UNIVERSITY OF
KWAZULU-NATAL

IMMUNE-MEDIATED DISORDERS OF THE PERIPHERAL NERVOUS SYSTEM IN HIV, WITH SPECIAL FOCUS ON THE FOLLOWING:

- MYASTHENIA GRAVIS
- MOTOR NEURON SYNDROME
- CHRONIC INFLAMMATORY DEMYELINATING POLYNEUROPATHY
- PURE MOTOR LUMBOSACRAL POLYRADICULOPATHY
- AUTOIMMUNE NODOPATHIES
- COMBINED CENTRAL AND PERIPHERAL DEMYELINATION :

  A NOVEL CASE OF CCPD IN 2 SIBLINGS : GENETIC OR IMMUNE MEDIATED?

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DECLARATION

I**, Dr Kaminie Moodley**, declare that this dissertation submitted for Doctoral Degree of Philosophy (PhD) to the University of KwaZulu-Natal has not previously been accepted in substance for any degree and is not being concurrently submitted in candidature for any degree. This thesis is the result of my own investigations, except where otherwise stated. Other sources are acknowledged by giving explicit references. I hereby give consent for my thesis, if accepted, to be available for photocopying and for inter-library loan, and for the title and summary to be made available to outside organizations.

This is a thesis in which the chapters are written as a set of separate manuscripts with an overall introduction, methods and final summary and conclusion.

Dr Kaminie Moodley

RESEARCH OUTPUT:

FIRST AUTHOR PUBLICATIONS SUPPORTING THIS THESIS

This thesis is inclusive of material covered in the following six first author publications.
Each chapter begins with a summary section which indicates how one or more of these publications fit in the context of that chapter.

The publications and research output are as follows:

1. A comparative study of CIDP in a cohort of HIV-infected and HIV-uninfected patients.
   Neuroinflammation, 2017, doi: 10.1212/NXI.0000000000000315
   Impact factor: 11.35
2. Nodal-paranodal antibodies in HIV-immune mediated radiculo-neuropathies: Clinical phenotypes and relevance, JPNS, 2023, doi.org/10.1111/jns.12596.
   Impact Factor: 5.2
3. Motor lumbosacral radiculopathy in HIV-infected patients, Southern African Journal of HIV Medicine, 2019; 20(1), a992. https://doi. org/10.4102/sajhivmed. v20i1.992
   Impact factor: 2.85
4. A comparative study of motor neuron disease in a cohort of HIV-infected and HIV-uninfected patients, Journal of Neurological Sciences ,2018, doi.org/10.1016
   Impact factor: 3.34
5. A comparison of clinical, electrodiagnostic, laboratory and treatment outcome differences in a cohort of HIV-infected and HIV-uninfected patients with Myasthenia Gravis, Frontiers in Neurology, 2021
   Impact factor: 4.86
6. CCPD: Immune mediated or genetic: Case report and review of the literature
   Practical Neurology, JNNP. 2024
   Impact factor: 3.7

Others:

1. A comparative study of motor neuron syndrome in a cohort of HIV-infected and HIV-uninfected patients, NASA Congress, 2018

DEDICATION

To my daughters:

Diya and Bhavya

“Is this the real life? Is this just fantasy?
Caught in a landslide, no escape from reality
Open your eyes, look up to the skies and see
I'm just a poor boy, I need no sympathy
Because I'm easy come, easy go, little high, little low
Any way the wind blows doesn't really matter to me, to me

Too late, my time has come
Sends shivers down my spine, body's aching all the time
Goodbye, everybody, I've got to go
Got to leave you all behind and face the truth
I don't want to die
I sometimes wish I'd never been born at all”

‘Bohemian Rhapsody” by Queen

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1. My supervisors (Prof Simon Rinaldi, Prof AA Moodley, Prof VB Patel)

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   My children: Diya and Bhavya: The sky is your limit. Reach for your dreams. May God bless both of you with good health and happiness. Love you to the moon and back.
   My Shetland Collie, Skye: Your almond eyes have always kept me calm and focused.
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I have enjoyed this project, learnt many life lessons and met wonderful people along the way. This has truly been an amazing, life changing experience, worth more than the doctoral degree itself. Thank you, God for making this experience possible.

PLAGIARISM DECLARATION:

I, Dr Kaminie Moodley declare that:

i) The research reported in this dissertation, except where otherwise indicated is my original work.

ii) This dissertation has not been submitted for any degree or examination at any other university.

iii) This dissertation does not contain other person's data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons.

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Dr Kaminie Moodley

Date 20 July 2023

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LIST OF ABBREVIATIONS

ART	Antiretroviral Therapy
ALS	Amyotrophic Lateral Sclerosis
ALSFRS	Amyotrophic Lateral Sclerosis Functional rating scale
AIDP	Acute inflammatory demyelinating polyneuropathy
AZA	Azathioprine
ANS	Autonomic nervous system
AChR AB	Acetylcholine Receptor Antibody
ADAM 22	Disintegrin and Metalloproteinase Domain- containing Protein 22
AnkG	Ankyrin G
Ax	Axon
Bral1	Brain Link Protein 1
C	Collagen
CNTN-1	Contactin1
CV	conduction velocity
CASPR	Contactin associated protein
CNTNAP1	Contactin-Associated Protein-1
CIDP	Chronic inflammatory demyelinating polyneuropathy
CP	Cyclophosphamide
CSF	Cerebral Spinal fluid
CST	Corticosteroid therapy
CCPD	Combined central and peripheral demyelination
CMV	Cytomegalovirus
CMAP	Compound Muscle Action Potentials
CT scan	Computed tomography scan
CB	Conduction Block
CMV	Cytomegalovirus
CV	Conduction velocity
DRG	Dorsal root ganglionopathy
DML	distal motor latency
DILS	Diffuse Infiltrative Lymphocytosis
DM	Diabetes Mellitus type 2
EBV	Epstein Barr Virus
ERM	Ezrin–Radixin–Moesin
FSGN	Focal segmental glomerular nephritis
FN	Fibronectin
GM1	Ganglioside GM1
Glm	Gliomedin
HAART	Highly Active Antiretroviral Therapy
HIV	Human immunodeficiency Syndrome
HIMRN	HIV immune mediated radiculo-neuropathies

HERV	Human endogenous retroviruses
HSV	Herpes simplex viruses
HTLV1	Human T cell lymphocytic virus type 1
IVIG	Intravenous immunoglobulin
IQR	inter quartile range
IL	interleukin
Kv1.4	Voltage gated potassium channel
KZN	Kwa-Zulu Natal
LRP4	Low-Density Lipoprotein Receptor-Related Protein 4
LEMS	Lambert-Eaton Myasthenic Syndrome
LLN	Lower limit of normal
MAG	Myelin associated glycoprotein
MTX	Methotrexate
MND	Motor Neuron Disease
MMN	Multifocal motor neuropathy
MG	Myasthenia Gravis
MGN	Membranous glomerular nephritis
MuSK	Muscle-Specific Kinase
MMF	Mycophenolate Mofetil
MMS	Minimal Manifestation Status
MNS	Motor Neuron Syndrome
ND	Not Documented
Nr-CAM	Neuronal Cell Adhesion Molecule
NF	Neurofascin
NF155	Neurofascin 155
NF186	Neurofascin186
NrCAM	Neuronal Cell Adhesion Molecule
NRTI	Non-nucleoside reverse transcriptase inhibitors
Nav	Sodium Channels
ODSS	Overall disability sum score
PLEX	Plasma exchange
PNS	Peripheral nervous system
PSD	Postsynaptic Density Protein
PMLR	Pure motor lumbosacral radiculopathy
RT-PCR	Real time polymerase chain reaction
RTX	Rituximab
RyR Ab	Ryanodine Antibody
SC	Schwann Cell
SNAP	Sensory nerve action potential
TAG1	Transient axonal glycoprotein
TNF	Tumour necrosis factor
UK	United Kingdom
VGSC	Voltage-gated sodium channels
VRR	Ventral root radiculopathy
ZO-1	Tight junction protein 1

ABSTRACT:

Background:

Literature regarding the prevalence, clinical features, CSF changes, laboratory investigations, response to therapy, and pathophysiology of autoimmune disease in HIV-associated lower motor neuron syndromes is limited. The above topic is broad and for the purpose of this thesis, has been restricted to chronic immune mediated polyradiculoneuropathies (chronic inflammatory demyelinating polyneuropathy (CIDP), autoimmune nodopathies, pure motor lumbosacral polyradiculopathy (PM LSP)),motor neuron syndrome (MNS),and myasthenia gravis(MG) as these are the commonest HIV-immune mediated lower motor neuron syndromes seen at our neuromuscular unit. Neuropathies due to HIV vasculitis and diffuse infiltrative lymphocytosis (DILS) were excluded as they are rare. Muscle disorders forms part of planned prospective work.

Objective:

To describe the differences in clinical presentation, electro-diagnostic findings, cerebrospinal fluid (CSF) changes, radiology (where applicable), treatment outcomes and pathophysiology of disease in HIV-infected immune mediated polyradiculoneuropathies, MNS and MG and to compare the above to the HIV-uninfected category. Additionally, we tested for nodal/paranodal and ganglioside antibodies in patients with HIV-associated chronic immune mediated radiculoneuropathies and MNS and explored their pathogenetic potential. We also explored genetic and immune factors that are potentially implicated in CCPD in two siblings, one HIV infected. Nodal/paranodal antibodies have not previously been described in an HIV-infected cohort of patients or in the African population in general and similarly genetic testing for acquired demyelination, has not been performed in South Africa.

Methods:

An insight into the virology, epidemiology, clinical aspects and immunology of HIV is essential in understanding the complexities of immune mediated neurological conditions which occur in people living with HIV infection and is discussed in chapter 1. Chapter 1 also provides background literature of the various neurological syndromes in the HIV-uninfected population, equipping the reader with the necessary tools to understand the clinicopathological correlates in the context of HIV. Available worldwide literature is discussed and analysed in chapter 2, which is a scoping review of the above topic. A retrospective analysis of medical records of all patients meeting the diagnostic criteria for chronic immune mediated radiculoneuropathies (CIDP,DRG,PM LSP), MNS/D, and MG from our neuromuscular unit in Durban, Kwa-Zulu Natal (KZN) between 2003 and 2020 was performed (see individual manuscripts).Clinical, demographic, laboratory, electrophysiological and treatment

outcome data were extracted and compared in the 2 arms of the study, namely the HIV-infected and HIV-uninfected cohorts. In addition, HIV-infected patients who met the inclusion criteria for chronic immune-mediated radiculo-neuropathies (IMRN) and MNS were prospectively screened for IgG antibodies directed against nodal (neurofascin (NF)186) and paranodal (NF155, contactin1 (CNTN1) and contactin-associated protein-1, (Caspr1) cell adhesion molecules, using a live, cell-based assay. This has not been previously done in South Africa and is therefore novel. Further testing was performed to determine pathogenicity of the antibodies using myelin co-culture screens and addition of complement. Other novel antibody detection methods included myelin co-culture screens, immunoprecipitation experiments and mass spectrometry. Whole exome sequencing for a potential novel mutation, and genetics for known inherited disorders such as CMT, mitochondrial disease and leukodystrophy was performed in the surviving sibling with CCPD.

Results:

Manuscript 1: A comparison of clinical, electro-diagnostic, laboratory and treatment outcome differences in a cohort of HIV-infected and HIV-uninfected patients with Myasthenia Gravis:

One hundred and seventy-eight (178) patients fulfilled the clinical criteria for MG. Twenty-four (13.4%) were HIV-infected and 154 (86.5%) were HIV-uninfected. There were 116 (65%) females, median 45 years, (IQR 40-62), 90 (50.5%) black African, 66 (37%) Indian, 20 (11.2%) white and two (1.1%) of mixed ancestry. In the HIV-infected cohort, 20 (87%) had generalised MG, 12 (50%) bulbar and 14 (60.9%) respiratory onset MG, 12 (50%) presented with MG Foundation of America (MGFA) class 5 disease at diagnosis, 6 (25%) presented with MG crisis during the 5-year follow up. Thirteen (54%) of the HIV-infected group required rescue therapy using (plasma exchange or IV immunoglobulin) combined with pulse cyclophosphamide compared to 17 (11%) in the HIV-uninfected cohort respectively. At five years, eight (33%) of the HIV-infected group remained refractory to treatment compared to 10 (6.5%) HIV-uninfected cohort respectively. No adverse events were documented in HIV-infected patients receiving combination rescue therapy (PLEX or IVIG combined with IV cyclophosphamide). In conclusion HIV-infected MG patients are more likely to require combination rescue therapy with PE/IVIG and IV cyclophosphamide compared to those who were HIV-uninfected. No side effects were documented in the HIV-infected group receiving the above therapy.

Manuscript 2: A comparative study of HIV-infected and HIV un-infected patients with motor neuron syndrome:

One hundred and thirty-six patients were included in the study, 101 (76%) were HIV-uninfected and 35 (26%) were HIV-infected. Ninety four percent of the HIV-infected cohort were under 50 years, median 41, IQR (33-45), $p<0.001$, had median ALS functional rating scale revised score (ALSFRS-RS) of 28, IQR (24-30) and 40% of these patients on anti-retroviral therapy (ART) survived longer

than 10 years. Ninety one percent of the HIV-uninfected cohort were over 50 years, median 66 years, IQR (57-74), p<0.001, had median ALSFRS-RS score of 44 (IQR 42-45) and 93% died within 5 years of their illness.

Manuscript 3: A comparative study of HIV-infected and HIV un-infected patients with CIDP:

Eighty-four patients were included in the study. Amongst HIV-infected patients 61% were female, median age was 37 years (IQR 30-42), 87.2% presented with a monophasic progressive illness, median CSF lymphocyte count was 5.75 (IQR 0-7.2), 86% were corticosteroid (CST) responsive and 76% were in remission within 6- 12 months requiring no further treatment. HIV- uninfected patients were predominantly male (64%), older (median age was 53years (IQR 29-66)), 53% had a relapsing remitting course, median CSF lymphocyte count was 0 (IQR 0-2), 22% were corticosteroid responsive, 95% required combination therapy and 33% were not in remission by 18 months follow-up.

