains unclear.

2.3 Incidence and prevalence worldwide

The reported worldwide incidence and prevalence rates of MND vary little (Chiò, 2013) Most cases of MND are sporadic. About 5 to 10 % of MND is familial, with a Mendelian pattern of inheritance. MND affects people worldwide, but the exact incidence of the disease is not known. The incidence of sporadic MND in the 1990s was reported to be 1.5 to 2.7 per 100,000 population/year (average of 1.9 per 100.000/year) in Europe and North America, with a fairly uniform incidence across these countries. The prevalence ranges from 2.7 to 7.4 per 100, 000 in western Countries (Wijesekera, 2009). Males are affected more than females, with a male to female (M: F) ratio of 1, 5:1. A review published in 2001 found that the mortality rates of MND in the 1990s ranged from 1.54 to 2.55 per 100.000/year in the US population (Shaw, 2005).

The mean age of onset of sporadic MND varies between 55-65 years depending on the study, with a median age of onset of 64 years (Haverkamp, 1995). Only 5% of cases have an onset before the age of 30 years. Bulbar onset is commoner in women and in older age groups, with 43% of patients over the age of 70 presenting with bulbar symptoms compared to 15% below the age of 30 (Beghi, 2007).

The incidence and prevalence peaks in older people between the ages of 65 and 74 years (Chiò, 2013). It decreases rapidly after 80 years of age (Logroscino, 2008). The incidence of MND is said to be increasing, but this is probably the result of improved diagnosis, better awareness of the disease and an ageing population. Death occurs in most patients within two to five years of the diagnosis (Rowland, 2001).

In familial MND (fMND), the mean age of onset is a decade earlier than in sporadic cases, around 47-52 years. It affects males and females equally, and has a shorter survival (Veltema,

1990). The inheritance is usually autosomal dominant, although autosomal recessive and X-linked modes of inheritance may be seen in some pedigrees.

During World War II an unusual frequency of MND combined with Parkinsonism and dementia was noted in the Pacific Island of Guam and the Kii peninsula of Japan. The prevalence in Guam has been reported to be 50 to 100 times higher than the prevalence anywhere else. The cause of MND associated with Parkinsonism and dementia in Guam is unknown, but it has been postulated that there might have been environmental or dietary triggers. However, since the late 1980s, the incidence in Guam has fallen to that of other regions of the world.

There are reports of conjugal and other non-consanguineous clusters of MND outside the Western Pacific, with rates much higher than would be expected by chance. Examples include people who work or live in close proximity, play in the same sport teams, have been war evacuees, share an environmental peculiarity such as high soil selenium, or a common exposure such as leather or textile workers (Roman, 1996).

2.3.1 Incidence and prevalence in Africa

We have no reliable epidemiological information about the incidence and prevalence of MND in Africa. To my knowledge, there have been few studies to date reported in the literature from the African Continent. Radakhrisnan *et al*. studied the epidemiology of MND in Libya (Radhakrishnan, 1986). Their found that of 32 patients diagnosed with MND, 17 had ALS, 4 had PMA and 2 had PBA. The male to female ratio was 2, 3:1. The average incidence was 0.89/100.000 population/per year. The median age was 57 years. The median duration of illness for 5 patients after the onset of the disease was 30 months. The median survival time for all MND cases combined was 42 months. In a study by Iman and Ogunniyi from Nigeria, they found 16 cases diagnosed with MND. The mean age of onset was 38,6 years, there was a male preponderance (15 males and 1 female), all subjects had ALS, and trauma was the most frequent risk factor identified (Imam, 2004). In 1971, a study by Osuntokun from Ibadan, Nigeria, found that the incidence of MND was 0.35 per thousand; the mean age was lower compared with a Caucasian population in Nigeria, and had a better prognosis (Osuntokun, 1971). Another study by Tekle-Haimanot *et al*. in 1990 described the prevalence of MND in Ethiopia as 5 per 100,000 of the population, but this and the Nigerian study included juvenile spinal muscular atrophy in the diagnosis of MND (Tekle-Haimanot, 1990).

Abdullah 1997, in Sudan found 28 patients with MND, 19 of whom had ALS, 7 had PBP and 2 patients had PMA. A family history of the disease was found in 4 patients and those presented mainly with bulbar symptoms. Sudanese patients had an earlier age of onset and a better prognosis of the disease than their Caucasian counterparts (Abdulla, 1997).

2.4 Diagnostic Criteria of MND

The diagnosis of MND is made clinically using:

1. the revised El Escorial criteria (EEDC) (Brooks, 2000).
2. clinical features of both upper and lower motor neurone degeneration.
3. electrophysiological examinations, neuro-imaging and clinical laboratory tests to exclude potential mimics and other treatable conditions.
4. repetition of clinical and electrophysiological examinations to ascertain evidence of progression.
5. neuropathological examinations where available.

2.4.1. The El Escorial diagnostic criteria (EEDC)

MND is a clinical diagnosis. Thus far there are no specific biological markers for a definitive diagnosis. Clinical electrophysiology is the main supportive diagnostic aid when MND is suspected. The electrophysiological tests can demonstrate subclinical MND changes, in muscles considered not affected clinically (De Carvalho, 2005). It was Lambert and Mulder in 1957 who first suggested that electrophysiology could assist in the diagnosis of MND and in the exclusion of mimics of the disease. In 1969 they published a set of electrophysiological criteria for MND diagnosis (Lambert, 1969). The World Federation of Neurology (WFN) sub-committee on motor neurone disease convened a workshop in EL Escorial, Spain, in 1990, to discuss and develop international clinical and electrophysiological criteria to diagnose MND (Brooks, 2000). These El Escorial diagnostic criteria (EEDC) criteria were revised in 1998 at Airlie House, Virginia (Brooks, 2000). The revised El Escorial criteria (EEDC) are summarized in table 2. The EEDC define four body regions to be evaluated: the (1) bulbar region, (2) neck and upper extremities, (3) trunk and abdominal wall, (4) lumbar spine and lower extremities. The EEDC categorize patients into four levels of diagnostic

probability: clinically definite MND, clinically probable MND, clinically probable-laboratory supported MND and clinically possible MND.

Table 2: El Escorial diagnostic criteria for MND (EEDC) (Brooks, 2000)

Clinically definite MND ▪ presence of both lower and upper motor neurone signs in at least 3 body regions.
Clinically probable MND ▪ presence of both upper and lower motor neurone signs in at least 2 regions, with some UMN signs above the region of LMN signs.
Clinically probable – laboratory supported MND ▪ presence of both upper and lower motor neurone signs in one region only or ▪ UMN signs in one region only and LMN signs defined by EMG criteria in at least two limbs, but only when mimics are excluded
Possible MND ▪ presence of UMN and LMN signs in one region only or ▪ presence of UMN signs in two or more regions only or ▪ presence of LMN signs found above the UMN signs

The EEDC were based on the originally proposed criteria by Lambert. However the category clinically probable-laboratory supported MND is included in EEDC. In the EEDC, fasciculations on electromyography (EMG) are not considered as evidence of LMN dysfunction (Brooks, 2000). This, despite the fact that Lambert had previously stated that "The EMG discloses fasciculations so regularly in ALS that one rarely accepts the diagnosis unless fasciculations are present" (Lambert, 1969).

The diagnosis of MND requires evidence of LMN and UMN degeneration as well as progressive spread of symptoms or signs within a region or to other regions. The latter is ascertained by an extensive history and a thorough clinical, electrophysiological and, ideally,

neuropathological examination. There must be an absence of electrophysiological and pathological evidence of mimics of MND.

Clinical neurophysiology tests are regarded as equivalent to clinical signs in diagnosing MND. Neurophysiology tests are important in making an early diagnosis, to monitor disease progression, and may be used in future to monitor responses to treatment. The core electrophysiological features of MND are based on: needle EMG, which assesses LMN involvement; nerve conduction studies (NCS), to asses axonal degeneration and to exclude mimics of MND, and transcortical magnetic stimulation (TMS), to asses UMN involvement. The latter, however, is not used widely by neurologists and neurophysiologists. The EEDC electrophysiological criteria for the diagnosis of MND are summarised in table 4 (Miller, 1999).

The muscles recommended for needle EMG evaluation are: bulbar muscles, facial muscles, tongue muscle, masticator muscle, thoracic paraspinal muscles and rectus abdominis muscles. The EEDC has limitations: the EMG findings and clinical findings can't be in the same limb and fasciculations are not considered as active denervation. This can lead to a delayed diagnosis in a patient suspected clinically of having MND. In December 2006, the International Federation of Clinical Neurophysiologists (IFCN) met on Awaji Island, Japan to discuss the EEDC limitations, and to discuss how to use neurophysiology to facilitate earlier diagnosis (Desai, 2000). Their conclusions have been published (De Carvalho, 2008), and have become known as the "Awaji Criteria". They are based on the El Escorial criteria (Dengler, 2010), with a few modifications. The fasciculation potentials in the Awaji criteria are considered a sign of "active denervation" in the absence of fibrillations and positive sharp waves and in the right clinical context. Secondly, the EEDC category "probable MND-laboratory supported" was removed from the Awaji classification, as it regarded the signs of denervation on EMG as equivalent to clinical signs of LMN dysfunction (Dengler, 2010). These new criteria consider patients with no fibrillations or positive sharp waves in muscles, but with fasciculations and chronic neurogenic changes, as having LMN dysfunction. This method would improve sensitivity and allows an earlier diagnosis. A study by Shaw *et al*. concluded that "the new criteria allow early diagnosis of MND, without increasing the false positive rate" (Shaw, 2010). Other studies comparing the EEDC and the new Awaji criteria also suggest an improved sensitivity from 28% to 61% with no change in specificity, which remains at 96 % (Noto, 2012). The new criteria classify MND patients into three categories: clinically possible, clinically probable and clinically definite (Duleep, 2013). The Awaji

criteria are summarized in table 3. They have not yet been widely used or accepted by neurophysiologists and neurologists (Chen, 2010).

