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Clinical heterogeneity, diagnostic features,  
Outcomes of  
Guillain–Barré syndrome spectrum  
disorders – An analysis of IGOS UK data

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# Abstract:

**Introduction:** GBS has a highly diverse clinical course and outcome. Currently available literature suggests that despite treatment about 20 % of patients remain disabled at one year and about 5 % patients die. These data come from clinical trials conducted between 1984 and 2006. Most of these studies included severe GBS cases. We conducted a multicentre prospective observational study looking at clinical and biological determinants of prognosis of GBS. As part of this study, I had an opportunity to analyse the data collected from 15 UK centres; looking at clinical and treatment patterns, various outcomes including ability to walk at 12 months, pain and quality of life. We also analysed Electrophysiological data from our local centre (Glasgow); compared newly published electrophysiological diagnostic criteria with existing criteria to determine whether serial studies are required for final electrophysiological diagnosis. Finally, to identify the patients with poor prognosis early in the disease course, we attempted to validate the currently available clinical prognostic models.

**Method:** We conducted a multicentre prospective observational study named IGOS (International GBS Outcome Study) with a web-based entry system. It aimed to study at least 1000 patients over 3 years. The study included two modules: 1) core module which consist of a) acute clinical data collection at 0, 1, 2, 4 weeks and follow up data at 6 and 12 months b) serum samples collection at each clinical data entry point c) electrophysiology studies within 2 weeks 2) optional modules included additional electrophysiology studies at 4 weeks, CSF studies and long term outcome data at 2 and 3 years. As the study still ongoing, I analysed the data of 122 GBS patients recruited from 15 UK centres between May 2012 and Jan 2015.

**Results:** In our cohort about 20 % patients remained disabled at 1 year, 18 %

required mechanical ventilation (MV), 5 % died. Pain continued to remain a major disabling symptom in more than half of the patients however unable to perform usual activity was the most disabling QoL domain affected at 12 months and was an important contributing factor affecting quality of life. Intravenous immunoglobulin was the most commonly prescribed treatment followed by plasma exchange. Immunotherapy was not beneficial in mildly affected GBS patients. Currently available electro diagnostic criteria are not very sensitive in identifying final EP subtypes and newly published Rajabally's criteria potentially addresses this issue and should be used in clinical practice to establish final EP diagnosis. Existing prognostic models EGOS and mEGOS performed well in our cohort and showed good discriminatory capacity

**Discussion:** Despite wider availability of immunotherapy prognosis of GBS has not changed in last 20years, which highlights the urgent need of more effective treatments in these patients. However new therapy can be expensive and can be only beneficial if the patients with poor prognosis are identified early in course. This can only be achieved by developing good prognostic models. Our results show that existing available models EGOS/MEGOS validates well and provides a proof of the concept that prognostic model can be used to identify patients with poor prognosis when the treatment is most beneficial. GBS continues to remain a clinical diagnosis. While there are drawbacks of existing EP criteria, newly developed Rajabally's criteria are sufficient to establish final EP diagnosis.

# Acknowledgements

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# Author's declaration

I declare that, except where explicit reference is made to the contribution of others, this dissertation is the result of my own work and has not been submitted for any other degree at the University of Glasgow or any other institution.

Signature \_\_\_\_\_

Printed name \_\_\_\_\_

# Definitions/abbreviations

A-CIDP	Acute Onset Chronic Inflammatory demyelinating Polyneuropathy
AGA	Anti Ganglioside Antibodies
AUC	Area under the Curve
AFP	Acute Flaccid Paralysis
AIDP	Acute Inflammatory Demyelinating Polyneuropathy
AMAN	Acute Motor Axonal Neuropathy
AMAN with CB	Acute Motor Axonal Neuropathy with conduction block
AMSAN	Acute Motor and Sensory Axonal Polyneuropathy
BBE	Bickerstaff Brainstem Encephalitis
CB	Conduction Block
CMAP	Compound Muscle Action Potential
CSF	Cerebro Spinal Fluid
CV	Conduction velocity
dCMAP	distal Compound Muscle Action Potential
DML	Distal Motor Latencies
EAN	Experimental Allergic Neuritis
EP	Electrophysiology
EPS	Electrophysiology Study
EGOS	Erasmus GBS Outcome Study
GBS	Guillain–Barré syndrome
GBSD	GBS Disability Scale
GFAP	Glial Fibrillary Acid Protein
HA	Hospital Admission
HLA	Human Leucocyte Antigen

IGOS	International GBS Outcome Study
LPS	Lipopolysaccharide
mEGOS	Modified Erasmus GBS Outcome Study
MFS	Miller Fisher Syndrome
MRCSS	Medical Research Council Sum Score
MV	Mechanical Ventilation
NCS	Nerve Conduction Studies
NF	Neurofilaments
NINDS	National Institute of Neurological Disorders and Stroke
ODSS	Overall Disability Scale
ONLS	Overall Neuropathy Limitation Scale
OSS	Onset of Symptoms
OOW	Onset of weakness
PCB	Pharygeal Cervical Brachial
PE	Plasma Exchange
QoL	Quality of Life
REC	Research Ethics Committee
RODS	Rasch Overall Disability Score
SIADH	The syndrome of inappropriate antidiuretic hormone (ADH) secretion
SNAP	Sensory Nerve Action Potential

# **Chapter 1 Introduction**

Guillain–Barré syndrome (GBS) is a spectrum of post infectious monophasic autoimmune disorders of peripheral nerves with a highly diverse clinical course and outcome. It was first described by French neurologists Georges Guillain, Jean-Alexander Barré and Andre Stroll in 1916.(1) After eradication of poliomyelitis, GBS has become the most common cause of acute flaccid paralysis worldwide. A typical GBS is characterised by symmetrical ascending weakness with minimal sensory symptoms and signs. Several regional clinical variants have been well described. One such variant is Miller Fisher Syndrome, a syndrome characterised by ataxia, ophthalmoplegia and areflexia, originally described by American neurologist Charles Miller Fisher.(2) Other regional variants include Pharyngeal-Cervical-Brachial (PCB) weakness and Bickerstaff Brainstem encephalitis (BBE).

### ***1.1 Historical Perspective:***

The concept of ‘acute paralysis caused by peripheral nervous involvement’ emerged in 1843, when Robert Graves, during a widespread epidemic, described various cases of acute polyneuropathies presenting as acute pain, paresthesia followed by paralysis.(3) In 1859, this concept evolved even further, when French physician Jean Baptiste Octave Landry de Thezillat described 10 cases of acute ‘ascending’ paralysis and of which, some patients also developed respiratory paralysis within 2 weeks of onset of symptoms. (4) Two of these 10 patients died and when autopsies performed, no central nervous system abnormality found, which led him to conclude that the disease must be a peripheral in origin. The disease was originally known as ‘Landry’s paralysis’. In 1890, Osler published another case series of patients with ascending paralysis and respiratory involvement.

In 1916, during the first world war, French neurologists Georges Guillain, Jean-Alexandre Barré and Andrew Strohl described two cases similar to ‘Landry’s paralysis’ in French soldiers, but they resisted calling it ‘Landry’s paralysis’ as they thought their cases had better prognosis while Landry’s cases had poor prognosis, moreover both of patients had elevated cerebrospinal fluid protein level with normal

cell count, a finding later on became one of the very important characteristics of the disease. Interestingly, Strohl's name was dropped in subsequent publications and disease was widely referred as "Guillain–Barré syndrome". In 1949, based on nerve biopsy reports Haymaker and Kernohan provided first ever pathological proof of underlying demyelination and this coupled with further report by Waksman and Adam describing animal models of extrinsic allergic neuritis led to the notion that GBS was a demyelinating disorder of peripheral nerves. (5,6) However this view was challenged by Feasby et al when they published the first report of "Acute Axonal GBS" in 1986.(7) McKhann et al later on substantiated this view and described a case series of Chinese GBS patients with predominant axonal degeneration as an underlying pathology. Clinically and pathologically, GBS now widely considered being a spectrum of post infectious polyneuritis.

## ***1.2 Epidemiology:***

Most epidemiological studies in GBS literature have been performed in Europe and North America. A recent review of all published epidemiological studies performed worldwide showed incidence of GBS were higher in prospective studies compared to retrospective studies, which probably represents the true incidence of GBS in western world.(8) Most prospective studies, showed the incidence rates of 1.11 to 1.66 cases per 100000 populations per year. Moreover most studies being performed using NINDS criteria for the diagnosis and therefore did not include MFS cases and none of these studies has systematically studied clinical and electrophysiological subtypes and therefore it is difficult to determine true incidence of each clinical GBS subtype.

Unfortunately there is no epidemiological data available from developing world. Lack of appropriate research infrastructure and resources to perform such studies remain major challenges in developing world. Since most developing countries have now successfully eradicated Poliomyelitis, GBS is emerging as a major cause of acute flaccid paralysis (AFP). In a recent study Islam et al systematically analysed the surveillance data on reported AFP cases from Bangladesh and have shown that although Bangladesh has been successful in eradicating poliomyelitis since 2000, non-polio AFP cases are continue to occur, with the incidence rate of 3.25 per 100,000

children less than 15 year of age and a great proportion of these non -polio AFP cases are diagnosed as GBS with crude incidence rates of GBS in children varied from 1.5 to 5 per 100,000 per year.(9) Thus the study demonstrated that, GBS rates are at least 2 to 3 times higher than what has been reported in Americas and Europe, and the burden of GBS in developing country could be much higher than previously thought.

The incidence of MFS has been reported to be considerably higher in eastern world with 19 % in Taiwan and 26 % in Japan.(10,11) Incidence of even rare variant, Bickerstaff Brainstem Encephalitis (BBE) has not been studied in western world, but one such study from Japan has reported the incidence of BBE is about 0.07 per 100000.(12)

### ***1.3 Clinical Spectrum of GBS:***

Clinically, GBS can be divided into two broad categories: Typical GBS and Variants. While typical GBS has been widely described as predominant motor syndrome with minimal sensory involvement, recognition of pure motor form of GBS in china has given a very interesting dimension to this area. As further research continue in this area description of other disease variants continued to emerge. Understanding of various clinical subtypes is very important in order to get better insight of underlying immune pathogenesis, pathophysiology and prognosis of GBS.

#### **1.3.1 Typical GBS:**

A typical GBS is a monophasic illness characterised by rapidly progressive symmetrical ascending weakness with minimum clinical sensory symptoms, however involvement of sensory fibre on electrophysiology is very frequent. Clinically the disease course can be broadly divided into four stages 1) stage of invasion 2) stage of progression 3) Plateau stage and 4) stage of recovery.

1. Stage of invasion: This is the phase when the initial inflammatory process start to affect the peripheral nerves and often a typical GBS starts with sensory

symptom. Inflammatory involvement of nerve roots and giant 1a sensory afferent fibre involvement lead to back pain and areflexia. Radicular pain can be a presenting feature.

2. Stage of disease progression: As the disease progresses further, patient typically develops ascending limb weakness. Hip flexors are commonly affected and suggest underlying proximal nerve root involvement. In about 25 % of cases, involvement of phrenic nerve leads to respiratory muscle paralysis and requirement of mechanical ventilation. Cranial nerve involvement leads to diplopia and dysphagia requiring nasogastric feeding. Autonomic disturbances have been reported 65% cases. About half of the patients develop Syndrome of Inappropriate ADH secretion (SIADH). Majority of patients reach nadir within 4 weeks of symptom onset after that they enter into a plateau phase.
3. Plateau Phase: This phase is characterised by stabilisation of muscle weakness and can last up to 4 weeks.
4. Stage of recovery: This is the phase where the muscle weakness begins to improve and often one group of muscle improves earlier than others. It continues for 6 months to 2 years. As the recovery in muscle weakness continues painful paresthesia and fatigue can become prominent symptoms and often lead to significant long-term disability.

### **1.3.2 Clinical Variants:**

1. **Pure Motor GBS:** Although a typical GBS has very minimal sensory involvement, clinically and electrophysiologically, pure motor GBS cases without any sensory involvement have been reported especially in Southeast Asia. This form of GBS is characterised by acute onset of symmetrical limb, facial and oropharyngeal muscle weakness without any sensory loss. It was first described in children, infected with *C.Jejuni* in northern china. (13) Although most patients have absent deep tendon reflexes, small proportion patients have hyperreflexia. CSF typically shows elevated protein and normal cell count. Electrophysiology typically shows selective involvement of motor fibres without any sensory fibre involvement. Most studies show typical low CMAPs suggestive of underlying motor axonal damage however sometimes

features of demyelination can be seen. Needle EMG shows diffuse denervation. Pattern of recovery can be variable, while some patients recover rapidly with complete reversal of EPS abnormality, others follow long protected course in which recovery process is complicated by on-going axonal degeneration on EMG.

2. **Sensory GBS:** Recently Uncini and Yuki have described a series of patients who presented with acute pure sensory symptoms and signs reaching their nadir within 6 weeks. (14) They suggested a new terminology “sensory GBS” based on the size of sensory fibres involved and the possible site of primary damage. They attempted to classify sensory GBS in to three different subgroups a) Acute sensory demyelinating polyneuropathy b) Acute large fibre axonopathy-ganglionopathy c) Acute small fibre neuroathy-ganglionopathy.

- (i) *Acute sensory demyelinating polyneuropathy:* This terminology was proposed for a subgroup of patients who present with acute sensory symptoms and signs with areflexia. Electrophysiology showed demyelinating features in both motor and sensory nerves, however no conduction blocks demonstrated in motor nerves.

- (ii) *Acute sensory large fibre axonopathy-ganglionopathy:* This encompasses two different clinical entities a) ataxic GBS and b) acute sensory ataxic neuropathy. Richter first proposed “Acute ataxic GBS” term in 1962. He described a case with acute cerebellar type ataxia and areflexia without proprioceptive sensory loss and ophthalmoplegia. Later on, few more cases have been described in the literature with similar presentation. Electrophysiology typically does not show any abnormality. There has been an ongoing debate as to origin of the ataxia in these cases. Some propose that ataxia is centrally mediated while the others think that it is an incomplete form of miller fisher syndrome without ophthalmoplegia and ataxia is due to GQ1b antibody mediated damage to group 1a afferents along their path from muscle spindles to spinal cord, which may explain normal electrophysiological finding as routine sensory nerve conduction studies do not examine group 1a fibres. (15–17) *Acute sensory ataxic neuropathy* term has been proposed to describe patients with acute sensory symptoms and ataxia

with loss of proprioception, areflexia and positive Romberg sign. EPS typically show selective involvement of sensory nerves with absent or reduced Sensory nerve action potentials. In some patients with ASAN, recovery may be incomplete leading to permanent ataxia indicates that the site of the lesion in this group of patients may be the primary sensory neurones, while in some patients there is rapid clinical recovery associated with reversal of electrophysiological findings suggest that the lesion may be in distal sensory nerve axons. Overall authors proposed that with selective afferent involvement of 1a afferent fibres, patients present with cerebellar type ataxia and normal EPS and with more confluent involvement of sensory fibres, patient present with sensory ataxia and abnormal EPS findings.

(iii) *Acute small fibre neuropathy-ganglionopathy*: In the same report Uncini and Yuki described 10 patients who presented with acute onset burning pain and numbness without ataxia and proprioceptive involvement. CSF showed cytoalbumin dissociation in 7 patients. (18,19) All patients reached their nadir within 6 weeks. In a few patients symptoms and signs were in non-length dependent fashion suggestive small fibre ganglionic involvement. Although the nerve conduction studies were normal, all the patients had abnormal thermal threshold studies. Authors suggested that these patients had acute small fibre neuropathy-ganglionopathy.

- 3 **Miller Fisher Syndrome:** Miller Fisher syndrome (MFS) is a clinical variant of GBS and characterised by clinical a triad of ophthalmoplegia, ataxia and areflexia. Fisher first reported the syndrome in 1956. (2) Apart from classical triad, patients also present with pupillary abnormalities, ptosis, bulbar and facial palsy. Occasionally patient presents with only limited form of MFS, with ophthalmoplegia or isolated bulbar symptoms only. MFS often overlap with classic GBS and patients go on to develop limb and respiratory weakness.
- 4 **Bickerstaff Brainstem encephalitis:** In 1951 Bickerstaff and Cloake first published report of three cases with asymmetric ophthalmoplegia, ataxia and impaired consciousness. (20) They used the term “mesencephalitis” to

describe the syndrome, the term subsequently changed to “Bickerstaff Brainstem encephalitis”. All these patients had preceding infection and CSF cytoalbumin dissociation and had benign outcome. Based on radiological and pathological findings, they argued that these patients had CNS pathology. However, some others argued that some of the cases described by these authors were typical of MFS and considered BBE as a variant of MFS, citing the presence of antecedent infection (92%), cytoalbumin dissociation (59 %) and presence of anti-GQ1b antibodies (66%) as features common to both disorders. (12)

- 5 **PCB:** This regional variant is characterised by rapidly progressive oropharyngeal and cervico-brachial weakness and upper limb areflexia. Some patients with PCB variant have associated ophthalmoplegia and ataxia and therefore it is widely considered as a variant of fisher syndrome. Most patients have antibodies against GT1a and these antibodies often cross react with GQ1b.

## ***1.4 Pathology and immune pathogenesis:***

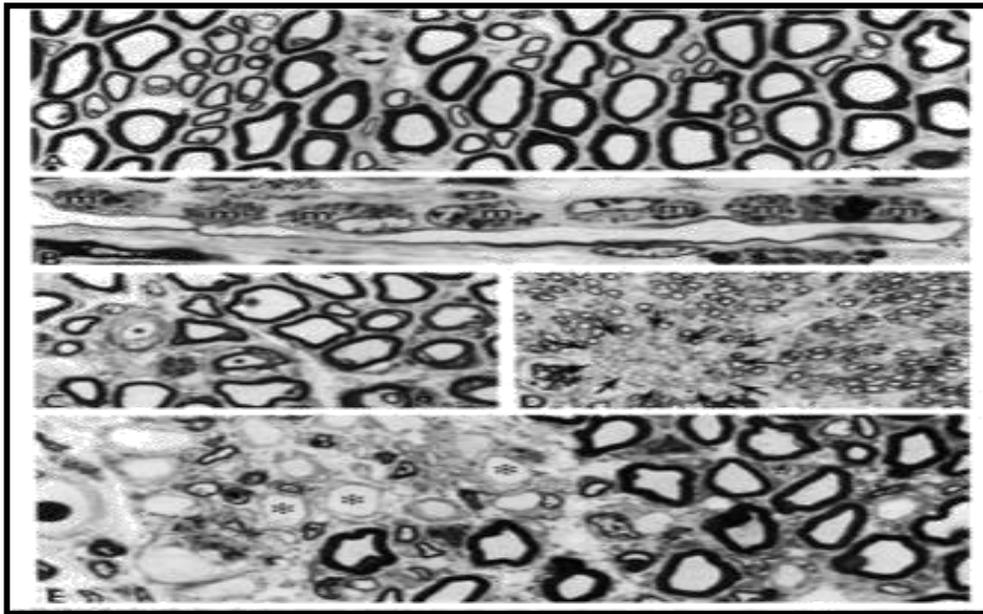
Pathologically, GBS can be classified into two different forms; A) demyelinating and B) axonal form. This classification was based on initial autopsy reports from GBS patients, and since the autopsies are rarely performed nowadays, electrophysiological markers is used to classify GBS in to demyelinating or axonal variants. Clinically, it is difficult to differentiate between these two variants, as motor, sensory and autonomic symptoms are common to both pathologies.

### **1.4.1 Pathology in Acute Inflammatory Demyelinating Polyneuropathy (AIDP)**

Landry's original report published in 1859 was instrumental in setting the direction for further pathological studies involving peripheral nerves in GBS. (4) In his autopsy report, he noticed that patients did not have any pathology in spinal cord and brain, concluding that pathology most likely to be in peripheral nerves of these patients. In their clinicopathological report of 50 fatal GBS cases, Haymaker and Kernohan in 1949 proposed that the nerve root oedema with subsequent myelin breakdown and lymphocytic infiltration was a primary pathological process in GBS. (5) Interestingly around the same time, in 1955, Waksman and Adams developed first experimental allergic neuritis (EAN) animal models by immunising Lewis rat with whole peripheral nerve myelin and complete Freund's adjuvant.(6) The pathological findings in these animals showed perineural oedema with lymphocytic infiltration and macrophage mediated demyelination. Similarly, in 1969, Asbury and his colleagues in their report of 19 GBS autopsies, found perivascular mononuclear infiltrates and segmental demyelination which led many researchers to believe that EAN was a counterpart of human GBS and demyelination was a primary pathology in GBS. (21)

Further pathological studies showed two patterns of demyelination; in the first pattern there is vesicular myelin degeneration causing soap bubble appearance on microscope followed by macrophage invasion into the myelin as scavenger cells, this was

associated with complement and immunoglobulin activation; the second pattern showed macrophage entry through the node or internodes in apparently normal nerve cell fibre. (22,23) Although pathologically the demyelinating process can be very widespread along the nerve fibre, it predominantly affects the proximal nerve roots and distal intramuscular nerve terminals where the blood nerve barrier is deficient. Electrophysiologically this is evident by absent F waves and prolonged distal motor latency.



**Figure 1-1 GBS Pathology**

From Hafer mako et al, Different degree of inflammatory response in sequential manner.  
A) Day 3 occasional demyelination B) on day 7 foamy macrophages (m) C) advanced perivascular demyelination D) complete demyelination

### **1.4.2 Pathology in Axonal GBS:**

In 1986, Feasby et al described 5 GBS cases with inexcitable nerves and autopsy showing widespread axonal degeneration without any evidence of demyelination. (7) They proposed that primary pathology in these cases was axonal degeneration. However this concept remained controversial until 1993, when McKhann and his colleagues published a first comprehensive autopsy reports of Chinese GBS patients showing wallerian type degeneration involving motor fibres without any demyelination in these patients and derived the conclusion that pathologically axonal GBS is a distinctive syndrome distinguishable from poliomyelitis and AIDP. (24)

Although the initial pathological reports showed predominant motor fibre involvement, a further study showed that these changes were not fibre specific, and depending on the involvement of sensory or motor fibres clinical phenotype varies. In AMAN these changes are seen predominantly in motor fibres and in AMSAN, changes seen in both motor as well as sensory fibres.

Antibody mediated attack to axolemmal membrane leads to either reversible conduction failure or completely irreversible axonal injury, which can be explained clinically by two different types of outcomes in axonal GBS patients.

### **1.4.3 Immune-pathogenesis: T cell or B cell?**

Understanding of underlying immunopathogenesis is of paramount importance in selecting the correct therapeutic approaches for any immunological disorder. As far as GBS is concern, whether it is a T cell or B cell mediated disorder has been continuously debated. In recent years significant progress has been made in our understanding of this spectrum of disorders. While immunopathogenesis in Axonal GBS and FS has been very well understood, the underlying mechanism of AIDP remains elusive.

Traditionally, GBS was thought to be a T cell mediated disorder and this was based on close resemblance of pathological findings from human autopsy from GBS patients and T cell mediated “experimental allergic neuritis” (EAN) animal models. (6) These T cell mediated EAN animal model were developed by injecting myelin protein components. Therefore, identification of antigenic myelin protein components has remained a major focus of the research in this field for long time. Unfortunately, research in this field has failed to identify any myelin protein related antibodies so far.