Manuscript 4: Motor lumbosacral radiculopathy in HIV-infected patients:

Eleven black African patients met the inclusion criteria. There were 6 females. The median age was 29 years, interquartile range (IQR), 23-41 years, median duration of symptom progression was 6.5 IQR (3-7.5) months. The median CD4 count of 327 cells/μL, IQR (146-457). The CSF median polymorphocyte count was 0 cells/μL, IQR 0-2, lymphocyte count was 16 cells/μL IQR 1-18 cells/μL, glucose was 3.1 mmol/L, IQR 2.8-3.4 mmol/L and protein was 1.02g/dl, IQR 0.98-3.4 g/dl. All patients tested negative for nodal (neurofascin (NF)186) and paranodal (NF155, contactin1 (CNTN1) and contactin-associated protein, Caspr1) cell adhesion molecules, using a live, cell-based assay and ganglioside antibodies, using ELISA. All patients were treated with corticosteroid therapy. Ninety one percent (91%) recovered fully within 6 months of treatment, median time for recovery was 3.4 months, IQR (1.8- 5.6). There were no relapses during the 18-month follow up period.

Manuscript 5 : Nodal-paranodal antibodies in HIV-immune mediated radiculo-neuropathies: Clinical phenotypes and relevance:

Twenty-four HIV-infected patients with IMRN were included in the study, 15 met the EFNS/PNS clinical and diagnostic criteria for CIDP, 4 had ventral root radiculopathies (PM LSP) and 5 had dorsal root ganglionopathies (DRG). Five patients with CIDP had combined central and peripheral demyelination. Three patients (12.7 %) tested positive for Neurofascin IgG1 antibodies in the following categories: 1 patient with PM LSP was NF186 positive and 2 patients were NF155 positive with DRG and mixed sensory motor CIDP with optic neuritis respectively.

Manuscript 6: Case report of CCPD in siblings:

Despite the above cases being fully worked up, they remain cryptic as various immune and genetic tests performed in this study have been inconclusive.

Conclusion

The 1st study shows that MG patients present with more severe bulbo-respiratory signs requiring supportive care in ICU. The study also suggests that immunosuppressive drugs, including IV cyclophosphamide may be safe and efficacious in HIV-infected patients. In the 2nd study, HIV-infected MNS patients were younger, had more severe disease at presentation and survived longer if treated with ART with possible reversal of the disease process, compared to patients with MND which is likely neurodegenerative. The findings of the 3rd study are that HIV-infected CIDP patients were younger, female predominant, had a CSF lymphocytosis, displayed slowly progressive disease, were highly steroid responsive and went into remission within 6-12 months of CST initiation compared to HIV-uninfected patients. This may argue for a different disease pathogenesis in this cohort. In the 4th study HIV-infected patients with PM LSP, had a pure motor presentation and responded to CST with no relapses during the 18 month follow up period. This cohort tested negative for NF186 and GM1 antibodies which are usually implicated in pure motor syndromes. The 5th study highlights the fact that nodal-paranodal antibodies occur at a similar frequency in HIV-infected and HIV-uninfected IMRN. However, interpretation of results in the context of HIV infection, especially with IgG1 subtypes and low antibody titres is challenging as many antibodies occur as an epiphenomenon in HIV and may therefore be non-specific and non-pathogenetic. Pathogenicity was not established using myelin co-cultures or complement assays. The 6th manuscript describes a rare and cryptic entity of CCPD in siblings and serves as a platform for future genetic, epigenetic and immune studies.

The above retrospective and prospective studies add valuable new background information to the medical literature as they describe our clinical experience in complex and rare HIV- related patient conditions where international experience in managing such patients is scant. In addition our work, provides a crude but invaluable direction for future clinical management and basic science research protocols.

Iqoqa

Lolu cwaningo luhlanganisa imibhalo eseyishicilelwe ngaphambilini engafakiwe noma eyimfihlakalo. Lubonisa uxilongozimpawu, ukuphatha, nezindlela zokwelapha ezihlobene ne-HIV, ukuxabana kwendlela ejikelezayo ehlanganiswe amasosha omzimba, okuchaza imithi emisha okuhloswe ngayo ukuthola okuliswana negciwane elihlobene ne-HIV radiculoneuropathies ngokusebenzisa amasu amasha kanye nawesimanje. Lokhu kubalwa kukho izivivinyo ezencike kumaseli, izibuko zemyelin-co-culture, i-immunoprecitation ngokusebenzisa izinhlelo zokusebenza ezinobuzibuthe kanye nokuthola okuhlosiwe ngokusebenzisa ispectroscopy esikhulukazi. Luvuma ukuthi ineurofascin 155, 186, icontactin-1 kanye neCaspr kwenzeka ngenjwayelo efanayo emaqoqweni angathelelekile nge-HIV, kodwa esigatshana se-IgG1. Lokhu kungahlobana nesifundo sokusebenza kwamasosha omzimba kwi-HIV noma eqoqweni elikhethekile. Ukusebenza kokwelashwa kanye nemiphumela yezinye izinhlanganisela zezinkomba ezingejwayelekile ezihlobene ne-HIV okubalwa kuzo imyasthenia gravis, iventral root radiculopathies, imotor neuron syndrome kanye nedemyelination ejikelezayo nakho kuyenekwa, kodwa kuseyimfihlo. Yize noma kunjalo, lolu cwaningo lunikeza inkundla yokusebenza okuyinhlanganisela kwesikhathi esizayo kwamasosha omzimba futhi okulufuzo, ngenhloso enqala yokuthuthukisa ukwelashwa okuhlosiwe hhayi kuphela kwe-HIV kodwa isifo samasosha omzimba sokulwa nokungaba usizo emzimbeni kanye kanye.

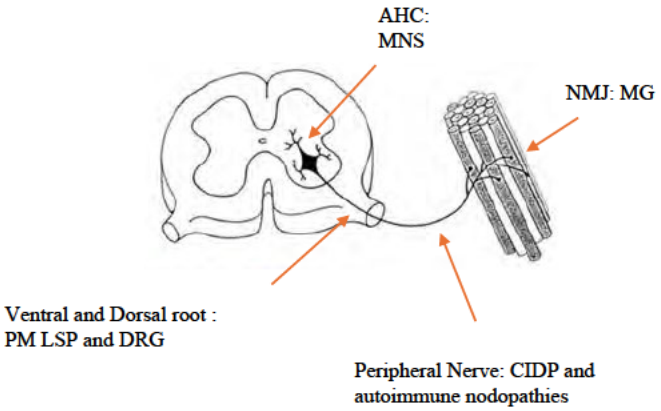
CHAPTER 1:

1.1 INTRODUCTION

Neurological disorders are common in HIV infection[1]. They occur at all stages of HIV and affect all levels of the neuroaxis. The aetiology of peripheral nervous system disorders are expansive and include autoimmunity, co-morbid illnesses, nutritional deficiencies, opportunistic infections (tuberculosis (TB), herpes viruses, HTLV-1, syphilis); malignancies (lymphoma) and side effects of drug therapy (antiretroviral therapy (ART))[2,3,4].

Peripheral nervous system disorders affect the anterior horn cell (AHC), dorsal root ganglia (DRG), nerve roots (ventral and dorsal roots), peripheral nerve, neuromuscular junction and muscle (Figure 1.1). This thesis is restricted to the AHC, nerve root, peripheral nerve and neuromuscular junction.

Figure 1.1: Peripheral nervous system diseases discussed in this thesis:



AHC=anterior horn cell, MNS=motor neuron syndrome, VRR=ventral root radiculopathy, NMJ =neuromuscular junction, MG=myasthenia gravis, CIDP=chronic inflammatory demyelinating polyneuropathy

A summary of peripheral nervous system disorders are described in Table 1.1[5]. The above conditions arise at different stages of HIV for example; CMV and lymphoma occur in advanced disease and AIDP commonly during seroconversion. Less well characterised immune disorders include; CIDP, PM LSP, DRG and MNS[2,6]. Muscle disorders include HIV-associated polymyositis, inclusion body myositis (IBM), nemaline rod myopathy, and ART induced rhabdomyolysis[7,8]. Neuromuscular junction disorders such as MG and Lambert Eaten myasthenic syndrome (LEMS) are also described in HIV[9]. The pathogenesis of many of the above diseases remains largely unknown.

Table 1.1.1 Summary of HIV-associated peripheral nervous system disorders[1, 3, 4]

Root/AHC/DRG	Peripheral Nerve	Muscle	Neuromuscular Junction
Infection: TB, syphilis, HSV, CMV,HTLV1	Infection: CMV, HSV, hepatitis C [4]	Inflammatory: Polymyositis, IBM	Myasthenia Gravis
Malignancy: Lymphoma	Small fibre Neuropathy/Autonomic Neuropathy (HIV, ART)	Nemaline Rod Myopathy	Lambert Eaton Myasthenic Syndrome
PM LSP	Distal sensory neuropathy: symptomatic, asymptomatic[3]	ART induced rhabdomyolysis	
Dorsal Root ganglionopathy	Mononeuritis multiplex (HIV vasculitis)	Mitochondrial	
Immune Mediated: AIDP,CIDP	Diffuse interstitial lymphocytosis		
Motor Neuron Syndrome (HIV, HTLV1)	Immune mediated: AIDP ,CIDP		
	Nutritional deficiencies		

Little is known regarding ART crossing the blood-nerve barrier or HIV in its active or latent forms being found in peripheral nervous system tissue. The direct influence of the retrovirus on neuromuscular disorders (NMD) remains uncertain despite a few isolated reports of HIV viral particles present in muscle and nerve histology specimens[10, 11].The pathophysiology of HIV-associated CIDP, DRG, PM LSP and MNS is unclear and whether they are due to the direct effect of HIV on neural tissue, exaggerated CD8 T lymphocyte responses (CTL responses), humoral mediated or a combination of the above is uncertain, however many aspects of the immune response are likely part of the pathogenesis. With regard to peripheral nerve in HIV, apart from isolated reports of sulphatide antibodies and neurofascin-155 antibodies binding to nodal regions and HIV or ART itself possibly causing distal sensory peripheral neuropathy, there is little described [12, 13]. The precise pathogenesis is also unclear in other humoral mediated conditions such as HIV-associated MG and LEMS. Available literature regarding clinical presentation and response to therapy in immune mediated syndromes is analysed and discussed in the scoping review in chapter 2.

Systemic chronic immune activation, immune dysregulation, immune destruction and loss of tolerance has emerged as an important component of HIV-associated autoimmune disease [14, 15]. In addition to neurological diseases, other autoimmune diseases have been reported in HIV as early as 1981 as listed in table 1.2.1. According to Zandman-Goddard and Schoenfeld's proposed model for the development of autoimmunity, at the early phase of the infection, the immune system is initially preserved. Later in the disease, chronic infection is established with progressive decrease of lymphocytes, and eventually, AIDS [16]. In each of these stages, autoimmune disorders that develop are different. For instance, CD8-mediated diseases such as psoriasis, spondyloarthropathies, or infiltrative lymphocytosis typically appear when there is a severe inversion of the CD4:CD8 ratio [16, 17].

Immune recovery after initiation of ART may trigger autoimmunity such as immune reconstitution inflammatory syndromes seen in tuberculosis, cryptococcal meningitis, progressive multifocal leukoencephalopathy, MG and sarcoidosis [18]. HIV infection also results in early immunosenescence [19]. HIV immunological changes and breakdown of immune tolerance are discussed in more detail in chapter 1.

The above thesis attempts to explore and describe rare immune mediated neurological syndromes that may arise in the context of HIV. This includes syndromes restricted to the lower motor neuron (figure 1.1). The neurological conditions include; MNS,PM LSP, CIDP, autoimmune nodopathies and MG. The aim of this thesis is to identify and fill gaps in the literature, recognise clinical syndromes in HIV, and understand immunopathogenesis of these autoimmune diseases in HIV. The background literature and scoping review are essential for more in-depth understanding of the above topic, to identify gaps in the existing literature and form a platform for future prospective research. Exploring the pathophysiology of autoimmunity in HIV will ultimately allow for the development of targeted and effective immunotherapy in HIV-infected patients which is often complex and challenging.

Background Literature on HIV infection

An insight into the virology, epidemiology, clinical aspects and immunology of HIV is essential in understanding the complexities of immune mediated neurological conditions which occur in people living with HIV infection. Such insights further underpin the importance of designing basic science and clinical research in order to delve into these complexities.

1.2.1: History and epidemiology of HIV and impact of ART

Human immunodeficiency syndrome due to HIV was discovered in clusters of young patients presenting with Kaposi's sarcoma and Pneumocystis pneumonia as reported by the CDC in a weekly morbidity and mortality report in 1981[20]. The causative link to AIDS was made by Gallo and Montagnier in 1983[20]. The official discovery of HIV in 1980 brought the disease to the fore. This was made more apparent by famous celebrities being afflicted, each with their own unfortunate story in an era before adequate ART. The story of Freddie Mercury, Rock Hudson and many others became part of mainstream HIV awareness.

HIV-1 and HIV-2 are phylogenetically related to Simian Immunodeficiency Virus (SIV) in the Chimpanzee and Sooty Mangabey respectively[20]. SIV may have jumped species to humans due to bushmeat hunting practices and exposure of humans to infected blood and body fluids of primates due to disruption of the ecology of Central Africa during French and Belgian colonization. The above practices may have accelerated Simian to human spread during the height of colonial power in Central Africa especially Belgian Congo. In these areas, primates may additionally serve as a source of food, suggesting routes of transmission to humans, which correlate with phylogenetic data implying cross-species infection or zoonotic transmission[20].

SIV does not cause disease similar to AIDS or even depletion of CD4 cells in the natural host despite very high SIV viral loads. Studies which analysed polymorphisms in MHC genes suggest that present day animals, which have SIV infection but no disease, may in fact represent the survivors of an ancient retroviral pandemic[21]. However, transmission of SIV to unnatural hosts, such as rhesus macaque, causes a progressive loss of CD4 cells and a high degree of susceptibility to opportunistic infections in addition to dysimmune responses[22]. Studies have shown that the "lymphopenic" or dysregulated state can serve to provide both clinical benefit or clinical autoimmune disease depending on the stage of the disease and the nature of the host proteins that are recognized during this process[23]. These studies show that the HIV/SIV env binding region of the primate CD4 protein is highly variable, both within and across species, and suggests that this diversity has been maintained by balancing selection for millions of years to confer protection against primate lentiviruses[24]. Therefore, comparing and contrasting SIV in the Chimpanzee and the rhesus macaque, studying of ancient viruses and their evolution over time, as well as adaptation of the host to retroviruses over thousands of years may serve as important disease models for HIV in humans and may lend clues to

understanding the pathogenesis of HIV-induced autoimmune disease that arise during different stages of HIV infection. Table 1.2.1 describes a time line for the emergence of autoimmune disease in HIV infection.