Table 3: Awaji diagnostic criteria for MND (Shaw, 2010)

Clinically definite MND • presence of both lower and upper motor neurone signs in at least 3 body regions.
Clinically probable MND • presence of both upper and lower motor neurone signs in at least 2 regions.
Clinically possible MND ▪ presence of UMN and LMN signs in one region only or ▪ presence of UMN signs in two or more regions only or • presence of LMN signs found above the UMN signs

The use of neuro-radiology in the diagnosis of MND has been mainly to exclude potentially treatable diseases. Its use is complex and expensive (Kalra, 1999).

However, the magnetic resonance imaging (MRI) technique of diffusion tensor imaging (DTI) has recently gained prominence as a non-invasive method that might provide valuable information about UMN dysfunction (Agosta, 2010). It can be used to identify UMN involvement and may even predict disease duration in ALS (Wang, 2006). A number of DTI studies have been performed in ALS (e.g. Sage, 2007), often with tentative conclusions. A recent meta-analysis of a number of case-control studies using DTI suggests that the diagnostic accuracy of DTI, taken in isolation, lacks sufficient discrimination (Foerster, 2013). More studies in this area are needed and are likely to follow.

Table 4 : Electrophysiological evidence for the diagnosis of MND (EEDC) (Miller, 1999).

Signs of active denervation • fibrillations • positive sharp waves
fasciculations
Signs of chronic partial denervation • motor unit potentials of increased duration, amplitude and high proportion of polyphasia. • reduce interference pattern, usually with high firing rates e.g. higher than 10 Hz. • unstable motor unit potentials. • chronic partial denervation could also be demonstrated by other techniques, e.g. single fibre electromyography (SEFMG), macro electromyography (macro EMG), turns-amplitude analyses, quantitative motor unit potentials (MUP) analyses and motor unit number estimation (MUNE).
Features compatible with UMN involvement • low firing rates of motor unit potentials on effort. • $\geq$ 30% increase in central motor conduction time.
Features suggesting other disease processes • evidence of motor conduction block. • motor conduction velocity lower than 70% of lower limit of normal. • distal motor latency greater than 30% above the upper limit of normal. • abnormal sensory nerve conduction studies except in the presence of entrapment syndromes or co-existing peripheral nerve disease. • F-waves or H-reflexes latencies more than 30% above established normal values. • decrements greater than 30% on repetitive stimulation. • somato-sensory evoked potentials (SSEP) latency greater than 20% of established normal value. • significant abnormalities on autonomic functions or electronystagmography. • full motor unit potential interference pattern in a clinically weak muscle.

2.4.2. The clinical features of MND

In 1824, Bell (Kazi, 2004), Aran, Duchenne and Cruveilher made important observations that contributed to the understanding of the clinical and pathological syndrome (Aran, 1850 ;Duchenne, 1851 & Cruveilhier, 1853). However, it was Jean Marie Charcot (Charcot, 1869 & 1874) who solved the puzzle and published the frst clinico-pathological description of the disease.

The disease may be divided according to the first presenting symptoms into classical presentations: spinal onset, bulbar onset and a very rare respiratory onset. Furthermore, there are MND subtypes or variants, such as progressive muscular atrophy (PMA), "flail arm" and "flail leg" variants (Hu, 1998), and primary lateral sclerosis (PLS) (Gordon, 2006). Differentiation between variant phenotypes and the typical or classical phenotype is important with regards to prognosis and survival.

Table 5: MND presentations

Classical phenotype	**Variant phenotype**
Spinal onset	Progressive muscle atrophy
Bulbar onset	Primary lateral sclerosis
Respiratory onset	Flail arm and flail leg

The spinal onset is the most common presentation and occurs in 70% of patients. Typical symptoms include tripping, dragging of the foot and difficulty in tying buttons, turning keys or performing fine motor tasks. The onset is insidious, and it may start distally or proximally in the arms or the legs. Weakness is asymmetrical and occasionally exacerbated by cold. Rarely, patients can present with fasciculations, cramps or muscle atrophy years before the onset of weakness. Gradually these symptoms and signs spread to other regions such as the bulbar and respiratory muscles. The physical examination reveals focal muscle atrophy (figure 4), fasciculations visible in more than one muscle group, spasticity noted by increase tone and a supinator catch in the upper limbs, with patellar catch, sustained clonus, and hypertonia, in the lower limbs. Deep tendon reflexes are pathologically brisk, Hoffman sign may be positive and plantar responses are often extensor.

The bulbar onset occurs in 25% of patients. They present initially with dysarthria of speech, with dysphagia for solids and liquids occurring later. Limb involvement can occur later. The

clinical presentation is that of slow slurred speech or a nasal quality of voice, a brisk jaw jerk and an upper motor neurone- type facial weakness affecting the lower half of the face leading to difficulty in sealing the lips or blowing the cheeks. The gag reflex is brisk and the soft palate is weak. The tongue is wasted and slow in movement. The rest of the cranial nerves are normal. With the disease progression, patients usually develop the characteristic picture of a combination of UMN and LMN signs coexisting within the same CNS region and affecting bulbar, cervical, thoracic and lumbar areas (Wijesekera, 2009).

About 5% of cases of MND present with respiratory weakness, predominantly with type 2 respiratory failure i.e. dyspnoea, orthopnoea, disturbed sleep, morning headaches, excessive day time sleep, anorexia, decreased concentration and mood changes (De Carvalho , 1996 & Leigh, 1994).

MND is a slowly progressive disease. 50% of patients die within 30 months of symptom onset and about 20% of patients survive between 5 to 10 years after onset. Older age at symptom onset, early respiratory muscle dysfunction, and bulbar onset disease are associated with reduced survival, whereas limb onset disease, younger age at presentation, and longer diagnostic delay are associated with prolonged survival.

The less common variants of MND have clinical presentations, rates of progression and survival better than classical MND.

The syndrome of progressive muscular atrophy (PMA) accounts for 5 to 10% of patients. A LMN presentation of limb-onset MND without UMN or bulbar signs has a slowly progressive course, one that can sometimes be as long as 20 to 30 years. 50% of patients may develop a typical MND picture (Visser, 2008).

Primary lateral sclerosis (PLS) is a clinically progressive upper motor neurone syndrome with limb and bulbar dysfunction. It accounts for 2% of MND cases. It has a presentation 5 to 10 years earlier than classical MND, with an age of onset of 50 years. Legs are affected before arms, with symmetrical or asymmetrical spasticity, brisk reflexes and extensor plantar responses. Pseudo- bulbar palsy is common. There are no LMN signs but muscle atrophy can occur later in the disease (Kim, 2009). The disease is slowly progressive with a survival of up to 30 years. It has a better prognosis than classical MND. For this reason the diagnosis should only be made after the disease has been present for at least 3 to 4 years. Mimics of MND needed to be excluded (Kim, 2009).

In the flail arm variant (also known as the Vulpian-Bernhardt syndrome or brachial amyotrophic diplegia) the presentation is primarily of a LMN syndrome (Katz, 1999). The physical examination reveals weakness and wasting affecting the proximal upper limbs in a symmetrical pattern. This leads to severe wasting around the shoulder girdle with the arms hanging flaccidly on either side; hence the name brachial amyotrophic diplegia. The reflexes are depressed or absent, but reflexes on unaffected limbs are normal or brisk. The normal limbs remain strong for some years but eventually spasticity and wasting develops.

In the flail leg variant also known as the pseudo-polyneuritic form of MND, patients usually present with symmetrical LMN signs in the legs. The arms are normal. These two variants show slower progression than other forms of MND.

There are very rare atypical presentations of MND that occur years before the core features of the disease develop. These include weight loss, cramps, fasciculations, emotional liability and frontal lobe cognitive dysfunction (Lillo, 2011).

Cognitive symptoms and dementia were previously reported as uncommon symptoms of MND, but it is now recognized that patients with MND may exhibit a range of cognitive abnormalities (Lillo, 2011). 5% of patients have a frank fronto-temporal lobe dementia (FTLD) and 20-40% probably has an impaired frontal executive function. The latter presents clinically with judgment problems, impulsivity and impaired verbal fluency (Lillo, 2011).

The hallmark of the diagnosis of MND is progression of the disease to involve contiguous body parts. This results in increasing disability and eventually the patient becomes bed-bound with respiratory compromise. Death becomes imminent.

It was thought that MND only affects the motor system, sparing the sensory system and sphincters. However many studies have reported involvement of other regions of the nervous system; actually MND is a multi-system degenerative disease in which motor neurones are affected first and bear the brunt of the pathology (Shaw, 2005).

2.4.3. The mimics of MND

MND is currently irreversible and clinically heterogeneous. However, there are some MND-like presentations, some of which are treatable and do not carry such a grave prognosis

(Turner, 2013). There are key signs on examination that, in the right clinical setting, should make us consider or suspect MND. These signs are fasciculations, especially in the thoracic and abdominal muscles, bilateral wasting of the tongue, preferential wasting of the lateral border of the hand (split hand), head drop, pathological crying and cognitive or behavioural impairment, predominantly of the fronto-temporal type (Turner, 2013).

Mimics can be grouped into those presenting with either LMN or UMN signs or those with mixed signs. The mimics of MND are summarised in table 6.

Table 6: Mimics of MND (Turner, 2013)

Predominant signs	Mimic disorders
LMN	• benign fasciculations • multifocal motor neuropathy with conduction block • neuralgic amyotrophy • Kennedy's syndrome • motor-predominant chronic inflammatory demyelinating polyneuropathy • Inclusion body myositis
UMN	• hereditary spastic paraparesis • primary progressive multiple sclerosis
Mixed	• cervical myeloradiculopathy

2.5. Pathogenesis and risk factors

2.5.1. Pathology

As mentioned before, the EEDC helps with the clinical diagnose of MND, but an absolute diagnosis can only be made post-mortem. The major pathological features of MND are degeneration of LMN groups in the spinal cord, brainstem, and UMNs in the motor cortex with intra-neuronal inclusions in degenerating neurones and glia.
Macroscopically, atrophy of the spinal cord, the precentral gyrus of the brain and anterior motor roots is seen, with loss of the normal cervical and lumbo-sacral enlargements of the cord (Rowland, 2001 & Shaw, 2010).