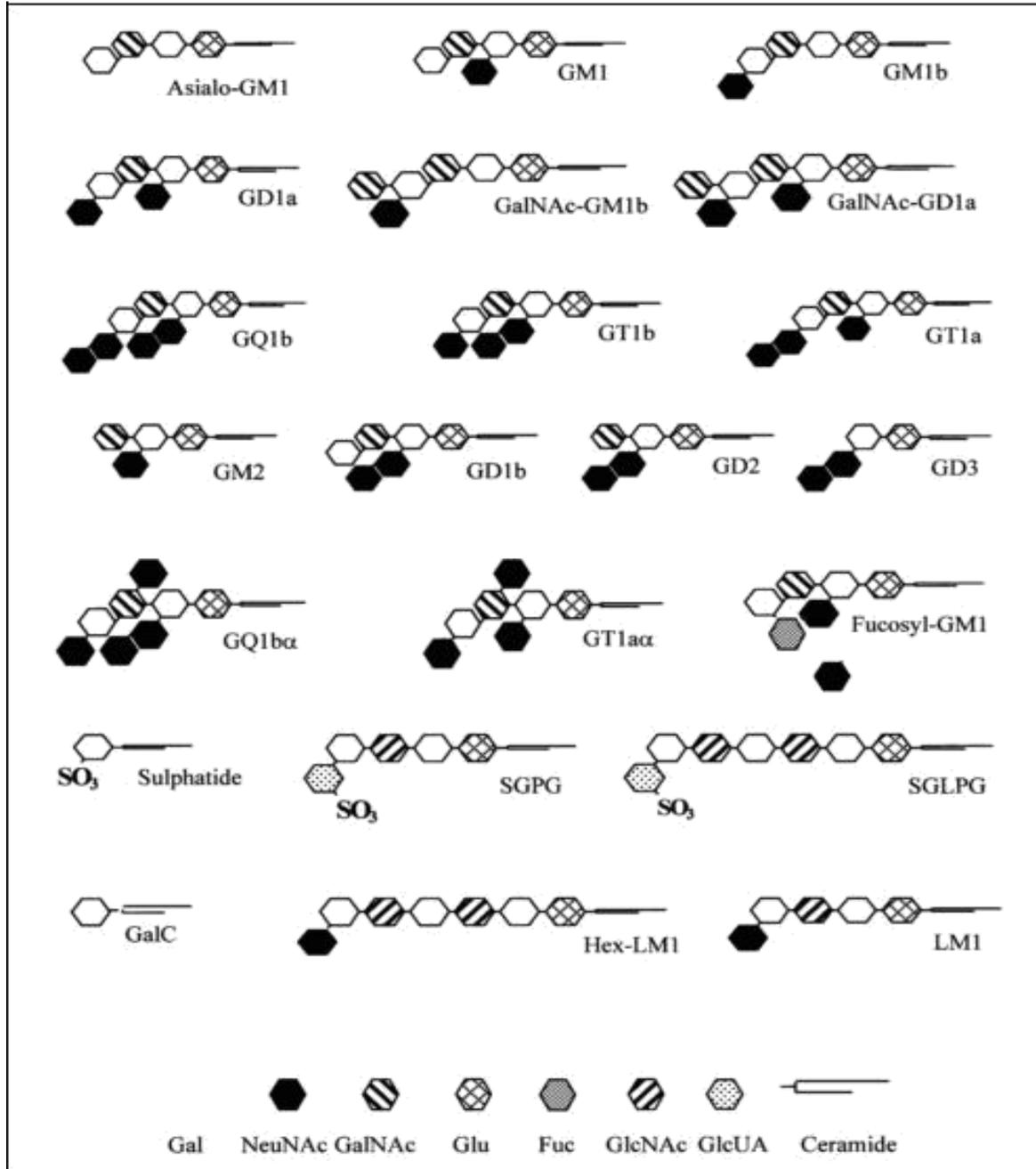
The concept of ‘T cell mediated immune damage’ has been challenged recently and there is growing body of evidence to suggest that GBS, at least in some forms, is a B cell or antibody mediated disorder, in which complement fixing IgG1/3 subclass anti-ganglioside antibodies play a major role. Thus, the focus of research has shifted from myelin proteins to Schwann cell and axolemmal membrane glycolipid molecules. (25)

Interestingly, identification of pathogenic role of anti glycolipid antibodies (AGA) is not a new concept, but it certainly has come round the circle after it being ignored for about 20 years. Ilyas et al. had first identified AGA back in 1988, and in fact glycoprotein induced EAN animal models have been described even before 1988, first by Nagai et al in 1976 and then by Saida et al in 1979. (26–28) The first significant breakthrough in this area was the identification of anti GQ1b antibody in MFS; a GBS variant, by Chiba and Kusunoki in 1992.(29) This was followed by the development of anti GD1b associated ataxic neuropathy rabbit models by Kusunoki et al in 1996 and anti GM1 antibody-associated rabbit models of axonal motor neuropathy by Yuki et al

in 2001. (30,31) These studies have compelled the researchers to refocus on anti-glycolipid antibodies and their role in development of some forms of GBS. Further studies have shown that various anti-glycolipid antibodies have been associated with specific forms of GBS. Anti GM1, anti GD1a, anti GM1b and anti GalNAcGD1a are associated with axonal GBS variants and anti Gq1b, GT1a and GD3 GD1b have been associated with MFS and chronic ataxic neuropathy. (25)

#### **1.4.4 Ganglioside Structure and mechanism of injury:**

Gangliosides, also a subtype of glycosphingolipids, are heterogeneous molecules composed of a ceramide, linked to one or more hexose sugar molecules and sialic acid. (32) The carbohydrate portion consists of variable backbone chain of neutral sugar linked to negatively charged sialic acid molecules, which defines ganglioside as a distinct subtype of GSL. There are more than 200 different gangliosides present in the body and the nomenclature is according to Svennerholm classification, in which GXyz represents G as ganglioside, X is a number representing the number of sialic acid molecule, y represent the number indicating the length of the neutral sugar and z indicates the isomeric form. (33) Gangliosides are present throughout the body; however they are highly enriched in nervous system and compose of 10-20 % of the total lipid of the outer neuronal membrane layer, which is ten times more compared to non-neural tissues. (34) This suggests their specific role in nervous system. The hydrophobic ceramide structure is immersed in the lipid membrane the hydrophilic sugar molecules are exposed outside the membrane, which may act as antigenic targets. Gangliosides are biosynthesised in the Golgi complex by sequential action of GalNAc- transferase enzyme. Gangliosides are concentrated in small dynamic membrane ‘rafts’, which plays an important role in cell signalling pathways.(35) Different nervous tissues have different expression of gangliosides, which may be responsible for different disease phenotypes. For example; GQ1b and GT1a are abundant in human extra ocular muscles compared to axial and limb muscles similarly GM1 gangliosides are relatively highly expressed in ventral nerve root compared to dorsal root ganglia, giving distinct phenotypic variation. Experimental studies indicate that any injury to nervous system alters the biosynthesis of complex gangliosides and as the regenerating axon express complex gangliosides it may also affect the axonal regeneration and overall disease outcome. (36)



**Figure 1-2 Glycolipid targets for neuropathy-associated autoantibodies.**

Gal = galactose; GalNAc = N-acetylgalactosamine; Glc = glucose; GlcNAc = N-acetylglucosamine; NeuNAc = N-acetylneuraminic acid; GlcUA = glucuronic acid; Cer = ceramide; LM1 = SPG, sialosylparagloboside; Hex-LM1 = SLPG, sialosyllactosaminyl- paragloboside; SGPG = sulfated glucuronyl paragloboside; SGLPG = sulfated glucuronyl lactosaminyl paragloboside.

### 1.4.5 Molecular mimicry:

Post infectious molecular mimicry has been widely described as underlying mechanism for Axonal GBS and FS.

In order to satisfy molecular mimicry, the disease must fulfil four criteria.

- 1) Strong epidemiological evidence between the disease and infection: In context of GBS, the possible association between GBS and infection was known long ago, but the foundation for molecular mimicry theory was laid by various observational studies performed by Rees et al in 1993 and 1995, which provided strong evidence for association between *C. jejuni* infections and GBS. (37–39)
- 2) Identification of pathogenic antibodies: Evidence related to possible pathogenic role of anti ganglioside antibodies in GBS started to emerge from an interesting observation from some European countries and north America, showing that some patients developed GBS after administration of ganglioside injections for non specific pain syndrome. (40, 41) Around the same time, Ilyas et al published the first report showing anti ganglioside antibodies in GBS patients. (42) Encouraged with these findings researchers started looking for AGA in GBS patient sera. Nobile Orazio et al found very high titres of anti GM1 antibodies in GBS patients. (43) Closing the gap, Japanese researchers started studying various strains of *C. Jejuni* in GBS sera and showed that Penner 19 strain was the most common strain associated with GBS. (44)
- 3) Identification of microbial mimicry of target antigen: Evidence for this came from a study performed by Yuki et al showing that axonal GBS results from cross reactivity between bacterial wall lipopolysaccharide and peripheral nerve components namely ganglioside.(44) The same group had further shown that bacterial LPS that generates immune response in GBS and FS patients shares structural similarity with GM1 and GQ1b gangliosides. Anti GM1 and anti GD1 antibodies have been shown to be reacting with LPS of few more *C. Jejuni* strains.
- 4) Reproduction of disease in animal: Evidence in support of this theory came from two separate studies showing a) development of AGA and AMAN in animals immunised with *C. Jejuni* LPS b) development of AGA and AMAN in animal models immunised with bovine brain gangliosides or GM1. (45)

Thus, above studies provided conclusive evidence that shared antigenic determinants between peripheral nerve fibres components and infective organisms like *Campylobacter Jejuni* (*C.Jejuni*) is responsible for molecular mimicry at least in AMAN cases. Antigenic stimulation derived from infective organisms generates antibody response, which in turn produce antibody-mediated damage to peripheral nerve. In most cases the shared antigenic determinants are gangliosides, however recent reports suggest that besides gangliosides, some axonal anchoring proteins may be the targets.

## ***1.5 Diagnosis of GBS and diagnostic challenges:***

GBS essentially remains a clinical diagnosis. CSF and EPS, when abnormal help aid the diagnosis however one of the major issues with these diagnostic markers is that both CSF and EPS can be normal especially in the early course of the disease. Identification of early diagnostic markers has remained a major challenge to the researchers. Early diagnosis of GBS is very important as it might influence the treatment and help guide better monitoring of GBS patients, especially in acute phase. In order to correctly identify GBS spectrum disorder both for research and epidemiological studies, various diagnostic criteria have been described. In this section I have discussed the development of various criteria and current issues related to these criteria.

### **1.5.1 Development of Diagnostic Criteria:**

After its first description in 1916, the term GBS was loosely applied to describe any form of acute polyneuropathies presented with acute flaccid paralysis. In 1960, Osler and Sidell, suggested that AFP cases with severe sensory involvement and sphincter disturbance should not be included and proposed a clinical criteria and suggested that the term 'GBS' should only be used to describe a relatively uniform group of disorder. (46) However 1966, McFarland and colleagues challenged this view and suggested that GBS is complex disorder the criteria proposed by Osler and Sidell was too narrow to include all clinical subtype, a view most researchers agree with nowadays. (47)

Further impetus to develop diagnostic criteria came from Centre of Disease Control (CDC), USA. This happened on the background of identification of thousands of GBS cases following administration of Swine flu vaccine in USA, which resulted in multimillion dollar lawsuits on federal government. In order to identify correct GBS cases US government and CDC, required an effective surveillance programme and as a part of this program CDC urged national Institute of Neurological and Communicable Disease (NINCD now NINDS) to developed a clinical criteria for GBS. In 1978 a panel of experts proposed diagnostic criteria based on clinical, laboratory and electrophysiological features. (48) As per these criteria, GBS can be diagnosed on

clinical ground only if a typical symmetrical motor weakness is present along with generalized areflexia. In an atypical case laboratory features such as raised CSF protein with normal cell counts and EPS features can aid the diagnosis. This criterion was developed for neurologist and non-neurologists physicians for the surveillance purpose and therefore GBS variants were not included in this criterion.

In 1990, Asbury and Cornblath modified NINDS criteria and proposed other criteria; this time they proposed specific EP parameters for demyelination. (49) These criteria have been extensively used for diagnostic work up and also in the research settings.

In 2009, the Brighton GBS working group made further such attempts in response to study the association of H1N1 vaccine and GBS. (50) The purpose of the criteria was to design a practical, sensitive and reasonably specific tool to identify most GBS cases, post immunisation. As per these, criteria GBS patients were divided into four different categories depending on the diagnostic certainty. Level 1; represented highest degree of certainty and included clinical features of bilateral flaccid weakness, areflexia and monophasic course and laboratory features of cytoalbumin dissociation and abnormal EPS. Level 2; represented the same clinical features as Level 1 but the presence of only one of the laboratory features. Level 3; represented only clinical features and level 4; represented the least diagnostic certainty based availability of any of the clinical and laboratory features in absence of any other diagnostic possibility. Recently Fokke et al performed an analysis of Dutch cohort 494 GBS patients from four different clinical trials and tried to classify them as per Brighton criteria. (51) The study showed that only 41 % patients met level 1 criteria, 36 % of patients met level 2 criteria, 3 % of the patients met level 3 and about 22 % of the patients met level 4 criteria. Of those who did not meet Level 1 criteria, about 25 % of patients did so due to normal CSF examination, 3 % of cases had progressive course more than 4 weeks and about 5 % cases did not have monophasic course. This study highlights the underlying clinical heterogeneity of the disease.

### **1.5.2 Anti Ganglioside antibodies- A new diagnostic marker of GBS?**

Both glycoproteins and glycolipids have been identified as putative antibody targets for GBS. Of all these putative antigens, gangliosides have generated very much interest over past two decades. The term gangliosides refers to the large family of glycosphingolipids that contains sialic acid linked to the oligosaccharide core, synthesised through addition of monosaccharides in a stepwise fashion by glycosyltransferases and sialyltransferases. (25) Gangliosides are present throughout the body but are highly concentrated in the nervous system. Auto antibodies to various gangliosides are present in up to 60% of GBS cases in acute phase.(52) While this observation does not provide the proof of their pathogenicity, the strength of this association has generated a considerable interest in this field of research.

#### ***1.5.2.1 GBS subtypes and associated antibodies:***

**AMAN:** IgG antibodies targeted against GM1, GD1a, GalNAc GD1a and GM1b gangliosides are frequently seen in patients with AMAN. In a recent joint Japanese-Italian study 66% of AMAN patients were positive for the antibodies against any of these four gangliosides. (53) Interestingly, in the same study about 30% of anti ganglioside positive patients who were originally classified as Acute Inflammatory Demyelinating polyneuropathy (AIDP) showed electrophysiological pattern consistent with AMAN on repeat examination at 3-6 weeks time. Taking this finding into consideration about 83% of AMAN patients had one of these four anti ganglioside antibodies. Thus testing for these antibodies may help in identifying motor axonal subtype of GBS. However, IgG anti GM1 antibodies are not specific to axonal variant of GBS and can also be found in patients with AIDP and chronic inflammatory neuropathies. (54)

**AIDP:** Unlike AMAN, association between auto antibodies and AIDP is not very clear and consistent. Occasionally serum auto antibodies against GM1, GM2, GM3, GD3, GT3 and galactocerebroside are found in AIDP. Antibodies against peripheral nerve myelin specific glycolipids such as LM1, Hex- LM1 and SGPG have also been found in various studies with very modest specificity. (55–57) In a recent study, Kuwahara et al

have shown that about 12% of GBS patients have antibodies against LM1.

**MFS:** A clear and constant association exist between anti GQ1b/GT1a and miller fisher syndrome. Anti GQ1bIgG antibodies are present in more than 90% of patients with MFS with high level of specificity. (25) Almost all anti GQ1b antibodies cross-react with the structurally similar ganglioside GT1a. (29) Some MFS sera also react to GD3 and GD1b. (58)

**BBE:** Because of the similarity in clinical presentation and identification of the common autoantibodies, antecedent infection, neuroimaging and neurophysiological findings BBE is now considered a variant of MFS with some central nervous involvement. Serum IgG Antibodies against GQ1b and GT1a gangliosides are found in 68% and 60% of BBE patients respectively. (12)

**Ataxic GBS:** Some patients with GBS present with acute ataxia without any limb weakness and ophthalmoplegia. IgG antibodies against GQ1b and GD1b gangliosides are found in some patients with ataxic GBS. (59) In a recent study Kaida et al. investigated the antibody reactivities of anti GD1b IgG positive sera against various GD1b containing complexes in GBS patients with and without ataxia. The study showed that reactivities of anti GD1b IgG were significantly inhibited by addition of other gangliosides in ataxic GBS patients indicating that antibodies highly specific for GD1b are strongly associated with ataxia in GBS. (60) This study further support the hypothesis that complex lipid environment in nerve membrane could affect the accessibility of anti ganglioside antibodies.

#### ***Antibodies against Ganglioside complexes:***

Since their first report in 2004 of anti GSC antibodies in GBS patients Kaida et al have subsequently shown that about 17% of GBS patients have antibodies against various gangliosides complexes and some of the GSC antibodies are associated with severe disability. (61,62) In a recent study, Kuwahara et al has shown that about 7% of the GBS patients had antibodies to LM1/GM1 complex and majority of these patients were classified to have AIDP on electrodiagnostic studies. (55) Antibodies against ganglioside complexes have also been reported in acute motor neuropathy. In a study

Kaida et al found IgG antibodies against GM1/GalNAc-GD1 complex are found in patients with acute pure motor neuropathy, characterised by early conduction block (CB) at intermediate segments, infrequent sensory and cranial nerves involvement and overall good recovery.(63) However there is an ongoing debate whether AMN with CB should be considered an AIDP or AMAN.

***New non-glycolipid antigenic targets in GBS:***

Nodal conduction failure plays an important part in GBS pathology however the precise mechanism leading to conduction failure has not been very well elucidated. Disruption of nodal sodium channel (Nav) cluster has been reported in both AIDP and AMAN and therefore lately the focus has been shifted to nodal proteins as target antigens in GBS. Languor et al studied the early changes in two animal models of AIDP and in one model they demonstrated that NF186 and gliomedin were selectively affected before the onset of demyelination and they were associated with serum antibodies against neurofascin and gliomedin and disruption of the Nav channels.(64)

Pruss et al investigated sera from 52 GBS patients and 44 healthy controls for antibodies against two nodal proteins, neurofascin and contactin and found that serum neurofascin IgG antibodies were significantly elevated in GBS patients compared to normal controls. (65) Thus, antibody screening against various gangliosides and nodal proteins can play an important role in early diagnosis of GBS and can be used as early diagnostic markers.

However this area of research has various limitations. 1) Historically researchers have tried to identify antibodies against a group of antigenic target gangliosides, with a concept that a single electrophysiological study is suffice to classify GBS either in to AMAN or AIDP. Recent studies have clearly challenged this concept and we now know that in GBS, EPS phenotype changes in first few weeks of disease onset. 2) Most electrophysiological and serological correlation studies have been performed using only a single EPS and therefore raises an important question about the validity of these correlations 3) further complexity in this field has been added by the discovery of ganglioside complex antibodies. Screening more than 200 ganglioside and their complexes is beyond the scope of currently available immunological methods such as

ELISA, and therefore newer techniques such as high throughput combinatorial microarray has been developed.

## ***1.6 Treatments:***

Treatment of GBS is usually supportive and broad-spectrum immunotherapy. There are no specific targeted treatments available yet. In this section various immunomodulatory therapies have been discussed.

### ***1.6.1 Corticosteroids:***

Based on animal models of allergic neuritis, initially, GBS was thought to be a cell mediated disorder analogue to experimental allergic encephalomyelitis (EAE). Since corticosteroids were effective in treatment of EAE models, it had been used for the treatment of GBS for many years with a little success. Most reports were based on either anecdotal reports or retrospective studies giving conflicting results. In 1978 RAC Hughes et al conducted the first randomised controlled trial of 40 patients comparing the efficacy of prednisolone with no treatment. (66) Twenty one patients in treatment arm were treated with prednisolone 60 mg for a week, 40 mg for a four days, and then 30 mg for three days and 19 patient in control arm did not receive any treatment. The primary outcome; an average improvement in a six-point disability scale at one, three and twelve month was considered. Although mortality rates were the same in both the groups, the trial showed that steroid treatment was not effective and in fact had detrimental effect on the outcome. About 20 % patients remained significantly disabled at the end of 1 year.

Since then two large multi centre double blind trials have been conducted. The first trial of 242 patients, treatment arm received high dose intravenous methyl prednisolone 500 mg daily for 5 days and control arm received placebo infusion. The results did not show any statistically significant difference in mean improvement in disability grade after 4 weeks and 3 months, reduction in duration of artificial ventilation and ability to walk

unaided. (67)

In 2004 Van Koningsveld et al conducted another multi centre study with 225 patients to assess the effect of methyl prednisolone combined with Intravenous Immunoglobulin (IVIG) vs. IVIG alone. This study did not show any significant difference in the outcome. (68)

Thus the above studies showed no evidence of beneficial effect of steroid on GBS outcome and therefore steroids have no role in GBS treatment.

### **1.6.2. Plasma Exchange:**

In 1978, Brettle et al published the first report of beneficial effect of plasma exchange (PE) in GBS. (69) Encouraged by this report and subsequent anecdotal reports showing the efficacy of plasma exchange in GBS, Greenwood et al conducted the first randomised placebo controlled trial in 1984. (70) This study did not show any beneficial effect of PE on GBS outcome, however in the same year, Osterman et al published another study with 38 patients comparing PE with supportive care, showed that 77 % patients who received PE recovered by more than one functional grade at 4 weeks as compared with only 30 % in control group with only supportive care.(71) Subsequently various studies, including one large multi centre study of 220 patients comparing PE with supportive care conducted by French cooperative group on PE in GBS showed significant improvement in both primary and secondary outcome measures including the rate of improvement in disability grade, median time to recover independent walking and disability rates at 1 year. (72) This group also showed that four sessions of plasma exchanges were better than two and effect of six session of PE was similar to four sessions. As a result of these studies PE was widely accepted as first line therapy in patients with GBS.

### **1.6.3. Intravenous Immunoglobulin:**

Initial report of Imbach and colleagues showing beneficial effect of IVIG in patients with immune thrombocytopenia, paved way for its use in other inflammatory conditions. (73) Encouraged by this, and subsequent observations that patients with CIDP showing beneficial effect of fresh frozen plasma, in 1988 Kleyweg et al conducted the first pilot study of eight GBS patients treated with intravenous gamma globulin showing beneficial effects in some patients. (74) The same group in 1992 conducted a randomised controlled trial 150 patients comparing IVIG with PE. (75) The results showed that after 4 weeks about 53 % patients improved by one grade on GBS disability scale compared to 34 % in PE group, and median time to improve by one functional disability grade was significantly shorter in IVIG group (27 days with IVIG and 41 days with PE). Although the result of this trial showed slight superiority of IVIG over the PE, subsequent study showed similar efficacy. (81) Since then due to ease in administration and relatively few complications involved with IVIG, it has now become the first line treatment in GBS.

### **1.6.4. Newer treatments:**

There is growing body of evidence to suggest that GBS is an antibody-mediated disorder in which complement fixing IgG1/3 subclass anti-ganglioside antibodies plays a major role in GBS pathogenesis. These antibodies are also directed against epitopes present on peripheral nerves and are induced through the mechanism of molecular mimicry with bacterial lipo-oligosaccharides. (25) The specificity of these antibodies largely determines the clinical spectrum (pure motor, sensory-motor, Miller Fisher syndrome, etc). Current research into the pathogenesis of GBS is primarily focused on identifying humoral immunity and downstream effector pathways, including complement activation. Recent experimental evidence suggests that complement activation plays a crucial role in the development of neuromuscular weakness in GBS making complement inhibitors and regulators attractive therapeutic targets. Indeed, when the effect of eculizumab- a humanised monoclonal complement inhibitor was studied in animal models of GBS, it showed that eculizumab prevented the formation of

membrane attack complex deposition on axonal surface and thus prevented antibody/compliment-mediated damage. (76) Encouraged by this finding Willison group has started a two-one randomised double blinded study of 30 patients comparing IVIG alone and IVIG with Eculizumab. This pilot study is essentially a safety and tolerability study but we will examine the efficacy of the treatment based on some secondary outcome measures. The study has been initiated in July 2014.