Table 1.2.1: History of HIV/AIDS and autoimmunity

(Extracted from KZN DOH Daily News Supplement (2004) with modifications)

Year	Highlights
Pre-1955	HIV was probably transferred before 1955 from a sub-species of chimpanzee infected with SIV.
1959	Earliest case of HIV was documented in 1959 in the Democratic Republic of Congo.
1969	First known case of HIV in USA was a teenager with Kaposi's Sarcoma who died of AIDS.
1980	31 HIV- related deaths in the USA.
1981	USA newspaper article announces, "A rare Cancer seen in 41 homosexuals". 55 cases of SLE reported in HIV-infected patients between 1981 and 2012 [25].
1986	37 061 AIDS cases reported in USA of which 16 301 died. First case of HIV- myositis reported [26].
1987	Zidovudine (AZT) was the first drug approved against HIV
1988	Human trials of HIV vaccine begin. 46 134 of 89 864 AIDS cases in USA die. South Africa reports 91 cases of AIDS.
1989	First cases of arthritis reported in HIV [27, 28].
1990	Reports of sero-negative MG in HIV [29].
1991	TB resurfaces in USA
1993	AIDS patients showing signs of AZT resistance, 1/250 adults worldwide infected with HIV and 1/40 in Africa
1996	USA FDA grants accelerated approval for Nevirapine. First HIV test which uses urine samples approved.
1997	Autoimmune hemolytic anemia reported in HIV, limited to few case reports [30, 31].
1998	Autoimmune Thyroiditis and Graves' disease reported after immune reconstitution[32].
1999	Sarcoidosis reported after commencement of ART [33].
2002	ITP reported in patients with CD4 counts < 200, related to GP111 antibodies [34].
2003	South Africa has 4.7 million people living with HIV (189 000 infants born with HIV). Worldwide-35.7 million adults and 2.1 million children living with HIV. GBS reported during seroconversion and IRIS [35, 36]
2004	South Africa: Largest roll-out of ARV's globally, emergence of virological and immunological failure
2005	Case reports of autoimmune hepatitis [37] in HIV related to IRIS and 8 cases of vasculitis from East Africa? Bechet's [38]
2023	8.4 million people in South Africa are infected with HIV, and approximately 5.5 million (65%) people receive ARVs

Epidemiology of HIV and ART

The World Health Organisation (WHO) statistics confirm that by the end of 2022, 39 million people were HIV- infected worldwide, and 30 million were on antiretroviral therapy (ART) [39, 40]. Sub-Saharan Africa is the only region where HIV is hyperendemic and constitutes the highest global burden with 20 million HIV-infected patients. High levels of disease are consistent and HIV prevalence rates among all adults of reproductive age are greater than 5% [41]. The largest number of infected patients, that is 1.8 million, reside in the province of KwaZulu-Natal (KZN) [42]. SA has the

largest number of people enrolled on the ART programme in the world, 1.6 million (88%) are currently on ART in KZN and 60% of newly infected patients are women[43].

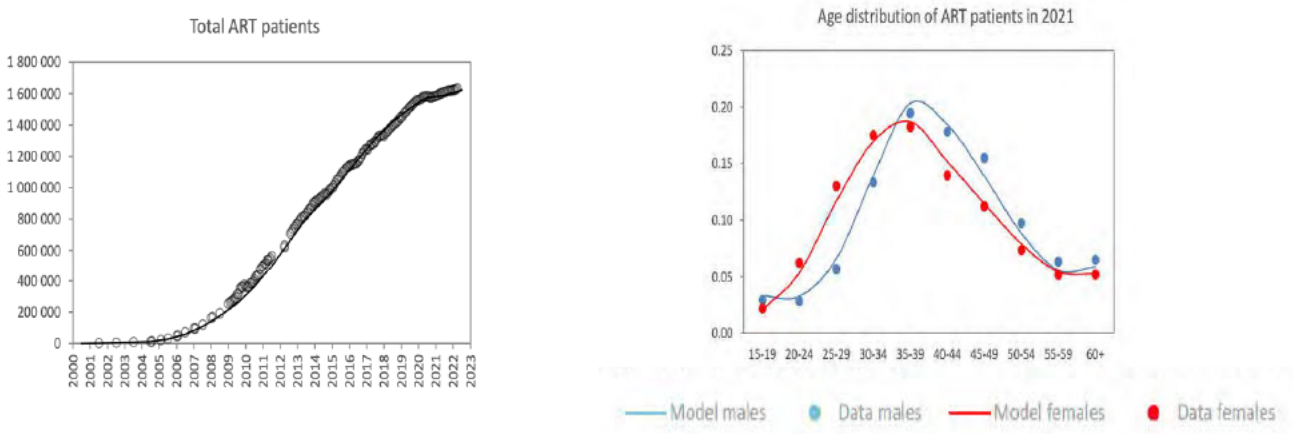


Fig 1.2.1: Total number of patients in KZN on ART and age distribution of patients in 2021. *(Adapted from Mid Term HIV Population Statistics SA, 2022)* [43]

The introduction of ART, in 1987, has decreased the spread of HIV, reduced opportunistic infection and to an extent "normalised" the immune system. SA has made great strides, in the post Thabo Mbeki era, in controlling the epidemic via increasing access to ART, implementation of HIV prevention programmes, prevention of mother to child transmission, community engagement programmes, restructuring policy frameworks, development of national strategic plans, and international collaboration. Several past celebrities have made significant contributions to HIV awareness in Africa and a few notable examples include Elizabeth Taylor, Princess Diana, Elton John, Annie Lennox and former US president Bill Clinton. All of the above, together with international organisations like UNAIDS, Global fund and US PEPFAR(US Presidents emergency fund for Aids relief) has provided funding for ART and awareness programmes. This has improved the quality and life expectancy of people living with HIV. ART has however, not been successful in normalising elevated markers of inflammation example CSF IL-1β, IL-6, and TNF alpha, possibly due to poor penetration into the central nervous system and other HIV reservoirs such as secondary lymphoid tissue, kidney and adipose tissue, or resistant viral clones [44, 45].

Currently there is no vaccine or cure for HIV. Neutralizing antibodies (NAbs) typically play a key role in controlling viral infections and contribute to the protective effect of many successful vaccines. In the case of HIV-1 infection, there is compelling data in experimental animal models, but not in humans, that NAbs can prevent HIV-1 acquisition. In recent years, however, the discovery of broadly neutralizing antibodies (bNAK) led to new optimism for a possible vaccine. Up to 20% of all infected individuals develop bNAK, but 2 years into disease. The target of bNAK is the viral spike of the HIV-1 viral envelope, a heterodimer consisting of trimeric gp120 and the transmembrane glycoprotein gp41[46] and may provide potential clues for new vaccines.

1.2.2 HIV biology:

HIV-1 and HIV-2 are enveloped RNA viruses belonging to the family Retroviridae. These viruses reverse transcribe their genomes to produce dsDNA which integrates into the hosts genome[47]. HIV-1, HIV-2 and SIV are members of the lentivirus genus characterised by cytopathogenicity, lack of oncogenicity, chronic viraemia and slow rate of pathogenesis. Among the 4 genetic groups of HIV-1 (M,N, O and P), group M viruses dominates the pandemic. Among the M group subtype C is most prevalent worldwide, however many circulating recombinants forms (CRF) exist[47].

Virus structure, genomic organization and replication:

The virus measures about 100-150 nm in diameter by electron microscopy[48].Mature viral particles are electron dense and have a conical core. The core is surrounded by a lipid envelope that is acquired as the virion buds from the infected cell. The virion (figure 1.2.2 A) contains 2 copies of single stranded RNA, each of which codes for the complete viral repertoire of proteins (structural , enzymatic and regulatory) listed in Table 1.2.2 The organization of the individual genes in a single strand of pro-viral DNA is shown in Figure 1.2.2 B

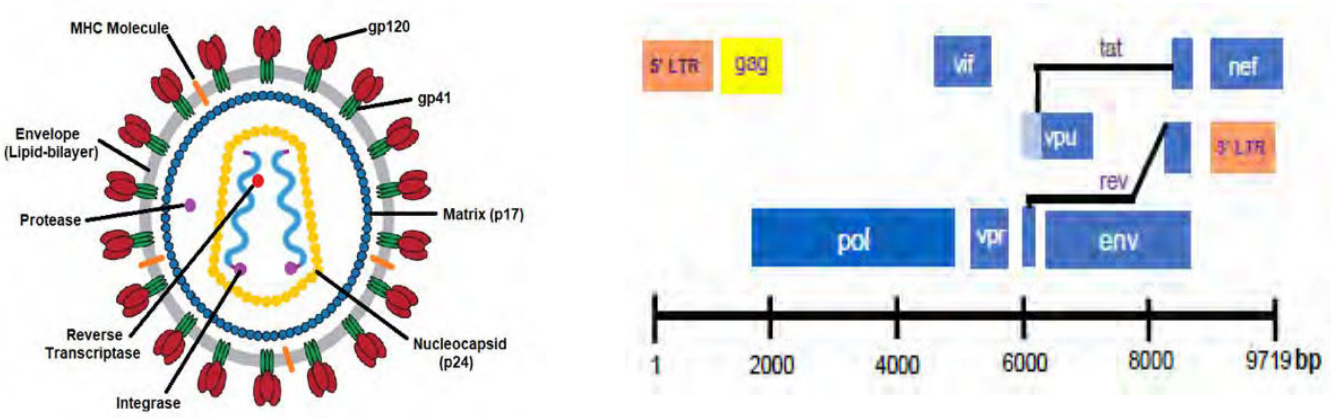


Figure 1.2.2: Diagram showing HIV-1 structure (A) and genome configuration of HIV-1 depicting a single strand of DNA, double strand of DNA is 10kbp (B)

Adapted from Human Immunodeficiency Virus. Clinical Virology 2016, Guatelli, JC [49]

Table 1.2.2: Major Structural and regulatory proteins

(Adapted from Human Immunodeficiency Virus. Clinical Virology 2016, Guatelli, JC [49])

Gene	Protein	Size	Function
Structural			
Gag	Matrix Capsid Nucleocapsid P6	P17 P24 P7 P6	Structural protein which recruits envelope glycoprotein Structural protein which forms conical core Binds viral RNA to encapsulate the genome Budding
Pol	Protease Reverse transcriptase Integrase	P12 P66/p51 P32	Viral enzyme: cleavage of polyprotein precursors Viral enzyme: reverse transcription Viral enzyme: integration of viral cDNA into host chromosome
Env	Surface glycoprotein Transmembrane	gp120 gp41	Viral envelope glycoprotein: receptor binding Viral envelope glycoprotein: fusion
Regulatory /Immune invasion			
Tat	Tat	P14	Transactivates viral transcription
Rev	Rev	P19	Transports unspliced mRNA to cytoplasm
Vif	Vif	P24	Promotes virion infectivity by degrading cellular cytidine deaminase
Nef	Nef	P27	Downregulates class 1 MHC and CD4, enhances viral infectivity, facilitates T cell activation
Vpu	Vpμ	P16	Promotes release of viral particles by counteracting BST2, induces degradation of CD4
Vpr	VPr	P15	Facilitates viral replication in dendritic cells and macrophages
Vpx	VPχ	P14	Facilitates viral replication in dendritic cells and macrophages by degrading the cellular enzyme SAMHD1

HIV replication strategy

The entire replication cycle is completed in 24hrs invitro and in-vivo. Replication is initiated by attachment of the virus to a target cell through the interaction of the viral envelope glycoprotein, gp120, with the cellular receptor molecule, CD4. The binding of gp120 to CD4 induces conformational changes in gp120, which enables binding to the cellular co-receptor molecules which are CXCR4 and CCR5. The natural ligand for CXCR4 is the stromal cell-derived factor 1 (SDF-1), and for CCR5 are the ß chemokines CCL3, CCL4, and CCL5 (formerly known as MIP-1a, MIP-1ß, and RANTES) respectively [47].