Figure 6 A normal spinal cord is shown compared with the cord of a patient with ALS to highlight the difference in size of the nerve roots. The atrophy is evident in the ALS cord. (Source): (The University of Utah Eccles Health Sciences Library, 2013)

Microscopically there is degeneration of Betz cells in the motor cortex (Brodman area 4), with reactive gliosis of the motor cortex and cortico-spinal tract. In the brain stem, there is loss of motor nuclei with sparing of the extra- ocular nuclei and motor nucleus of Onuf in the S2 spinal segment. The cytopathology of the degenerating neurones shows Bunina bodies, Skein-like inclusions, round hyaline inclusions and basophilic inclusions (Leigh, 1994).

Bunina bodies (Figure 7) are small eosinophilic hyaline intracytoplasmic inclusions that stain positive for cystatin and transferrin. These inclusions are seen in 95% of classical and variant

ALS, particularly in LMNs. The Bunina bodies are not pathognomonic of MND, as they are found in brains of older people (Kusaka, 1999). The round hyaline inclusions are argyrophilic inclusions seen in spinal cord motor neurones. They stain for phosphorylated and non-phosphorylated neuroflaments. They are associated with fMND and are rarely seen in sporadic MND. Skein-like inclusions (figure 7) are ubiquinated. They are most commonly found in MND. The exact composition of these inclusions is not known. They are mostly found in LMNs of the spinal cord and brain stem, and in cortico-spinal UMNs (Kawashima, 2000).

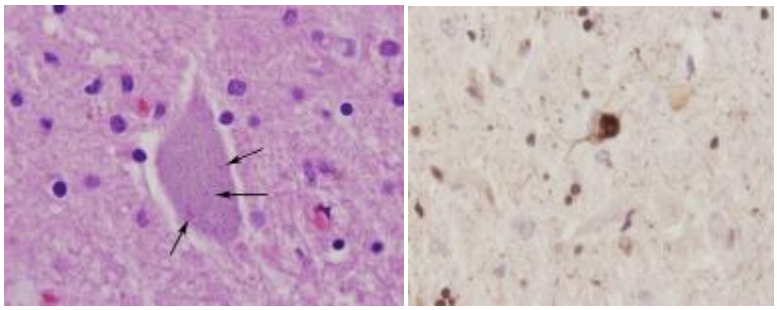

Figure 7 intra-neuronal inclusions (Buninas bodies, arrows) hematoxylin and eosin stain, x400 and skein-like inclusions/ubiquitin immunostain x200 (source) : (The University of Utah Eccles Health Sciences Library, 2013).

MND variants seem to share common pathological features with classical MND, through the findings of ubiquinated inclusions that are common to PLS, flail arm syndrome, and Guam motor neurone disease-Parkinson dementia complex (Guam MND-PDC).

2.5.2. Pathogenesis

We have limited understanding of the mechanisms underlying the pathogenesis of MND. However, several hypotheses have been proposed. These are summarised in table 7. These factors alone or in combination may lead to the cell death of motor neurones that resembles the process of apoptosis (McDermott, 2008).

Table 7: Pathogenic mechanisms that may contribute to MND (McDermott, 2008)

- genetic factors
- oxidative stress
- protein aggregation
- glutamatergic toxicity
- mitochondrial dysfunction
- impairment of axonal transport
- dysfunctional signaling pathways
- inflammatory cascades/contribution of non-neuronal cells

2.5.3. Aetiology and risk factors

The aetiology of MND has been a controversial issue for many years. Several associated risk factors for MND have been postulated (Chiò, 2009). For an easier understanding of the aetiology of MND, I shall classify the causes as being genetic or acquired (Wijesekera, 2009). The acquired causes are summarised in figure 8.

Genetics causes of MND

As previously indicated, MND is sporadic in 90 to 95% of cases and familial in 5 to 10%. Inheritance in familial MND is usually autosomal dominant, with rare exceptions being reported as autosomal recessive and X-linked inheritance (McDermott, 2008). The most commonly occurring gene for fMND is the ALS1-copper-zinc superoxide dismutase (Rosen, 1993). So far more than 6 dominantly inherited, adult-onset MND genes have been reported. The copper-zinc superoxide dismutase (SOD1) gene is situated on chromosome 21q22.1. SOD1 is a free radical scavenger enzyme; it is a metallo-enzyme of 153 amino acids. Its function is to convert intracellular superoxide free radicals (a toxic waste product of mitochondrial oxidative phosphorylation) to hydrogen peroxide, thus preventing the generation of reactive oxygen species (McDermott, 2008). SOD1 is widely distributed in the CNS, accounting for 1% of the protein in the brain. It is localized in the cytosolic compartment of the cells and in the inter-membrane space of the mitochondria. The

mechanism by which SOD1 leads to motor neurone death has not been fully elucidated, but it is believed that the mutant SOD1 protein leads to a toxic gain of function in the enzyme (Andersen, 2003). The exact mechanism of the toxic gain function has not been fully defined yet, but many other pathophysiological processes may be involved such as oxidative stress, mitochondrial dysfunction, protein aggregation and inflammation (Hadano, 2001). Different SOD1 mutations cause different syndromes. These differ with regards to age of onset, penetrance, SOD1 activity of erythrocytes, survival and clinical manifestations (Bruijn, 2004).

Of the other known genetic mutations, the transactive response DNA binding protein (TARDBP), fused in sarcoma (FUS), angiogenin gene (ANG) and optineuron (OPTN) gene mutations can all give rise to MND clinical syndromes (Vance, 2009). Mutations in SOD1 account for 20% of fMND and 5% of apparently sMND. Mutations in TARDBP account for 5 to 10 % of fMND (Kabashi, 2008). Mutations in FUS account for 5% (Vance, 2009), and ANG for 1% of fMND (Kiernan, 2011).

Acquired causes of MND

An environmental aetiology has been the cause of many debates amongst researchers, since the disease was first described (Mitchell, 2000). Various environmental risk factors for MND have been suggested; however none of them has been directly proven to be a cause of MND.

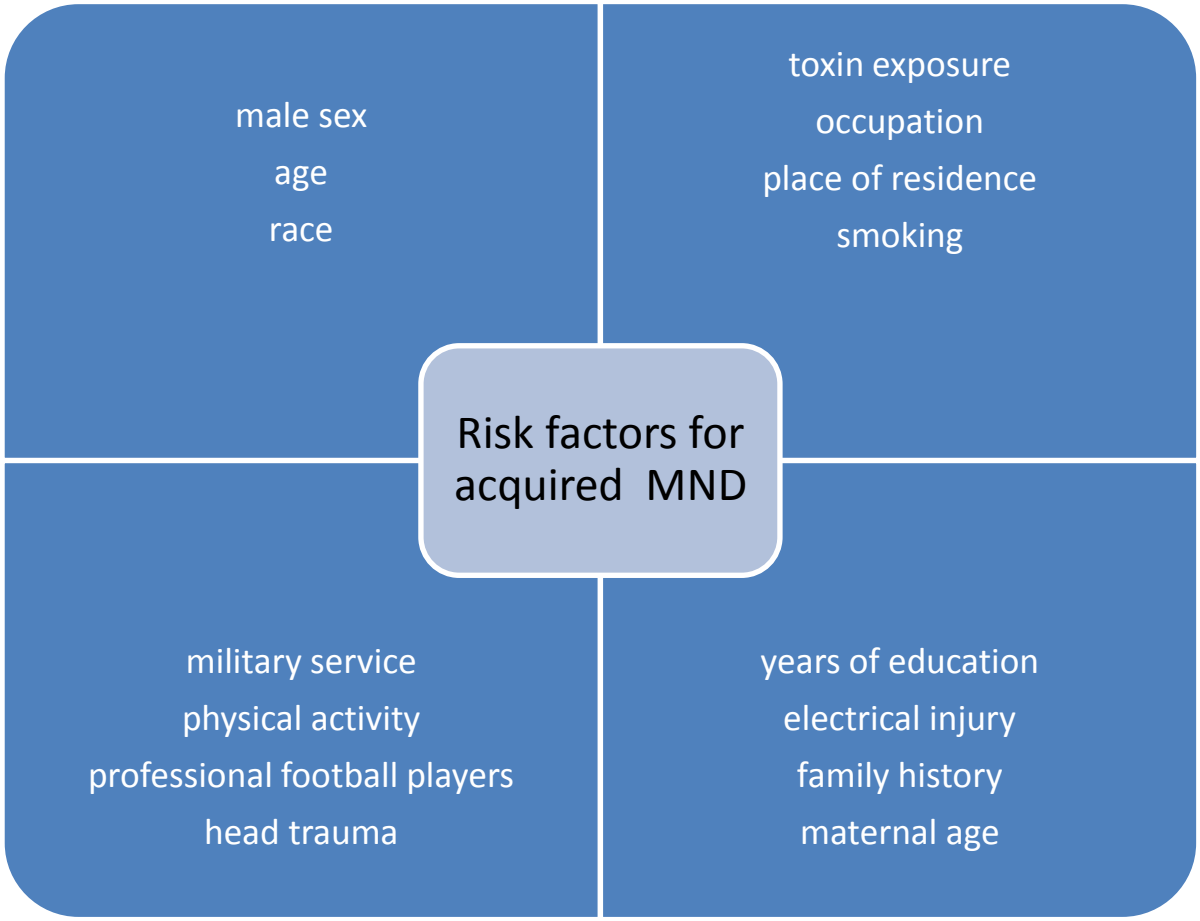

Figure 8 Risk factors for acquired MND

A number of epidemiological studies have assessed the associations between cigarette smoking, physical exertion, head microtrauma, diet, age, and male gender. The results, however, have not been consistent (Armon, 2003). A case-control study conducted by Pupillo *et al* to assess trauma and the risk of MND showed that previous trauma, repeated trauma and severe trauma may be risk factors for MND. He defined traumatic events as accidental events causing injuries requiring medical care (Pupillo, 2012). Savica *et al* conducted a community-based study, to evaluate if high school football players would have a higher risk of developing neurodegenerative diseases in later life (dementia, Parkinson's disease and MND). They found no increased risk of MND among 438 football players who played football between 1946 to 1956 (Savica, 2012). Another study of footballers in the Italian football series A (first division) showed a disproportionate number of MND patients. Either the physical activity of the sport, the trauma associated with it, or the fertilisers and toxic substances used to prepare the football pitches have been speculated to account for this association (Chio, 2005). A review by Armon in 2003 stated that the evidence did not support trauma, physical activity, residence in rural area and alcohol consumption as risk factors for

MND. However, it did confirm an association with exposure to cigarette smoking and a family history of MND. Exposure to lead and agricultural chemicals were possible risk factors. A large prospective study published in 2004 stated that there was limited evidence that current cigarette smoking might be associated with increased death rates from MND in women but not in men (Weisskopf, 2004). Another study also supported the findings that smoking increased the risk of MND and worsened survival in women but not in men (Alonso, 2010). A large population-based control study conducted from 1990 to 1994 in three counties of Western Washington State found that cigarette smoking was a risk for MND (Nelson, 2000).