## ***1.7 GBS outcomes, outcome measures***

Although the outcome of most appropriately treated GBS cases are considered to be favourable, about 20 % patients remain significantly disabled at 12 months. (77) Most of the GBS outcome data comes from clinical trials and some prospective observational studies conducted in western world. Due to lack of systematic studies and infrastructure required for registration and monitoring of the GBS cases outcome data from developing countries are not available. In this section, we discuss GBS outcomes and their predictors.

### **1.7.1 GBS Outcomes**

In context of GBS most widely studied outcomes are; *a*) Mortality (Death) *b*) Requirement of ventilation *c*) Disability; especially ability to walk independently at 3 months, 6 months and 12 months *d*) Pain *e*) Fatigue. However, systemic long-term outcome data are not available.

#### ***1.7.1.1 Mortality of GBS:***

GBS can be a life threatening disorder. Most common causes of death in GBS are respiratory failure, cardiovascular failure, pneumonia and autonomic disturbance. (39) Although, there were no large-scale prospective studies in Pre IVIG/PE era, some retrospective studies from western countries showed mortality rates of 2 to 5 % while around the same time, one study of 63 GBS patients from India showed mortality rate of 28 %. (78, 79)

Winer et al conducted the first well-designed prospective study in 1988. (39) In this study, authors recruited 100 patients with acute idiopathic neuropathy and followed them for 52 weeks. The study showed mortality of 13 %. The most common cause of death in this study was cardiac arrest. In post PE/IVIG era mortality data comes from various clinical trials performed after 1978. Meta analysis of five clinical trials comparing PE and placebo and a single trial of steroid comparing the placebo showed mortality of 5.9 % in the treatment group and around 5 % in the placebo group, suggesting that the availability of treatment like plasma exchange has not made a significant difference in the mortality rates. (80) Similarly trials performed comparing two widely available treatments namely PE and IVIG continued to show mortality rates between 2.4 to 6.3 %.(81) Recently a Dutch group has published the data of 527 GBS patients recruited in one observational study and three therapeutic trials showing mortality of 3.9 %.(82) Out of fifteen patients who died during the follow up, 20 % died during the acute phase, 13 % died in plateau phase and 67 % of patients died during the recovery phase. Most common causes of death were respiratory and cardiovascular complications.

#### ***1.7.1.2 Requirement of ventilation:***

Respiratory failure requiring mechanical ventilation (MV) is a serious complication of GBS. Respiratory failure in GBS is due to underlying inspiratory and expiratory muscle weakness. Often insidious in onset, it increases the risk of aspiration pneumonia and respiratory arrest. Various retrospective studies from pre PE/IVIG era showed that about 10- 33 % patients required MV. (39, 66) Data from various clinical trials comparing PE and placebo, IVIG and PE, continues to show the rates to be around 31 %. (80)

#### ***1.7.1.3 Disability:***

“Ability to walk independently” is the most common outcome measure has been studied in various clinical trials so far. In pre IVIG/PE era, one study showed that about 20 % patients remained significantly disabled i.e. able to walk but unable to perform any manual work at 12 months also a proportion of patient remained bed bound or chair bound. (78) Other studies performed during that period also showed residual disability

rates between 7 to 22 %. (39)

**Table 1-1 Outcome measures in various clinical trials**

<b>Outcome Measure</b>	<b>Number of Studies</b>	<b>Studies</b>
<i>Ability to walk at 6 months</i>	<i>1</i>	<i>GBS study Group 1985</i>
<i>Ability to walk at 12 months</i>	<i>2</i>	<i>Bernsen et al 2002 Van Koningsveld et al 2004</i>
<i>Full muscle strength at 12 months</i>	<i>5</i>	<i>Greenwood et al 1984 Osterman et al 1984 French Co-operative study 1987 Farkilla 1987 French CO-operative study group 1997</i>

In post PE/IVIG era, various controlled trials used different timings to measure the outcomes as outlined in the table below.

***Does ability to walk independently change with PE at 6 months?*** Only a single study performed comparing PE with placebo has used this outcome measure. A study performed by GBS study group in 1985 showed that about 82 % of patients were able to walk independently at 6 months in PE group as compared to 71 % in control group, suggest very modest benefit. (83)

***Does ability to walk independently change with PE or IVIG at 12 months?*** A Study comparing IVIG with IVIG and PE combination showed 84 % of patients were able to walk independently at 12 months. (81) Considering the fact that about 82 % of patients were able to do so within 6 months, this benefit is not very substantial. Unfortunately there is no 12 months outcome data available for this outcome in patients without immunotherapy treatments and therefore no conclusion can be drawn about long-term efficacy of immunotherapy.

***Recovery of full motor strength at 12 months:*** French cooperative study group trial performed in 1987, comparing PE with no treatment, showed recovery of complete muscle strength in 52 % of untreated patient at 12 months, compared to 72 % in PE group. (72) However another study showed this rate to be 58 % only in treatment group, giving conflicting results. (84)

***Is immunotherapy with PE/IVIG useful?*** : Whilst above studies show only modest improvement in the long-term outcome, all the studies showed significant improvement in short-term disability measures. One such measure used in all trials is GBS disability (GBSD) score, also known as Hughes Disability score which scores disability from 0-6, where score of 0 is normal; 1 patient is capable of running with minor symptoms; 2 is walking independently; 3 is walking with assistance; 4 is bed/chair bound; 5 is ventilated and 6 is Dead. In all the trials GBSD score measured at the time of entry and 4 weeks after the starting of treatment, showed significant proportion of the patients improved by 1 grade in treatment group compared to untreated group. This clearly shows that immunotherapy hastens the recovery.

**Table 1-2 Proportion of patients improved by 1 grade on GBSD with immunotherapy**

	<b>Plasma Exchange Group</b>	<b>No Treat ment</b>
	(% of patients improved by >1 grade of GBSD at 4 weeks)	(% of patients improved by >1 grade of GBSD at 4 weeks)
<i>Greenwood et al, 1984</i>	50 %	40 %
<i>Osterman et al, 1984</i>	77 %	30 %
<i>The GBS Study Group, 1985</i>	59 %	39 %
<i>French Cooperative Study Group, 1987</i>	61 %	36 %
<i>French Cooperative Study Group, 1997</i>	50 %	30 %
<b>Average</b>	59.4 %	35 %

#### **1.7.1.4 Relapse rates:**

Data from 5 trials showed that about 3.8 % of GBS patients have clinical relapses within 12 months of disease onset.

#### **1-3 Relapse rates in various clinical trials**

<b>Study</b>	<b>Relapse at 12 months</b>
<i>Greenwood et al, 1984</i>	<i>1/14</i>
<i>Osterman et al, 1984</i>	<i>1/18</i>
<i>The GBS Study Group, 1985</i>	<i>2/122</i>
<i>French Cooperative Study Group, 1987</i>	<i>6/109</i>
<i>French Cooperative Study Group, 1997</i>	<i>14/361</i>
<i>Average</i>	<i>24/624 (3.8 %)</i>

#### **1.7.1.5. Pain in GBS:**

Ruts et al performed a prospective study of 156 GBS patients and found that in about 36 % of patients, pain was a presenting symptom irrespective of type of GBS. (85) Different types of pain reported by patients, which included radicular pain, meningism, painful paresthesia/dyesthesia, and myalgia. With regard to long term outcome about 38 % of patients complained of pain at 12 months.

#### **1. 7.1.6 Fatigue**

Fatigue can be the most disabling symptoms in patients with immune mediated neuropathy and can occur even after apparent physical recovery, which may have profound impact on patients' quality of life. In a study performed by Merkies et al on 113 patients with immune mediated neuropathy, which included 80 GBS patients, 80 % of patients reported fatigue as amongst three most disabling symptoms. (86) Only two studies have been performed looking at the long term fatigue data in GBS patients and both studies have shown that severe fatigue persists for long time in this group of patients even after apparent physical recovery. (87, 88)

### **1.7.2 Outcome Measures:**

The major focus of modern day clinical practice is evidence based medicine. Evidence generated by sound research techniques has huge implications for not only clinical practice but also for health policies. In clinical research focus is largely on measurement of a particular outcome with or without a specific intervention. In context of GBS, "disability" is the most widely measured outcome. Unfortunately, there is no consensus-based definition of disability; however various conceptual models available which help guide the measurement.

#### ***1.7.2.1 Defining Disability and Disability measurement in GBS:***

Various conceptual models available, these range from medical model of disability to social model, and some with intermix of both. As per world health organisation (WHO), disability is defined as an umbrella term for any impairment, activity limitation or participation restriction which limits functioning within personal and environmental factors. In context of GBS, disability has been traditionally defined by "ability to walk" and very little emphasis has been given on other medical or social parameters.

### 1.7.2.2 GBS Disability Scale:

The very first attempt to develop a GBS disability scale was made by Hughes et al in 1978. They designed a scale also called as ‘Hughes Disability scale’ or ‘Hughes functional scale’, in which they measured disability on a categorised scale of 0 to 6 where 0: healthy; 1 minor symptoms but capable of running; 2: walking unaided but unable to run; 3: able to walk with a stick, appliance or support; 4 confined to bed or chair; 5 ventilated and 6: dead. (66)

**Table 1-4 GBS disability scale**

<b>GBS disability score</b>	
0	<i>Healthy</i>
1	<i>Minor symptoms but able to run</i>
2	<i>Able to walk independently</i>
3	<i>Unable to walk 10 meters independently</i>
4	<i>Bed bound or chair bound</i>
5	<i>Ventilated</i>
6	<i>Dead</i>

This scale has been widely used in clinical trials. While on the one side it has an advantage that each of the categories has been very well defined and is clinically relevant, which significantly increases intra rater agreement, a quality needed for a good multi centre study, the other side it has some disadvantages 1) GBSD is lower limb specific, which means it does not capture any upper limb disability 2) It is less sensitive in capturing small clinical change in early stages of GBS. This was first demonstrated by Ropper et al in 1988 in a series of 96 GBS patients treated with PE and out of those 96 patients 10 patients had early relapse following the first PE, evident by decrease in muscle strength and vital capacity. (89) These patients were subsequently treated with additional course of PE and showed significant improvement. One of the interesting

points noted by Ropper et al was that some of these patients with obvious clinical deterioration noted on muscle strength did not have any change in their GBSD scale, highlighting the fact that GBSD scale was relatively insensitive in identifying a small clinical change especially in acute settings. A similar observation was by Kleyweg et al. (90) In their study authors introduced a new score; MRC sum score which is a summation of the strength of 6 muscle groups on each side and the score ranges from 0 to 60 where 0 represents complete paralysis and 60 represents full muscle strength. They compared MRC sum score with GBSD scale in patients participated in Dutch GBS trial and measured the sensitivity of GBSD and MRC sum score by simultaneously measuring these two scores at various disease stages and showed that when patient with clinical deterioration has simultaneous decline in their MRC sum scores but not in GBSD scale. One of the major issues with the ordinal measurement scales is that their nonlinearity and GBSD being inherently an ordinal scale, the distance between different categories is not the same. For example on a measurement scale can the distance between dead (GBSD 6) and being ventilated (GBSD 5) be the same as the distance between being healthy (GBSD 0) and able to run (GBSD 1) ? And the answer is probably not and therefore using these values for parametric calculations defies the principles of measurement science.

### **1.7.2.3 Overall Neurological Disability Scale (ODSS)**

In 1999, based on Guy's neurological disability scale, Sharrack et al developed *Overall Neurological DisabilitySum Score (ODSS)*, which included both upper limb and lower limb disability with a total score ranging from 0(no disability) to 12 (Severe disability). (91) In this scale various upper limb functions were graded as being "not affected", "affected but not prevented" and "prevented", these results were then translated into numbers ranging from 0 to 5, for upper limb disability. Similarly lower limb functions were graded depending on ability to walk but not run, these results then translated into lower limb scores of 0 to 7.

In order to be clinically useful, any measurement scale must be able to satisfy all clinimetric properties such as validity, reliability and responsiveness. Validity here is defined by the relation between the disability and the scale used to assess the disability, which usually relies on expert judgements, by establishing high correlation between the scale and a gold standard. Reliability assesses the internal consistency in a multi item scale with good intra and inter observer agreement also called K value. Responsiveness of the scale is defined by scale's ability to detect any minor clinically meaningful change when evaluating the benefits of a particular medical intervention.

In a study involving 113 immune mediated polyneuropathy Merckies et al, showed that ODSS was very simple to use, met all the clinimetric requirement and compared to Hughes disability score it was shown to have captured wide range of disability and therefore was better at monitoring the clinical progress of the patient. (92) In 2006, the same authors compared five different disability scales with short form health survey (SF-36) in patients with immune neuropathies and showed that, compared to other scales ODSS was better at relating to patient's own perception of disability. (93) However this scale also had some limitations such as "ability to run" was not included in the scale and again being an ordinal scale it deemed not suitable for parametric calculations. (94)

**Table 1** The overall disability sum score (ODSS)

Arm disability scale – function checklist	Not affected	Affected but not prevented	Prevented
Dressing upper part of body (excluding buttons/zips)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Washing and brushing hair	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Tuning a key in a lock	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Using knife and fork (/spoon—applicable if the patient never uses knife and fork)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Doing/undoing buttons and zips	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
<b>Arm grade</b>			
0 =	Normal		
1 =	Minor symptoms or signs in one or both arms but not affecting any of the functions listed		
2 =	Moderate symptoms or signs in one or both arms affecting but not preventing any of the functions listed		
3 =	Severe symptoms or signs in one or both arms preventing at least one but not all functions listed		
4 =	Severe symptoms or signs in both arms preventing all functions listed but some purposeful movements still possible		
5 =	Severe symptoms and signs in both arms preventing all purposeful movements		
Leg disability scale – function checklist	No	Yes	Not applicable
Do you have any problem with your walking?	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Do you use a walking aid?	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
How do you usually get around for about 10 metres?			
Without aid	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
With one stick or crutch or holding to someone's arm	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
With two sticks or crutches or one stick or crutch and holding to someone's arm	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
With a wheelchair	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
If you use a wheelchair, can you stand and walk a few steps with help?	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
If you are restricted to bed most of the time, are you able to make some purposeful movements?	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
<b>Leg grade</b>			
0 =	Walking is not affected		
1 =	Walking is affected but does not look abnormal		
2 =	Walks independently but gait looks abnormal		
3 =	Usually uses unilateral support to walk 10 metres (25 feet) (stick, single crutch, one arm)		
4 =	Usually uses bilateral support to walk 10 metres (25 feet) (sticks, crutches, two arms)		
5 =	Usually uses wheelchair to travel 10 metres (25 feet)		
6 =	Restricted to wheelchair, unable to stand and walk few steps with help but able to make some purposeful leg movements		
7 =	Restricted to wheelchair or bed most of the day, preventing all purposeful movements of the legs (eg, unable to reposition legs in bed)		
Overall disability sum score = arm disability scale (range 0–5) + leg disability scale (range 0–7); overall range: 0 (no signs of disability) to 12 (maximum disability).			
For the arm disability scale: allocate one arm grade only by completing the function checklist. Indicate whether each function is "affected," "affected but not prevented," or "prevented."			
For the leg disability scale: Allocate one leg grade only by completing the functional questions.			

**Figure 1-3 Overall Neurological Disability Sum Score**

#### ***1.7.2.4 Overall Neuropathy Limitations Scale (ONLS)***

Considering the shortcomings of ODSS, especially scale's inability to assess functions like running and climbing stairs, and to reduce the ceiling effect, Graham et al developed another scale called Overall Neuropathy Limitations Scale (ONLS) (95). ONLS was assessed for inter rater reliability and content validity and responsiveness. Analysis showed that ONLS had better content validity and in terms of construct validity. ONLS correlated very closely with ODSS and other measures of impairment such as MRC sum scores, limitation, handicap and health related quality of life scores. ONLS also showed very good inter rater agreement and similar responsiveness to ODSS with retaining its simplicity.

Name:  
Date:

### Overall Neuropathy Limitations Scale (ONLS)

**Instructions:** The examiner should question **and** observe the patient in order to determine the answers to the following questions. Note should be made of any other disorder other than peripheral neuropathy which limits function at the foot of the page.

#### ARM SCALE

Does the patient have any symptoms in their hands or arms, eg tingling, numbness or weakness? Yes  No   
(if "no", please go to "legs" section)

Is the patient affected in their ability to:	Not affected	Affected but not prevented	Prevented
Wash and brush their hair	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Turn a key in a lock	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Use a knife and fork together (or spoon, if knife and fork not used)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Do or undo buttons or zips	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Dress the upper part of their body excluding buttons or zips	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
If all these functions are prevented can the patient make purposeful movements with their hands or arms?	Yes <input type="checkbox"/>	No <input type="checkbox"/>	Not applicable <input type="checkbox"/>

#### Arm Grade

- 0=Normal  
 1=Minor symptoms in one or both arms but not affecting any of the functions listed  
 2=Disability in one or both arms affecting but not preventing any of the functions listed  
 3=Disability in one or both arms preventing at least one but not all functions listed  
 4=Disability in both arms preventing all functions listed but purposeful movement still possible  
 5=Disability in both arms preventing all purposeful movements

SCORE= \_\_\_\_\_

#### LEG SCALE

	Yes	No	Not applicable
Does the patient have difficulty running or climbing stairs?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Does the patient have difficulty with walking?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Does their gait look abnormal?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
How do they mobilise for about 10 metres (ie 33 feet)?			
Without aid	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
With one stick or crutch or holding to someone's arm	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
With two sticks or crutches or one stick or crutch holding onto someone's arm or frame	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
With a wheelchair	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
If they use a wheelchair, can they stand and walk 1 metre with the help of one person?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
If they cannot walk as above are they able to make some purposeful movements of their legs, eg reposition legs in bed?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Does the patient use <b>ankle foot orthoses/braces?</b> (please circle)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/> if yes (please circle) right/left

#### Leg grade

- 0=Walking/climbing stairs/running not affected  
 1=Walking/climbing stairs/running is affected, but gait does not look abnormal  
 2=Walks independently but gait looks abnormal  
 3=Requires unilateral support to walk 10 metres (stick, single crutch, one arm)  
 4=Requires bilateral support to walk 10 metres (sticks, crutches, crutch and arm/frame)  
 5=Requires wheelchair to travel 10 metres but able to stand and walk 1 metre with the help of one person  
 6=Restricted to wheelchair, unable to stand and walk 1 metre with the help of one person, but able to make some purposeful leg movements  
 7=Restricted to wheelchair or bed most of the day, unable to make any purposeful movements of the legs

SCORE= \_\_\_\_\_

Overall Neuropathy Limitation Scale=arm scale (range 0 to 5)+leg scale (range 0 to 7);  
 (range: 0 (no disability) to 12 (maximum disability))

TOTAL SCORE= \_\_\_\_\_

Is there any disorder, other than peripheral neuropathy, which affects the above functions Yes  No   
 if yes please describe:

### 1-4 Overall neuropathy Limitation Score

#### ***1.7.2.5. Development of New outcome measures:***

As described earlier, most outcome measures used in GBS are ordinal, multi-item, composite scores and have been designed using “classic test theory”. Data collected using such measures are qualitative and traditionally the numbers have been assigned to each descriptive category and based on these numbers, parametric calculations are being performed, assuming the linearity of the scales. Interestingly, most clinical trials have been conducted using these ordinal scales, which hampers the interpretations and results of clinical trials. To mitigate the shortcomings of currently available scales new outcome measures, which are based on modern clinimetric measurement techniques have been proposed. In the new technique measurements are performed at interval and ratio level, which in turn increases the precision. Not only that, the distance between two response categories is known and therefore parametric calculations can be easily performed. One such modern techniques is called “Rasch model” has generated huge interest. This probabilistic model has been designed using “Item Response Theory” and is based on logical assumption that a patients with high ability is more likely to give affirmative response to a particular “Task” or “Item” compared to a patient with less ability. (96) The probability of a patients to give affirmative response is also depends on “Task” or “Item” difficulty. Thus patient’s ability and “Task” or “Item” difficulty having logarithmic relations are measured using a logit scale and thus ordinal data can be converted into an interval scale. (97)

##### **1.7.2.5.1 Rasch Based Overall disability scale:**

A generic, Rasch based measure, ACTIVLIM has been designed to capture and monitor disability in neuromuscular disorders in children and adults. (98) However item weights obtained from this scale were significantly varied from those obtained from inflammatory neuropathies such as GBS, CIDP and multifocal motor neuropathies, highlighting the need of disease specific Rasch based outcome measure. In one such attempt to develop a disease specific outcome measure, van Nes et al constructed a preliminary scale with 146 items obtained from WHO international

classification of functioning, disability and health and patient questionnaire.

This preliminary scale was further assessed in 294 patients using various Rasch parameters and finally a 24-item Rasch based overall disability scale was constructed.

(100)

R-ODS was further compared to ODSS and as outlined in the figure, it was shown to have good representation of wider range of item difficulties and therefore was better at targeting patients with different disability levels. R-ODS was also shown to have good reliability and validity. Thus, development of this linearly weighted scale was a remarkable step forward in terms of outcome measures in peripheral neuropathies (PN), one of the limiting factors is its disease specificity. When R-ODS was analysed in Multifocal Motor Neuropathy (MMN) subgroup, it strongly differed to other PN, in terms of item difficulties. This was due to selective and asymmetric, especially distal involvement of different muscle groups in MMN and items such ‘turn a key’ and ‘making a sandwich’ were considered more difficult in this subgroup compared to other PN. The other limiting factor was applicability of this scale to different geographically and culturally diverse populations as this scale was designed using a patient cohort from a single country. Thus further studies are needed to assess its responsiveness and cross cultural applicability.

## 1.9 Conclusions:

GBS is a spectrum of post infectious inflammatory polyneuropathy with various clinical, electrophysiological and regional variants. It remains a clinical diagnosis. While we have made considerable progress in our understanding of underlying immunopathogenesis, this has not yet translated into better diagnostic and therapeutic avenues. Clearly, the discovery new antigenic targets and new EP variants has generated significant interest in this area of research, however their precise role as diagnostic tools is yet to be established. Understanding various immunological factors and their association with different clinical and EP variants is of paramount importance and holds the key for development of new therapeutic targets.

Similarly, due to heterogeneous nature of the disease identification of underlying predictive factors also holds key for development of good prognostic models so that individual patients with poor prognosis can be identified early and can be targeted with novel treatment. New monoclonal antibodies against complement activation have raised hopes for availability of better therapeutics targets.

In order to study these issues in greater detail, systematic, well organised, international observational studies are needed

## 1.10 Aims

As outlined earlier in this chapter, GBS remains a clinical diagnosis with highly diverse clinical course and outcome. Currently available literature suggests that about 20 % of patient remained severely disabled at 1 year and about 5 % of patient die. Current research in GBS focuses on two major issues: 1) identification of early prognostic markers of poor outcomes 2) identification of early diagnostic biomarkers of the disease.

Early identification of patients with poor prognosis is very important, and if identified early, this group of patients may benefit from additional treatment when it is likely to be most effective. This can be achieved by developing good prognostic models and biomarkers based on clinical, serological and genetic information on patients. At present there are no readily applicable and validated prognostic models available to identify this group of patients with poor prognosis. (133) Previous studies have identified age, type of preceding infection, extent of nerve damage; immune factors including anti glycolipid antibodies and genetic polymorphisms as important prognostic markers and based on this information various prognostic models have been designed. However these studies have been performed on a small number of relatively homogenous patient population and therefore the results of these studies need to be validated by a systemic large multi centre study.

Identification of anti glycolipid antibodies in a proportion of GBS patients has generated considerable interest in the field of biomarker development; however their

diagnostic role has been constantly debated. Moreover recent discovery of anti ganglioside complex antibodies has added further complexity to this field and therefore a systematic, well designed, clinico-serological association study is required to investigate their diagnostic role in GBS.