Binding of gp120 to these co-receptors is obligatory for the fusion of virus with the host cell. Natural or synthetic ligands for these molecules can block the infectivity of HIV-1. Some primary isolates of HIV-1 can utilize either CXCR4 or CCR5 as a coreceptor for entry, but many may utilize CCR5 exclusively. Binding of gp120 to the coreceptors allows exposure of a fusogenic motif in the amino-

terminal ectodomain of gp41, which leads to fusion of the lipid bilayer of the virion with the host membrane. Subsequently there is release and integration of the viral genome into host cellular DNA using viral enzyme integrase. Subsequently viral RNA replication and translation into viral proteins occurs. The viral RNA and HIV proteins form new HIV viral particles, which are released from the host cell via budding and maturation. [47, 50]

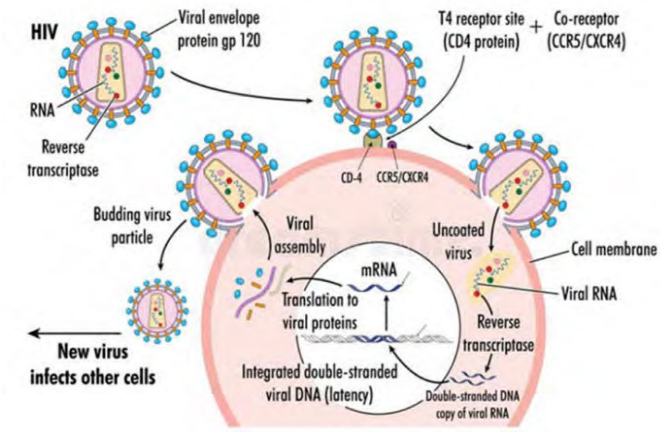
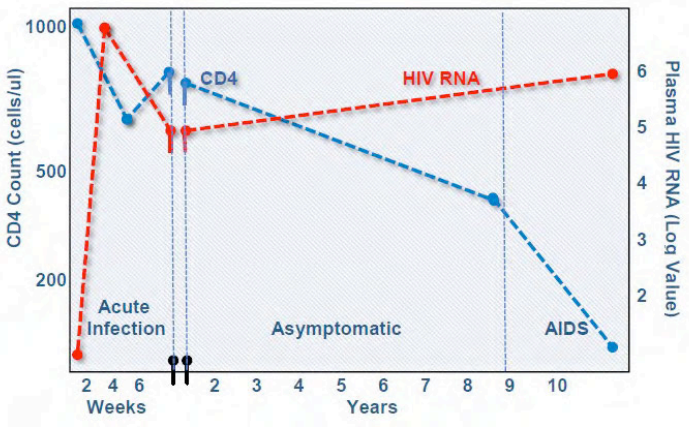


Fig 1.2.3: HIV Replication adapted from: Pathogens, 2020, 9(4), 25

1.2.3 Clinical and immunological stages of HIV infection:

HIV infection results in complex immune dysfunction that affects all arms of the immune response (innate and adaptive), due to infection and destruction of, predominantly, macrophages and T-helper cells resulting broadly in immune dysfunction, immune suppression and activation[51] . Viral replication occurs mainly in the peripheral lymphoid tissue, especially the spleen, lymph nodes, and gut associated lymphoid tissue. However, HIV can infect CD4+ cells in any tissue. Target cells include:

a. Mature CD4+ T cells in lymphoid tissue
b. Developing T cells in the thymus and ubiquitous tissue
c. Macrophages and dendritic cells (which express low amounts of CD4 and co-receptors CCR5 and CXCR4)



Adapted from Human Immunodeficiency Virus. Clinical Virology 2016, Guatelli, JC [49]

Figure 1.2.4: Immune changes and clinical stages of HIV-infection

The cardinal manifestation of HIV-1 infection is the progressive loss of CD4+ T lymphocytes. The resulting defect in cellular immunity leads to development of the opportunistic infections and malignancies that characterize AIDS. In addition, certain organ-specific syndromes may be caused directly by the virus itself. A comprehensive discussion of the myriad complications of HIV-1 infection is beyond the scope of this chapter. Immune dysregulation, however, can occur during varying stages of HIV as described by the Zandman-Goddard and Schoenfeld's proposed model for the development of autoimmunity in the introduction. There is uncertainty and insufficient literature available as to when during HIV, the chronic immune mediated neurological diseases discussed in this thesis, occur, and their relation to treatment with ART.

Acute Infection results in disruption of cellular immunity

(High viraemia and decreasing CD4 count)

The disruption of cellular immunity is characterized by robust cytotoxic T lymphocytes responses (CTL) and Th1 responses during acute infection. Virus-specific CD8 cells (CTL responses), appear early and are a critical host factor in the control of acute HIV-infection. The Th1 response produces, mainly IL-2 and IFN-γ, promoting cell mediated responses. CTL responses initially control the acute high-level viraemia by lysing infected cells and decreasing viral replication via release of cytokines. There are reports of AIDP during this phase of disease, which may be related to molecular mimicry induced by HIV. However, insufficient literature exists to draw reliable conclusions [52].

The rate of CD4 T cell loss exceeds the rate of production. Potential mechanisms for a quantitative decrease in CD4 cells include:

a. Direct infection of antigen active CD4 cells. Productively-infected CD4 cells die within a few days from the cytopathic effects of infection [49].
b. Destruction of infected CD4 -infected cells by CD8+ CTL responses.[49]

c. Non-productive infection of resting cells in a latent state may also experience death due to pyroptosis. In these cells the virus is detected by the innate system which triggers programmed cell death pathways (PCD).[49]
d. Decreased production due to accelerated involution of the thymus, gut associated lymphoid tissue and lymph nodes[53].
e. Decreased production due to bone marrow suppression[54]

The relevant CD4 subsets which are affected include the Th1 and Th2 subsets, Th17 , Treg , T follicular helper cells and memory T cells. The functions of the different subsets and repercussions of their quantitative or qualitative loss differ.

In brief, Th1 predominates in early HIV infection resulting in a CTL response promoted by the secretion of IFNγ and IL2. CTL target the intracellular virus in an attempt to control the acute viraemia. The Th2 response (IL4, IL5,IL 10) occurs later in the disease, promoting disease progression and chronic inflammation [49].

Th17 is responsible for mucosal immunity[49]. Profound depletion occurs in HIV, associated with increased bacterial translocation, persistent chronic inflammation and increased immune system activation.

The T regulatory subset is characterized by the expression of fork-head box protein 3 (FOXP3) markers. They maintain immune homeostasis via the secretion of IL-10 and TGF-β. In HIV infection, the absolute numbers are decreased, with an imbalance of the Treg/Th17 ratio, playing a significant role in breakdown of T cell immune tolerance and hence autoimmunity [49].

The T follicular helper cells are located in the germinal centres of lymph nodes in close proximity to B cells. They are regulators of mature, long-lived plasma cells which allow protective antibody responses. These T-cells appear to be relatively preserved in early HIV, and decrease later in the disease. They affect regulation of long-lived plasma cells and hence immunoglobulin production and class switching. HIV-IgG1 subclasses being predominant during advanced disease, may reflect their decline in advanced HIV[55]. This may impact on humoral mediated neurological syndromes such as MG and autoimmune nodopathies discussed later in the thesis

Central memory T cells harbour latent virus which can persist indefinitely in multiple T cell subsets (naive, stem cell memory, central memory, effector memory, transitional memory) as intact or defective virus and in various transcriptional states, even when ART is administered. This is due to several reasons which include latency which is established in long-lived naive and central memory T cells, which are designed to persist for life. These cells can undergo antigen specific clonal expansion after re-exposure to antigen. Clonally expanded cells can produce infectious virus and can account for >50% of the HIV reservoir. They can produce virions that can be detected as low-level viraemia, even in the face of excellent adherence to ART.

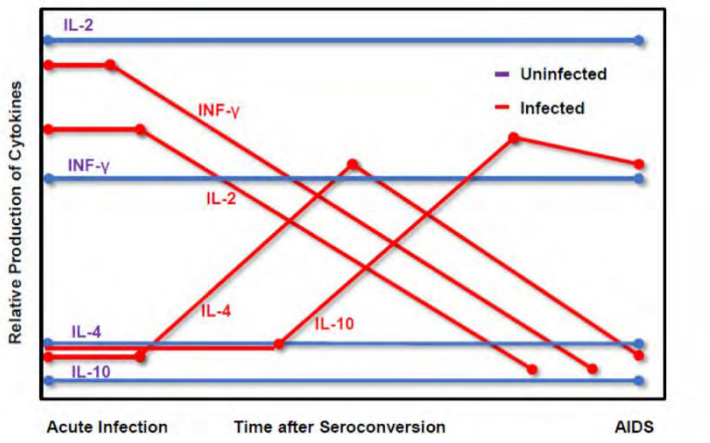
They are the fundamental reservoir of the virus since low levels of infected central memory cells are associated with the phenotype of the HIV elite controller. Their depletion is associated with progression to AIDS.

Other CD4 cells affected by HIV includes macrophages and dendritic cells which have low levels of CD4 receptors and hence may also play a role in immune dysregulation.

Depletion of the CD4 compartment characterizes AIDS. However, the extent of immunosuppression is variable during the course of HIV depending on the degree of dysregulation of the different CD4-T cell subtypes mentioned above and hence the emergence of different diseases due to chronic immune activation and immune dysregulation during the early stages of HIV and immune suppression during AIDS.

Disease progression and switch to TH2 response (Clerici and Shearer Hypothesis) [56]

A long asymptomatic period follows acute infection. This is followed by progression to clinical immunodeficiency and eventually AIDS. The Th1-Th2 switch (Clerici and Shearer Hypothesis) [56] is a significant event during disease progression as shown in Figure 1.2.5.



Adapted from Human Immunodeficiency Virus. Clinical Virology 2016, Guatelli, JC [49]

Fig: 1.2.5 Kinetics of cytokine production in stimulated PBMCs (peripheral blood mononuclear cells) from asymptomatic HIV-infected individuals demonstrating Th1 and Th2 response.

Th2 clones produce IL-4, IL-5, IL-6 and IL-10. These cytokines influence B-cell development and augment humoral responses resulting in chronic immune activation and non-specific B cell hyperstimulation.

Other factors promoting disease progression include host genetic factors (long term non-progressors carry class 1 MHC HLA B507), virological factors, antiviral immune responses and environmental factors.

Viral evasion of the immune system occurs via the following mechanisms which promotes viral replication[48]:

a. Antigenic variations (substitutions in envelope protein, switch from M (macrophage) tropic to T tropic, allows for binding to CXCR4 receptor more than CCR5 receptor) [57]
b. Carbohydrate masking of target epitopes [58]
c. Conformational changes by viral envelope to mask the neutralizing target[59]
d. Downregulation of Host HLA[60]
e. Viral latency in infected cells and antigen presenting cells[61]
f. Cell-cell transmission [62]
g. Destruction of HIV-specific T lymphocytes[63]
h. Incorporation of host proteins, including CD55,CD59,CD46, ICAM-1(intercellular adhesion molecule) and HLA[64]

Chronic Immune Activation [49]:

The primary cause of the chronic immune hyperactivation seen in untreated HIV-1 infection remains unclear. One theory suggests that microbial translocation from the gastrointestinal tract contributes to the activation [49] Continued exposure to HIV and to other viruses, such as CMV are likely to contribute. In addition to the depletion of CD4+ T cells, qualitative defects in the function of the surviving CD4+ T cells may likely impair B-lymphocyte function. While normal absolute numbers of circulating B cells are found in HIV-1-infected individuals, circulating levels of immunoglobulins are high, reflecting polyclonal B-cell activation. However, the precise mechanisms for the above remain obscure. Factors promoting chronic hyperstimulation are likely multiple and include those listed in table 1.2.3

Table 1.2.3: Sources of chronic immune activation during HIV infection [48]

Mechanism of immune activation	Proposed Consequences
Massive loss of mucosal CCR5 and CD4+ memory T lymphocytes after acute infection	Immune activation which occurs indirectly through chronic homeostatic imbalance results in progressive exhaustion of T-lymphocyte reserves.
Impaired intestinal mucosal defences after acute infection	Progressive translocation of microbial products and toxins results in chronic immunological activity through persistent stimulation of innate immune receptors.
HIV depletion of CD25+ CD4+ Treg populations	Loss of regulatory responses contributes to uncontrolled immunological activity autoimmunity, and impaired immune tolerance.
Excessive CD25+ CD4+ Treg response	Immunosuppressive response promotes viral persistence resulting in chronic viral replication and immune activation.
Influence of interferon α produced by activated plasmacytoid dendritic cells.	Upregulation of the TNF-related apoptosis-inducing ligand (TRAIL) death molecule on CD4 T lymphocytes results in elective death of HIV-exposed CD4 T lymphocytes.
Direct immunological stimulation from viral gene products (Nef, Tat, gp120)	Viral proteins interact with innate receptors to promote persistent immunological stimulation.
Molecular mimicry with HLA and other self-proteins	Autoactivation and alloactivation of immune response. Selective immunosuppression of immune responses related to host HLA results in autoimmunity.

Adapted: Cadogan M, Dalgleish AG. HIV immunopathogenesis and strategies for intervention. Lancet Infect Dis 2008;8:675-684.

1.2.4 Physiological immune tolerance: [51]

Immunologic tolerance is an active, but carefully regulated response of lymphocytes to self-antigens. Normal individuals are tolerant to their own antigens (self-antigens). Even though many self-antigens have free access to lymphocytes, lymphocytes do not normally mount an immune response against self-antigens. This self-tolerance is maintained by several mechanisms that prevent the maturation and activation of potentially self-reactive lymphocytes. Immunologic tolerance occurs in two forms: central and peripheral. The comparison of central and peripheral immunologic tolerance with respect to B cell and T cell participation includes many tiers such as clonal anergy and deletion (Table 1.2.4). Tolerance requires priming of B and T cells which occurs centrally (bone marrow and thymus) and peripherally in lymphoid tissue (spleen & lymph nodes). This results in lymphocytes which become immune competent or tolerant towards self-antigens. Breakdown of tolerance occurs as a series of events and is seldom due to a single genetic or environmental factor. The breakdown of immune tolerance and non-clearance of the virus promotes chronicity and perpetuation of autoimmune disease like CIDP.

Table 1.2.4 :Multiple Tiers of Tolerance [65]

Tolerance	Cell Type	Site	Mechanism
Central Component			
Central Tolerance	T Cells	Thymus	Primarily deletion, anergy & editing
	B Cells	Bone Marrow	Editing, anergy, deletion
Peripheral Component			
Immature B Cell Tolerance	Transitional B Cells	Periphery	Deletion and anergy upon activation
Peripheral Anergy	B and T cells	Secondary lymphoid organs and peripheral tissue	Inadequate signal induces cell inactivation
Ignorance	T and maybe B cells	Peripheral and secondary lymphoid tissue	Insufficient self-antigen or co-stimulation
Inaccessible self-antigen	B and T cells	Peripheral organs	Sequestration
Regulation	B and T cells	Secondary Lymphoid organs	Suppression by regulatory cells and cytokines
Clonal deletion following activation	B and T cells	Secondary lymphoid tissue	Apoptosis
Cytokine deviation	T Cells	Secondary lymphoid tissue	Differentiation towards less pathogenetic Th subsets
Post somatic hypermutation	B Cells	Germinal centre	Insufficient CD4 T cell help, deletion via *FAS*
Tissue resistance	B and T cells	Peripheral tissue	Inhibitory intercellular signals and cytokines
Innate mechanisms			
PRR rearrangement	Innate cells	Site of inflammation	Simple mechanism for self-non self-discrimination
Suppression of adaptive immune responses	Dendritic Cells	Site of inflammation	Activation of T reg cells
Clearance of apoptic cells	Complement & phagocytes	Peripheral tissue	Removal of pro-inflammatory material and self-antigens
Complement mediated effects on adaptive responses	Lymphocytes/ innate cells	Secondary lymphoid tissue	Modulation of activation

Adapted: Nat Immunol 2017, Multiple pathways to Autoimmunity, Vol. 18 Issue 7 Pages 716-724 [65]

1.2.5: HIV, autoimmunity and tolerance

HIV induces autoimmunity through postulated mechanisms which include: molecular mimicry, allo-activation and disruption of the multiple tiers of immune mechanisms of tolerance described in table 1.2.4.