In 1972, Kurland suggested an association between MND and ingestion of the seeds of *Cycas micronescica*. Spencer *et al*. were the first to test this hypothesis experimentally (Spencer, 1987). They found that monkeys fed large quantities of cycad seeds developed symptoms similar to those of the MND-dementia and Parkinsonism complex of Guam. Cox *et al*. observed that people in Guam consumed two species of flying foxes (bats). These bats were consumed especially during certain celebrations (Cox, 2003). The bats themselves ingested cycad seeds, thus lead to an accumulation of β-methylamino-L-alanine (BMAA) in their tissues in higher concentrations than in the plant. This led to a suggestion that consumption of these bats might be a risk factor for developing MND-PDC complex in Guam. BMAA is a neurotoxic non-proteiogenic amino acid produced by cyanobacteria isolates. Cyanobacteria, also called blue-green algae, are bacteria that obtain their energy through photosynthesis. Cyanobacteria can be found in oceans, fresh water, damp soil, deserts, rocks and hot springs. Cyanobacteria produce a variety of bioactive substances and toxins (neurotoxins, cytotoxins, hepatotoxins). The cyanobacteria species that produce neurotoxins are *Anabaena, Microcystis, Oscilatoria, Nostoc*, *Anabaenopsis and Nodularia* species. Different toxins known to be produced by cyanobacteria include anatoxin-α, homoanatoxin-α, anatoxin-α(s), and saxitoxins. The BMAA neurotoxin produced by cyanobacteria of the genus Nostoc are mainly found in the cycad tissues and in contaminated water supplies (Banack, 2007). The concentration of protein-bound BMAA is greater than free toxin. BMAA present in the seeds and flour are eaten by many animals such as flying foxes, pigs and deers, leading to biomagnification up the food chain in Guam (Bradley, 2009). Cyanobacteria are distributed worldwide, so it is possible that many humans are exposed to small amounts of cyanobacterial BMAA. Protein-bound BMAA in human brains is a potential reservoir for chronic neurotoxins that could lead to motor neurone degeneration. However, the mechanism(s) by which this might occur are not yet known.

In Africa and particularly in South Africa, BMAA neurotoxin has been isolated from dams used for agricultural and recreational activities and as well from raw water sources for potable water production (Esterhuizen, 2011).

2.6. Management

There are no specific biochemical or pathological biomarkers for MND. The aim of electrophysiological, imaging and laboratory investigations is to exclude MND mimics or to provide electrophysiological support for the clinical diagnosis.

The low molecular weight microtubule- associated protein, tau, has recently been studied as a possible biomarker of MND. Tau is found primarily in axons. Its main function is to stabilize and to support polymerization of microtubules. Ryberg and Bowser (2008) reviewed 41 research studies in which a total of 49 proteins were analysed in the plasma, serum and CSF as potential biomarkers in ALS. They found conflicting results amongst the studies, which led them to suggest that "sporadic ALS cannot at present be considered a tauopathy". More recently, Grossman *et al*. (2014), using a cross-validation prediction procedure, found that the ratio of phosphorylated to total tau protein in the CSF might serve as both a sensitive and specific biomarker of ALS.

MND remains an incurable disease. However in the last two decades, its management has evolved rapidly. Symptomatic relief of MND is the mainstay of treatment especially for the respiratory and bulbar complications. A team of workers including a neurologist, specialist nurses, and an ICU specialist in respiratory complications, psychologist, dietician, physiotherapist and speech therapist should ideally be involved in the management plan and follow up of MND patients.

The treatment strategy of MND is aimed at:

- delaying progression of the disease and preventing further loss of motor neurones, especially in the early stages of the disease
- treatment to alleviate symptoms of the disease and to maintain a reasonable quality of life.

Riluzole is the only drug licensed to treat MND. Although the drug reduces glutamate-induced toxicity, its exact mechanism of action in MND is unknown. Riluzole prolongs the

survival by 2 to 3 months, but does not improve the quality of life. The symptomatic management includes treating hyper- salivation and drooling, spasticity, cramps, fasciculation, anxiety, depression, dyspnoea, pain, sleep disturbances, constipation, spasticity, emotional liability, communication difficulties and fatigue.

Management of dysphagia includes modification of food and fluid consistency, postural advice and parenteral feeding. A percutaneous endoscopic gastrostomy (PEG) placement is indicated for those who have symptomatic dysphagia or significant weight loss. Patients and their families should be suitable counselled regarding the benefits and risks of the procedure.

Symptoms of respiratory insufficiency may be subtle and develop insidiously. Patients may report dyspnoea, orthopnoea, morning headaches, fatigue, and sleep fragmentation, due to hypoventilation. Respiratory muscle weakness is an independent predictor of quality of life, and respiratory failure is the most common cause of death in MND patients. Non-invasive ventilator support maybe used to relieve symptoms of dyspnoea and to aid respiration when sleeping.

The ultimate goal of MND research is not only to halt neuronal degeneration, but also to restore the original structure and function of the motor nervous system (Donnelly, 2012). Stem cells that are capable of differentiating into different cell types are potential candidates for this restorative function. However stem cell technology is still at an early stage of experimental development and large scale clinical trials are likely to be a long way off (Silani, 2004).

Another promising approach to the treatment of MND might be through the use of antisense oligonucleotide (ASO) technology. Spinal muscular atrophy (SMA) is a genetic neurodegenerative disease of children and infants associated with mutations or deletions in the survival motor neurone 1 (SMN1) gene. ASO-based strategies have been used to block a regulatory element of a related gene called SMN2. The regulatory element is known as intronic splicing silencer N1 (ISS-N1). ASO, by blocking ISS-N1, is able to restore the function of SMN2 in such a way that it compensates for the abnormal SMN1 gene (Silvanesan 2013). This technique has already moved from experimental laboratory work to clinical trials. The ASO can be delivered intrathecally into humans and the first human clinical study of an antisense drug delivered in this way has already demonstrated excellent tolerability with predictable pharmacokinetics (Miller, 2013). This study should enable future studies of similar anti-sense drugs to be carried out in familial SOD1ALS, other genetic forms of ALS, as well as possibly sporadic MND (Federici, 2012).

2.7. Summary

MND is a rare neurodegenerative disorder that affects upper and lower motor neurones of the nervous system. It is a relentlessly progressive disorder with an average duration of disease from time of onset of symptoms to death of around 3 years.

MND is the third commonest neurodegenerative disorder after Alzheimer's and Parkinson's disease. The worldwide incidence of MND is said to be around 2 per 1000 000 per year with a prevalence of 6 per 100 000.

The cause of MND is unknown. Most cases are sporadic, with few familial inherited cases. Environmental factors such as potential neurotoxins, cigarette smoking, exposure to agricultural chemicals, and head trauma have been postulated as risk factors for acquired causes of MND, but conclusive evidence is lacking. The role of cyanobacteria and the BMAA neurotoxin they produce has become a recent subject of interest in the search for potential environmental factors. In South Africa there are environmental programmes to monitor BMAA toxins in the water.

Little is known about the incidence and prevalence of MND on the African continent.

Chapter 3: Aims and hypotheses

Aims:

1. To characterise the cases of MND seen at GSH from 2005 to 2010 with respect to :

    - Age of onset of the disease
    - Duration of symptoms
    - Sex
    - MND type / form of disease
    - Possible exposures to toxins
    - Place of residency
    - Creatine phosphokinase plasma levels
    - Erythrocyte sedimentation rate (ESR), white cell count (WCC), cerebrospinal fluid (CSF) protein concentration

2. To compare the demographic characteristics of our patients with those reported in the literature.

Hypotheses:

1. The age of onset in MND is earlier in the Western Cape population compared with other population studies. Also, black African patients have a younger age of onset of MND than populations of European ancestry.
2. There is a male preponderance of the disease.
3. A disproportionate number of cases is seen from the West Coast region compared with others areas of the Western Cape.
4. Younger onset disease has a shorter duration of symptoms to death.
5. The age of onset of familial MND is younger than sporadic MND and the disease duration is shorter.
6. Bulbar onset disease has a shorter survival compared with limb-onset disease.
7. Occupations that involve exposure to agricultural and other chemicals will be disproportionately represented in the MND patients.

8. CPK levels will be higher in MND patients than normal control laboratory values.
9. High CPK levels will be associated with faster disease progression and time to death.
10. Both peripheral blood and CNS inflammatory markers will be raised in MND patients.
11. Cigarette smoking is associated with an increased risk of MND.

Many of these hypotheses are based on previously reported findings or putative associations suggested in the world literature. Hypotheses 1 and 3 are based on anecdotal observations by clinicians in our hospital but they require a more rigorous examination.

This pilot study will serve as the basis for future larger prospective studies on MND prevalence and aetiology in South Africa. It may be interesting to explore the question of the role of cyanobacteria in the disease, especially if geographic clustering of cases is observed.