As GBS is relatively a rare disorder, we conducted an international, prospective, observational study called 'IGOS' to address above issues. It aimed to study at least 1000 patients worldwide over 3 years. The study provides an excellent opportunity to study various clinical and biological factors in a systematic way with an aim to validate existing prognostic models of GBS and also to develop new prognostic models. IGOS has generated a large clinical and serological database, which will enable screening of these sera for new diagnostic biomarkers of the disease.

Thus, the overall aims of this study (IGOS) were:

- To identify clinical and biological determinants and predictors of disease course and outcome in individual patients with GBS, as early as possible after onset of disease
- To identify new serological diagnostic markers
- To validate already existing prognostic models and develop new prognostic models

Due to various limitations in access of the international data, we first sought to analyse IGOS UK database of 122 patients with following aims:

- To perform a pilot analysis IGOS UK cohort of GBS patients with an opportunity to identify questions of interest for the main study and to learn lessons from this
- To describe the clinical features and current treatment trends in the UK
- To study the short term as well as long term outcomes including mortality, disability and QoL
- To externally validate the existing prognostic models- EGOS and MEGOS
- To compare newly developed rajabally's EP criteria with existing criteria and to determine whether single study performed using Rajabally criteria is sufficient to establish final EP diagnosis

# **Chapter 2 Materials and methods**

## ***2.1 Introduction:***

This chapter provides a detailed description of the general methods used in the International GBS outcome study. Methodology, protocol and endpoints relevant to this study have been discussed. While recruitment into IGOS study continues at the time of writing this thesis, I analysed clinical data collected between May 2012 and January 2015 at 15 different UK centres.

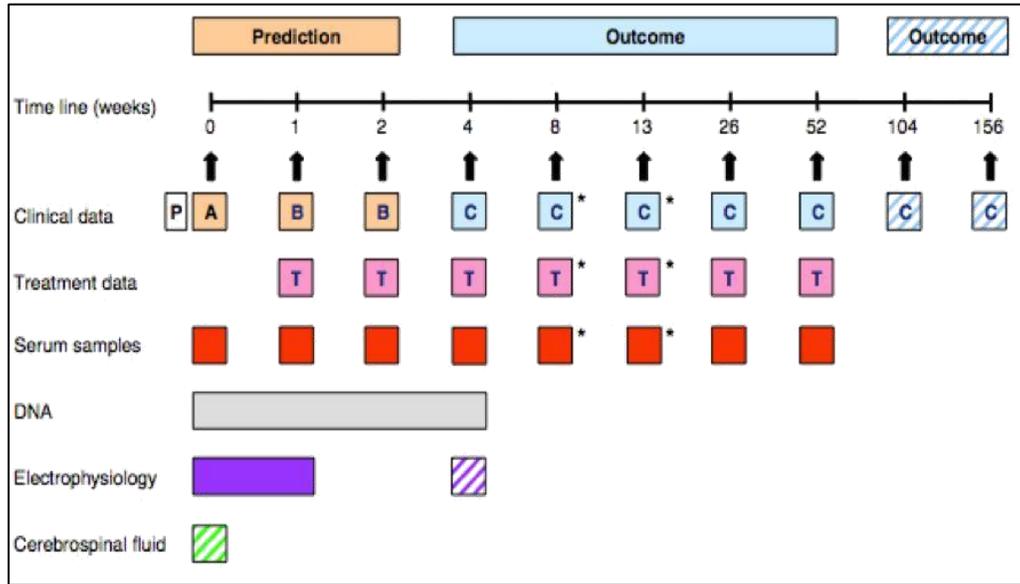
Govind Chavada and Hugh J Willison were the country co-ordinators for IGOS UK. Individual principal investigator at different UK centres collected patient's recruitment, clinical and serological data. Clinical data was transferred on to IGOS website ([www.gbsstudies.org](http://www.gbsstudies.org)). All the serological samples were stored at University of Glasgow.

## ***2.2 International GBS Outcome Study (IGOS)***

### **2.2.1 Study design**

IGOS was a prospective observational international multi-centre study. It aimed to recruit at least 1000 patients with GBS or variants of GBS, including the Miller Fisher syndrome (MFS) and overlap syndromes with a follow-up period of one year with an option to extend this period to two and three years. As part of the study a detailed and standardised database on clinical features, treatment, and diagnostic electrophysiology, serum samples and DNA samples were obtained at specific visits. There were two

optional modules of the study (1) to collect cerebrospinal fluid (CSF) during routine diagnostic work-up for proteomic studies, and (2) to conduct an extended follow-up of two and three years (Figure 2.1).



**Figure 2-1 International GBS Outcome Study (IGOS) database and bio bank.**

*The striped blocks refer to optional sub studies. Clinical data will be obtained using standard forms regarding personal data (P-form), clinical characteristics at study entry (A-form), at 1 and 2 weeks (B-form), and at later visits (C-form). At the same visits data are collected about treatment interventions and intensive care (T-form). Blood samples are obtained as indicated for serial serological studies and for DNA extraction (single sample in the first month). Routine diagnostic electrophysiology will be conducted in the first week, and is possible at 4 weeks.*

*\*Admitted patients at 8 weeks and 13 weeks will have a full examination and serum sampling; discharged patients at these visits a telephone assessment only and no serum sampling.*

The clinical, treatment and electrophysiology data and the biomaterial obtained in the first two weeks of inclusion were used to predict the clinical course and outcome in individual patients at four weeks and at later time points during follow-up.

The first two weeks after entry reflected the time window in which additional therapy was likely to be most effective. Questionnaires for the first three visits have focused on potential prognostic factors, including the clinical features used in previously designed prognostic models such as the EGOS and MEGOS. Questionnaires for later visits has focused on clinically relevant endpoints, including: extent of weakness, autonomic dysfunction, respiratory insufficiency, treatment-related fluctuations, recurrences, complications, disability, pain, fatigue, and transition to chronic inflammatory demyelinating polyneuropathy (CIDP).

Serum samples were collected and will be tested for infection serology and for the presence of antibodies to gangliosides, which may be related to the clinical course and outcome. In addition, sera will be tested for the presence of antibodies to the combination of different gangliosides (ganglioside complexes), which frequently occur in GBS. Antibodies to other peripheral nerve will also be determined.

In patients treated with intravenous immunoglobulin (IVIg), the serum samples will be used to define the variation in pharmacokinetics in relation to outcome. The serum samples will also be used to determine other potential biomarkers for inflammation and peripheral axonal and myelin damage.

DNA extracted from a single blood sample obtained in the first month will be used to determine the relation between gene polymorphisms and disease susceptibility, clinical course and outcome.

In addition to this obligatory core study of the IGOS, there was an option to participate in two additional sub studies. It was anticipated that these studies would be performed only in a proportion of the patients and centres. These optional additional studies were:

*(1) Cerebrospinal fluid (CSF) biomarkers.*

Most patients will undergo a spinal tap as a routine procedure in the diagnostic work-

up of GBS. The observed number of cells and concentration of protein in CSF was registered in the core study. In addition there was an option to collect an extra sample of CSF to determine biomarkers for nerve damage, which are related to outcome. These biomarkers will be examined by advanced proteomics, which require a special procedure for conservation and storage of this extra CSF sample.

*(2) Long-term outcome*

Most recovery occur in the first year after onset of disease, but further recovery has been reported in a proportion of patients. To define the long-term outcome of GBS and to be able to predict this outcome, there was an option to conduct an extended follow-up in which patients are re-assessed at two years and three years after onset of disease.

**2.2.2 Patient Inclusion criteria**

- Fulfil the diagnostic criteria for GBS of the National Institute of Neurological Disorders and Stroke (NINDS) (as outlined below)
- In addition all patients with Miller Fisher syndrome (MFS) and other variants of GBS, including overlap syndromes can be included, for which additional diagnostic criteria will be provided. (as outlined below)

- Inclusion of all males and females patients over the age of 18 years, independent of disease severity and treatment Inclusion within two weeks of onset of weakness (or other symptoms attributed to GBS)
- Opportunity to conduct a follow-up of at least one year

### **2.2.3 Diagnostic criteria for Guillain-Barré syndrome (GBS)**

#### **Features required for diagnosis**

- Progressive weakness in both arms and legs (might start with weakness only in the legs)
- Areflexia (or decreased tendon reflexes)

#### **Features that strongly support diagnosis**

- Progression of symptoms over days to 4 weeks
- Relative symmetry of symptoms
- Mild sensory symptoms or signs
- Cranial nerve involvement, especially bilateral weakness of facial muscles
- Autonomic dysfunction
- Pain (often present)
- High concentration of protein in CSF
- Typical electrodiagnostic features

#### **Features that should raise doubt about the diagnosis**

- Severe pulmonary dysfunction with limited limb weakness at onset
- Severe sensory signs with limited weakness at onset
- Bladder or bowel dysfunction at onset
- Fever at onset
- Sharp sensory level
- Slow progression with limited weakness without respiratory involvement (consider subacute inflammatory)
- demyelinating polyneuropathy or CIDP)

- Marked persistent asymmetry of weakness
- Persistent bladder or bowel dysfunction
- Increased number of mononuclear cells in CSF ( $>50 \times 10^6/L$ )
- Polymorphonuclear cells in CSF

#### **2.2.4 Diagnostic criteria for Miller Fisher Syndrome (MFS)**

##### **Features required for diagnosis**

- Bilateral ophthalmoparesis or ophthalmoplegia
- Ataxia
- Areflexia (or decreased tendon reflexes)
- Features that support diagnosis
- Progression of symptoms over days to 4 weeks
- Relative symmetry of symptoms
- Mild limb weakness (in case of prominent limb weakness, consider GBS-MFS overlap syndrome)
- Mild sensory symptoms or signs (in case of prominent sensory symptoms or signs, consider GBS-MFS overlap syndrome)
- Facial palsy and/or bulbar palsy
- Presence of serum IgG antibodies against ganglioside GQ1b Nerve conduction studies: no changes in extremities
- High concentration of protein in CSF, cytoalbuminologic dissociation

##### **Features that should raise doubt about the diagnosis**

- Alterations in consciousness
- Corticospinal tract signs
- Fever at onset
- Marked persistent asymmetry of weakness

### **2.2.5 Criteria for inclusion of patients in the optional research modules of the IGOS:**

Additional informed consent was required for each optional research module:

- CSF biomarkers: additional volume obtained at diagnostic spinal tap
- Long-term outcome: additional clinical assessments at two and three years

To limit selection bias as much as possible there are no exclusion criteria to be included in IGOS.

### **2.2.6 Clinical Assessments**

Clinical assessments were performed according to a standard protocol at eight visits using specific data entry forms for each visit (Figure 2). Forms A and B focused on the clinical predictors of outcome; Form C focused on outcome measures which included the GBS disability Score, MRC sum score, Rasch-built MRC score, Overall Neuropathy Limitations Scale (ONLS), Rasch-built Overall Disability Scale (R-ODS), Fatigue Severity Scale (FSS) (and Rasch-built FSS), and the EuroQol EQ-5D health questionnaire. In addition information regarding involvement of cranial, sensory and autonomic nerves, pain and complications were collected. If a patient has been recruited at a time point (within 14 days of onset) when they were already recovering, additional information about the historical peak disability and the date it was reached was collected.

All patients had a full neurological examination at entry, and at 1 week, 2 weeks, 4 weeks, 26 ( $\pm 2$ ) weeks and 52 ( $\pm 2$ ) weeks after entry. Patients who were still admitted to the hospital at 8 ( $\pm 1$ ) weeks and 13 ( $\pm 1$ ) weeks were examined additionally at those time points, but once discharged, patients at these visits were not been seen and had a telephone assessment of the GBS disability score, ONLS, R-ODS, FSS and EuroQol EQ-5D only.

### **2.2.7 Collection of treatment data and admission to intensive care unit data**

Information concerning treatment and complications of treatment, admission to ICU, intubation and artificial ventilation was updated at all visits during follow-up (Figure 2). This information was collected via Form T . These data will be used to define the current practice of treatment of GBS in various centres and countries and the side effects of treatment. Some patients with poor prognosis received a second dose of IVIg as a routine practise. Therefore, information collected from form T will be used to compare the functional outcome between the patients who would have received a single dose of IVIg and those who would have received a second dose of IVIg. (I-SID GBS study). This will be one of many specific research areas. Some patients would have not received any treatment and would have not required ventilation or admission

to an ICU, these patients were still recruited into the study, and in these patients this questionnaire remained empty.

### **2.2.8 Routine diagnostic electrophysiology**

Most patients with GBS have routine diagnostic electrophysiology examinations to confirm the diagnosis and to specify the electrophysiological subtype of GBS. Although a specific EPS protocol was designed and recommended, due to pragmatic nature of this study, the raw data of the electrophysiological examination was collected. Diagnostic guidelines of GBS recommend conducting a diagnostic electrophysiology study in the first week of hospital admission on at least four peripheral nerves in arms and legs. Many clinics routinely perform a second diagnostic electrophysiology at four weeks, since the electrophysiological subtype of GBS frequently changes in the acute phase of disease. In these cases the raw data from routine EPS was also collected.

### **2.2.9 Collection of blood samples to obtain serum and DNA**

Blood samples were obtained at all visits when the patients had a full clinical assessment, which is at entry (before start of treatment when possible), and at 1 week, 2 weeks, 4 weeks, 23 weeks, and 52 weeks after entry (Figure 2). Additional samples were collected at 8 weeks and 13 weeks in patients still admitted to the hospital. No serum samples were obtained from patients who were discharged at the 8 weeks and 13 weeks time points, since these patients had telephone assessments only. Blood samples were drawn to obtain serum (24 ml at entry and 16 ml at later visits) and DNA (4 ml at any visit in the first month). All the blood samples were obtained using a standard operating procedure. The first serum sample was obtained before the start of treatment. Later serum samples were collected anytime during the treatment. All the blood samples were sent to the Coordinating Centre of the country or region.

### **2.2.10 Collection of CSF for biomarker studies**

Most patients have a spinal tap to examine CSF for routine diagnostic work-up of GBS. In case of a diagnostic puncture there was an option to collect an additional volume of 2 ml of CSF for biomarker studies. If the patient did not have a diagnostic puncture, or if the puncture was performed before inclusion in the IGOS, no additional spinal tap was performed only to be able to participate in this research module. Special handling of the CSF samples required for advanced proteomic studies. A standard operating procedure for the CSF sampling, transport and storage was provided. In short, CSF samples were centrifuged within one hour after the spinal tap. The supernatant (without pellet) is removed and immediately stored in a polystyrene tube at  $-20^{\circ}\text{C}$  until it is transferred to the coordinating centre of the country. Additional informed consent is required to participate in this optional research module.

### **2.2.11 Extended follow-up of two and three years**

To determine the long-term residual deficits, the patients had a telephone assessment at two additional visits: at 2 years ( $104 \pm 4$  weeks) and at 3 years ( $156 \pm 4$  weeks) after onset of disease. The clinical assessments at 2 and 3 years were performed using Form

C and clinical outcome measures as in the assessments at the visits at 4 weeks to 1 year (see Figure 3 and Website questionnaires). This questionnaire contains the following clinical assessments scores: GBS disability score, ONLS, R-ODS, FSS, and EuroQoL EQ-5D. There was also an option to neurologically examine the severely patients at these visits to evaluate the residual cranial nerve and sensory involvement, pain, autonomic dysfunction and complications. Additional informed consent is required to participate in this research module.

#### **2.2.12 Definition of first day and follow-up time points**

IGOS data were compared with data derived from previous trials. Therefore, the first day of the IGOS was defined as the day of the start of this treatment. The patients had a full examination on the same day and this data was registered on the website.

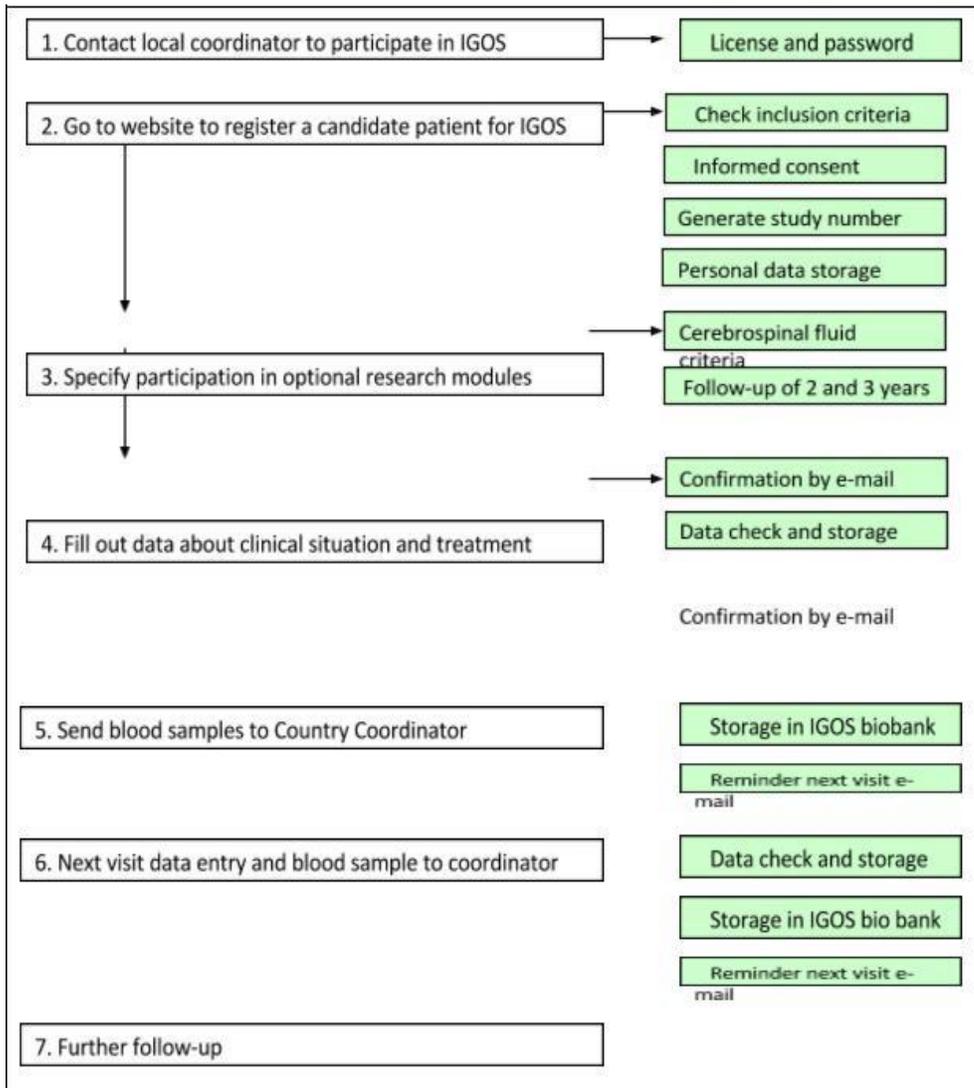
Patients with mild GBS, who did not receive any treatment, in which cases the first day of the IGOS was defined as the day of their registration into the study.

Patients who already had received the treatment, for instance in another hospital from which the patient was transferred, the first day of the IGOS was defined as the day of their registration into the study.

### ***2.3. Development of IGOS website***

IGOS was a multicentre registry database and therefore for ease of administration a web based data entry system was developed with help from professional web designers. The website was designed to inform patients and researchers and to assist and control data entry. This web based data entry system met the highest standards for security and privacy. The IGOS database and bio bank was strictly anonymous: all registered patients were assigned a unique number. Privacy-sensitive personal data including name, address and date of birth of the patients were obtained by a distinct Form P and this information was available only to the local participants and the

country co-ordinators, and was stored separately from the data for research. The website was constructed in a highly secured environment that can be entered by licensed researchers only. Continuous backup of central database was undertaken and the data was continuously monitored for any inconsistencies. The website also provided the time-stamped electronic audit trails to identify what, when and by whom changes were made in the records. Patients were first checked for inclusion criteria. Eligible patients received a unique identification code, which was used throughout the study to ensure the anonymity. Patient information forms and informed consent forms in native languages were available at the website. The coordination centres provided the most common translations, but other translations (and back translations) were developed in collaboration with the participants. Data entry at the eight visits were standardized by using predefined questionnaires for each visit provided by the website. The website also provided explanations for several items and, if needed, the participant could ask for further help by e-mail to the Coordination centre. The website performed instant and automatic quality control of the data during input. At entry a schedule was provided with the dates for the next standard visits. The form of visit 1 (entry) needed to be completed on the same day, otherwise it was considered to be a missed visit. For visit 3, and 4 (on week 1, week 2 and week 4) a maximum of 1 week of window available to fill in the form, otherwise it was considered to be a missed visit. For visit 5 and 6 (on week 8 and week 13) there was a maximum of 2 weeks to fill in the form and for visit 7 en 8 (on week 26 and week 52 there is a maximum of 4 weeks. Reminders for data entry were sent to the researcher automatically by e-mail before the next visit. Instant updates of the patients included were provided.



**Figure 2-2 IGOS Structure**

## **2.4 Organisation**

The members of Inflammatory Neuropathy Consortium conducted IGOS. A steering committee was formed, who supervised the development of study protocol and subsequently monitored the progress of the study. the committee was chaired by Dr Bart Jacobs. To develop sound prognostic models with sufficient statistical power a study with large number of patients was required. Due to low incidence of the GBS, international collaboration required. Members of Peripheral nerve society were informed and country coordinators were identified for each country. Each country coordinator, in turn was responsible for development, monitoring and support of local country network. The main co-ordinating centre was located in Rotterdam, who supported the country coordinators when required. 88 centres from 14 countries had participated in the study. By end of April 2016, 1265 GBS patients were recruited into the study.

### **2.4.1 IGOS UK**

In the UK, three country Coordinators were identified (Prof Hugh Willison, Dr Mike Lunn, Dr John Winer). Dr Govind Chavada was appointed as clinical research fellow. Ethics application (11/WS/0118) to local and national Research and Ethic Committee (REC) was submitted to West of Scotland REC (4). Once ethical approval was obtained nationwide research and development approvals were sought for NHS England, NHS Scotland, NHS Wales and NHS Northern Ireland through a separate procedure. Individual applications were made to 4 national bodies. Once R&D approvals were in place, local principal investigators were identified.

Fifteen centres across the Scotland and England participated in the study. Between 1st May 2011 and 14 April 2016, 154 GBS patients were recruited into the study. For the purpose of analysis, only 122 patients recruited between May 2012 and January 2015 were considered as these patients had completed 12 months follow up visits at the time of analysis (January 2016). Out of 122 patients 8 patients were excluded from final analysis due to protocol violation and incorrect diagnosis. Forty one patients were recruited from Glasgow, 3 patients from Pinderfield, 2 from Leeds, 6 from Bradford, 5

from St Georges hospital London, 2 from National Hospital of Neurology, Queen Square, London, 8 from Kings College University Hospital of London, 9 from Cambridge, 4 from Sheffield, 1 from Ipswich, 9 from Newcastle, 1 from Birmingham, 13 from Oxford, 8 from Liverpool, and 2 from Middleborough. At all centres, patients diagnosed with GBS were notified to local research team by clinical care team (treating neurologist/direct care team), who in turn formally assessed all patients for the suitability of the study. Only treating neurologists had access to patient records without explicit consent in order to identify potential participants, and to check whether they met the inclusion criteria or to make the initial approach to patients. A member of the local research team took consents. Before obtaining consent participants were given both verbal and written information about the study.

Transportation and storage of bio samples constitute an important aspect of the IGOS. In the UK, we have designed a very easy and reliable transportation system in which co-ordination centre in Glasgow provided a welcome pack to each participating centre which contains pre-labelled bio sampling kit, with almost 100 % recollection rate. Similar approach was acquired at other coordinating centres.