Molecular mimicry and allo-activation

HIV-1 viral proteins in particular the *env* protein are homologous to human self-antigens [66]. This results in antibody production against various host self-antigens example T-cell receptors, CD4, CD95, complement, IgG, TNF, and other immune-related proteins. Autoantibodies may compromise the immune system via knockdown of key proteins. Over 500 human proteins contain pentapeptides or longer consensi, similar to HIV peptides [66]. This homology explains the viral-human interaction, related to the ability of viral homologues to compete with human counterparts as binding partners example antibodies against gp120 and gp41 bind to like-antigens in the glomerular basement membrane of the host or antibodies to GP IIIa are implicated in HIV-1–related thrombocytopenia. However, many of the auto-antibodies are low-affinity and polyreactive against self-antigens, and may not result in disease, as reflected in the IgG1 nodal-paranodal antibodies in chapter 8.

AIDS pathogenesis has been compared clinically and immunologically to graft-versus-host disease. Evidence suggests that HLA mimicry may present gp120 with the capacity to arouse the inherently reactive nature of the immune system towards alloantigen. Alloreactivity is the phenomenon in which a strong immune response can be generated against foreign allelic variants of major histocompatibility complex (MHC) molecules. Certain epitopes derived from regions of gp120 have been shown to stimulate alloreactive T lymphocytes, promote autoreactive T-lymphocyte activation, and induce CD8 T-lymphocyte suppressor responses against uninfected activated HLA-DR expressing cells. The conserved C5 region of gp120 is of particular interest because of its reported serological and structural homology with peptide-binding domains of HLA molecules resulting in allo-activation [48].

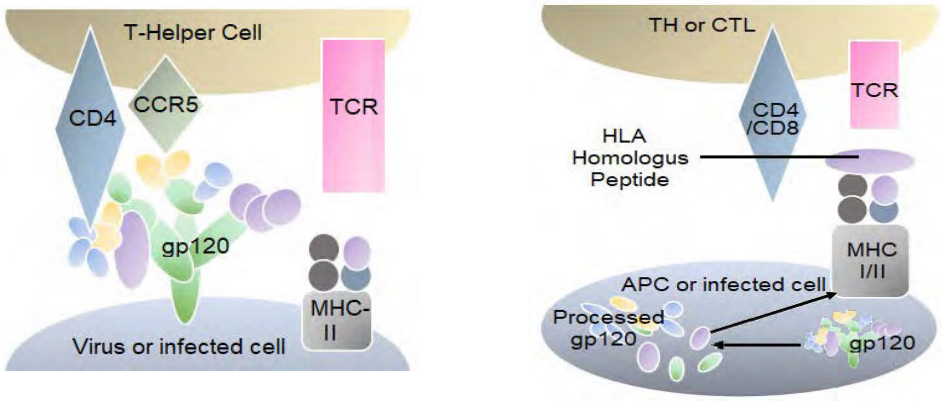


Figure 1.2.6: Models for HIV-induced alloactivation and autoimmune suppression [48]

(A) Direct alloactivation may result from immune recognition of HLA homologous structural domains of gp120 (purple). A close association with host HLA might be mistaken for an allogeneic or hybrid complex by the host.

(B) Processing of HLA homologous sequences from gp120 may result in presentation by host HLA on infected or uninfected cells and provoke indirect alloactivation. Activation of CD8 T lymphocytes by HLA homologous sequences may provoke an autoimmune reaction against native HLA-derived sequences when presented as a peptide of host self-repertoire.

Breakdown of B cell tolerance and generalised B cell immune dysregulation in HIV[67]

Subsequent to establishing cellular reservoirs of virus and chronic infection, HIV1 elicits generalized B cell immune dysregulation, including B-cell activation regardless of antigen specificity. This polyclonal B-cell activation does not result from direct viral infection as HIV-infected B cells are rarely observed in patients. Instead, chronic HIV-1 infection drives B-cell activation via systemic inflammatory signals including but not limited to interferons, TNF-family members, interleukins, and elevated B cell activating factor (BAFF) levels. This sustained B-cell dysregulation is permissive for the production of serum autoantibody that can result in autoimmune disease. Thus, the milieu of inflammatory cytokines (e.g. BAFF, TNF) present during chronic infection appears to relax B-cell tolerance mechanisms of clonal deletion, anergy and receptor editing .

Role of BAFF [67]

Unlike the central tolerance mechanisms, the stringency of transitional B-cell tolerance is plastic. Selection depends on the interplay between BCR-mediated signals and a B cell survival factor, called B cell activating factor (BAFF). BAFF is a soluble type II transmembrane protein that promotes key biological functions including peripheral B-cell survival and homeostasis . Its receptors include BAFF-R, TACI, BCMA which are key regulators of BAFF and B cell survival. Overexpression of BAFF has been linked to human autoimmunity. Recent data provide clues as to how excessive secretion of BAFF may allow the emergence of autoreactive B cells in mice and humans [67]. BAFF overexpression by key dendritic cell subsets during HIV infection might subsequently initiate the unexpected expansion of HIV cross-reactive B-cell clones and atypical memory B-cells [68].

Role of Toll-Like Receptors in B cell tolerance [48]

Toll-like receptors (TLRs) are pattern recognition receptors that recognize pathogen associated molecular patterns (PAMPs). TLR-mediated recognition of PAMPS leads to activation of innate immune cells . TLRs expressed by B cells can also recognize self-antigens released from host tissues that are damaged. Such self-recognition by the B cell intrinsic TLR can potentially promote the development of autoimmune disease by disruption of B-cell tolerance**.**

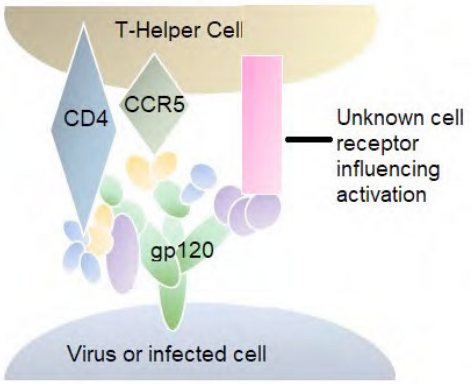


Figure 1.2.7 Inappropriate receptor-triggered immune activation: HIV gp120 structure is represented by unexposed/hidden regions (light green), exposed neutralising domains (yellow), alongside exposed non-neutralising regions (pink). The neutralising domains are shown interacting with target-cell receptors for viral entry including CD4 and the co-receptor CCR5. Chronic immune activation may result from the stimulation of an additional host receptor, such as a Toll-like receptor, that may influence disease progression [48]

Breakdown of T-Cell Tolerance in HIV

The breakdown of T-cell tolerance may occur centrally, peripherally or in both compartments. Central T-cell tolerance is disrupted by premature thymic involution and dysfunction which has been associated with rapid progression of HIV in infants infected perinatally with the virus[69]. In vitro evidence of thymic organ culture, thymic epithelial cell culture, the SCID-hu mouse system and simian HIV infection of primates have supported HIV-induced thymic damage. This has been confirmed on post mortem histology. The mechanisms underlying this are multiple, including direct thymocyte killing by the virus, apoptosis, or disruption of thymic stromal architecture which result in ineffective thymopoiesis and decrease in AIRE expression. [70]

The peripheral mechanism of T-cell tolerance is maintained through 5 mechanisms, which are disrupted by HIV, which are as follows [51, 71] :

1. **Ignorance**

T cells that are physically separated from their specific antigen, example, by the blood–brain barrier, cannot become activated. This is referred to as **immunologic ignorance.** Primary HIV infection and other opportunistic infections disrupt immunologic ignorance via breakdown of the blood brain barrier (CNS is an immunologically privileged site), hence exposing neural self-antigens example neurofascin and contactin to native T cells [72].

2. **Deletion**

Among the lymphocyte subsets, only activated T cells and natural killer cells express readily detectable levels of Fas ligand. Reactivation of previously activated T cells through T-cell receptors induces apoptosis. This phenomenon (activation-induced cell death) is mediated by means of the Fas-Fas ligand interaction, a process known as **deletion.** Deletion is subverted in HIV by increasing the expression of the Fas ligand by the macrophages it infects, thereby inducing apoptosis of T cells that encounter such macrophages [73]. The finding that HIV-infected cells are less susceptible to Fas-Fas killing means that HIV-infected cells become enriched when Fas-mediated apoptosis is the major death pathway.

3. Anergy

T cells that do not produce interleukin-2 on encountering their antigen (and therefore cannot be completely activated) are called anergic. In HIV infection, both CD4 and CD8 cells are non-responsive to nominal antigen. This state is due to the binding of HIV envelope glycoprotein moieties to CD4 molecules and chemokine receptors. The resulting decrease in antigen presenting cell function and the interference with functioning of positive and negative regulatory molecules involved in signal transduction have an "anergizing" effect on the immune system. This effect is exemplified by diminished production of interleukin-2 (IL-2) and interferon-gamma and reduced expression of IL-2 receptor by CD4 helper cells of HIV patients. These immune abnormalities lead to clinically relevant immunological phenomena such as Type-1 to Type-2 switch and decrease in delayed-type hypersensitivity dermal reaction.[74]

4. Inhibition

CD152 or cytotoxic-T-lymphocyte–associated protein 4, (CTLA-4) on T cells binds CD80 (B7-1) and CD86 (B7-2) on B cells with a higher affinity than the costimulatory receptor CD28. CTLA-4 also binds CD80 on antigen-presenting cells, thereby inhibiting the activation of T cells. CTLA-4 is preferentially upregulated on virus-specific CD4 T cells, and not CD8 cells. Therefore there is preferential expansion of HIV-specific CD4 T cells and increase CD4 activation. This provides additional targets for viral entry. Studies have shown that CTLA-4 expression correlates positively with disease progression and negatively with the capacity of $CD4^+$ T cells to produce interleukin 2 in response to viral antigen. Most HIV-specific $CD4^+$ T cells co-expressed CTLA-4 and another inhibitory immunoregulatory receptor, PD-1. *In vitro* blockade of CTLA-4 augments HIV-specific $CD4^+$ T cell function. This, indicates a reversible immunoregulatory pathway selectively associated with $CD4^+$ T cell dysfunction, and provides a potential target for immunotherapy in HIV-infected patients [51].

5. Suppression and deviation

Mediated by a protective CD4+ T cell compartment that participates in regulating and modifying auto immune response. This compartment is decreased in HIV.

The other mechanisms through which HIV disrupts T-cell tolerance include:

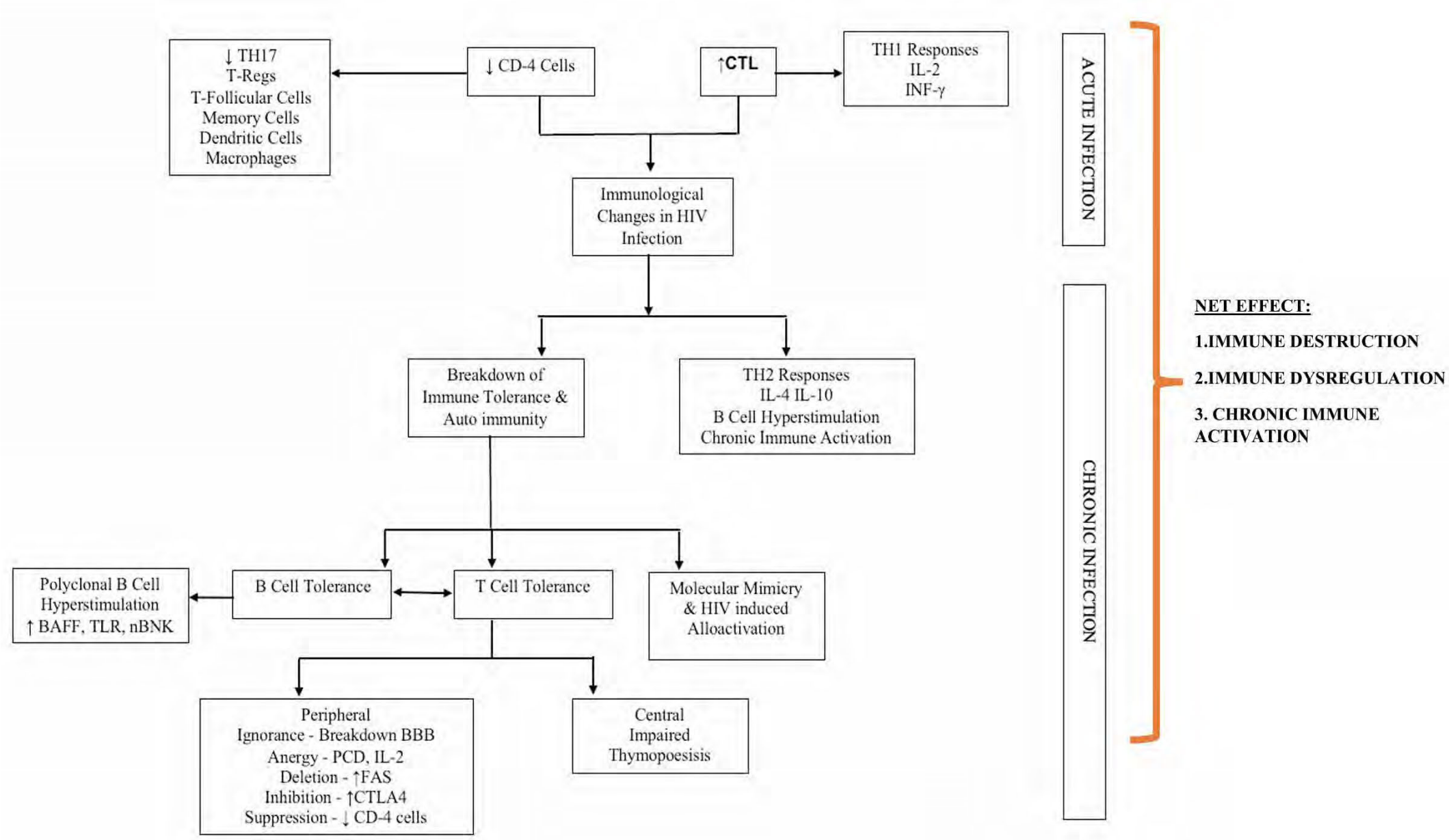
a. Upregulation of the inhibitory molecules such as programmed death-1 (PD-1)
b. Blunted T-cell signalling induced by T-cell receptor (TCR) cross-linking.
c. Impaired cross talk between T and B cells

Teasing out the complexity of the immuno-pathogenesis of autoimmunity in HIV requires a systems approach where one needs to examine cross-sectionally and longitudinally all aspects of the immune

response as immune responses vary with time and all arms are linked. Future prospective work is required to expand the concept of breakdown of immune tolerance in HIV and to possibly investigate the loss of tolerance in HIV by measuring subclasses of Treg cells, cytokines, PD-1 protein, autoantibodies, CTLA4 and chip technology to measure regulation of genes in the context of HIV. This may provide clues to autoimmune disease in general. The net effect of the immunological disruption by HIV is a state of immune destruction and suppression, immune dysregulation and paradoxical chronic immune activation as summarized in Figure 1.2.8.