Chapter 4: Methods

Patients included in this study were identified from the database of patients diagnosed with MND in the Division of Neurology, Department of Medicine, University of Cape Town (UCT) and Groote Schuur Hospital (GSH) from 2005 to 2010. This retrospective folder review study was approved by the Human Research Ethics Committee (HREC) of the UCT and GSH (HREC no 451/2013) (appendix 2). Permission to review the clinical notes was granted by Dr. B. Patel, medical superintendent of GSH (appendix 3).

4.1. Data collection

Data were collected from the clinical records. These were searched using the ICD5 code G12.0 and ICD.10 code G12.2 for MND. The search was conducted through the Bio-informatics Department at GSH, the main tertiary academic health care referral hospital in the Western Cape Province. Data were collected on an MND data sheet. These were based on my reading of the clinical records of the neurology clinic out-patient and in-patient admission notes.

Data collected included:

4.1.1. Clinical data

- date of birth
- date first seen
- date last seen
- age
- gender
- current residence
- occupational exposures
- smoking history
- alcohol history
- concomitant diseases
- family history of MND (at least one first degree relative)

- onset of first symptoms (from history notes)
- durations of symptoms (from year of onset of symptoms to year of death)
- date of death

The latter involved searching the data base of the registration of births and deaths of the Department of Home Affairs of South Africa. This was done through the South African Medical Research Council Burden of Diseases Unit.

I classified the cases at first presentation according to the El Escorial criteria (definite, possible and probable).

4.1.2. Laboratory data collected

1. Haematology data: full blood count (FBC) and erythrocyte sedimentation rate (ESR).

2. Biochemistry data: urea and electrolytes, thyroid functions, liver functions, glucose, venereal disease research laboratory (VDRL), human immunodeficiency virus (HIV), collagen screen, creatine phosphokinase (CK) and cerebrospinal fluid analyses (CSF).

3. Radiology data: Chest X ray and magnetic resonance imaging (MRI) of the brain and spine

4. Electromyography data: I read the reports of nerve conductions studies and electromyography looking specifically for:

- Associated neuropathies and conduction blocks.
- The presence of chronic neurogenic changes in affected and non-clinically affected muscles.

4.2 Statistical analysis

Most data were not normally distributed; therefore descriptive data are presented as means and interquartile range. Specific statistical tests used for the hypotheses testing are indicated in the results. Significance was set at the 0.05 level.

Chapter 5: Results

The raw data are included in appendix 1.

Forty-eight patients were identified with MND over a 5 year period at the Groote Schuur Hospital (01/01/2005 to 31/12/2010).

50 patients were identified using the hospital bioinformatics ICD code searches. However, 2 were excluded from the analyses: one had missing notes, and the other, on review, did not meet El Escorial criteria for the diagnosis of MND.

The clinical notes of 48 patients referred to Neurology Unit at GSH with suspected MND were reviewed. 29 (60.4%) patients were male, 19 (39.6) were female. There was an approximate 2:1 male to female ratio. The mean age of onset of symptoms was 54 years, the youngest patient being 30, and the oldest 70 years. The mean duration of illness (age at onset of first presentation to death) was 2 years, with a range of less than a year to 10 years. All cases reviewed had MND, the commonest form was limb onset (38 patients); bulbar onset was seen in 7 patients, and PMA was seen in 3 patients. The mean age of death was 56 years, with the youngest patient at 30 and the oldest patient at 79 years. 8 (16.7%) patients were "white", 11 (22.9%) were black African and the majority of patients in this study were of mixed ancestry 29 (60.4%). The descriptive data are summarised in table 5.1.

Using the revised El Escorial diagnostic criteria (EEDC), 15 (31%) patients had "clinically definite" diagnosis of MND, 24 (50%) patients had a "clinically probable" MND and 9 (19%) patients had "clinically possible" MND.

Table 8 Demographic characteristics and MND subtypes of 48 patients included in the study.

Characteristics (n)	**n=48 (100%)**
Male sex, n(%)	29 (60.4)
Population classification, n (%)	
"White"	8 (16.7)
Mixed ancestry	29 (60.4)
Black African	11 (22.9)
Age of onset of symptoms (years), median (IQR)	54.0 (47.5-63.0)
Age of death (years), median (IQR)	56.0 (47.0 – 65.0)
Duration of disease (years) (age of onset of first symptoms to age of death), median (IQR)	2.0 (1.0 – 3.0)
Subtypes of MND (n, %)	
ALS	48 (100)
Bulbar	38 (79.2)
PMA	7 (14.6)
PLS	3 (6.3)
EEDC	
Clinically definite	15 (31.0)
Clinically probable	24 (50.0)
Clinically possible	9 (19.0)

Hypotheses (H1 to H11)

H1: **the age of onset of MND is earlier in the Western Cape population compared with other population studies. Also, black African patients have a younger age of onset of MND than populations of European ancestry.**

Our study: median age of onset in years (IQR) 54 (47.5 – 63.0)

We do not have data from any other region of Africa and therefore we could only compare our results with that published in the international (mostly European and North American) literature. The reported mean age of onset for sMND in the international literature varies between 55 and 65 years.

Table 9 The age of onset of disease and duration of symptoms amongst population groups.

Population classification		Age of onset of Symptoms (Years)	Duration of Symptoms (Years)
"White" [n=8, 16.7%]			
	mean	58.8	3.6
	min-max	50-70	1-7
	IQR 25th-75th	51.5-67.8	2-5.8
Mixed ancestry [n=29,60.4%]			
	mean	56.3	3.6
	min-max	31-74	1-11
	IQR 25th-75th	51-65.5	1-4.5
Black [n =11, 22.9 %]			
	mean	45.9	2.3
	min-max	30-73	1-5
	IQR 25th-75th	34-59	1-4

For testing these hypotheses, a Kruskall Wallis statistic test was used, which showed no significant statistical difference in the duration of symptoms compared by population groups ($Chi^2 = 1.902$, $d.f = 2$, $p = 0.39$).

For testing differences in the age of onset, a Kruskall Wallis statistic test also showed that there was no statistically significant difference in the age of presentation by population group (Chi^2 *=5.742, d.f= 2, p=0.057).* However the latter *p* value is just over the limit of the statistic significance (*p<0.05*). Patients classified as "Black" had a lower mean age of onset compared with other groups, and this difference came close to being significant.

H2: there is a male preponderance of the disease

The proportion of males in the Western Cape is 48.98% (Wikipedia, 2014). In a sample of 48 MND patients there were 29 males or 60.4%. To test if the proportion of males in the MND sample is higher than expected I used the formula $z = (p'-p)/\sqrt{(p\ (1-p)/n)}$. In this case, z = 1.583 and p = 0.0567. This p value is only just above the 5% significance level. 5% significance means that the probability of a chance result is 5% or less. This means that the hypothesis that MND sufferers are more likely to be male was not proven. However, there was a tendency for a male preponderance that came close to significance (p=0.056).

H3: a disproportionate number of cases is seen from the West Coast region compared with other areas of the Western Cape

The population of the Western Coast region of the Western Cape is 6.73% (Western Cape Government, 2014). Three (3) of the sample of 47 Western Cape MND sufferers were from the West Coast area, or 6.383%.

In this case, z = -0.0973 and p = 0.46. This p value was well above the 5% significance level. That means that MND sufferers who presented to our hospital were not likely to come disproportionately from the West Coast region of the Western Cape.

H4: younger onset disease has a shorter duration of symptoms to death

To test these hypothesis two different methods were used:

1. The Pearson Product Moment correlation between age of onset of MND and the duration from first symptoms and death.

   N = 41, R = -0.12 and p>0.05.

2. Since the duration of disease symptoms was not normally distributed, I used a Spearman rank correlation.

   r = -0.25 p = 0.113

   This means that it is more than 5% likely that the correlation is due to chance. So I have to reject the hypothesis that there is any association between the age of onset of MND and the disease duration. H4 is therefore not proven.

H5. The age of onset of a familial MND is younger than sporadic, and the disease duration is shorter.

This hypothesis was tested using the t-statistic for differences between means. The mean age of onset for the familial group (n=5) was 55.8 and for the sporadic group 54.21. The t-statistic was t=0.361, degrees of freedom 46 and p>0.25. This means that familial MND was not associated with earlier onset of symptoms than sporadic MND. The age of onset of familial MND was not younger than that of sporadic MND.

Table 10 Comparison between ages of onset and disesase duration in fMND *vs* sMND

Comparison	U (value)	p (value)
fMND *vs* sMND	55	0,416

Table 11 Disease duration and age of onset of symptoms in sporadic *vs* familial MND

Form	***n***	**Age of onset of symptoms Mean±SD**	***n***	**Duration of disease (years) median±IQR**
sporadic	43	54.0 ± 12.2	37	2 ± 1
familial	5	55.8 ± 8.9	4	1.5 ± 1

H6. Bulbar onset disease has a shorter survival compared with limb-onset disease.

Table 12 Comparing age of onset of different subtypes of MND

Comparison	t	Df	p Value
1 *vs.* 2	-1.00	45	0.322
1 *vs*. 3	-0.42	37	0.673
2 *vs.* 3	0.128	3	0.906

1=ALS 2= bulbar 3= PMA

The age of onset of disease was not significantly different between the 3 subtypes of MND.

Table 13 Comparing duration of symptoms to death: Mann-Whitney U test (data not normally distributed)

	U value	p Value
1 *vs.* 2	23.5	0.364

There was no significant difference between the duration of the disease in the ALS *vs* the bulbar patients. There were only three patients with PMA, and one was still alive at the time of writing. The age of onset for the surviving case was 59, and he had survived at least 5 years. The PMA subtypes were not statistically compared due to the small number of cases (only 3). Although statistical tests do not suggest differences in survival of patients per subtypes of the disease, the small sample size may have limited the power for proper inferences by statistical analysis. Thus, the hypothesis under study, while not proven, needs further investigation.