Since the study was ongoing at the time of my data analysis was limited to UK patients only. The website was managed by the main co-ordination centre in the Netherlands and release of the data required permission from steering committee and a complete data quality check, since neither of these been done at the time of analysis, I was unable to assess the complete data set. However, as a country co-ordinator, I was able to assess the UK data and therefore all the relevant data was manually transcribed from the IGOS website to an excel sheet for the analysis. Moreover the data quality check had not been performed at the time of this analysis.

#### **2.4.2 Collection of controls sera**

GBS is a post infectious inflammatory disorder. IGOS aimed to develop new biomarkers of the disease and to validate these biomarkers; we needed to compare them with samples (at least 1000) from healthy volunteers (pre-post vaccination) (300) and patients with recent infection (300) and other neurological diseases (300). Therefore, blood samples were collected from healthy volunteers (pre and post vaccination), patients with recent infection and other neurological disorders after appropriate consenting procedure.

Inclusion criteria for controls include: 1) Healthy volunteers (pre and post vaccination) 2) patients with recent infection 3) patients with other neurological disorders other than GBS 4) any adult above age of 18 and ability to give informed consent.

Most control samples were collected at Glasgow site. Patients attending neurology outpatients were approached for OND controls. For pre-post vaccination healthy controls, participants (NHSGGC staff) attending annual flu vaccination clinics were approached and pre vaccination and 2 weeks post vaccination samples were collected. Post infectious controls were identified from infectious disease units.

### ***2.5 Statistical analysis***

Statistical methods used for the analysis of IGOS UK data have been described in detail in individual chapter. In general the statistical analysis presented in this thesis was performed mainly with IBM SPSS statistics (SPSS Chicago, Illinois, USA v.21)

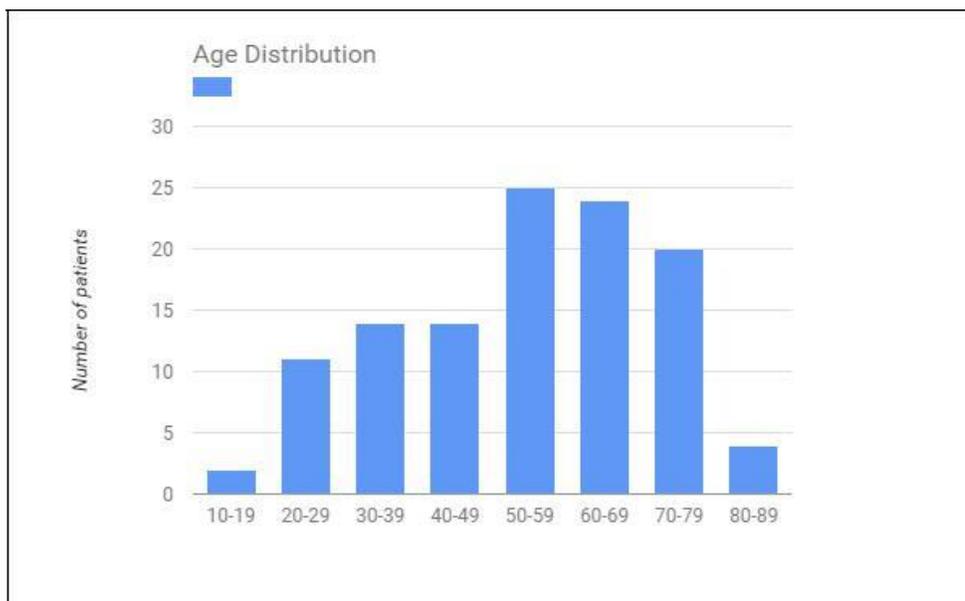
# **Chapter 3 IGOS UK database-Cohort description**

### ***3.1 Introduction:***

This chapter describes basic demographics, clinical features and treatment patterns of UK GBS patients. I first probed the IGOS UK database to determine the baseline demographic including age, sex, preceding infections, presenting symptoms and signs, speed of onset and recovery. We also looked at the CSF characteristic and frequency of pain in acute phase of the disease. All together 122 patients from 15 UK centres were recruited between May 2012 and January 2015. Out of 122 patients only one patient had incorrect diagnosis. This patient was initially diagnosed to have MFS, however subsequently diagnosis was changed to brainstem demyelination. Seven patients with GBS were excluded from analysis due to protocol violations. All these patients were included beyond two weeks of onset of symptoms namely weakness. Altogether data from 114 GBS patients was analysed.

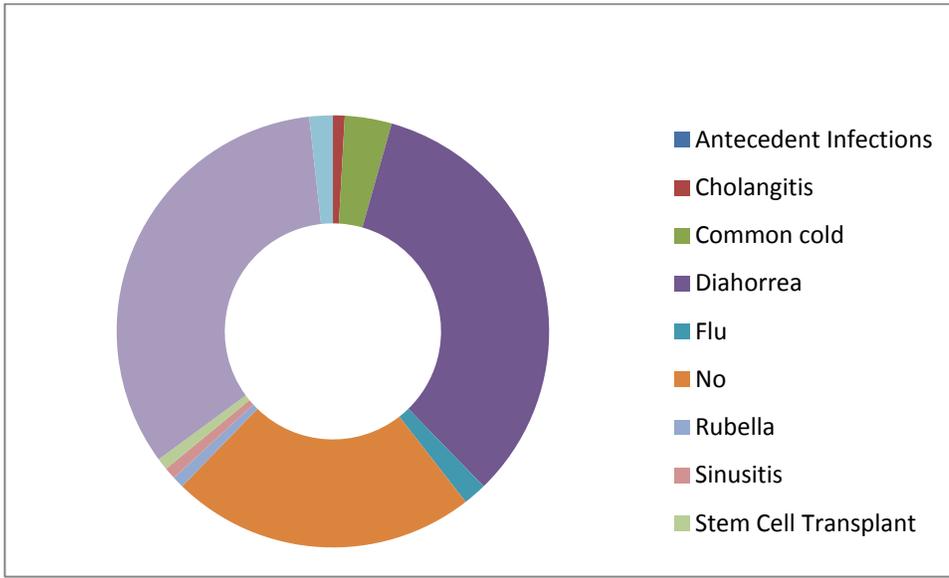
### ***3.2 Age, Sex, Preceding infections & other clinical features:***

Fifty six % patients were male and 44 % of the patients were female. Age distribution of all patients ranged from 18 to 83 years. Age demographic chart showed patients from all age groups being affected but more so in the fifth, sixth and seventh decade.



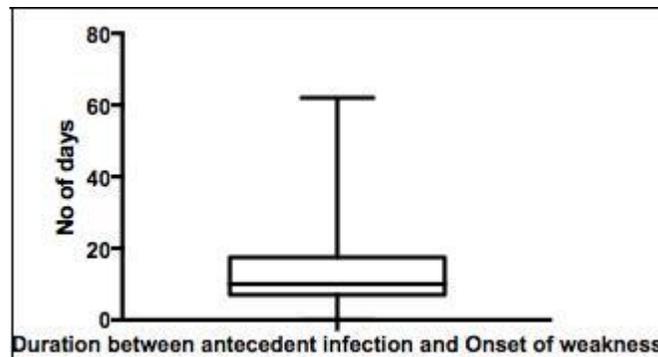
**Figure 3-1 Age distribution of IGOS UK cohort**

Seventy eight % of patients had preceding infections. One patient had GBS following stem cell transplant. Twenty two % of patients did not have any infectious trigger. Amongst the patients with preceding infections, 33 % had diarrhoea, 33 % had upper respiratory tract infections, and other infections group had common cold (3.5 %) and flu like illness (1.8 %). About 2.6 % patient had GBS following vaccination.



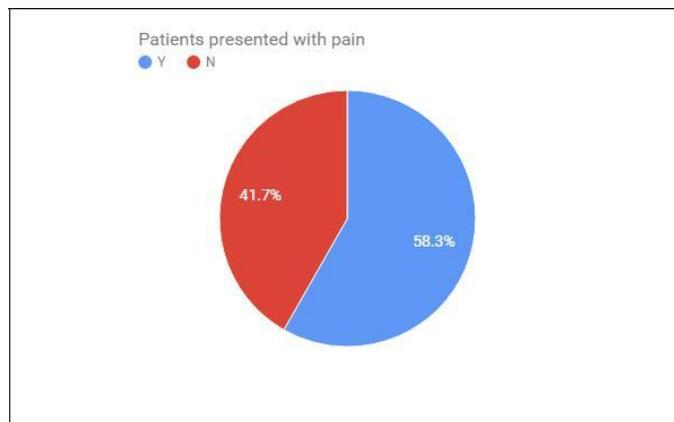
**Figure 3-2 Frequency of antecedent Infection**

Mean duration between infection and onset of symptoms was 15 days and median duration was 10 days with a range of 0-62 days.



**Figure 3-3 Median duration between preceding infection and onset of weakness**

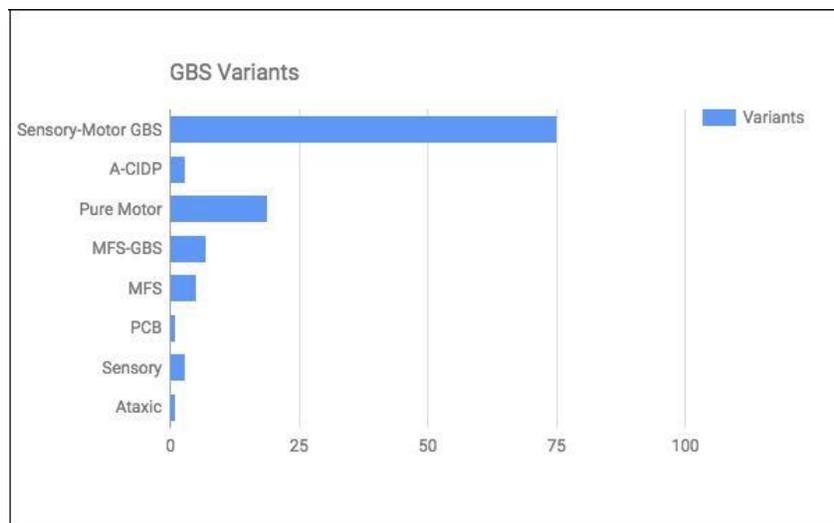
About 58 % of patient had pain at the time of onset of symptoms and about 54 % of GBS patients had cranial nerve involvement. CSF analysis was performed in 93 patients (81.57 %). Mean duration of OOW and CSF study was 4.9 days (range 0-13 days). Mean CSF white cell count was 2.8/cumm (range 0-18). Mean CSF protein was 1.07 gm/L (Median 0.67 gm/L, Range 0-11.65 gm/L). There was statistical difference in CSF protein values in patients CSF study performed within 7 days of OOW and after 7 days of OOW (Mean 0.93 gm/l within 7 days Vs Mean 1.54 gm/l after 7 days P 0.03). Out of 22 patients, who did not have CSF studies performed, only one investigator had a set pattern of not performing CSF in any patient recruited in that centre.



**Figure 3-4 Proportion of patients with pain**

### 3.3 Frequency of clinical Variants:

Symmetrical ascending weakness with very minimal sensory symptoms and signs is a typical feature of GBS. For clinical classification, we considered this type of GBS- a sensory motor GBS. To determine the frequency of different clinical variants we first probed the database and looking at the entry visit and subsequently compared it with other visits to check for consistent recording of clinical variants. Sixty six % of patients had typical sensory motor GBS, 16 % had pure motor GBS, 6.1 % had MFS-GBS overlap syndrome, 4.3 % had pure MFS, 2.6 % pure sensory and only 1 % had ataxic variant. About 2.6 % of patients had acute onset CIDP. Of interest, two patients who were clinically classified as “Pure motor GBS” had sensory symptoms and also sensory deficit on examination at the entry point.



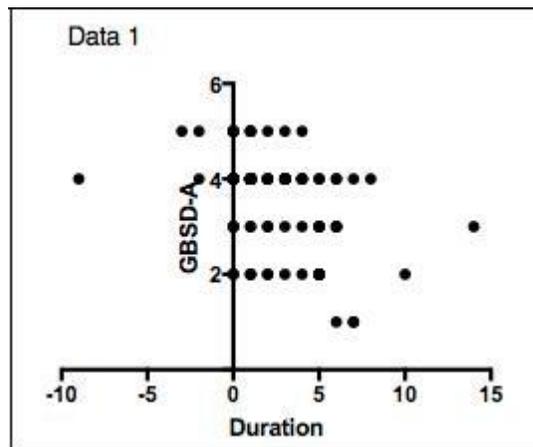
**Figure 3-5 Frequency of clinical Variants**

Mean age for sensory-motor GBS patients was 56.2 years and mean age for pure motor variant was 54 years. There was no statistical difference in age of both groups (P 0.72). However there was significant association identified between preceding infection and type of GBS. All pure motor GBS patients (100 %) had preceding infection as opposed to 70 % patients with sensory motor GBS had preceding infections (P 0.005). Patients with Pure Motor GBS had either diarrhoea or upper respiratory tract infection while patients with sensory motor GBS had other infections such as common cold, flu and cholangitis. None of the patients with A-CIDP had preceding infection.

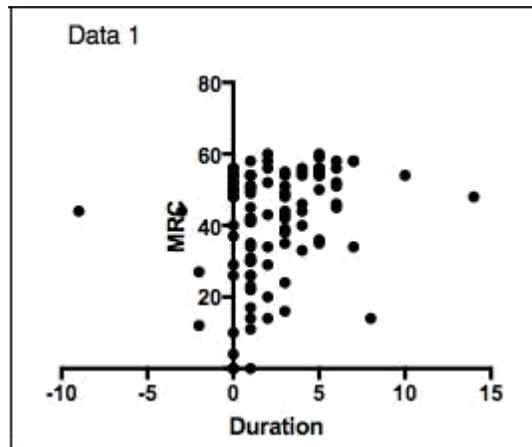
All five patients of MFS had preceding infections (3 URTI, 1 Diarrhoea, 1 Rubella). Similarly all patients with MFS-GBS variant had either diarrhoea or URTI as preceding infections. Two out of 3 sensory GBS patients had preceding infections. Mean CSF protein value of sensory Motor GBS was 1.25 gm./l whereas mean CSF protein for Pure motor GBS was 0.63 gm./l. There was no statistical difference between the two values (P 0.179). However, overall CSF protein was elevated in both variants. Similarly CSF protein was elevated in MFS and MFS-GBS variants as well (Mean 0.90 gm./l)

### ***3.4 Speed of onset, Progression and recovery patterns in acute phase:***

Out of 114 patients; 18.4 % had mild GBS, defined by GBSD < 3 at nadir and 81.57 % patients had severe GBS defined by GBSD > 2 at nadir. Mean duration of onset of symptoms (OOS) and hospital admission (HA) was 3 days (Range 0-11 days). There was weakly negative correlation between duration between onset of symptoms (OOS) and date of hospital admission (HA) and GBS disability scores (R -0.39 CI -0.21 to -0.54). Similarly there was weakly positive correlation between duration of OOS and HA and muscle power graded by MRC sum scores. (R=0.26 CI 0.04 to 0.40).



**Figure 3-6 Correlation between GBSD and the duration between date of onset of symptoms and hospital admission.**



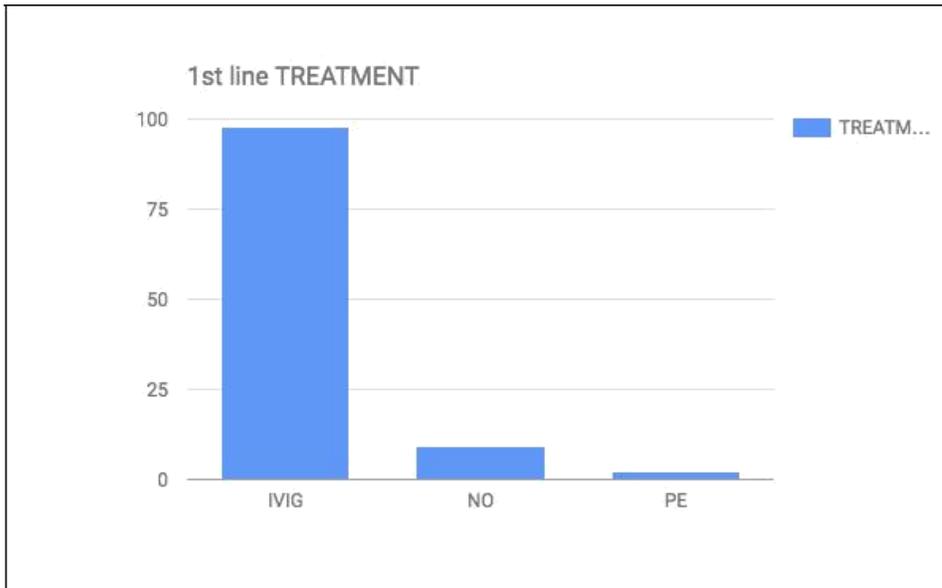
**Figure 3-7 Correlation between MRC sum score and the duration between onset of symptoms and hospital admission.**

Mean duration between OOS and entry into study was 7 days. All the patients reached their peak disability within 4 weeks of entry into the study. This suggests that all the patients reached their nadir within 5 weeks of onset of symptoms. Only 3 patients deteriorated beyond 4 weeks of entry into the study and out of these three patients two had A-CIDP.

Clinical recovery process (as defined by improvement in GBSD by 1 grade) started in 18 % patients within the first week of entry into study and about 24 % patient within 4 weeks of entry into the study. About 58 % patients did not have any functional recovery up to 4 weeks. While the frequency of sensory motor GBS was similar across the England and Scotland, there was significant difference between the frequency of MFS (England 6.3 % Vs 0% Scotland) and pure Motor GBS (12.7 % England Vs 25 % Scotland) patients across the Scotland and England.

### 3.5 Treatment patterns

About 90 % of patients were treated with intravenous immunoglobulin (IVIG), and only 1.8 % patients received plasma exchange. Only two patients had received PE as the first line treatment. Reason for this was not clear in one patient recruited elsewhere, while in one patient recruited at local centre, PE was given due to concerns regarding renal failure. About 8 % patients did not receive any treatment.



**Figure 3-8 Treatment patterns of GBS patients in the UK**

About 16 % of patients received 2nd course of immunotherapy. Out of 19 patients 12 received second course of IVIG and 6 patients received PE. One patient had been concomitantly recruited into ICA GBS trial and had received either placebo or eculizumab. Six patients received the third course of treatment.

### ***3.7 Missing data:***

Most patients had well documented acute phase data. However about 20 % patients did not have 4 weeks outcome data, 25 % patients had 6 months missing data, and only 58 % patients had 12 months outcome data collected.

### ***3.8 Discussion:***

This study represents the first prospective observational study since wider availability of immunotherapy in the UK. Previous population based studies were performed in 1988 and 1998. (39,101) Although, randomised control clinical trials have been performed previously, it only included severely affected patients. In this study, adult GBS patients irrespective of their clinical severity were included. Our study continues to show slightly male predominant pattern with ration of 1.3/1.

With regard to preceding infections, previous studies have shown that about 68 % of GBS patients have clinical or serological evidence of preceding infection within 4 weeks prior to symptoms onset. In our study, about 78 % patients had antecedent infections within 8 weeks prior to GBS onset, this is consistent with biological plausibility. The median time between OOS and antecedent infections was only 15 days in our cohort, which suggests that in majority patients, with infectious trigger; this occurs in following 2 weeks time. The range of duration between OOS and antecedent infection was 0-62 days. One patient had symptom onset and onset of infection at the same time, this patient was diagnosed with Rubella infection at the time of GBS onset. This may need further clarification from the local investigator as the patient may have been infected prior to clinical symptoms onset due to 2 weeks incubation time. One patient had symptoms of URTI 62 days prior to onset of GBS which is slightly out in keeping with strict 8 weeks (56 days) definition. In most cases primary immune response initiates within 5-7 days of antigenic trigger and this followed by class switch and exponential rise in the concentration of antibodies. Since

the median time between most infections and OOW was 14 days, this can be well explained by antibody mediated pathogenesis; however why some patients took almost 8 weeks to develop GBS after initial trigger needs further explanation. Further clinical –serological correlation studies will be very helpful to get better understanding of this issue.

Clinically, GBS can be classified in to different variants. In a typical GBS, generalised ascending muscle weakness is a major feature and sensory symptoms are relatively minor component. In our cohort, 66 % patients had both sensory motor symptoms followed by 16 % patients had pure motor symptoms. Some patients characterised as “pure motor GBS” had abnormal sensory signs on examination. Whether these patients with subclinical sensory fibre involvement should be classified as sensory- motor or pure motor variants based on symptoms only needs further clarification and should be incorporated in the future studies. The current study protocol does not clarify this issue and hence patients were classified at examiners’ discretions.

Only 10 % patients with pure motor variants had cranial nerve involvement as oppose to 100 % patients with MFS and MFS-GBS variants and 49 % patients with sensory motor GBS. Frequency of respiratory muscle involvement was similar in both sensory motor and pure motor forms (17 % in pure motor GBS Vs. 20 % in sensory motor GBS). One patient with MFS- GBS overlap syndrome had respiratory failure. One patient with PCB variant required MV; however this well may be due to dysphagia rather than respiratory muscle involvement.

CSF analysis is considered to be the part of routine diagnostic process in GBS. However at some centres this is not being performed routinely and diagnosis was made mainly on clinical grounds. Our cohort continues to show previously well documented observation of cytoalbumin dissociation with mean CSF white cell count of 2.8/cu mm and raised mean CSF protein of 1.08 gm/L. One patient had CSF protein of 11 gm/L. In our study about 18.4 % patient did not have CSF studies performed and most of these patients had sensory motor GBS. CSF can be normal in GBS patients especially if it is performed early in the course. In our study, mean duration of OOW and CSF study was 4.6 days with a range of 0 to 13 days. About 29.8 % of patients had normal CSF studies. The mean duration of OOW and CSF study in this group was 3.5 days.

There was no statistical difference in duration of OOW and CSF study, between the groups of patients with elevated and normal CSF protein (3.5 days normal vs 5.3 days elevated CSF protein, P 0.79)

Treatment of GBS has changed significantly in last 30 yrs. In a study performed by Winer et al in 1988, only 10 % patients had received PE and none of the patients received IVIG. (39) Subsequent population based study performed by Rees et al in 1998 showed only 66 % patients received immunotherapy and out of these patients, 46 % patients received IVIG as opposed to only 6 % patients received PE. This trend has clearly changed now and our cohort study showed that about 90 % patients received IVIG and only 1.8 % patients received PE. Out of 114 patients 24 had mild GBS and 90 patients had severe GBS (defined by inability walk more than 10 meters independently). Sixteen out of 24 mild GBS patients (66.6 %) received IVIG. This is an important finding as immunotherapy is generally not recommended in mildly affected GBS cases. Two out of 90 patients did not have treatment data recorded however all 88 severely affected patients received either IVIG or PE. One patient who did not receive any immunomodulatory treatment despite being unable to walk had MFS. This trend suggests that IVIG is the most commonly prescribed treatment in GBS patients. However, due to the cost of IVIG treatment and poor affordability of patients, this may not be the trend in developing world and it would be interesting to compare the results of our cohort with IGOS data from any other developing countries. One of the important findings from our study is that about 66.6 % of mildly affected GBS patients still being treated with immunotherapy. Whether this makes any difference in overall outcome requires further analysis and if this is the case, it would have significant implication on current treatment practice.