The knowledge and insight into HIV structure, replication, role of genome products, in addition to a brief overview of the epidemiology, relevant clinical aspects and the immunology of HIV forms the basis of understanding the complexities of immune mediated neurological conditions which occur in people living with HIV infection.

Figure 1.2.8: Summary of Immunological Changes in HIV Infection



1.3:Background literature on neurological syndromes:

CIDP, Nodopathies, PM LSP, MNS and Myasthenia Gravis

The above neurological diseases occur both in HIV-infected and HIV-uninfected patients. An overview of the epidemiology, clinical features, diagnostic laboratory, electrophysiological findings and pathophysiology of disease in HIV-uninfected patients forms the basis of understanding the disease in the context of HIV.

1.3.1:CIDP

Epidemiology and introduction:

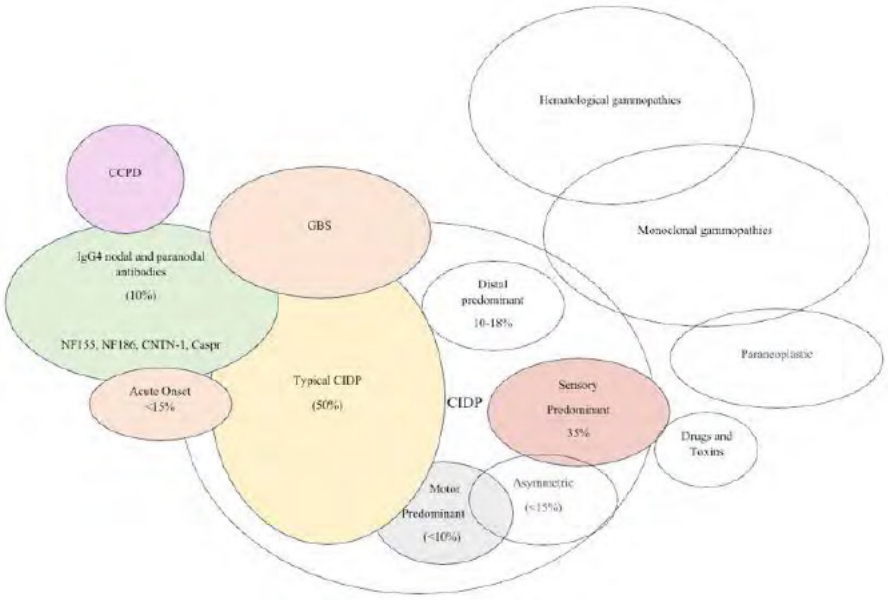
CIDP is the most common treatable chronic neuropathy worldwide, with a prevalence ranging from 1-9 cases per 100 000 [75, 76]. There are no African epidemiological or genetic studies to date. Most of our knowledge regarding the disease is from the western literature. Although CIDP is described as an autoimmune disorder in which an aberrant immune response is directed towards components of the peripheral nerve sheath causing demyelination and axonal damage, the exact mechanisms underlying the immunopathology remains poorly understood [77]. In addition, considerable variation in clinical presentation and response to therapy makes identification of the pathogenic mechanisms complicated, as they may vary among individuals.

Clinical Features and Clinical Phenotypes of CIDP in the HIV-uninfected population:

CIDP is broadly classified into typical CIDP and CIDP variants. Typical CIDP occurs between 20 and 70 years of age, with average onset at 50 years, with a slight female predominance. The disease is usually characterised by symmetric quadriparesis, paraesthesia, sensory disturbances, and ataxia, which evolve over at least 8 weeks [77]. Tendon reflexes are reduced or absent in affected extremities. Cranial nerves are rarely affected but optic neuritis, bifacial weakness, and vocal cord paralysis can sometimes occur. Respiratory failure and autonomic dysfunction are rare. Tremor is seen in a subset of patients and CNS involvement is rare[78, 79].The course of the disease is often monophasic with stepwise progression or relapsing with spontaneous remissions. CIDP symptoms usually peak at 2-3 months from disease onset, but universally. This contrasts with AIDP which maximally evolves over 4 weeks. Approximately 15% of CIDP patients have a subacute onset of disease and a monophasic course that falls between the time frame of AIDP and CIDP. A few patients with CIDP experience a more acute onset and peak within 4 weeks of onset, resembling AIDP. Distinguishing AIDP from acute-onset CIDP, is challenging because their time courses overlap. However acute onset CIDP is unlikely to present with bulbo-respiratory failure, autonomic dysfunction or bifacial weakness or be precipitated by preceding infection.[80] However, there has been recent reports of acute onset CIDP, post COVID vaccination[81]. Other clues include a second deterioration 8 weeks after initial presentation or when deterioration occurs 3 fold.[82]

Phenotypic variants of CIDP are listed in the table 1.3.1.2 and figure 1.3.1 [83]. The entity of *nodo-paranodopathies* or autoimmune nodopathies accounts for < 10-20% of patients initially diagnosed with CIDP. They are now classified as a distinct entity with distinct underlying pathological mechanisms.

Figure 1.3.1.1 Diagrammatic representation of CIDP and its variants and mimics:



Adapted from Haruki et al, May 2020, Neurology and therapy

Table 1.3.1.1: CIDP variants [77]

CIDP Phenotype	Estimated Prevalence	Onset	Clinical Symptoms	Distribution
Typical CIDP	51%	Chronic	Sensory + Motor	Symmetrical, Proximal + Distal
Sensory CIDP	4-35%	Chronic	Sensory Predominant	Symmetrical, Proximal + Distal
Chronic Immune Sensory Polyradiculopathy (CISP)	5-12%	Chronic	Sensory ataxia	Symmetrical, Proximal + Distal
Lewis-Sumner variant (MADSAM)	6-15%	Chronic	Sensory + Motor	Asymmetrical, Upper limb onset
Focal CIDP	1%	Chronic	Sensory + Motor	Focal, may become diffuse over time
DADS	2-16%	Chronic	Sensory + Motor	Symmetrical, distal
Acute onset CIDP	2-16%	Acute	Sensory + Motor	As per typical CIDP
Motor CIDP	4-10%	Chronic	Motor Predominant	As per typical CIDP
Combined central and peripheral demyelination (CCPD)	rare	Chronic	Motor and sensory	As per typical CIDP + CNS demyelination
Nodopathies (Distinct entity)	<10%	Acute, subacute or chronic	Sensory Ataxia	Distal > proximal

CIDP=chronic inflammatory demyelinating polyradiculoneuropathy, DADS=distal acquired demyelinating symmetrical neuropathy, MADSAM=Multifocal acquired demyelinating sensory and motor neuropathy

The above table highlights the varied and heterogenous clinical variants of CIDP. Creating homogenous cohorts of patients with pure motor, pure sensory and CCPD will assist in identifying a single or panel of immunogenetic biomarkers may help refine pathology and predict for specific therapy in each category (Prospective studies, chapter 12)

Pathophysiology [77, 84]

Unraveling the pathogenesis of HIV-infected CIDP is complex and challenging. Literature is available regarding possible pathogenesis of HIV-related CNS demyelinating disorders such as ADEM and PML [85], however it is lacking in peripheral nerve disease. The abiding pathogenesis of CIDP in HIV-uninfected patients is that cell-mediated and humoral mechanisms act synergistically to cause damage to peripheral nerves. There are several lines of evidence to support the conclusion that CIDP is an autoimmune disease mediated by humoral and/or cellular immunity against as yet undefined Schwann cell or myelin antigens . Although some patients have reported antecedent infections prior to onset of symptoms, neither the target(s) nor the trigger for the autoimmune response has been identified and no infectious agent has been consistently linked with initiation of disease. However, the autoimmune aetiology is supported by the efficacy of treatments that target the immune system, including IVIg, plasma exchange and corticosteroids, and by evidence of an inflammatory response in the blood, CSF and peripheral nerves.

Figure 1.3.1.2: Proposed model for the immunopathogenesis of chronic inflammatory demyelinating polyneuropathy : The putative antigen is presented by antigen presenting cells to autoreactive T cells in the peripheral immune compartment : *Adapted from Mathey et al , JNNP, 2015* [77]

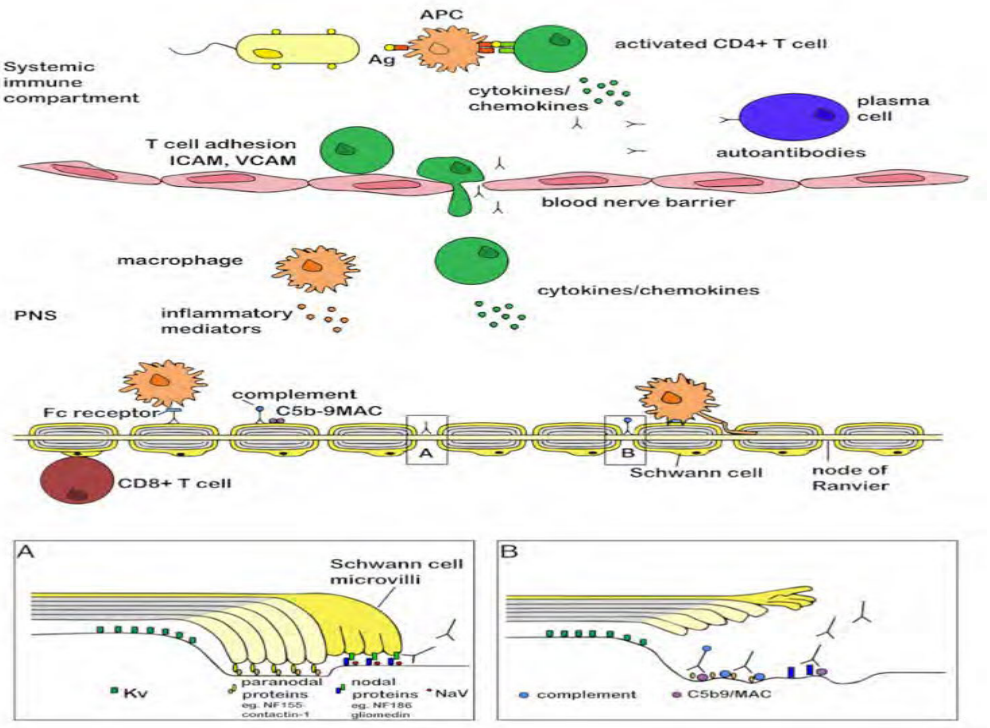


Figure 1.3.1.2 shows activated T cells, undergoing clonal expansion and release of inflammatory mediators that cross the blood-nerve barrier (BNB). Breakdown of the BNB allows humoral factors such as autoantibodies access to the endoneurium. Further damage may be caused by macrophage-mediated demyelination, complement deposition, deposition of C5b-9/membrane attack complex (MAC), subsequent cell lysis and $CD8^+$ direct lysis of cells.

Inset (Figure 1.3.1): Effects of antibody binding at the node of Ranvier. (A) Binding of an autoantibody to the node of Ranvier could block the function of nodal molecules interfering with saltatory conduction. (B) Binding of an antibody followed by fixation of complement and deposition of the MAC leading to disruption/destruction of the node and surrounding areas. This may result in an autoimmune nodopathy. However most nodopathies are usually IgG4 mediated and do not activate complement, example NF155 antibodies are usually of the IgG4 subtype. Nodopathies are now classified as a distinct entity and no longer CIDP. The molecular anatomy, functional domains, electrophysiology and clinical findings are discussed in section 1.3.3.

The pathophysiology of disease is difficult to study in HIV-infected individuals due to the presence of confounding factors in HIV, such as persistently elevated markers of chronic inflammation in blood and CSF, polyclonal gammopathy and non-specific antibody production due to B cell hyperstimulation and chronic immune activation, comorbid infections, and the effects of therapy example ART. Robust animal models of HIV-peripheral nerve may be helpful to dissect the underlying pathogenesis of this disorder and direct the development of new therapeutic strategies. SIV-infected Macaque Model of HIV peripheral neuropathy have been widely used to study host and viral aspects of HIV infection in humans. [86] The appropriateness of such models is underpinned by the close resemblance of macaque immune responses during SIV infection to those of HIV-infected humans; and extensive homology shared by SIV and HIV, including the binding of SIV envelope glycoprotein gp120 to the host receptors CD4 and CCR5 for viral entry [86] In Chapter 7 and 8 , live cell- based assays and human induced pluripotent sensory neuron stem cell co-cultures were used to screen for nodal-paranodal antibodies and other peripheral nerve reactive antibodies in HIV-infected CIDP,DRG and PM LSP. Future studies using SIV-Infected Macaque Model or nerve tissue from HIV-infected patients which may harbour antigens not expressed in non-inflammatory stem cell cultures may be useful or creating a culture model that is specific to HIV.

Given the difficulties in nerve imaging and histological diagnosis, there is emerging interest in serum and cerebrospinal fluid biomarkers of peripheral neuropathy. These include neuronal biomarkers of axonal degeneration, glial biomarkers for peripheral demyelinating disorders, immunopathogenic biomarkers (antibodies or cytokines) and genetic biomarkers. Many, such as sphingomyelin, peripherin, and neurofilament light chains remain under evaluation as potential indicators of disease activity and treatment response. Autoantibodies which are immunopathogenic in demyelinating

neuropathies include ganglioside antibodies, nodal/paranodal antibodies, paraprotein, IgM MAG antibodies and paraneoplastic antibodies Table 1.3.1.1[87]. Useful electrophysiological prognostic tests and follow up investigations include motor unit number index (MUNIX).