H7. Occupations that involve agricultural chemicals and other chemical industries will be disproportionately represented in the MND patients

According to government statistics 6.7% of Western Cape adults are employed in agriculture, forestry and fisheries, and 6.7% in manufacturing (of whom 20% work with chemicals) (Western Cape Government, 2014). So the overall percentage of the adult population exposed to chemicals in their work is 8.05%. In the MND sample 9 of the 48 (18.75%) worked in comparable industries.

In this case, $z = 2.725$ and $p = 0.0032$. This p value is well below the 5% significance level (and also the 1% level of significance). That means that we do not reject the hypothesis that work exposure to chemicals is associated with MND. We do have a disproportionate number of MND patients (relative to the general population), in the Western Cape who worked in the chemical and agricultural industries.

H8. CPK levels will be higher in MND patients compared with normal control laboratory values

Normal levels of CPK are regarded as being between 26-140 IU/ml in males and 38-174 IU/ml in females (The National Health Laboratory Service (NHLS), 2014). The mean CPK level of this MND sample was 230.6 (and standard deviation 204.9). None had a CPK level below 10 but 25 or 52.1% had a CPK level above 140.

I assumed that 95% of the population fell within normal ranges. To test this hypothesis I compared the proportion of the MND sample that fell within the normal range with the expected 95%.

In this case, $z = 14.97$ and $p<0.0001$. That means MND is associated with higher than normal CPK levels. H8 is therefore proven overall; MND is associated with higher CPK levels compared with the normal control population.

H9. High CPK levels will be associated with faster disease progression and time to death.

This hypothesis was tested using the Pearson Product Moment correlation between CPK levels and duration of MND.

N = 41, r = 0.21 and $0.05<p<0.10$. The p value is above 5% but below 10% so the probability of the association being due to chance is between 5% and 10%. Accordingly, the likelihood that CPK levels are associated with the duration of MND is weak. Also the direction of the correlation is in the opposite direction to that proposed in the hypothesis i.e. if anything, higher CPK levels are weakly associated with longer durations of MND. H9 is not proven and the inverse, in fact, maybe true.

H10. Both peripheral blood and CNS inflammatory markers will be raised in MND patients

Table 14 Inflammatory CNS markers

Blood marker	**Distribution** Skewed (s)/normal (n)	**N=48** Mean ± SD	**NHLS Lab reference range**
CPK	S	230.6 ± 204.9	Male: 26-140 IU/ml Female: 38-174 IU/ml
WCC	S	68.0 ± 4.20	$4\text{-}10\times10^{9}$/l
ESR	S	0.0 ± 13.3	Male: 0-10 mm/hr Female: 0-15mm/hr
CSF protein	S	0.36 ± 0.31	0.15-0.45 g/l

Normal ESR levels are 0 -10 for male and 0-15 mm/hr for females. 16.7% of the MND sample had ESR levels above 20. In this case, z = 3.719 and p=0.0008. That means that the MND sample tended to have higher than normal ESR levels.

Normal WCC levels are 4 to 11 × 10^9/l. 2.1% of the MND sample had WCC levels below 4 and 14.6% had WCC levels above 11. In total 83.33% of the MND sample fell with the normal WCC range. In this case $z = 3.719$ and $p=0.0008$. This means that the MND sample has WCC levels outside the normal range – some too low and more too high.

Normal CSF protein levels are 0.2 to 0.45g/l. 20.8% of the MND sample had CSF protein levels below 0.2 and 27.9% above 0.45. Therefore 51.3% of the MND sample had normal CSF protein levels. In this case $z = 13.9$ and $p<0.0001$. This means that the MND sample frequently had CSF protein levels outside the normal range – both too low and too high.

H11. Cigarette smoking is associated with an increased risk of MND.

In the Western Cape, 44.7% of the men and 27% of the women smoked (Chopra, 2007). In the MND sample 75.9% of the men and 68.4% of the women smoke. In this case $z = 3.38$ and $p=0.0004$ for men, and $z = 4.065$ and $p<0.0001$ for women. This means that the MND sample have significantly higher smoking rates than expected, for both men and women.

Table 15 Results by hypothesis

Results by Hypothesis
H1: The age of onset of MND was the same as that published in the literature. There was no difference in the age of onset and duration of symptoms amongst various population "groups". However, it tended to be slightly younger in Black African patients.
H2: There was a male preponderance of the disease.
H3: There was no clustering of MND patients from the West Coast.
H4: There was no correlation between the age of onset of the disease and duration of symptoms to death.
H5: The age of onset of familial MND was not earlier than sporadic MND in my sample.
H6: There was no difference in the age of onset amongst MND subtypes.
H7: Working in the chemical and agricultural industries might be a risk factor for MND.
H8: MND patients had higher CPK levels, compared with those of the normal laboratory ranges.
H9: Higher CPK levels were not associated with faster disease progression.
H10: CNS inflammatory markers tended to be raised in some, and lower in other, MND patients, compared with the normally defined laboratory ranges.
H11: A higher proportion of patients with MND smoked, compared with the "normal" Western Cape population.

Chapter 6: Discussion

I set out the aims and hypotheses H1 to H11 in chapter 3. I shall review the descriptive data first, and then I shall discuss the specific hypotheses.

In this study, I described the demographic and clinical characteristics of cases of MND seen at GSH between 2005 and 2010. It was a retrospective study; I collected data from the patient's records. I compared data with worldwide published literature. Duration of symptoms was defined as time from first onset of symptoms to date of death. Time of onset of symptoms was based on the patient's and the patient relative's history. It was not the same as the time of first presentation to a doctor. A precise time of onset for MND is, of course, impossible to formulate. The onset of symptoms was rounded off to the nearest year. MND sub-type or form of the disease was classified according to the first symptoms with which the patient presented. This is the first study describing the demographic and clinical characteristics of 48 patients with MND in South Africa and probably the first well conducted retrospective study in the African continent.

Descriptive data and demographic characteristics

The mean age of onset of symptoms in my study was 54 years (IQR: 47.5 – 63.0). This was similar to studies published in the international literature (Logroscino, 2010). The mean duration of illness was 2 years, with a range of less than a year to 10 years. This finding does not differ much from that reported in the literature (Rowland, 2001). The commonest phenotype was limb onset (79%), followed by bulbar onset (15%) and then progressive muscular atrophy (6%). There were no cases of primary lateral sclerosis. However, the latter could be due to the fact that these cases would not have been recognised as MND sub-types in the first place. They may well have been regarded as cases of idiopathic myelopathy. I suspect that primary lateral sclerosis is an under diagnosed entity. Also, LMN signs might have evolved years later, after UMN signs had been present for several years.

In our study we found that the majority of patients presented with limb-onset MND subtype. In the literature it has been reported that the commonest form of MND subtype presentation is limb-onset. Our findings are similar to those reported in the literature.

The distribution of MND amongst different population "groups" in the Western Cape probable reflects the demographics of the region, where people of mixed ancestry

predominate. No particular group would appear to be disproportionately represented in the MND patients, relative to their expected proportions in the overall Western Cape population.

Using the EEDC, the majority of patients were classified as clinically probable MND. This finding may reflect the difficulty, in a retrospective study, of obtaining accurate information. I had to interpret the written clinical notes of doctors to be able to classify them, and some of them were not absolutely clear.

All the patients in the study were admitted to the Neurology Ward of Groote Schuur Hospital. Thus the diagnoses would have been made by experienced neurologists, backed up by electrophysiological investigations. It is unlikely that many cases would have been missed or mis-diagnosed in this specialist unit. It is, however, possible that cases seen in other clinics or other departments would not have been evaluated with the same diagnostic rigor. However, other departments are likely to have referred cases with unusual neurological presentations to the neurology unit. On balance, I think I would have captured the majority of MND cases at GSH in the time period 2005-2010.

Discussion of Hypotheses

H1: The age of onset of MND was not different in our Western Cape study compared with other populations.

Anecdotally, we thought that we were seeing many younger-onset MND cases at GSH. However, the statistical analyses showed that our mean age of onset (54 years; IQR: 47.5-63.0) was not different from that reported in the literature. A study in Nigeria in 2004 showed a mean age of onset of 38.6 years (Imam, 2004). However, many cases of spinal muscular atrophy may have been included in their study. Anecdotally, the neurologists also thought that they were seeing more young black African patients with early onset MND. The mean age of onset in "African" patients (45.9 years) was lower than that in patients with European ancestry (58.8 years). This result approached, but not quite reach, statistical significance ($p = 0.057$). With larger number of participants, we might have demonstrated a significant difference. These results might then support the findings from Nigeria that MND onset in black Africans is, on average, lower than that described in studies from Europe and North America. A population-based mortality study from Cuba found that disease rates were 60% lower in their population compared with European and North American populations (Zaldivar, 2009).

H2: There is a male preponderance of the disease

Worldwide studies have shown a male:female ratio ranging from 1, 5: 1 to 2.6:1. In my study, the ratio was much the same as that reported elsewhere. The reason for the male preponderance is not known. Perhaps males are more exposed to the occupational hazards that are associated with MND e.g. work in the chemical industries, and professional sportsmen. There is a variant of PMA (LMN motor neurone disease) known as Kennedy's syndrome or bulbo-spinal muscular atrophy, which is linked to a trinucleotide expansion repeat in the testosterone receptor gene (Grunseich, 2014). Hormonal influences may therefore also be important in explaining the gender differences in MND.

H3: The West Coast region was not disproportionately represented in my study.

Anecdotal evidence from neurologists has suggested that there might be clustering of cases in this particular area of the Western Cape Province. Our analyses showed that the proportion of patients from this area was consistent with the proportion of people from the Western Cape who live in the West Coast area. That is, they were not disproportionately represented in the MND patients. However, another important factor is the drainage area of the Groote Schuur Hospital. There is one other large academic hospital in the Western Cape with a tertiary neurology service: the Tygerberg Hospital in the Belville/Parow area. It probably drains a larger number of patients from this area. Ideally, we should perform a larger study that includes cases in the same period seen at both Groote Schuur and Tygerberg Hospitals. In so doing, we should then be able to derive true prevalence data of MND in the Western Cape. Another factor is the stated place of residence of the patients. This was taken from the stated address in the hospital folder. However, people migrate and there may be patients, who live in, say, the Mitchell's Plain district of the Western Cape now, who might have spent most of their lives in the West Coast. Again, in an ideal prospective study, we should ascertain not just their current address, but the places they lived in for the majority of their lives. These data will also be important if we wish to further examine the question of cyanobacteria in the environment and its possible association with MND.