### ***3.9 Conclusion:***

Demographic description of our cohort shows that sensory-motor GBS remains to be the major clinical phenotype in the UK. Rate of clinical antecedent infections remains very consistent at 78 % especially preceding 8 weeks of GBS onset and this requires further serological correlation studies to establish the link between antecedent infections and type of GBS. Although, CSF studies are routinely performed in most centres, at some centres this is not being done routinely. While IVIG has replaced PE as a first line treatment for GBS in the UK, it is also being prescribed for mild cases too and whether that translates in to any clinically meaningful outcome remains to be seen and requires further analysis,

# **Chapter 4 Outcome of IGOS**

## **UK cohort**

## ***4.1 Introduction:***

Despite immunotherapy GBS remains a disabling disorder in about 20 % patients and about 5 % patients die. These data comes from various clinical trials and some prospective observational studies performed in western world. Outcome in developing world may not be the same. One of the purposes of IGOS was to study GBS patients across the wide geographical areas including some developing countries. IGOS will provide us a unique opportunity to compare outcomes in various geographical areas. Since this data is still being collected at the time of writing this thesis we sought to analyse the outcome of IGOS UK cohort.

## ***4.2 Mortality***

Out of 78 patients, who had 12 months outcome 4 died, with an overall mortality rate of 5.1 %. Out of 4 deaths, one patient died of aspiration pneumonia, one due to ischemic colitis, and one due to peritonitis. Cause of death was unclear in the fourth patient. Three patients died within 4 weeks of entry in to the study and one patient died in the recovery phase.

## ***4.3 Respiratory failure***

Twenty-one out of 114 patients (18 %) required mechanical ventilation (MV). Out of 21 patients, 14 patients (66 %) required MV within 2 weeks of symptoms onset, 5 patients (23.8 %) required MV within 3 weeks of symptoms onset and only 2 patients required MV within 6 weeks of symptoms onset. Only 2 patients continue to require MV beyond 6 months. Out of 21 patients 10 had their 12 months outcome data available, and 2 out of 10 patients died with an overall mortality of 20 %.

## ***4.4 Disability***

In order to compare the outcomes of our current study with previous studies we used proportion of patients achieved a particular functional outcome at 4 weeks, 6 months and 12 months as the primary outcome measures.

We first divided all patients in two categories; Mild GBS as defined by GBSD < 2 and Severe GBS cases as defined by GBSD > 3 at the entry point into the study. Out of 111 cases 89 had severe GBS and 22 had mild GBS.

### **4.4.1 Outcome of severely affected GBS cases:**

#### **Proportion of severe GBS patients improved by one grade on GBSD scale at 4 weeks:**

Most of the clinical trials have used this outcome as a primary end point. In our cohort, out of 89 (79 %) severe GBS cases 17 patients did not have 4 weeks outcome data and these cases were excluded from analysis. Thirty-four patients (**45.9 %**) out of 72 cases improved by 1 grade on GBSD at 4 weeks. All these patients were treated with either IVIG or PE.

#### **Ability to walk without support at 4 weeks:**

Although most clinical trials have used “proportion of patients improved by 1 GBSD” as the primary endpoint, as clinicians what we need is the information about prediction of ability to walk as that what matters to most of the patients. In our cohort out of 72 patients, 24 (**33.3 %**) were able to walk independently at 4 weeks.

#### **Ability to walk without support at 6 months:**

Out of 89 severely affected cases 70 patients had 6-month outcome data. All together 78 % patients were able to walk independently at 6 months time with standard treatment.

#### **Ability to walk without support at 12 months:**

Only 59 severely affected patients had 12 months outcome and about 85 % patients were able to walk unaided at this time point.

#### **Complete Recovery at 12 months:**

19 out of 59 patients (22 %) had complete neurological recovery as defined by GBSD of 0. About 32 % patients continued to have minor symptoms and 32 % patients were able to walk independently but unable to run.

#### **4.4.2 Outcome and subgroup analysis of severely affected GBS cases:**

We divided severe GBS in to two further sub groups moderate GBS defined by GBSD of 3 at entry and severe GBS defined by GBSD of 4 and 5 at entry.

Moderate GBS (GBSD of 3): 20 patients had entry GBSD of 3. Out of 20 patients, 16 patients had their 4 weeks outcome data available. Eight out of 16 patients (50%) improved by 1 grade on GBSD, similarly 8 out of 16 (50%) patients were able to walk independently at 4 weeks time. Eighteen out of 19 patients (94.7 %) were able to walk independently at 6 months time and almost all 19 patients (100 %) were able to walk independently at 12 months time. Only 36.8 % patients had complete recovery defined by GBSD of 0.

Severe GBS (GBSD of 4 and 5): Out of 69 patients, 56 patients had their 4 weeks outcome data available. Twenty six out of 56 patients (46.4 %) improved by 1 grade on GBSD, 16 out of 56 (28.5 %) patients were able to walk independently at 4 weeks time. Thirty six out of 52 patients (69.2 %) were able to walk independently at 6 months time and 33 out of 45 patients (69.2 %) were able to walk independently at 12 months time. Only 15. 5 % patients had complete recovery defined by GBSD of 0.

**Table 4-1 Subgroup analysis of moderate and severe GBS**

	Moderate GBS	Severe GBS	P Value
<i>Improvement by 1 grade on GBSD at 4 weeks</i>	8/16	26/56	0.802
<i>Proportion of patients able to walk independently at 4 weeks</i>	8/16	16/56	0.1096
<i>Proportion of patients able to walk independently at 6 months</i>	18/19	36/52	<b>0.025*</b>
<i>Proportion of patients able to walk independently at 12 months</i>	19/19	33/45	<b>0.012*</b>
<i>Proportion of patients with complete recovery at 12 months</i>	7/19	7/45	0.060

#### **4.4.3 Outcome of mild GBS cases:**

All together we had 24 mild GBS cases (21 %). Two mild GBS cases had acute onset CIDP and they were excluded from analysis. Amongst 22 cases, 3 had GBSD of 1 and they were not treated with IVIG, out of the rest of 19 patients, 14 were treated with IVIG. Out of 19 cases, two patients had clinical deterioration within the first week of entry.

#### **Outcome at 4 weeks:**

Fourteen patients with GBSD of 2 had outcome data available at 4 weeks. Out of fourteen patients, 3 patients did not receive IVIG and 11 patients received IVIG. Only one patient out of 3 had improvement of more than 1 grade on GBSD (33 %) as opposed to 54 % patients in treatment group had improvement of more than 1 grade at weeks suggestive of faster recovery.

#### **Outcome at 6 months:**

Only 1 out of 9 IVIG treated patients had complete recovery (11 %) while none of the patients in non-treatment group had complete recovery.

#### **Outcome at 12 months:**

This analysis was confounded by very small numbers of patients. Altogether 10 patients had outcome data available at 12 months. All the patients with entry GBSD of 1 had complete recovery. While patients with entry GBSD of 2, 20 % had complete recovery in IVIG treated group as opposed to 50 % patients had complete recovery in non-treatment group.

#### ***4.5 Quality of life measurement:***

Apart from mortality and mobility, outcome of GBS patients can be affected by various other determinants such as pain, fatigue, functional impairment related to upper limb disabilities and mental health. (102) All these factors in combination can affect quality of life (QoL) in GBS patients. In this study we measured QoL using newly developed preference based generic tool EQ-5D-5L. The tool divides health related QoL in to five dimensions; Mobility, self care, usual activity, pain/discomfort and anxiety/depression. It also includes a visual analogue scale (VAS), ranges from 0 (worst health) to 100 (best health), which is self reported by patients depending on their perception of overall health status.

All together 67 patients had EQ-5D-5L and VAS data recorded at 12 months time. Response level frequencies for each dimension are shown in the table 4.2.

Difficulty with usual activity was the most problematic domain amongst all. Overall, 62.12 % patients reported some degree of problem with their usual activities at 12 months. This was followed by pain with 54.55 % patients reported slight to severe pain at 12 months time. Mobility continued to remain as a problem in about 51.52 % patients. Only 9.9 % patients reported severe problem with their mobility. This was followed by anxiety and depression with 33.33 % patients reported slight to severe mental health issues at 12 months time. Self care was the least affected domain of all, with only 27.27 % patients had problem with this domain at 12 months time.

Table 4-2 Percentage of participants reporting level 1-5 in each EQ-5D-5L dimension at 12 months

EQ-5D-5L dimensions	Distribution of response	Percentage frequency
<b>Mobility</b>		
No Problem	32	48.48
Slight	14	21.21
Moderate	12	18.18
Severe	6	9.09
Unable to walk	2	3.03
<b>Self care</b>		
No Problem	48	72.73
Slight	6	9.09
Moderate	8	12.12
Severe	3	4.55
Unable to walk	1	1.52
<b>Usual Activity</b>		
No Problem	25	37.88
Slight	18	27.27
Moderate	15	22.73
Severe	6	9.09
Unable to walk	2	3.03
<b>Pain</b>		
No Pain	30	45.45
Slight	17	25.76
Moderate	15	22.73
Severe	4	6.06
Extreme	0	0.00
<b>Anxiety/Depression</b>		
No Problem	44	66.67
Slight	14	21.21
Moderate	6	9.09
Severe	2	3.03
Extreme	0	0.00

### Relationship between each domain and overall QoL visual analogue score:

In general, QOL VAS represents patient's own view of their health and therefore it gives very useful information about wider dimensions of patients' health other than 5 dimensions of EQ-5D-5L.(103) In order to identify most important domain of QoL affecting the perception of good quality of health of GBS patients, we analysed response level frequencies and compared with QOL VAS. Patients were divided in to "no problem" and "slight to extreme" groups. Mean QoL VAS calculated using unpaired t tests. As described in table only problems with "usual activity" was associated with statistically significant difference in QOL VAS suggestive of most important domain remained affected at 12 months time in GBS patients responsible for poor perception of quality of health in this group of patient.

**Table 4-3 Relationship between EQ-5D-5L domains and QoL VAS**

	Mean QoL VAS		
	No	Yes	P value
Mobility	71.06	88.72	0.8525
Self Care	85.1	65	0.5648
Usual Activity	91.72	72.24	<b>0.0415</b>
Pain	90.47	70.58	0.2272
Anxiety/ Depression	84.2	70.45	0.3812

#### 4.6 Comparison between GBSD, MRC sum scores, ONLS and RODS

##### Sensitivity of GBSD and MRC sum score in detecting small clinical change in muscle strength:

We compared MRC sum scores and GBSD to ascertain the sensitivity of both scales in detection of small clinical change. As described in figure 4.1, there was a strong negative correlation ( $R = -0.74$ , CI  $-0.7962$  to  $-0.6723$ ,  $P < 0.0001$ ) between GBSD and MRC sum scores, i.e. Higher the GBSD lower the MRC sum scores. However the variation in MRC sum scores was high in GBSD categories 4 and 5, which suggests that GBSD is not very sensitive measure to detect small change in muscle strength. However MRC sum score also has disadvantage for example MRC sum score can be normal in patients with pure sensory GBS as well as MFS where patient can be severely disabled due to ataxia. Similar negative correlation observed between rasch MRC sum scores and GBSD ( $R = -0.71$ , CI  $0.77-0.64$ ,  $P < 0.001$ ).

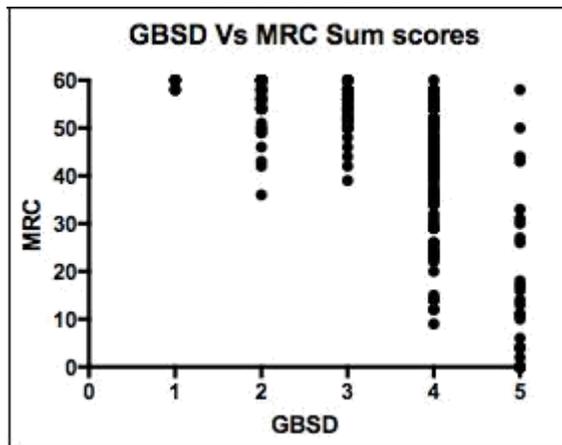


Figure 4-1 GBSD Vs MRC sum score in detecting small clinical changes

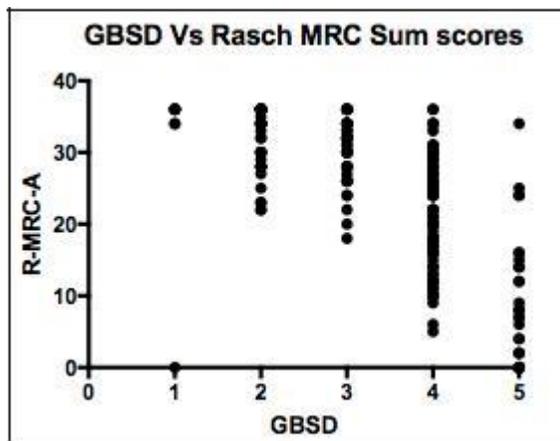


Figure 4-2 GBSD Vs Rasch MRC sum score

### Comparison between GBSD, ONLS and RODS:

Similarly we compared GBSD scale with ONLS and RODS and as shown in Figure 4.3 and 4.4 respectively, an increasing value of GBSD was broadly associated with a higher value of ONLS though the range of ONLS values obtained for each participant for the lower GBSD categories was wider implying a weaker relationship between the two measures at lower GBSD than higher GBSD categories. This analysis suggests that in acute setting MRC sum score and rasch MRC sum score are very useful in detecting minimal change in clinical condition while RODS is useful in capturing wide range of disabilities in the later stage of the disease.

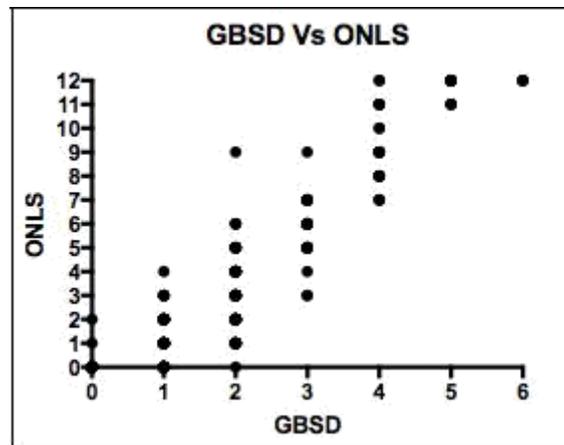


Figure 4-3 GBSD vS ONLS

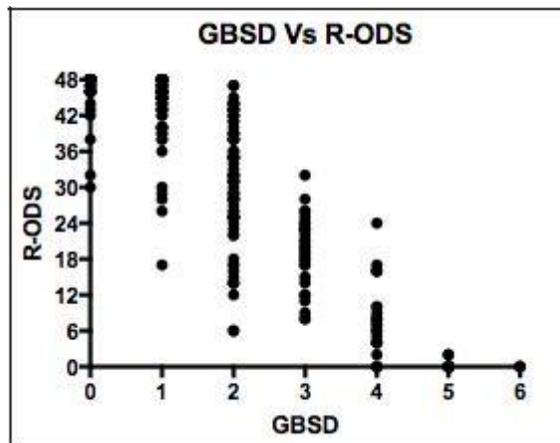


Figure 4-4 GBSD Vs RODS

#### ***4.7 Discussion:***

IGOS is the first prospective multi centre observational study looking at various outcomes in GBS spectrum disorders. As part of this study, we had an opportunity to study various outcomes in GBS patients recruited in the United Kingdom between 2012 to 2015.

Our study showed that despite wider availability of immunotherapy, GBS mortality rate has not changed significantly. This clearly highlights the need for more effective therapeutic options in the area. While aspiration pneumonia remains to be an important cause of death, in our study two patients died due to gastroenterological complications namely ischemic colitis and peritonitis. While venous thromboembolic events are common complication of GBS due to immobility and prothrombotic nature of IVIG treatment, arterial thromboembolic complications have not been widely reported in the literature. Whether this was a coincident or was related to GBS remains to be seen.

Compared to previous studies, the rate of patients requiring MV was slightly lower at 18 % and those who required MV, they did so in the first 3 weeks of GBS symptoms onset. This finding suggest crucial role of respiratory monitoring in GBS patients especially during the first 3 weeks. Only around 10 % patients required MV after 3 weeks of symptom onset. Mortality rate was higher in patients requiring MV compared to overall mortality rates in GBS patients (20 % vs 5%).

With regard to disability, we first compared the percentages of severely affected GBS patients improved by 1 grade on GBSD at 4 weeks. This is the most common primary outcome measure has been used in previous clinical trials involving sever GBS patients. Our study showed that about 45.9 % patients had improvement in their GBSD by 1 grade. This is slightly lower than average of 59.4 % of five studies involving PE, however better than average of 35 % of patient with no treatment.

In our study, 33 % severely affected GBS patients were able to walk independently at 4 weeks, 78 % were able to walk independently at 6 moths and 85 % patients were able to walk independently at 12 months. These rates are very much similar to findings

from previous clinical trials. Only 22 % patients had complete recovery at 12 months time, these findings suggest that long term outcome of severely affected GBS has not changed significantly.

Analysis of outcomes mildly affected GBS was somewhat cofounded by small number of patients and also by the treatment variation. However, our study showed that short term outcome at 4weeks was slightly better in patient who had received immunotherapy. However, the long term outcome was better in patients who did not receive any immunotherapy. This finding requires further study with a larger cohort.

Analysis of QoL data using EQ-5D-5L scale showed that although mobility and pain remained to be major issues at 12 months, inability to perform usual activity was the most commonly affected dimension of life, which also resulted in perception of poor QoL to GBS patients.

With regard to available outcome measures our analysis of comparison of impairment scales and outcome scales showed that MRC sum score was better at capturing minimal clinical change in acute phase of the disease compared to GBSD scale. However, we recommend using both in acute phase, as MRC sum score is very much dependent on muscle weakness, which is absent in sensory/ataxic GBS cases. For the recovery phase monitoring although ONLS captures wide range of disabilities, due to its ceiling effect and non linearity we recommend using RODS.

#### ***4.8 Conclusion:***

IGOS is the first observational study conducted after almost 30 years, providing an excellent opportunity to study GBS outcome in the UK. Previous study conducted by Winer et al showed mortality rate of 13 %, about 67 % patients had complete recovery at 12 months and 20 % patients remained significantly disabled. Out of 100 patients only 10 % patients received plasma exchange and about 12 % patients received steroid treatment.

Our study shows that there has been significant change in the mortality rates ( 13 % vs 5.1%) due to wider the availability of immunotherapy. However, the long term outcome at 12 months has not changed significantly highlighting the need for better therapeutic options other than broad spectrum immunotherapy such as IVIG.

# **Chapter 5 Role of EPS in GBS**

## **5.1 Introduction**

Traditionally GBS was considered to be a demyelinating disorder. However after identification acute motor axonal variants, this view has been challenged. Electrophysiologically, GBS now is being classified into two variants - axonal or demyelinating form; depending on the nerve fibre involvement. Axonal variants can be further reclassified into acute axonal motor (AMAN) and acute motor and sensory (AMSAN) variants. Recognising these subtypes is important as different subtypes may have different immunopathological basis and therefore their treatments and prognosis may also be different. Thus electrophysiology (EP) may have both diagnostic as well as prognostic value.

### **5.1.1 GBS electrophysiological diagnostic criteria**

Lambert and Mulder published the first report of EP abnormalities in GBS patients in 1964. (104)The study showed that about 14 % of the patients had normal studies, 61 % of the patients had abnormal motor conduction velocities (CV) and about 25 % of the patient had prolonged distal motor latencies (DML). At this time motor nerves conduction slowing in distal segment along with prolonged latencies were considered the criteria for demyelination. In 1976, King and Ashby first described that measurement of F wave abnormality (using the technique developed by Kimura) could represent underlying proximal demyelination and can be used as an early diagnostic marker of the disease. (105,106)

The first consensus based EPS criterion was published by Asbury and colleagues in 1978. (48) In their report they described following EPS features; 1) CV is usually less than 60 % of normal; 2) about 80 % of patients have motor conduction slowing or block at some point during the course of illness; 3) DML can be prolonged up to three times of normal and 4) about 20 % of patients have normal studies.

In 1985, Albers and colleagues reviewed sequential EPS from 180 GBS patients and described normal sural and abnormal median nerve findings pattern. In this report they

also proposed quantitative cut off values for CV, DML and F wave latency.

Criteria suggestive of demyelination in the electrodiagnostic evaluation of acute inflammatory demyelinating polyradiculoneuropathy.
Demonstrate at least one of the following in two or more nerves (exceptions noted): A. Conduction velocity less than 95% of lower limit of normal if amplitude exceeds 50% of lower limit of normal, less than 85% if amplitude less than 50% of lower limit of normal. (1) B. Distal latency exceeding 110% of upper limit of normal if amplitude normal, exceeding 120% of upper limit of normal if amplitude less than lower limit of normal. (2) C. Evidence of unequivocal temporal dispersion or a proximal to distal amplitude ratio less than 0.7. (2,3) D. F-response latency exceeding 120% of upper limit of normal. (1,2)
Exceptions: 1. Excluding isolated ulnar or peroneal nerve abnormalities at the elbow or knee, respectively. 2. Excluding isolated median nerve abnormality at the wrist. 3. Excluding the presence of anomalous innervation (e.g., median to ulnar nerve crossover).

**Figure 5-1 GBS electrodiagnostic criteria (Alber et al 1985)**

In 1990, Cornblath et al proposed a new criteria set for detection of primary demyelination.(49). These criteria required minimum of three features of demyelination as oppose to only one required in Albers criteria, making it more specific but less sensitive for GBS diagnosis. (107,108)

Recognition of axonal GBS cases in 1995, required researchers to amend previously existing criteria, and therefore Ho et al published a new criteria, which included parameters for primary axonal degeneration.(109) Hadden et al further modified this criteria and proposed a new set of criteria to diagnose AIDP and AMAN. (110) One of the major differences between these two criteria was consideration of CB in Hadden's criteria while it was not considered in the criteria proposed by Ho. Also Ho et al considered temporal dispersion as one of the features of demyelination while Hadden et al did not include TD in their criteria.

Criteria for AMSAN have been described. Rees et al first in 1995, and subsequently Capasso et al in 2011 described following EP features; 1) No evidence of demyelination 2) dCMAP <80 % of lower limit of normal in motor nerves 3) reduction in sensory nerve action potential <50 % of normal in at least two nerves. (111,112)

### 5.1.2 Pitfalls in existing EP criteria

One of the major issues involving EPS in GBS is that there is no consensus based, universally accepted criteria. AIDP criteria vary in terms of cut off values for various demyelinating parameters. For example for CV, various values ranging from 60 % to 95 % of lower limit of normal have been used in GBS literature. The Cut off value of 60 % has been used in various studies based on assumptions that slowing in CV below 60 % occurs due to demyelination based on some studies performed on amyotrophic lateral sclerosis and Charcot Marie Tooth diseases patients.(113) The evidence for other cut off values are not available in the literature. As a result of different cut off values used in different criteria, diagnostic sensitivity of GBS varies depending on the criteria used. Alam et al studied 43 american GBS patients and their EPS and compared the diagnostic sensitivities of six different available criteria and found that sensitivities varied from 21 % to 72 %. (105) In a similar study performed on Indian cohort of 51 GBS patients, Kalita et al found that diagnostic sensitivities of different criteria varied from 39 to 88 %. (108)

Criteria for AMAN and AMSAN have been proposed, however they were based on traditional view that axonal GBS represents underlying simple axonal degeneration. Recently, Kuwabara and colleagues have challenged this view. (114) In their study, they demonstrated that three AMAN patients with positive anti ganglioside antibodies had low distal CMAPs and on repeat studies CMAPs became normal. Similarly, in another group of antibody positive patient, who were classified as having AIDP based on only prolonged DML or only CB on the first EPS, complete reversal of EPS abnormality occurred in the repeat study, without development of any TD or polyphasia, which are the markers of re-myelination. Patients in both groups had almost complete clinical recovery. It is likely that these patterns of reversible EP abnormalities in antibody positive patients with subsequent complete clinical recovery reflect underlying functional rather than structural block due to Na-channel dysfunction. (115) Interestingly, these features were not seen in antibody negative AIDP and AMAN patients with on-going degeneration and poor clinical recovery. Hiraga et al performed a similar study of 25 antibody positive GBS cases and found that in some patients with complete recovery had reversible isolated F wave

abnormality. (114)

Above studies suggest three different EP patterns in axonal GBS; 1) Simple wallerian axonal degeneration 2) transient CB in intermediate or conduction slowing at distal motor nerve terminals reflected by prolonged DML and 3) transient prolongation of F wave latency. Considering these features Uncini and Kuwabara have coined a new terminology calling it 'Reversible Conduction Failure' (RCF). (117) These patterns also reflect in the clinical recovery pattern seen in axonal GBS; as some axonal GBS with typical wallerian degeneration make poor recovery while others with RCB make almost complete recovery. Not only that, these studies also highlight the importance of serial EPS, in order to identify appropriate GBS subtype, as some of these features can be seen in otherwise classical AIDP in the early stages and these patients can be wrongly classified as having AIDP based on single EPS.