Table 1.3.1.2 Immunopathogenic antibodies in demyelinating neuropathies

Immunopathogenic biomarker	Clinical syndrome
Anti- GM1(IgM)	Multifocal motor neuropathy (MMN)
Anti- GQ1B (IgG)	Miller Fischer Syndrome
Anti - GD 1a (IgG)	GBS (axonal)
Anti- GT1a (IgG)	GBS (Pharyngeal branchial cervical variant)
Anti - GD3/GD1b/GT1b (IgM)	Ataxic Neuropathy
Anti-MAG IgM	Anti-MAG /IgM paraproteinemic neuropathy
Paraprotein	Amyloidosis, POEMS, and haematological malignancies
Nodal/Paranodal	Autoimmune nodopathies
Paraneoplastic antibodies	Demyelinating neuropathy

Many of the above may mimic CIDP such as amyloidosis, POEMS syndrome (polyneuropathy, organomegaly, endocrinopathy, M-protein and skin changes), autoimmune nodopathies and demyelinating paraneoplastic neuropathies.

Diagnostic Criteria for CIDP [88]

Refer to EFNS/PNS diagnostic criteria

Treatment for CIDP [83, 89-91]

Current treatments aim to suppress the underlying immunopathology. This prevents further nerve injury, facilitates functional recovery, and decreases relapses. In practice, patient specific and pragmatic considerations usually determine first line therapy. Co-morbidities, rate of progression, disease severity and practical considerations are important factors to consider, alongside quality and weight of evidence.

Established Treatments for HIV-uninfected patients include [83] [92]:

First Line Therapies

a. Corticosteroid Therapy: In our unit CST is first line therapy unless contra-indicated example poorly controlled Diabetes, HIV-infected with low CD4 counts, rapidly progressive disease and pure motor syndromes that clinically resemble multifocal motor neuropathy.
b. IVIG: Overall the highest level of evidence in CIDP is for IVIG. Data from 5 randomised trials show that IVIG is significantly more likely than placebo to improve outcome at 1 month[93]. Overall about 80% of patients respond to IVIG [94]
c. Plasma exchange or plasma adsorption: This modality is reserved for patients who have not responded to CST and/or IVIG due to the impracticalities of administration.

Second Line therapy or CST sparing agents/ Escalation therapies

More potent forms of immunosuppression are usually considered in the context of markedly inadequate response to first line therapies. Disease modifying therapy is used in patients who have had a response to CST but have side effects on CST or the response is inadequate. This includes:

a. Disease modifying therapy or corticosteroid sparing therapy: example Azathioprine, Methotrexate, Mycophenolate Mofetil , Cyclosporine, Tacrolimus: Choice of therapy is a clinical decision dependent of availability of the drug, adverse effects and patient factors
b. B cell depleting agents: example Rituximab especially for cases of autoimmune nodopathies or paraproteinemic states
c. Autologous haemopoietic stem cell therapy: Despite promising results, morbidity and mortality risks are high, mainly related to infections and long-lasting immunodeficiency.
d. Immuno-adsorption: Allows for selective removal of IgG only. This modality may have primary importance in some subtypes as IgG is unlikely to be the sole pathological agent in CIDP

To date there are no established guidelines regarding use of immunosuppressant therapy in HIV-infected patients or interactions with antiretroviral therapy.

Little is known about the pathogenesis, course of disease and response to therapy in HIV or in the African population where the prevalence of HIV and other infections are high and genetic factors vary[83]. There is uncertainty as to whether demyelination is a direct effect of HIV, humoral mediated example an autoimmune nodopathy or due to CTL responses.

1.3.2: Pure motor lumbosacral polyradiculopathy in HIV-infected patients:

Lumbosacral polyradiculopathy is a well described complication of HIV infection and is usually due to opportunistic infections such as CMV (Cytomegalovirus), herpes simplex virus (HSV), varicella zoster (VZV), Epstein barre virus (EBV), syphilis, tuberculosis and less commonly malignancies such as lymphoma [95-97]. Immune mediated causes of lumbosacral polyradiculopathy include CIDP and AIDP. More recently there has been a case report from Japan of neurofascin-155 paranodopathy presenting with root involvement in an HIV-infected patient[13], however patients had sensory symptoms and weakness. Whether, this patient had a nodopathy or CIDP remains uncertain. As mentioned in subsequent chapters, interpretation of antibody results post IVIG and in HIV is challenging.

AIDP is a well-recognised presentation complicating HIV, usually occurring at seroconversion. Naidoo et al described 39 HIV positive patients with AIDP [98]. Compared with HIV negative AIDP patients HIV positive AIDP patients were more likely to have sensory symptoms and axonal features on electrophysiology. CIDP in the setting of HIV is described in chapter 4 and characterised by mixed motor sensory symptoms. None of the patients with HIV-associated AIDP or CIDP were reported to have a pure motor presentation.

Four similar cases of pure motor lumbosacral radiculopathy in HIV infected patients have been described by Benatar et al [6]. Benatar et al reported spontaneous improvement in 3 out of 4 reported cases supporting the diagnosis of AIDP. All 4 patients described by Benatar et al had axonal changes on electrophysiology and were described as a variant of AIDP. The optimal treatment approach remains largely unknown.

In article 2 we report 11 cases of PM LSP described as possible CIDP due to CST response and duration of disease. Other possibilities include a distinct entity such as an autoimmune nodopathy rather than AIDP, CIDP or MNS.

Treatment, pathogenesis and predilection for the ventral root are discussed in the subsequent chapters and recommendations for future research.

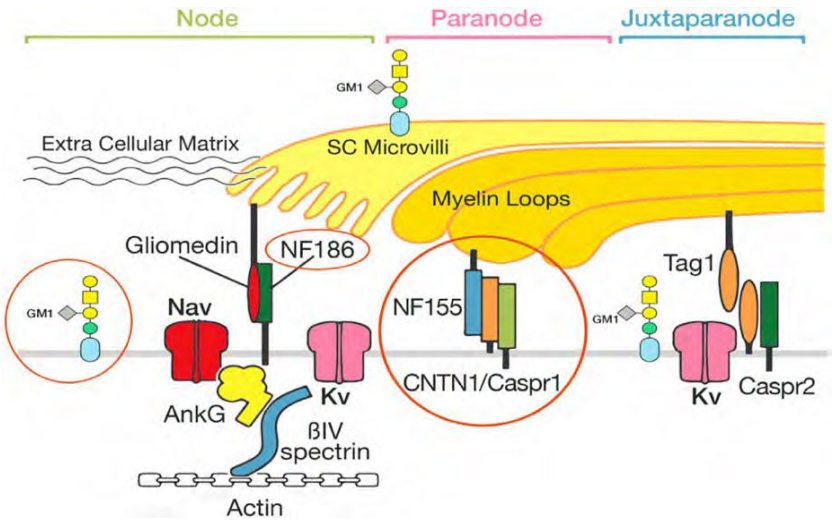
1.3.3. Autoimmune Nodopathies: Anatomy and clinical features of nodal disease:

Autoimmune nodopathies are a distinct group of antibodies mediated peripheral and central disorders that target the node and flanking regions. This includes a small percentage of patients initially classified as GBS or CIDP. Devaux et al in 2012 reported IgG antibodies from 43% of GBS and 30% of CIDP patients that immunolabelled the nodes of Ranvier and flanking regions of myelinated axons[99]. Susuki et al demonstrated that complement mediated dysfunction and disruption of the node of Ranvier occurred in experimental neuropathy models with antibodies to GM1, GD1a and GD1b gangliosides [100]. The nodal region has emerged as a hot spot in the field of autoimmune neuropathies and the category of "nodo-paranodopathy" or "autoimmune nodopathies" has recently been proposed, to characterise neuropathies in which the nodal region is crucial in the pathogenesis of inflammatory neuropathies [101, 102].

Autoimmune nodopathies include disorders that, although not showing pathological evidence of segmental demyelination, present with the electrophysiological features characteristic of demyelinating or axonal neuropathies. They, can however, exhibit reversible conduction failure and rapid recovery, contrary to the common notion of an axonal neuropathy. Currently, autoimmune nodopathies account for < 10% of all immune mediated inflammatory neuropathies. This prevalence arises from studies in the West and Asia, with limited or no testing done in the African population or among HIV-infected patients[103]. With more knowledge and awareness of the above entity, greater availability of antibody testing and refined laboratory methods the reported statistics and the clinical spectrum may expand in the future. Understanding of the anatomical and molecular structure of the node and flanking regions is essential to understanding of this disease entity.

Organisation and function of myelinated axon domains :

During the development of the human peripheral nerve, Schwann cells myelinate peripheral axons and oligodendrocytes central axons larger than 1um in diameter. Both PNS and CNS nodes consist of three domains: the node, the paranode and the juxtaparanode. Each of these domains has a distinct molecular architecture. Each internode is flanked by nodes of Ranvier, where the axolemma is most exposed to extracellular fluid and where saltatory conduction occurs (Fig 1.3.3.1). Complex highly structured axoglial interactions shape the node and flanking regions. The axonal and glial components of each domain are distinct, and our understanding of this region, its contribution to disease, and impact on immune-modulatory therapy has expanded over the last decade[104].



Simplified Structure of the Node :

Figure 1.3.3.1 : Simplified molecular organization at the nodal region, *adapted from Uncini, A: JPNS, 2023* [105]. Encircled in red are the best studied and clinically relevant target antigens of autoimmune nodo-paranodopathies and their molecular organisation which includes NF186, NF155,CNTN1 and CNTN1/CaspR-1 complex [105]. Gangliosides such as GM1 may also be present in this region, as indicated.

Detailed molecular structure of the node and flanking regions:

The node is surrounded by Schwann cell microvilli (PNS), perinodal astrocytes (CNS) and oligodendrocytes (CNS). The paranode is characterized by the presence of axoglial septate-like junctions, which attach the terminal myelin loops to the axon. The juxtaparanode is located within the myelin loops and acts as a boundary between the internodal space and axon. The proteins that are present at the node can be divided into four main functional categories namely a)ion channels and ion channel-related proteins, b) cellular adhesion and extracellular matrix molecules, c) signal transduction proteins, d) and cytoskeletal/structural proteins. Many of the proteins are also found in locations other than the node of Ranvier, such as the internode and internodal compact myelin. In addition, the axonal initial segment expresses several molecules that are found at the nodal and juxta-paranodal regions. This molecular anatomy is highly complex with multiple adhesion molecules and ion channels, not yet discovered or understood (Fig 1.3.3.2). Multi-structural changes restricted to the node or paranode may induce significant demyelination[106, 107]. Detachment of myelin loops lead to massive leak of current, and conduction failure.

Adhesion molecules (NF186, Nr-CAM, CNTN-1, NF155, Caspr1, Cntn2, Caspr2 and MAG) mediate axoglial attachment. Ion channels (KV7.2/7.3, KV1.1/1.2 and NaV1.6) mediate action potential propagation. Both adhesion molecules and ion channels are linked to the cytoskeleton; by proteins, which include ankyrin-G/B, PSD-95/93, 4.1B, and spectrins. Glm, versican V2, brevican, phosphacan, neurocan, Bral1 and tenascin-R are extracellular matrix constituents and stabilize the structure of the nodal area. Syndecan-3/4, dystroglycan, laminin-2/10 and ADAM 22 are involved in cell signalling.

Connexins are gap junction proteins, claudins and ZO-1 are tight junction proteins, and cadherin-1 and catenin β-1 are adherens junction proteins. *(Fig 1.3.3. 2)*[108]. These various adhesion molecules, ion channels, gap, adheren and tight junction proteins are potential antigenic targets.

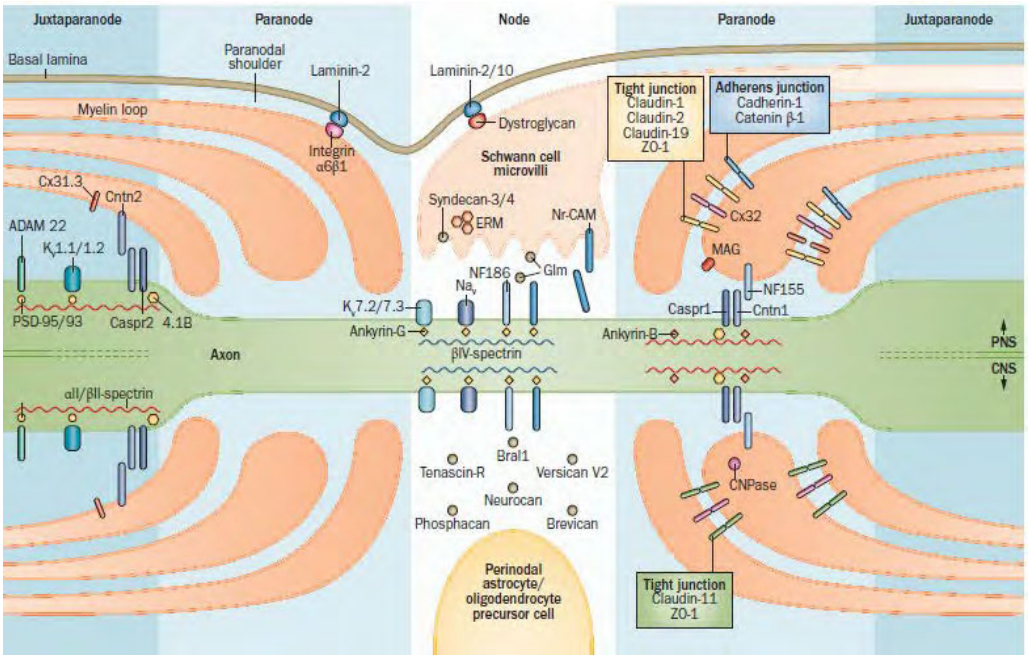


Figure 1.3.3 2: Molecular Anatomy at the Node of Ranvier: *Nat Rev Neurol 2011; 7(5): 250-1*
Abbreviations: ADAM 22=disintegrin and metalloproteinase domain-containing protein 22; Bral1=brain link protein 1; Caspr = contactin-associated protein; Cntn = contactin; Cx=connexin; ERM =ezrin–radixin–moesin; Glm=gliomedin; KV=voltage-gated potassium channel; MAG = myelin-associated glycoprotein; NaV=voltage-gated sodium channel; NF= neurofascin; Nr-CAM = neuronal cell adhesion molecule; PSD= postsynaptic density protein

Structure of Nodal antigen and molecular changes that occur with autoantibody mediated nodal attack
Neurofascin 186

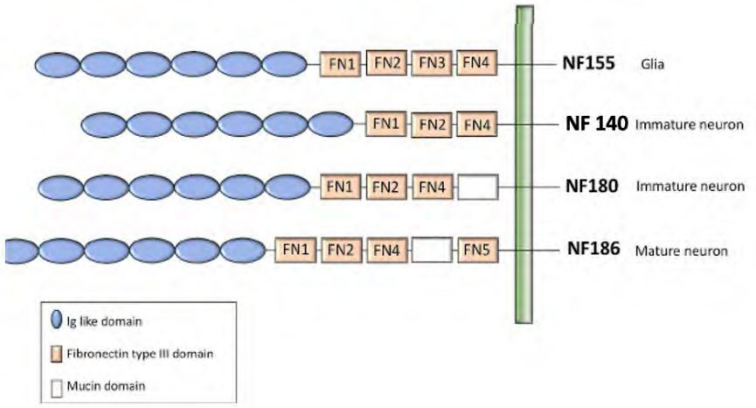


Fig 1.3.3.3: Schematic representation of Neurofascin:
Kriebel M, Wuchter J, Trinks S, Volkmer H. Neurofascin: a switch between neuronal plasticity and stability. *Adapted : Int J Biochem Cell Biol 2012; 44(5): 694-7.*

Four major NF polypeptides are expressed in nervous tissues namely NF186, NF180, NF140 and NF155 (Fig 1.3.3.3)[109]. These polypeptides consist of six immunoglobulin-like domains, up to 5 fibronectin type III (FN) domains, transmembrane domains, and a short cytoplasmic domain. NF180 and NF140 are immature neuronal proteins. In the mature state, neuronal isoform NF186 and glial isoform NF155 are predominant [109].