H4: Younger onset disease was not associated with a shorter duration of symptoms.

The fact that younger onset disease was not associated with a shorter duration of symptoms to death has previously been reported by Li *et.al*. in 1988 (Li, 1988). Again, larger numbers might have shown this association, but our study did not support H4.

H5: The age of onset of familial MND was not younger than that of sporadic MND; nor was the duration of disease shorter.

We had 5 familial out of 48 cases i.e. patients with a first degree relative with MND. This is 10% of all our cases. A similar proportion of familial MND cases have been reported worldwide. Study by Li *et.al*. (Li, 1988) showed an earlier age of onset, and a shorter duration of disease, in familial compared with sporadic MND. We have not confirmed these

results. We did not perform any genetics studies on the familial cases to screen for known gene mutations associated with familial MND. Familial cases are rare and the study is probable not sufficiently powered to show significant differences in age of onset and disease duration in familial *vs.* sporadic MND. Also, family histories may be incomplete and many familial cases may not be recognised as such.

H6: Bulbar-onset disease did not have a shorter survival compared with limb onset disease.

MND patient can present with bulbar, respiratory or spinal/limb onset. Bulbar-onset MND is said to have a worse prognosis compared with the other sub-types (Wijesekera, 2009). In this study there were no significant differences between all three subtypes of MND. The reason for the difference between my studies and the other studies may relate to: selection bias i.e. bulbar-onset disease may progress rapidly to death, so they might be poorly represented in my sample. Also, ascertaining the true time of onset of the disease is not easy, especially if the patient is anarthric. Furthermore, classification of the patients into one or the other group may not be straight-forward especially if bulbar and limb symptoms started simultaneously.

H7: Occupations that involve agricultural chemical, or other chemicals industries may be risk factors for MND.

Many studies have published an association between toxin and chemical exposure and the risk of MND. Most, however, have been inconclusive**.** The toxicity could be acute or slowly progressive over the years. An extensive review by Armon *et al*. concluded that exposure to lead and agricultural chemicals might be risk factor for MND (Armon, 2003). It has been suggested that exposure to agricultural chemicals might explain the apparent association between MND and professional footballers. We compared the proportion of MND patients in my study, working in the agricultural chemical or chemical industry, with that known for the Western Cape population. Ideally, however, we should perform a case-control study in which age and geographically matched cases are compared to a similar population of controls (without MND). We could then compare the two groups with respect to their occupations, both present and past. Also, if these factors are important in MND, the exposure may be short lived but very intense, and a short exposure e.g. to heavy metals, chemicals or toxins, may be less well remembered than a long period in a safer job. Conversely, there may be a recall bias. Patients with MND are more likely to remember their exposure to chemicals or toxins than those who are healthy and do not have MND. Our results, nevertheless, support the idea that chemical exposure might be an aetiological or risk factor for MND. It should, however,

also be pointed out that potential patients from the major agricultural districts of the Western Cape (Paarl, the Winelands, Franschoek, Elgin River Valley) are more likely to "drain" to the Tygerberg Hospital in Parow, our main tertiary sister hospital in the Western Cape. The association between agricultural chemicals and even possible cyanobacteria exposure in groundwater or wells may be not be obvious in the population served by Groote Schuur Hospital.

H8: CPK levels were higher in MND patients compared with normal control laboratory values.

CPK laboratory levels have been reported to be higher in MND than in the normal population. This finding was confirmed in my study. Chronic muscle denervation and atrophy would explain the rise in CPK: as muscles fibres disintegrate secondary to denervation, they release CPK which enter the systemic circulation.

H9: **High CPK levels were not associated with faster disease progression and time to death**.

Higher CPK levels are described in MND patients in the literature, but it was not clear that high CPK levels are associated with a faster progression of disease to death. In this study there was no association between CPK levels and disease progression. However, CPK levels could vary with disease duration. We might expect higher CPK levels with more advanced muscle wasting. More advanced cases, by definition, are nearer to death. Our results suggest that CPK levels are not reliable markers of disease progression and cannot be used as a prognostic factor.

H10: Peripheral blood inflammatory markers and CSF protein concentrations were both higher and lower than normal values.

These non-specific markers did not add much useful diagnostic information. They were both higher, and lower, in MND patients compared with the normally defined laboratory ranges. This might indicate that there was a systemic inflammatory component to the disease in some patients, but not in others. The numbers were too small to do post-hoc analyses of a possible association between the degree of systemic inflammation and the progression of the disease. One could speculate that a more protective systemic inflammatory response might better "contain" the disease. On the other hand, a greater systemic inflammatory response could

indicate more severe disease, with greater "spill-over" of the pathology into the systemic circulation.

H11: Cigarette smoking is associated with an increased risk of MND.

This subject was reviewed by Armon (Armon, 2003) and Alonso (Alonso, 2010). Armon concluded that smoking might be a risk factor for MND. Alonso evaluated the association between smoking and MND incidence and survival in a cohort study. He found that the risk of MND was greater in smokers than non-smokers, and that it was associated with poorer survival in women and not in men. I showed that the proportion of smokers in the MND cases (76% of the men; 68% of the women) was much higher than the rates of smoking in the Western Cape population in general (45% of men, 27% of women). My results support the hypothesis that cigarette smoking might be a risk factor for MND. My data on whether a patient smoked or not was obtained from the clinical notes. I did not have accurate information about when they smoked, for how long they smoked, the number of pack years. Or for how long they might have stopped smoking. A prospective questionnaire for future MND patients should be designed with these points in mind.

Study limitations and suggestion for future work

The data were collected retrospectively from clinical records. Not surprisingly, there was some missing information and the clinician's notes often had to be interpreted. Information on patient's co-morbidities was limited. Obviously, if a prospective structured questionnaire was used for future new cases of MND, many of these problems could be circumvented.

Some cases of MND might have been missed. I know of one patient, for example, who presented as an idiopathic myelopathy with UMN signs in his limbs, in 2010. Only 2 years later did he develop the classical picture of MND with additional LMN signs.

I did not have a true control group of age-matched participants. These might have provided better controls than using statistical data from the Western Cape population in general, which I was forced to do.

A perfect but practically impossible study would have been a long term cohort study with thousands of participants, who are normal at baseline, and who could be followed up prospectively over several years for the development of MND. In this way one could ascertain the presence of true risk factors i.e. factors present in the participants that preceded the advent of the disease. Such a study would, however, be enormously expensive and

labour-intensive. Since MND is a relatively rare condition, we would have needed to follow up a million participants to obtain 10 cases of MND.

A more practical suggestion would be to combine the MND data of patients admitted to both our hospital (Groote schuur Hospital) and those of the other main tertiary neurology unit in the Wester Cape, i.e. at the Tygerberg Hospital. If the same study was conducted over the same time period in both hospitals, we would probable capture the majority of cases of MND in the Western Cape. We would then better be able to ascertain incidence and prevalence rates in the Western Cape. With larger numbers, many of our data that tended to significance e.g. the younger age of onset of MND in African patients, might became significant. Furthermore, we would be able to determine whether clustering occurs in the West Coast area. This would be a very interesting finding that could lead on to questions about cyanobacteria in the soil and water of these areas, compared with the other areas of the Western Cape. There are marine and environmental biology departments, e.g. cyanobacterial research group, Nelson Mandela Metropolitan University, Port Elizabeth, South Africa, who are able to perform these assays. Despite these limitations, however, the study has been one of only a few conducted in our continent.

Chapter 7: Conclusion

My study of 48 cases of MND at Groote Schuur Hospital, in the Western Cape, has reproduced many of the findings in the literature. The age of onset of the disease was similar and I showed a similar male preponderance. Smoking and toxic chemical exposures were identified as possible risk factors. The disease may have an early age of onset in black Africans. A combined prospective study with the two major tertiary referral centres in the Western Cape will strengthen our ability to definitively answer the hypotheses.

My study will, I hope be the basis on which future studies of motor neurone disease in southern Africa could develop.