Stressing importance of serial nerve conduction studies, Uncini et al performed a study of 55 GBS patients with serial EPS recording, using two well-described criteria.(118) The study showed that about 65-67 % of patients were classified as having AIDP, 18 % were classified as having axonal GBS and 14-16 % were classified as equivocal on the first examination. On repeat examination, 24 % of the patients' EPS classification changed; only 58 % had AIDP, 38 % had axonal GBS and only 4 % of patients had equivocal EPS. This study highlights the critical role of serial nerve conduction studies in identification of final GBS subtypes, which is again very crucial for correct serological-clinical-EPS correlation. However performing serial EPS can be time consuming and impractical in some clinical settings. Also a recent study performed by Rajabally et al showed that the demyelinating cut off values used in current EP criteria (Ho and Hadden) may partly be responsible for incorrect EP diagnosis of GBS. (122) Using their modified criteria Rajabally et al showed that a single EP study may be adequate to establish final EP diagnosis.

IGOS provides a unique opportunity to study these issues in detail. We conducted a pilot analysis of prospectively collected EP data at a single centre (Glasgow).

## **5.2 Aims**

The aim of this study was to analyse prospectively collected EP data from a single centre (Glasgow) to determine whether using modified EP criteria developed by Rajabally et al is sufficient to achieve the final EP diagnosis. We also compared various criteria to determine sensitivity of each criterion and finally performed a clinical- electrophysiological correlation of entire IGOS UK database.

## **5.3 Methods**

### **Patients:**

Electrophysiological data from 32 prospectively recruited GBS patients at local centre (Glasgow, Scotland) was analysed. Out of 32 patients 24 patients had repeat EP studies performed. At the local centre we first circulated IGOS EPS protocol to all five neurophysiology consultants and data was collected using this protocol when feasible. Electrophysiological examination was performed at two separate time points in 24 patients: the first within 7 days of admission or registration in IGOS, and the second at four weeks after admission or registration in IGOS. The data included sensory studies in legs and arms (3-4 nerves), motor studies with F wave (3-4 nerves), tibial H-reflexes and EMG of a proximal and distal muscle in an arm and leg. Normative data and pictures of the waveforms were also being included in the report. Following parameters were measured: conduction velocities (CV), distal motor latencies (DML), distal and proximal compound muscle action potential (dCMAP and pCMAP), minimum F wave latencies, sensory nerve distal latency and sensory nerve action potential (SNAP). When possible, data was collected from non-dominant side.

**Normal values for local lab (Glasgow):**

For the analysis, I compared different criteria using following normative local lab (Glasgow) values:

**Table 5-1 Electrophysiology normal values (Glasgow lab)**

	<b>Median</b>	<b>Ulnar</b>	<b>C Peroneal</b>	<b>Tibial</b>
<i>CMAP</i>	4	6	2	4
<i>DML</i>	4.1	3	6.1	6.1
<i>CV</i>	48	48	40	40
<i>F</i>	30	31	55	55

**Electrophysiological criteria:**

Following EP diagnostic criteria were assessed.

Alber Criteria:

**Table 5-2 Alber's electrodiagnostic criteria set**

<b><i>AIDP</i></b>
Evaluation should satisfy at least three of the following in motor nerves
<ul style="list-style-type: none"><li>• MCV &lt; 75 % LLN ( two or more nerves)</li><li>• DML &gt; 130 % ULN ( two or more nerves)</li><li>• F wave latency &gt; 130 % ULN ( one or more nerves )</li><li>• Proximal CMAP/distal CMAP ratio &lt; 0.7 ( one or more nerves)</li></ul>

Hadden criteria:

**Table 5-3 Hadden electrodiagnostic criteria set**

<i><b>AIDP</b></i>
<ul style="list-style-type: none"><li>• At least one of the following in each of at least two nerves, or at least two of the following in one nerve if all others are inexcitable and dCMAP <math>\geq</math> 10 % LLN<ul style="list-style-type: none"><li>○ Motor conduction velocity &lt; 90 %LLN (85 % if dCMAP is &lt; 50 % LLN)</li><li>○ Distal Motor Latency &gt;110 % (&gt; 120 % if dCMAP is &lt; 100 % LLN)</li><li>○ Amplitude of proximal CMAP /dCMAP ratio &lt; 0.5 and dCMAP <math>\geq</math> 20 % LLN</li><li>○ F wave latency &gt; 120 %</li></ul></li></ul>
<i><b>AMAN</b></i>
<ul style="list-style-type: none"><li>• None of the features of demyelination in any nerves defined as above except one demyelinating feature allowed in one nerve if dCMAP is &lt; 10 % LLN</li><li>• dCMAP &lt; 80 % LLN in at least two nerves</li></ul>

Rajabally criteria:

**Table 5-4 Rajabally's electrodiagnostic criteria set**

<i>Normal</i>
<p>All the following in all nerves tested</p> <ul style="list-style-type: none"> <li>• DML <math>\leq</math> 100 % ULN</li> <li>• F wave latency <math>\leq</math> 100 % ULN</li> <li>• MCV <math>\geq</math> 100 % LLN</li> <li>• Distal CMAP <math>\geq</math> 100 % LLN</li> <li>• Proximal CMAP/distal CMAP ratio <math>&gt;</math> 0.7 (excluding tibial nerve )</li> </ul>
<i>AIDP</i>
<p>At least one of the following in at least two nerves</p> <ul style="list-style-type: none"> <li>• Motor conduction velocity <math>&lt;</math> 70 %LLN</li> <li>• Distal Motor Latency <math>&gt;</math>150 %</li> <li>• F wave latency <math>&gt;</math> 120 % ULN or <math>&gt;</math> 150 % ULN ( if dCMAP <math>&lt;</math> 50 % of LLN)</li> </ul> <p>OR</p> <p>Absent F waves in two nerves with dCMAP <math>\geq</math> 20 % LLN with an additional parameter in one other nerve</p> <p>OR</p> <p>pCMAP/dCMAP ratio <math>&lt;</math> 0.7 in two nerves with additional parameter in one other nerve</p>
<i>AMAN</i>
<p>None of the features of demyelination in any nerves defined as above (except one demyelinating feature allowed in one nerve if dCMAP is <math>&lt;</math> 10 % LLN) and at least one of the following</p> <ul style="list-style-type: none"> <li>• dCMAP <math>&lt;</math> 80 % LLN in at least two nerves</li> <li>• Absent F waves in two nerves with dCMAP <math>\geq</math> 20 % LLN in absence of any demyelinating parameter in any other nerve</li> <li>• pCMAP/dCMAP ratio <math>&lt;</math> 0.7 in two nerves</li> <li>• Absent F waves in ONE nerves with dCMAP <math>\geq</math> 20 % LLN</li> </ul> <p>OR</p> <p>pCMAP/dCMAP ratio <math>&lt;</math> 0.7 in ONE nerves with in addition dCMAP <math>&lt;</math> 80 % LLN in one other nerve</p>
<i>Inexcitable</i>
<p>If dCMAP absent in all nerves or present in only one nerve with dCMAP <math>&lt;</math> 10 % LLN</p>
<i>Equivocal</i>
<p>Abnormal range findings however not fitting criteria for any other group</p>

In order to identify sensitivity of different EP criteria, first data was entered into a standard template using excel software. This data was probed using different sets of EP criteria using local normal values. Statistical analysis was performed using SPSS software when required.

For clinical- EP correlation, we collected clinical and electrophysiological data from IGOS website from the patients recruited at 15 different centres in the UK, between May 2012 and January 2015. Clinical characteristics such as sensory motor or pure motor or MFS variants were identified and correlated with their electrophysiology record. Statistical analysis was performed using fisher's exact test.

## **5.4 Results**

I included 32 GBS patients at the local centre. There were 19 male and 13 female patients with a mean age of 58.3 years ( range 28-87 years). Median interval between the symptom onset and EPS was 8 days (range 1-21 days).

### **5.4.1 Sensitivity of existing criteria:**

Since Albers' was the most commonly used criteria for the early detection of AIDP cases, we first probed EPS database (Glasgow) using this criteria to see how many patients met the EPS criteria for AIDP. These criteria allowed one of the demyelinating abnormalities in two motor nerves. Using these criteria, 62.5 % patients could be diagnosed with AIDP.

We then probed the database using Dutch GBS study criteria, and found that 25 out of 32 patients (78 %) could be diagnosed as having AIDP. Since both Albers and Dutch GBS study group criteria did not define axonal variants, we probed our data using Hadden criteria, which included axonal variants. Using Hadden criteria 78 % patients

met AIDP criteria. Interestingly none of our patients were classified as having axonal GBS.

**Table 5-5 Sensitivity of different GBS EP criteria set**

<b>GBS Electrodiagnostic criteria</b>	<b>AIDP</b>	<b>Equivocal</b>	<b>Normal</b>
<i>Alber</i>	81.3 %	12.5 %	6.3 %
<i>Dutch GBS</i>	78 %	15.6 %	6.3 %
<i>Hadden</i>	78 %	15.6 %	6.3 %

As highlighted in the table 5.6, when I performed clinical correlation, 44 out of 75 patients with sensory motor GBS had AIDP, 2 patients with clinical Sensory Motor GBS were classified as having AMAN on EPS. Repeat study performed in 3 weeks in one of these patients continued to show findings in keeping with AMAN with on-going active denervation.

Out of 19, clinically pure motor GBS cases, 7 were classified as having AIDP and 8 patients were classified as having AMAN and 1 patient was classified as having equivocal on EPS.

Out of four MFS patients three patients had normal EPS, and EPS was not performed in one patient. Three patients out of nine patients with MFS-GBS overlap syndrome were classified as having AIDP and 3 had equivocal findings. One patient with pure sensory GBS and one patient with pure ataxic GBS were classified as having AIDP while all patients with acute onset CIDP patients had typical findings of AIDP on electrophysiological studies.

#### 5.4.2 Does EP characteristics change with serial studies using conventional Hadden criteria or a single study using modified Rajabally criteria suffice?

To address above question I first analysed the EP data of 32 patients from the local centre (Glasgow) using both Hadden and Rajabally criteria. As highlighted in table 5.5 using Hadden's criteria set 81.3 % patients were classified as having AIDP, 12.5 % were classified as having equivocal and 6.3 % patients were classified as having normal electrophysiology. Application of Rajabally criteria showed 56.3 % patients having AIDP, 21.9 % patients having AMAN, 15.6 % patients having equivocal and 6.3 % patients had normal studies.

**Table 5-6 Comparison of Hadden and Rajabally criteria in 32 patients with a single study**

	AIDP	AMAN	Equivocal	Normal
<i>EPS Criteria</i>				
<i>Hadden</i>	81.3 %	0 %	12.5 %	6.3 %
<i>Rajabally</i>	56.3 %	21.9 %	15.6 %	6.3 %
<i>P Value</i>	0.03	0.005	0.72	1

#### **Analysis of serial EP data using Hadden's criteria set:**

Analysis of serial EP data collected from 24 patients showed that using Hadden's criteria 83.33 patients were classified as having AIDP, 8.33 % equivocal and 8.33 % had normal study on the 1st EPS. Repeat EP analysis of this cohort using the same criteria proportion of AIDP patients decreased to 62.5 % and about 20.8 % patients were identified as having AMAN. The classification shift occurred mainly in AIDP group and 5 (20 %) patients who

were initially classified as having were reclassified as AMAN (4 patients) and Equivocal (1 patient). On the repeat study, the proportion of equivocal cases was increased to 12.5 % and the proportion of normal EPS reduced to 4.1 %.

EP classification shift occurred in 29 % cases and mostly in AIDP cases (71 %). Out of 5 AIDP cases in which classification shift occurred, 4 cases were classified as having AMAN and 1 case was classified as having equivocal. Overall, there was a statistically significant proportion of patients changed the classification to AMAN (P 0.01).

**Table 5-7 Comparison of Hadden and Rajabally criteria in 24 patients with serial studies**

	<b>AIDP</b>	<b>AMAN</b>	<b>Equivocal</b>	<b>Normal</b>
<i>EPS Criteria</i>				
<i>Hadden-1</i>	83.33 %	0 %	8.33 %	8.33 %
<i>Hadden-2</i>	62.5 %	20.8 %	12.5 %	4.1 %
<i>P value</i>	0.10	0.01	0.60	0.54
<i>Rajabally-1</i>	54.1 %	20.8 %	12.5 %	12.5 %
<i>Rajabally-2</i>	54.1 %	16.6 %	20.8 %	8.3 %
<i>P value</i>	1	0.74	0.44	0.63

**Analysis of serial EP data using Rajabally’s criteria set:**

Analysis of serial EP data collected from 24 patients showed that using Rajabally’s criteria 54.1 % patients were classified as having AIDP, 20.8 % as

AMAN, 12.5 % as equivocal and 12.5 % as normal on the 1 st EPS. Importantly, the proportion of the patients classified as having AMAN with Rajabally’s criteria on the first study was similar on the repeat studies performed using Hadden’s criteria.

Repeat EP analysis of this cohort using the same criteria proportion of AIDP patients remained the same at 54.1 % and about 16.6 % patients were identified as having AMAN. The classification shift occurred mainly in AMAN and normal group where one patient in each group was reclassified as having equivocal study.

EP classification shift occurred in 25 % cases (total 6 out of 24 cases). Two cases of AIDP became equivocal and 2 equivocal cases became AIDP. One case classified as normal changed to equivocal and one AMAN case changed to equivocal on the repeat testing. However, overall proportions of each classification remained the similar with no statistical differences.

**Comparison between 2<sup>nd</sup> Hadden and 1<sup>st</sup> Rajabally study:**

We then compared 2<sup>nd</sup> EP results using Hadden criteria and 1<sup>st</sup> EP results using Rajabally’s criteria to determined whether single study using Rajabally is sufficient to achieve final EP diagnosis.

**Table 5-8 Compariosn between Hadden-2 and Rajabally criteria**

	AIDP	AMAN	Equivocal	Normal
<i>EPS Criteria</i>				
<i>Hadden-2</i>	62.5 %	20.8 %	12.5 %	4.1 %
<i>Rajabally-1</i>	54.1 %	20.8 %	12.5 %	12.5 %
<i>P Value</i>	0.55	1	1	0.29

Our results showed that there was not any statistically significant difference in the proportion of each EP subtype.

## ***5.5 Discussion:***

Above findings suggest that clinical variants are not good discriminator for underlying EP abnormality and highlight the pivotal role of EPS for correct sub classification of GBS patients. Electrophysiological demyelinating GBS cases can present with various clinical subtypes including ataxia, pure motor, pure sensory and sensory motor GBS. Our study confirms the earlier finding that EP remain normal in most MFS cases and if the EP becomes abnormal they most likely to have GBS-MFS variant. However, this analysis was performed on the data collected from IGOS website and also on basis of single EPS and therefore further clinical-electrophysiological analysis using normative values from individual lab will be very useful in order to confirm this finding.

Our study also highlights the drawback of existing EP criteria (including the most widely used Hadden's criteria) showing variation in identification of various EP subtypes especially if the study is performed early in the course. Interestingly, without even considering "RCF" Hadden's criteria failed to identify any AMAN cases when EP is performed early in the course. However on the serial study, EP shift occurred in about 29 % cases, mostly AIDP cases. This result confirms the earlier finding that existing Hadden's criteria is not sufficient for the identification of final EP subtype, especially performed early in the course and serial EP is required.

However, when we applied Rajabally's criteria to first set of EP results, the proportion of each EP subtype was similar to the proportion identified by serial studies using Hadden criteria and therefore provides further support to the notion that using different demyelination cut off values and considering RCF, single EP study, even performed early in the course is sufficient to achieve final EP diagnosis.

Having "gold standard" EP criteria although sounds ideal but practically may not possible for variety of reasons. As shown in our study, even in genuine cases of GBS, in about 4-8 % cases, EP remains normal, even after 3 weeks. Similarly, due to RCF, a proportion of patients including AMAN cases become normal and therefore 2<sup>nd</sup> study also cannot be used as a gold standard. In proportion of cases, demyelination occurs

quite late in the clinical course which makes serial EPS even more challenging and therefore unhelpful. Demyelination, primary axonal degeneration or secondary degeneration is pathological diagnosis and since biopsies are rarely performed nowadays, EP is being used as surrogate marker to identify and sub classifies GBS into pathological categories. Also it is hard to believe that a single nerve can have only either demyelinating or axonal changes, considering the fact that inflammatory changes are patchy it is quite possible that EP may not be sensitive enough to capture all underlying pathologies.

EP literature so far has been very confusing. As one can see from this study there are no consensus based criteria for demyelination and also the evidence for using specific cut offs are also lacking. However if we were to identify specific antibodies associated with specific EP abnormality, EP may hold a key for better understanding of this otherwise heterogeneous disorder. Moreover, if we identify that different EP patterns associated with different treatment response then performing early study may be beneficial for therapeutic decision-making and also for predicting prognosis.

## ***5.6 Conclusion:***

Our study highlights the pivotal role of EPS in identification and understanding of underlying GBS pathology. Existing EP criteria has various limitations and are not sufficient to achieve final EP diagnosis especially applied early in the course of GBS. Based on the result of this study, also in absence of a gold standard for diagnosis of EP subtype, I recommend using Rajabally criteria for future research analysis as well as in the wider clinical practice for early identification of final EP subtypes.



# **Chapter 6**

## **Outcome predictors and Validation of existing prognostic models- EGOS and mEGOS**

## **6.1 Introduction**

Clinician's ability to predict the outcome plays a crucial role in deciding treatment and also having this information early in the disease course may be vitally important for patients and their relatives so that they can make educated choices about their own treatment. GBS is a monophasic illness and although about 80 % of GBS patients make good functional recovery, about 20 % remain significantly disabled after 12 months, and about 30 % require mechanical ventilation (MV), which in self is a bad prognostic marker and therefore identification of early prognostic factors that predict long term recovery and requirement of MV, has remained a major focus of GBS research. Identification of prognostic factors is critical in identifying patients who require closer monitoring in acute phase and also help clinicians to identify patients with poor prognosis, who may benefit from expensive and potentially dangerous immunomodulatory treatment which otherwise may not be necessary in patients with good prognosis. This chapter describes various clinical, EPS and biological prognostic factors and this is followed by detailed description of the methodology used to probe UK IGOS database to validate existing prognostic models. We also discuss the drawbacks of existing models and make suggestions at the end for further improvement of these models.

## **6.2 Overview**

Early reports can be traced back to 1960, when Osler and Siddell, first claimed that raised CSF white cell count in GBS carried poor prognosis however subsequent studies did not substantiate this claim. (39,46,78) McLeod et al suggested that appearance of denervation on needle EMG did not carry favourable prognosis.(117) Similarly, some early reports suggested severe weakness involving all four limbs in acute phase of the disease also did not carry a favourable prognosis. (78,121,122)

In 1988, Winer et al conducted first ever well designed prospective observational study of 100 GBS patients and found that time taken to become bed bound, requirement of

MV, age greater than 40 years and small or absent median nerve CMAP were associated with poor prognosis at 12 months. (39)

As clinical trials continued to show beneficial effect of IVIG and PE on overall outcome of the disease, further clinical, EPS and biological factors were identified.

### **6.2.1 Clinical predictors for long term outcome:**

Analysis of American GBS clinical trial data comparing PE with control, showed older age (>60 years), speed of onset of weakness (less than 7 days) and requirement of MV were associated with poor prognosis at 6 months. (83) Treatment with PE itself was proven to be a very significantly good prognostic factor in the study. Similar analysis performed on the data from The PE/Sandoglobulin trial, showed preceding diarrhoea, severe arm weakness and age > 50 were associated with poor 12 months prognosis defined by inability walk independently or death. (123)

### **6.2.2 Electrophysiological Predictors of long term Outcome:**

EPS, when abnormal, plays very crucial role in diagnosis of GBS. It also helps with categorising GBS in to various EPS subtypes. Role of EPS in prognosis has been well described. McLeod et al first described that presence of denervation on needle EMG was associated with incomplete recovery.(117) The American PE clinical trial data showed mean distal CMAP of < 20 % of lower limit of normal was the most powerful predictor of poor outcome.(83) PE/Sandoglobulin trial performed in 2001, showed inexcitable nerves on EPS was associated with poor outcome at 12 months. (123) Outcome was similar in axonal and demyelinating forms of GBS.

## **6.2.3 Biological predictors of outcome:**

### ***6.2.3.1 Immunological factors:***

GBS is an immunological disorder. While some factors such as level of immunological tolerance, level of complement activation and regulation and types of auto antibodies influence the disease expression; factors like anti-glycolipid antibodies, auto antibodies subtypes and response to IVIg may influence the overall outcome of the disease.

Antibodies against GM1, GM1b, GD1a and GalNAc GD1a antigens are closely associated with AMAN subtype. Prognostic role of anti GM1 antibodies is widely debated. Bech et al showed that patients with short lasting elevation of anti GM1 IgG antibodies levels had faster recovery and long lasting elevation had poor recovery suggesting that monitoring of anti GM1 may predict the outcome. (126) In another similar study Koga et al showed that the IgG1 subclass of anti-GM1 antibody is a major subtype indicative of slow recovery, whereas isolated elevation of IgG3 subclass antibody titre suggests rapid recovery and the variation in subclass pattern may depend on the pathogens precipitating GBS. (127) Recently Lardon et al demonstrated that fine specificity of IgG anti GM1 antibodies is associated with disease severity. (128)

Since its first description in 2004, serum antibodies reacting against anti ganglioside complexes (GSC), made up of two different gangliosides but not to a single ganglioside, anti GSC antibodies have generated a huge interest in this field of biomarker research. (61) Kaida et al showed that patients with Anti-GD1a/GD1b complex antibody positive patients with GBS tend to have severe disabilities and cranial nerve deficits. (63)

IVIg is a proven effective treatment for GBS. Most GBS patients receive standard dose of 2 gm/kg. Despite its efficacy, about 20% of the patients still remain unable to walk unaided after 6 months. Mechanism through which IVIG works in most immune mediated disorders remains complex. Similarly, the question of why some GBS patients respond to IVIg and others have partial response remained unanswered. It may

be pharmacokinetics of IVIg have a role to play and in first ever such attempt Kuitwaard et al performed a retrospective study of 174 GBS patients and showed that patients with a minor increase of serum IgG level after standard single IVIg dose had considerably slower recovery suggesting that those patients who metabolise IVIG quickly, do not respond favourably and may benefit by additional dose of IVIG. (129) However, this finding needs to be substantiated by a large study in order for it to be used in routine clinical practice.

#### ***6.2.3.2 CSF markers of axonal damage:***

There is evidence to suggest that axonal damage especially in the acute phase of the disease adversely affects the long-term outcome. (132) The extent of axonal damage can be assessed by electrophysiological studies however, results may be inconclusive in the early phase of the disease, indicating the need for additional early markers of the axonal degeneration. A prospective study performed on 38 GBS patients, Petzold and colleagues showed that high CSF Neurofilaments (NFHs) levels at admission in GBS patients were associated with poor outcome indicating that NFHs, a biomarker for axonal damage were of prognostic value in GBS. (131) In this study patients with neurophysiological evidence of axonal damage had higher CSF NFHs levels compare to those with demyelinating GBS. This information is very helpful in the GBS, which has highly variable clinical course, and these biomarkers could be used in the early phase of the disease to identify those who would require more intensive forms of treatment. Besides axonal proteins like NF, detection of some glial proteins in the CSF of GBS patients may have prognostic roles. Jin et al analysed CSF samples of 26 GBS patients, studying CSF tau protein and compared the outcomes of these patients at 6 months time. (132) The authors showed that CSF tau protein concentrations were significantly high in patients with poor prognosis. Similarly elevated levels of CSF

glial fibrillary acidic protein (GFAP) and S100 have been described in GBS patients however these observations have not been studied to provide any conclusion about their prognostic roles.

#### **6.2.4 Genetic factors:**

Genetic factors are widely recognised to be associated with increased host susceptibility in various inflammatory disorders. GBS is a heterogeneous disorder and reports of familial GBS and recurrent GBS in the same patient coupled with the observation that not all patients with the same preceding infection develop clinical GBS suggest that host susceptibility factors play a crucial role in development of GBS.(131) However, recent studies have failed to show such relationship between susceptibility of GBS and certain HLA class II alleles or CD1 gene polymorphisms.(132) With regard to prognosis, some immunoregulatory genes have been shown to influence the inflammatory response and thereby influence the prognosis. Recently some studies have showed that single-nucleotide polymorphisms of genes encoding Fc̄-receptor-III, matrix metalloproteinase-9, and tumour necrosis factor-̄ and mannose-binding lectin are associated with the severity of GBS. (77)

### ***6.3 Development of GBS clinical Prognostic models:***

As shown in previous section, based on various clinical trials, variety of clinical, biological and EP predictors have been identified for GBS outcome. One of the key factors in development of any predictive prognostic model is the easy availability of predictive factors and in that respect, a model developed based on easily available clinical information is desirable. Biological factors may show strong correlation with poor outcome availability of laboratory tests may not be universal and even it is available it may take some time to get the results back. Similarly EP can be normal in early phase of GBS and therefore may not provide any useful prognostic information, when the urgent decision of treatments to be made in clinical settings.

Attempts have been made to develop such clinical prognostic models for GBS outcome. In an analysis performed on 388 GBS patients from two randomised controlled trial and one pilot study, Van Koningsveld et al found that age, preceding diarrhoea, and GBS disability score at 2 weeks, were the main predictors of poor outcome at 6 months. (135) Based on this analysis the authors have proposed a clinical prognostic model of GBS called EGOS. EGOS accurately predict the prognosis at 6 months. However, one of the limitations of EGOS is that it cannot be applied earlier than two weeks of hospital admission. Early identification of patients with poor prognosis is very important in early therapeutic decision making as in the early phase, treatment is considered to be more effective and the chances of nerve regeneration are maximum.

Walgaard et al subsequently produced a modified version of above model called mEGOS which can be used at the time of hospital admission and 1 week after the admission to predict the outcomes at 4, 12 and 26 weeks post admission.(136) One of the limitations of mEGOS model is its limited applicability to western world population, as the model was developed and validated on European patients and therefore may not be applicable to all other GBS patients with different demographic background.

In this section, I have attempted to validate both clinical predictive models using IGOS UK data.

## ***6.4 Aims:***

One of the many aims of international GBS Outcome Study was to validate existing clinical prognostic models. We probed our IGOS UK database

1. To validate EGOS model at two weeks
2. To validate mEGOS model at admission

## **6.5 Methods:**

Validation of EGOS model: We first analysed data to identify patients who had their 6 months outcome recorded. EGOS model predicts the patient's ability to walk at 6 months based on acute phase data of GBS disability scores at 2 weeks post entry into the study, preceding diarrhoea and age of the patient. The score ranges from 1 to 7, with three categories for age, two for diarrhoea and five categories for GBS. Based on this model, prediction of inability to walk independently at 6 months ranges from 2 to 83 %. We first calculated EGOS score of all our patients at 2 weeks post study entry and based on their EGOS values, predicted probabilities were calculated using EGOS statistical model  $1/(1+\exp[8.2-1.4*EGOS])$ . External validation was carried out using calibration and discrimination technique. For calibration predicted probabilities and observed probabilities were compared and plotted graphically on the table. For the model discrimination area under the curve (AUC) was calculated.

Validation of mEGOS model at 7 days post admission: We first analysed data to identify patients who had their 6 months outcome recorded. mEGOS model predicts the patient's ability to walk independently at 6 months based on acute phase data of MRC sum scores, preceding diarrhoea and age of the patient. mEGOS can be used at the time of admission however performance of this model was best at 7 days post admission and therefore we analysed 7 days post admission model in our study. MEGOS score ranges from 1 to 12, with three categories for age, two for diarrhoea and four categories for MRC sum score at 7 days post admission. Based on this model, prediction of inability to walk independently at 6 months ranges from 0 to 66 %. We first calculated mEGOS score of all our patients at 7 days post admission and based on their mEGOS, predicted probabilities were calculated using statistical model  $1/(1+\exp[-4.5681 + (0.4364*mEGOS) ])$ . External validation was carried out using calibration and discrimination technique. For calibration predicted probabilities and observed probabilities were compared and plotted graphically on the table. For the model discrimination area under the curve (AUC) was calculated. Statistical calculations were performed using SPSS v 21.

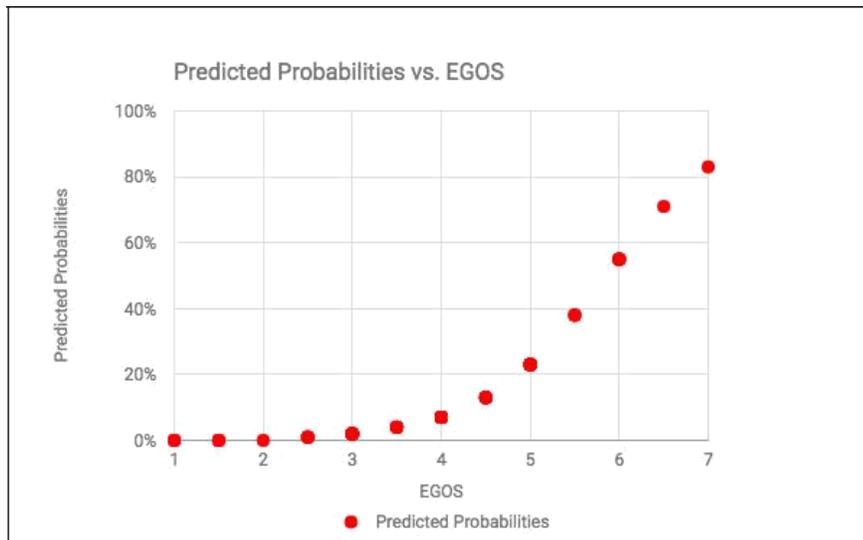
## 6.6. Results:

### Validation of EGOS model:

Out of 114 IGOS UK patients, outcome data available was available in 72 patients.

**Table 6-1 Erasmus GBS Outcome Score**

	<b>Categories</b>	<b>Score</b>
Age at onset (years)	> 60	1
	41-60	0.5
	< 41	0
Diarrhoea	Absence	0
	Presence	1
GBS disability score	0-1	1
	2	2
	3	3
	4	4
	5	5
<i>The Erasmus GBS Outcome Score</i>		



**Figure 6-1 Predicted probability of inability to walk at 6 months based on EGOS score**

**Table 6-2 Percentage of patients who were unable to walk independently at 6 months in the derivation set and validation set as per EGOS score**

<b>EGOS</b>	<b>Predicted</b>	<b>Observed</b>
1-3	0 %	0 %
3.5-4.5	6.6 %	8.6 %
5	20 %	15 %
5.5-7	60 %	63 %

As described, in the table 6.2, EGOS model calibrated well in IGOS UK cohort. For calibration purpose we compared the frequency of outcome in four different EGOS score categories. In the score category 1-3, only 0.5 % patients of derivation set had

poor outcome as defined by inability to walk independently at 6 months' time, in IGOS UK validation cohort none of our patient in that category had poor outcome. In the score category 3.5-4.5, only 7 % patients of derivation set had poor outcome as opposed to 8.6 % in our validation cohort. In the score category 5, 27 % patients of derivation set had poor outcome as opposed to 15 % in our validation cohort. Frequency in this group showed statistical significant variation ( P 0.02). In the score category 5.5-7, 52 % patients of derivation set had poor outcome as oppose to 63 % in our validation cohort.

We then analysed discriminatory capacity (figure 6.2) of EGOS model in IGOS UK cohort. AUC for prediction of outcome at 6 months using EGOS model was 0.87 suggestive of very good discriminatory power of this model.

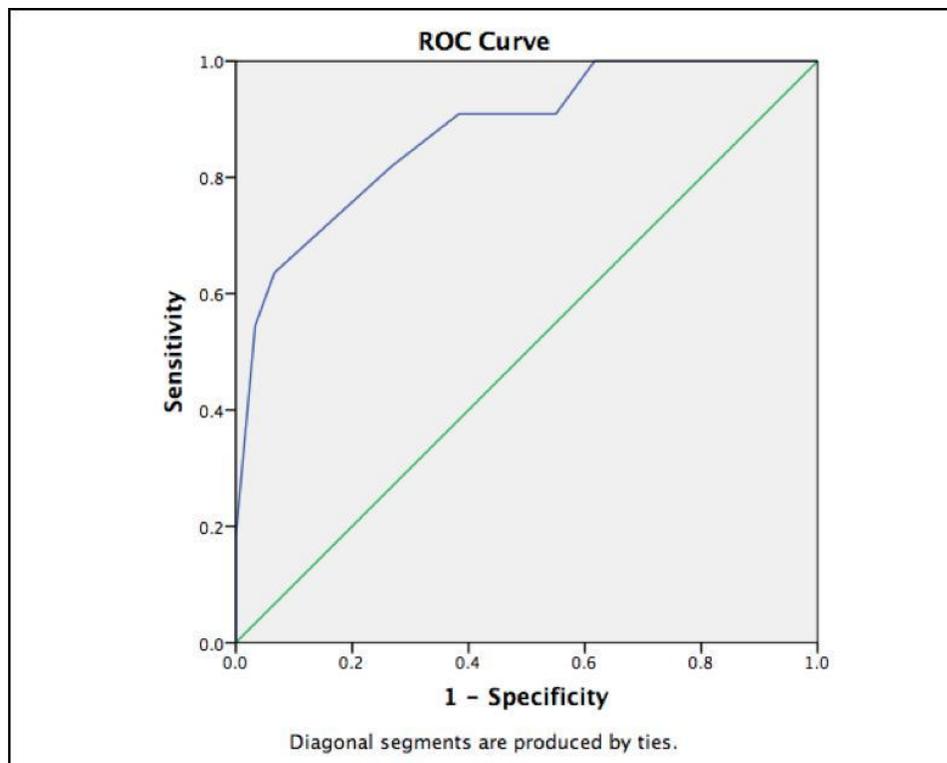
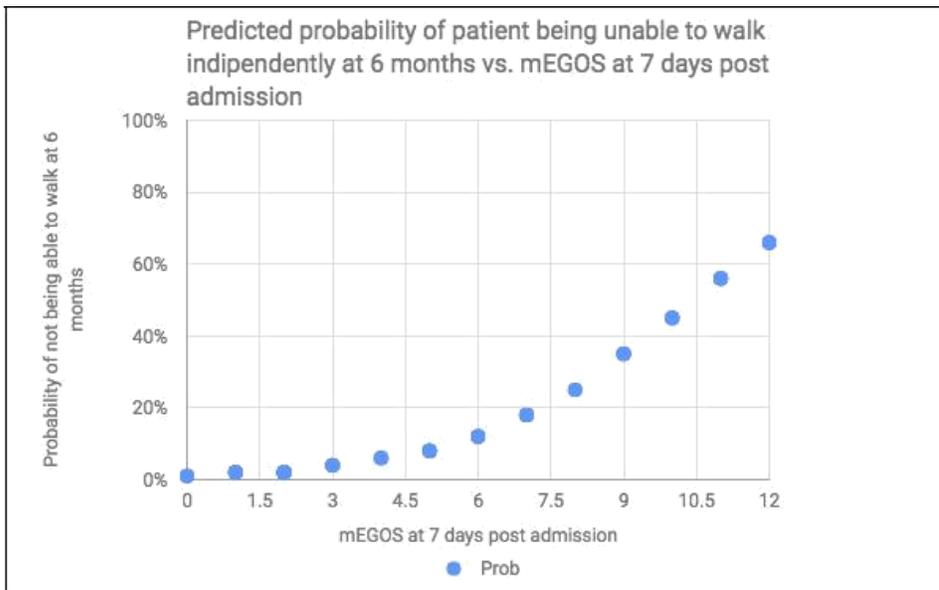


Figure 6-2 Area Under Curve (AUC) of EGOS model

## Validation of mEGOS model at 7 days post admission:

**Table 6-3 Modified Erasmus GBS Outcome Score Model**

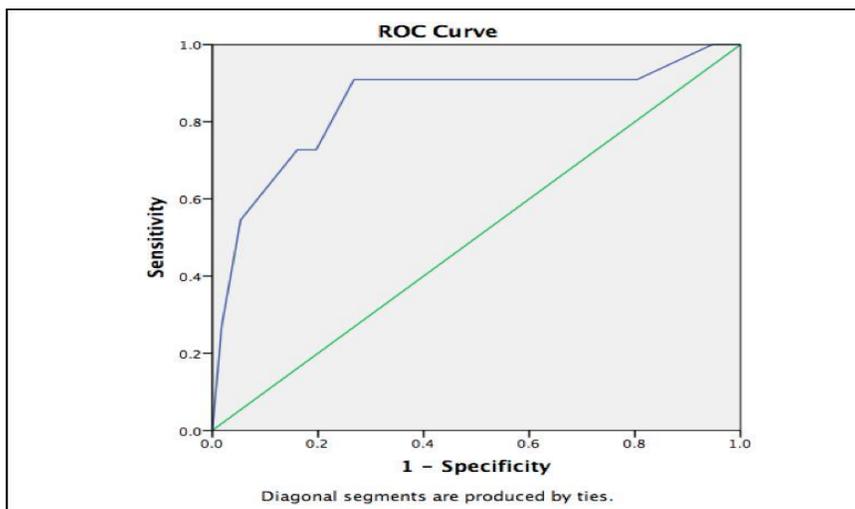
	Categories	Score
Age at onset (years)	> 60	2
	41-60	1
	< 41	0
Diarrhoea	Absence	0
	Presence	1
MRC sum score at 7 days post admission	51-60	0
	41-50	3
	31-40	6
	0-30	9
MEGOS		0-12



**Figure 6-3 Predicted probability of patient being unable to walk independently at 6 months Vs mEGOS at 7 days post admission**

**Table 0-4 Percentage of patients who were unable to walk independently at 6 months in the derivation set and validation set as per EGOS score**

mEGOS at 7 days post admission	Predicted (%)	Observed(%)
1 to 5	0	2.9
6 to 7	15	18
8 to 9	30	16
10 to 12	55	53



**Figure 6.4 AUC of mEGOS model**

As shown in table 6.3, mEGOS model calibrated well in IGOS UK dataset. There was no significant difference in observed and predicted frequencies of patient unable to walk at 6 months using mEGOS model. Similarly, AUC (Figure 6.4) for mEGOS model was 0.84 in IGOS UK cohort, suggestive of very good discriminatory power of this model

### ***6.7 Discussion:***

Prediction of prognosis is an essential part of clinical practice. In context of GBS, which is a heterogeneous disorder, it even becomes more important. While general prognostic information has been available for long time, prediction of prognosis of individual GBS cases has not been available. In first ever such attempt Koningsveld et al have developed a clinical prognostic model based on multivariate regression analysis of the clinical data available from 388 GBS patients who participated in two different clinical trials. EGOS model is based on simple clinical information such as age of the patient, preceding diarrhoea and disability at 2 weeks post entry into study. The model predicts individual patient's ability to walk unaided at 6 months' time. The predicted probabilities ranged from 2 to 82 %.

As shown in the result EGOS model performed well on our IGOS UK database. Observed probabilities in IGOS UK cohort agreed well with predicted probabilities of the model. Also model showed very good discriminatory capacity based on AUC of 0.87. These results show that EGOS can be extremely helpful in providing clinical prognostic information in acute phase of the disease. However one of the issues with EGOS model is that it can only be applied after 2 weeks of entry into clinical study. Most patients received treatment in first two weeks of time and therefore this model may not be clinically helpful in order to make early treatment decisions on patients.

In order to mitigate this problem, Walgaard et al proposed another model called mEGOS, which was based on clinical information such as age, preceding diarrhoea and MRC sum score at the time of admission and 7 days post admission. This model was essentially based on the same clinical database that was used for the development of EGOS model. MEGOS predicts patient's ability to walk unaided at 6 months. This model can be applied at two time points 1) at the time of admission and 2) 7 days post admission. The model performed very well at 7 days post admission compared to at the time of admission and therefore we applied this model to IGOS UK cohort using 7 days post admission clinical information. When applied to IGOS UK database, mEGOS model showed excellent discriminatory capacity with AUC of 0.84.

Overall the results of this study provide proof of principle that prognostic model like EGOS and mEGOS can be applied to GBS patients with different geographical area. One of the important requirements of prognostic model is that it should be useful and readily applicable in most clinical settings. MEGOS model uses MRC sum score available on admission and 7 days post admission to hospital to predict the 6 month outcome. This may have several limitations 1) timing of hospital admission depends on various factors such as availability of local medical services, this especially a major drawback in developing country as often patients do not get admitted to local hospitals until late in the disease course due to lack of finances or resources. 2) Using MRC sum score may be difficult for non neurologist physicians for example in the UK, most patients will first get admitted to district general hospital and performing MRC sum score can be difficult by non neurologist physicians 3) some patients with pure sensory GBS or MFS-GBS variants may not have weakness and in those patients MRC sum score would be normal, despite patient being significantly disabled.

In order to address above limitation we propose two modifications to the model 1) Along with factors like age and diarrhoea, model should use a parameter, which can be easily collected by any medical physicians without much neurological training. In this respect, I propose the future model should be designed using GBSD scale, which captures the disability very well irrespective of type of GBS 2) Timing of the collection of acute phase data should be standardized and we propose to use date of onset of symptoms as a reference point rather than admission date as date of onset of

symptoms will likely to remain constant irrespective of country of practice and therefore model will be applicable to wider geographical population. IGOS provides a unique opportunity to refine existing models. In IGOS most patients are recruited within 2 weeks of onset of symptoms and therefore two separate models can be developed on the patients who have been recruited in the first week of symptoms onset and second week of onset of symptoms. Also besides using “ability to walk unaided” as a standard outcome, future analysis of complete IGOS dataset should use other outcome scales like RODS, which will potentially increase the statistical power of the model due to linearity of the scale. Similarly, electrophysiological data could be included in the model; however factors such as lack of standardisation in performing EPS and delay in development of EP abnormality in some GBS patients will have to be considered very carefully before their inclusion in to prognostic models.

### ***6.8 Conclusion:***

Above results provide the proof of the concept that predictive models like EGOS and mEGOS can be useful in providing important prognostic information to GBS patients early in the course when the treatment decisions are critical. However there are certain limitations to these models and future models can be developed based on keeping these limitations in mind so that it can be applicable to wider geographical populations. This is important especially in the counties with limited resources where the aim is to direct the resources in appropriate direction using these prognostic models

# Chapter 7 Conclusions

IGOS represents the first international prospective observational study with an aim to study 1000 GBS patients around the world. It provides a unique opportunity to study various clinical, EP, serological and genetic factors in relation to outcomes in a wider geographical population.

As part of this thesis, we analysed the data from 114 patients from UK. We compared our outcomes with previous observational studies. The study shows that IVIG is now widely available and most commonly (90 % of total GBS patients) administered treatment in GBS patients in the UK. One of the interesting findings of our study is the patients with mild GBS (GBSD < 2) are also being treated with immunotherapy, without any significant long-term benefit. If this finding is validated with larger IGOS cohort this practice should be discouraged.

Despite wider availability of immunotherapy, GBS outcomes have not changed over the past 20 years. In our study, 5.1 % patients died, 18 % required mechanical ventilation, 15 % patients remain unable to walk at 12 months and only 22 % patients had complete neurological recovery at 12 months. These results highlight urgent need of more effective immunotherapies in these patients.

However, due to rarity of this condition, conducting multi centred RCT can be expensive and challenging and in these circumstances IGOS can provide an excellent historical outcome database to which newer therapies can be tested against. Also development of reliable prognostic models not only help patients and clinicians to guide the treatment decisions but also these models can be used early in the disease course to identify patients with poor prognosis, when the newer treatments can be more effective. In our study I validated existing prognostic models like EGOS and mEGOS. Our study shows that these models can help identifying and stratifying patients with poor prognosis, however , some refinement into these models required for their wider geographical applicability. This issue can be addressed by analysing large IGOS database.

In our study we also tried to address some electrodiagnostic issues related to GBS. Our study shows that currently available EP criteria are not sensitive enough to identify various EP subtypes. Some authors have suggested performing two sets of EPS, in order to achieve correct EP diagnosis. However performing two sets of EP can be challenging in resource poor countries. Rajabally et al suggested modified criteria, which probably mitigates the need of two EPS. In our study we probed this criteria and showed comparable results. However this finding needs to validate by larger EP database and IGOS will provide that unique opportunity to do so.

### **Future directions:**

#### **Identification of new target antigens and development of new diagnostic biomarkers of GBS:**

One of the major focuses of biomarker development in GBS is identification and characterization of anti-glycolipid antibodies. Antibodies against glycolipids, especially against various gangliosides are present in about 60% of the GBS patients in acute phase. More than 20 different anti-glycolipid antibody specificities have been shown to be associated with GBS so far. Furthermore the recent discovery of anti-glycolipid complex (GSC) antibodies that react with two different gangliosides structures in heteromeric complex has not only given a new dimension but also added more complexities to this area of research. Thus, from 20 single glycolipids, 190 1:1 complexes can be generated and if 3 or 4 glycolipids are combined, the number of complexes increases significantly which makes current low throughput ELISA screening techniques practically impossible in terms of time required to prepare the reagents and consumption of the reagents. Therefore, the Willison group has recently developed a novel miniaturized method called combinatorial glycoarray for assessing sera for anti-GSC antibodies. In this respect, IGOS will provide an excellent opportunity to screen GBS sera for anti GSC antibodies and also for antibodies against novel axo-glial proteins.

#### **Development of consensus based EP criteria:**

Work described in thesis highlights some important drawbacks of existing EP criteria. The current EP criteria do not consider the possibility of axonal conduction block and also the nodo-paranodopathies in axonal GBS and as a result some patients can be

wrongly classified to have AIDP. Correct EP classification is of paramount importance for correct serological and EP correlation. Systematically collected IGOS EP database will help study various factors responsible for these drawbacks and will help develop a universal consensus based EP diagnostic criteria.

#### **Validation of existing prognostic models of GBS:**

Work described in this thesis provides further proof of the concept that prognostic model help stratifying patients with poor prognosis early in the disease course. IGOS database will help validate these models in wider geographical population and if found to have good discriminatory capacity, will have potential to be used in routine clinical practice.

#### **IGOS-2**

As part of IGOS study, more than 1000 GBS patients have been recruited worldwide. For a relatively rare disease like GBS, this is a remarkable achievement by Inflammatory Neuropathy Consortium. However one of the major issues in a multicentered observational study like IGOS is data quality and missing data. These factors can have significant impact on statistical power of the study. IGOS will provide a better insight in to the factors leading to data quality compromise and missing data; once these factors are identified future observational study can be designed to minimize the missing data and to improve the quality of the data.

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