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Chapter 9: Appendices

Appendix 1 Motor neurone disease raw data

case no	DOB	GENDER	DOD	FP	age death	Race	EEDC	DS	MND type	occup	FMH	SMOKE	ESR	CPK	TSH	VDRL	HIV	B12	WCC	HPT	DM	arthritis	CSF prot	PEG
1	15.7.1965	M	15.03.2007	41	42	3	1	1	1	5	0	0	2	180	0,8	0	0	340	10,6	0	0	0	0,66	0
2	18.3.1956	F	21.08.2009	50	53	2	1	3	1	1	0	1	39	188	1,2	1	0	450	4,27	0	0	0	0	0
3	10.1.1929	F	01.08.2008	74	79	2	1	5	1	1	0	1	4	95	1,6	0	0	560	6,13	0	0	0	0	0
4	22.2.1935	M	alive?	71	?	2	3	8	3	1	1	1	1	120	2,7	0	0	230	9,65	0	0	1	0,45	0
5	19.4.1946	F	05.05.2012	62	66	2	1	4	1	1	0	0	7	80	0,5	0	0	437	8,2	0	0	0	0,22	1
6	14.12.1952	M	16.12.2008	55	56	2	2	1	1	6	0	0	4	120	1,2	0	0	547	4,5	0	0	0	0,56	0
7	13.7.1938	F	01.07.2009	70	71	1	1	1	1	1	0	1	10	100	1,4	0	0	678	16,1	1	1	1	0,33	0
8	31.10.1948	M	02.05.2009	52	61	2	3	9	3	2	0	0	1	80	1,6	0	0	554	8,84	1	0	0	0,45	0
9	21.7.1954	M	18.6.2009	53	55	2	1	2	1	7	0	0	76	80	1,3	0	0	440	29,8	0	0	0	0,45	0
10	15.3.1950	M	26.06.2006	53	56	3	2	3	1	1	0	1	35	120	0,9	0	0	248	9,56	1	1	1	0,31	0
11	19.4.1947	M	28.10.2008	59	61	2	2	2	2	1	0	1	20	474	0,9	0	0	550	10,49	0	0	0	0,23	0
12	26.8.1944	M	01.12.2008	62	64	2	1	2	1	1	0	1	12	181	0,6	0	0	321	9,76	1	1	1	0,64	1
13	26.1.1940	F	23.08.2009	68	69	2	1	1	1	1	0	1	6	115	0,7	0	0	320	8,05	1	0	0	0	0
14	23.1.1938	M	16.08.2006	67	68	2	3	1	1	1	0	1	5	85	1,3	0	0	400	9,7	1	1	1	0,65	0
15	04.6.1970	M	07.09.2011	37	41	3	3	4	1	6	0	0	4	68	1,75	0	0	440	6,19	0	0	0	0,3	0
16	26.11.1945	M	10.03.2010	62	65	2	1	3	1	8	0	1	5	554	1,9	0	0	540	4,5	1	0	0	0,21	0
17	22.8.1965	M	13.05.2012	39	47	2	2	8	1	1	0	0	15	432	2,1	0	0	640	7,26	0	0	0	0,47	0
18	21.7.1943	M	02.03.2010	64	67	1	2	3	1	4	0	1	2	403	0,8	0	0	546	7,08	1	0	0	0	1
19	3.11.1934	F	08.05.2008	73	74	3	2	1	1	1	0	0	4	203	0,9	0	0	358	11,2	1	0	0	0	0
20	27.7.1977	M	11.05.2013	31	36	3	1	5	1	7	0	0	4	237	4,8	0	0	549	5,07	0	0	0	0,54	0
21	3.3.1959	F	21.05.2011	48	52	2	1	4	1	1	1	0	20	320	0,9	0	0	432	6,8	0	0	0	0	0
22	24.10.1954	M	10.09.2009	54	55	2	1	1	1	7	0	1	12	223	1,97	0	0	542	8,26	0	0	0	0,45	1
23	21.1.1956	M	16.01.2011	53	55	1	2	2	1	1	0	1	2	86	0,8	0	0	326	10,9	1	0	0	0,35	0
24	6.12.1938	M	30.12.2012	69	74	1	3	5	2	1	0	1	5	56	1,96	0	0	269	7,93	1	0	0	0,45	1

case no	DOB	GENDER	DOD	FP	age death	Race	EEDC	DS	MND type	occup	FMH	SMOKE	ESR	CPK	TSH	VDRL	HIV	B12	WCC	HPT	DM	arthritis	CSF prot	PEG
25	24.12.1956	M	16.09.2013	50	57	1	2	7	1	6	0	1	3	123	1,2	0	0	365	7,04	0	0	0	0,7	0
26	24.9.1951	F	18.08.2009	56	58	2	1	2	2	1	0	1	6	120	1,3	0	0	360	9,13	0	0	0	0,33	1
27	14.3.1954	M	25.07.2010	54	56	2	2	2	1	7	1	1	9	56	1,42	0	0	420	5,73	0	0	0	0,33	0
28	3.6.1962	F	06.10.2007	44	45	3	1	1	1	1	0	0	3	43	1,4	0	1	438	14,6	0	0	0	0,41	0
29	4.9.1941	F	16.09.2008	66	67	2	2	1	1	1	0	1	6	95	1,2	0	0	548	11,7	0	0	0	0,28	0
30	21.6.1949	M	alive	59	?	1	3	6	3	6	0	1	2	78	1,3	0	0	350	7,1	1	0	0	0,53	0
31	14.3.1940	M	12.11.2006	65	66	2	2	1	1	1	0	1	5	222	0,7	0	0	438	6,5	1	0	0	0	0
32	3.3.1968	F	10.11.2012	42	44	3	2	2	1	1	0	0	4	183	0,8	0	0	569	6,3	1	0	0	0,23	0
33	31.1.1957	F	16.03.2010	51	53	1	2	2	1	1	1	1	5	58	1,4	0	0	339	7,89	1	0	0	1,9	0
34	28.3.1976	M	06.04.2011	31	35	2	1	4	2	4	0	1	5	233	1,37	0	0	544	13,7	1	0	0	0,34	0
35	11.8.1964	M	06.10.2005	40	41	2	2	1	1	1	0	1	2	848	1,5	0	0	440	6,8	0	0	0	0,21	0
36	15.7.1949	F	01.07.2005	55	56	2	2	1	1	8	1	1	4	88	1,3	0	0	659	5,7	0	0	1	0,45	0
37	13.4.1970	F	14.6.2005	34	35	3	2	1	1	1	0	1	23	780	1,03	0	0	443	4,8	0	0	0	0,19	1
38	15.5.1979	M	2013	30	34	3	2	4	1	3	0	1	29	560	0,7	0	0	560	12,04	0	0	0	0,34	0
39	31.10.1962	F	18.2.2005	40	43	2	2	3	1	8	0	0	2	320	1,55	0	0	453	8,4	0	0	0	0,23	1
40	23.02.1958	F	28.02.2008	47	50	2	2	3	1	8	0	1	4	252	3,32	0	0	655	3,01	0	0	0	0,71	0
41	27.6.1951	M	alive	52	?	2	3	11	2	7	0	1	6	259	1,4	0	0	440	6,7	0	0	0	0	1
42	24.7.1945	F	alive	60	?	2	3	9	2	1	0	1	30	105	1,2	0	0	320	9,8	1	0	0	0,31	1
43	22.8.1930	M	03.11.2008	68	78	2	2	10	2	1	0	1	4	818	1,6	0	0	555	6,2	1	0	1	0	0
44	1.9.1945	M	25.04.2007	61	62	3	2	1	1	1	0	1	15	99	0,8	0	0	445	12	1	1	0	0,47	1
45	28.8.1953	F	04.09.2007	53	54	2	3	1	1	1	0	1	5	222	1,2	0	0	666	12,1	1	0	0	0,41	0
46	29.11.1949	M	25.05.2010	59	61	3	2	2	1	1	0	1	2	248	1,4	0	0	553	6,74	0	0	0	0,31	1
47	22.9.1953	M	07.10.2010	54	57	1	2	3	1	4	0	1	9	120	1,6	0	0	448	6,1	1	1	0	0,43	0
48	31.5.1934	F	20.05.2005	70	71	2	2	1	1	1	0	1	3	540	1,2	0	0	420	5,87	1	0	0	0,23	1

Appendix 2 University of Cape Town ethics approval letter

UNIVERSITY OF CAPE TOWN

Faculty of Health Sciences
Faculty of Health Sciences Human Research Ethics Committee
Room E52-24 Groote Schuur Hospital Old Main Building
Observatory 7925
Telephone [021] 406 6338 • **Facsimile** [021] 406 6411
e-mail: sumayah.ariefdien@uct.ac.za
www.health.uct.ac.za/research/humanethics/forms

30 July 2013

HREC REF: 451/2013

Dr A Daude
c/o A/Prof M Combrink
Division of Neurology
E 7room 63
NGSH

Dear Dr Daude

PROJECT TITLE: A FOLDER REVIEW OF CASES OF MOTOR NEURONE DISEASE SEEN AT GROOTE SCHUUR HOSPITAL BETWEEN 2005 AND 2010

Thank you for submitting your study to the Faculty of Health Sciences Human Research Ethics Committee for review.

It is a pleasure to inform you that the HREC has **formally approved** the above mentioned study.

Approval is granted for one year till the 28 August 2014.

Please submit a progress form, using the standardised Annual Report Form, if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.

Please note that the on-going ethical conduct of the study remains the responsibility of the principal investigator.

Please quote the REC. REF in all your correspondence.

Yours sincerely

pp
PROFESSOR M BLOCKMAN
CHAIRPERSON, HSF HUMAN ETHICS

Federal Wide Assurance Number: FWA00001637.
Institutional Review Board (IRB) number: IRB00001938

sAriefdien

This serves to confirm that the University of Cape Town Research Ethics Committee complies to the Ethics Standards for Clinical Research with a new drug in patients, based on the Medical Research Council (MRC-SA), Food and Drug Administration (FDA-USA), International Convention on Harmonisation Good Clinical Practice (ICH GCP) and Declaration of Helsinki guidelines.

The Research Ethics Committee granting this approval is in compliance with the ICH Harmonised Tripartite Guidelines E6: Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95) and FDA Code Federal Regulation Part 50, 56 and 312.

Appendix 3 Authorization letter from Senior Medical Superintendent of GSH

DEPARTMENT of HEALTH
Provincial Government of the Western Cape

GROOTE SCHUUR HOSPITAL
Bpatel@pgwc.gov.za
Tel. (021) 404-4469
Fax: (021) 404-4304x number>
Private Bag, Observatory, 7935
www.capegateway.gov.za

Department Obstetric, Gynaecology & Neonatology

REFERENCE: Research
ENQUIRIES: Dr B Patel

For attention: Amina Ismael Daude

RESEARCH : **FOLDER REVIEW OF CASES OF MOTOR NEURON DISEASE (MND)**

Your recent letter to the hospital refers.

You are hereby granted permission to proceed with your research through review of folders at Groote Schuur Hospital in relation to the extension phase of your study.

Please note the following:-

a) Your research may not interfere with normal patient care.
b) Hospital staff may not be asked to assist in the research.
c) No hospital consumables and stationary may be used.
d) Please introduce yourself to Mr Weeder (telephone 021 – 4044056) before commencing. He will assist you in accessing the folders. Please note that you may not remove folders from the GSH premises.

I would like to wish you every success with your project.

Yours sincerely

DR B PATEL
SENIOR MEDICAL SUPERINTENDENT
Date: 30 September 2010