NF155 and NF186 are distinct in their extracellular domains; NF155 has FN3 while NF186 lacks this domain, and instead has a mucin domain between FN4 and FN5. Axonal NF186 interacts with ankyrin-G to cluster sodium channels at the nodes of Ranvier (Fig. 1.3.3.4)[110]. In the PNS, NF186 interacts with gliomedin in the matrix and in Schwann cell microvilli to promote axon-Schwann cell microvilli attachment (Figure 1.3.3.4)[108]. In the CNS, several extracellular matrix proteins may play similar roles to gliomedin[108]. Genetic ablation of NF186 results in loss of neuronal cell adhesion molecule (NrCAM), another axonal adhesion molecule that binds to ankyrin, and the Schwann cell adhesion molecule, gliomedin, leading to unclustering of sodium channels (Nav) and ankyrin-G at nodes in the CNS and PNS. This is accompanied by invasion of paranodal loops in the nodal region [108, 111]. This indicates that NF186-dependent assembly of the nodal complex acts as a molecular boundary to restrict the migration of paranodal loops into nodal areas[109].

NF186 and Nodal Organisation:

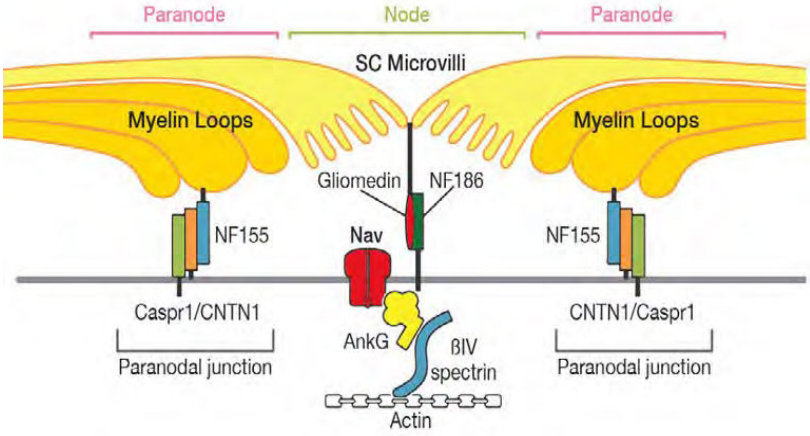


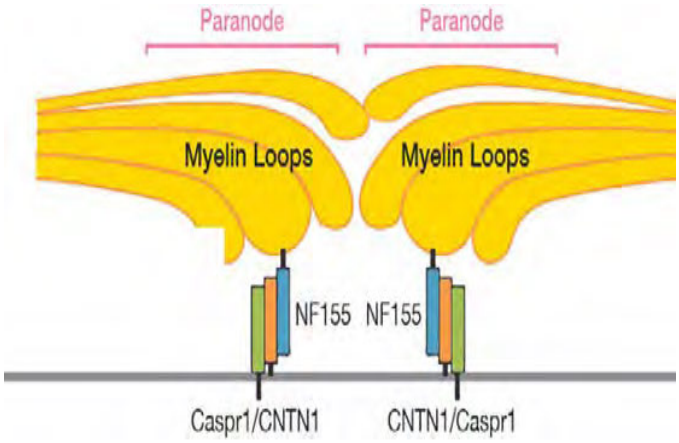
Figure 1.3.3.4: Neurofascin 186 (NF186) coordinates nodal organization by recruiting voltage-gated sodium channels (Nav) and Ankyrin G (AnkG) at node of Ranvier [105]

The dense concentration of voltage-gated sodium channels (VGSC) in the nodal region facilitates the generation of the inward current of the action potential, and the internodal compact myelin sheath reduces the internodal capacitance, enabling saltatory conduction. Nav1.2 later switches to Nav1.6 during myelination. In the PNS, 2 axoglial interactions are of importance, namely:

1. Gliomedin (GLDN)—a cell adhesion molecule—binds to neurofascin-186 (NF186) in the axolemma (Fig 1.2.3.4). NF186, recruits ankyrin G, that mediates the binding of NF186, NrCAM and VGSCs to the nodal cytoskeletal protein βIV-spectrin. NF186 null mice demonstrate disrupted axonal conduction highlights the role of NF186 in supporting the node. (Fig 1.3.3.3 and Fig 1.3.3.4)

2. Apposition of two adjacent paranodes during development, prevents the lateral diffusion of NF186, NrCAM and VGSCs from under the myelin sheath. This results in meeting of 2 adjacent internodes, and the two opposing hemi-nodes form one node.

Figure 1.3.3.5: NF186 deficiency[105]



When NF186 is deficient, Schwann cell (SC) microvilli disappear as indicated in Figure 1.2.3.5. This results in failure of Nav and AnkG to assemble and paranodal junction components are dislocated into the nodal gap. Additionally, there is decreased nodal length, with resultant complete occlusion by extensions of Schwann cell cytoplasm. This can be confirmed and seen histopathologically, fig 1.2.3.6 (B). Electrophysiological changes of conduction block, prolonged distal motor latency and conduction slowing occur because of the above, (fig 1.2.3.6 (A)) and potentially reverses with immunomodulatory treatment, as demonstrated at Day 27 as demonstrated in the figure below.

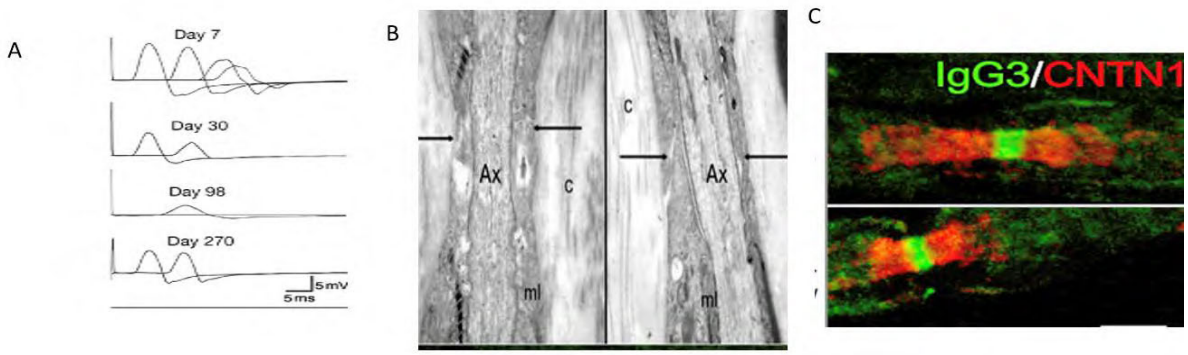


Figure 1.3.3.6 : Immunological and electrophysiological corelates in NF186 autoimmune nodopathies: adapted from Uncini et al, JPNS 2023[105].

A: Superimposed compound muscle action potentials (CMAPs) recorded from abductor pollicis brevis muscle after stimulation at wrist, elbow, axilla and Erb's point showing at serial recordings a partial conduction block (CB) progressing till to a complete CB without temporal dispersion at the clinical nadir (day 98). Coexisted reduction of distal CMAP amplitudes, increased distal motor latency and slow conduction velocity in the demyelinating range. On day 270, when patient had almost completely recovered, CB was not present any longer, distal CMAP amplitude had returned to normal, DML and CV were normal.

B: Electron microscopy, longitudinal sections, sural nerve biopsy. Left, normal control: at the node presence of several microvilli (arrows on each side of the nodal region). Myelin loops (ml) delineate the paranodal region. Bar = 500 nm Right, patient with ab with ab to NF140/186: disappearance of microvilli which are replaced by extensions of Schwann cell cytoplasm occluding the nodal gap (arrows). Ax, axon; C, collagen; MS, myelin sheath of an adjacent fibre. Bar = 200 nm.

C: Mouse sciatic nerve fibres stained with antibodies to Contactin1 (CNTN1) (red) and patient's IgG3 anti-NF186 (green) binding specifically to the node.

NF155 and NF186, which increases. Nevertheless, NF140, like NF186, results in clustering of voltage-gated sodium channels (Nav) at the developing node of Ranvier and can restore electrophysiological function independently of NF155 and NF186. This may suggest that NF140 complements the function of NF155 and NF186 in initial stages of the assembly and stabilization of the nodal complex. Further, NF140 is re-expressed in demyelinated white matter lesions of post-mortem brain tissue from human subjects with multiple sclerosis. This expands the critical role of the NF gene in the function of myelinated axons.

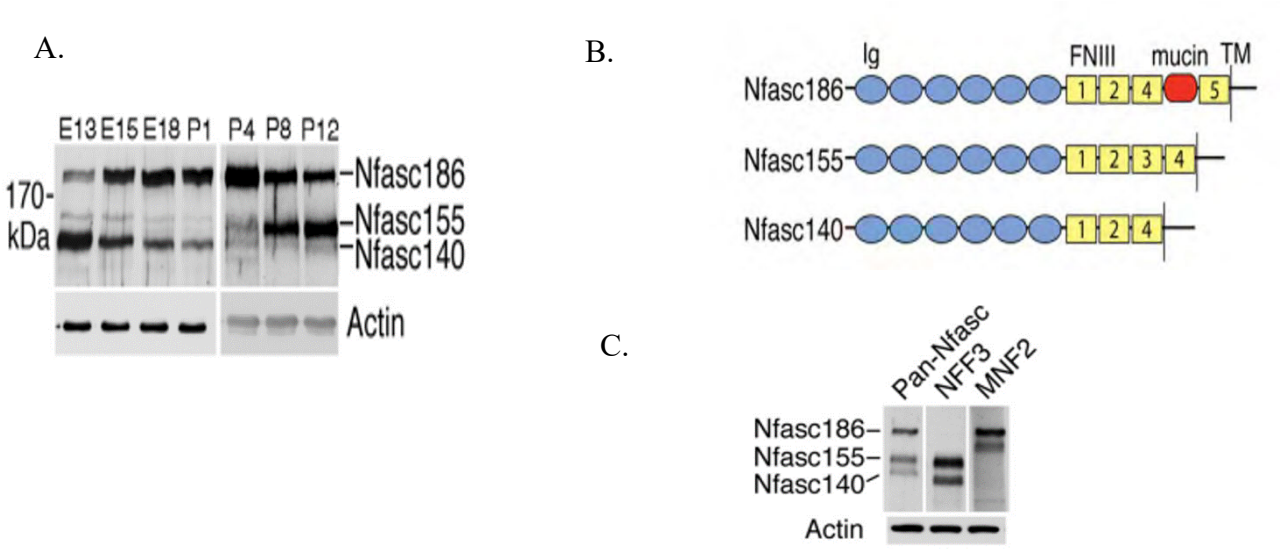


Fig 1.3.3.7 (A,B,C) : Western blotting of lysates from mouse hindbrains at different ages:
Zhang A et al. J Neuroscience. 2015 Feb 4;35(5):2246-54. doi: 10.1523/JNEUROSCI.3552-14.2015. PMID: 25653379; PMCID: PMC4315843.

A) Developmental Western blot of hind brain lysates on two separate gels using a pan neurofascin antibody (NFC) that recognizes the C terminus of the cytoplasmic domain shows abundant but declining expression of Nfasc140 relative to Nfasc186 in the embryo up until birth (E13-P1), which declines further postnatally at the onset of myelination (P4–P12) when glial Nfasc155 becomes prominent
B) The domain structure of the three neurofascin isoforms. Sequencing of the Nfasc140 RT-PCR product generated from E15 RNA showed that it lacked the mucin and FNIII5 domains characteristic of neuronal Nfasc186 or the FNIII3 domain unique to Nfasc155.
C) Western blots of hind brain lysates at P45 confirmed that Nfasc140 lacked the FNIII3 and mucin/FNIII5 domains found in Nfasc155 and Nfasc186 [112]

Western blotting of lysates from mouse hindbrains at different ages showed that a neurofascin band at 140 kDa was expressed in the embryonic mouse hind brain, whereas NF186, although also present, became more abundant at the onset of myelination when NF140 was downregulated (Fig. 1.3.3.7 (A)). Because the 140kDa band was abundant in mouse embryo , RT-PCR was performed to isolate full-length cDNA clones of NF140. These clones were sequenced, and the deduced protein domain

structure was compared with the two well-characterized neurofascin isoforms (Fig. 1.3.3.7). This confirmed that NF140 lacked the mucin and FNIII5 domains characteristic of neuronal Nfasc186, and the FNIII3 domain unique to Nfasc155 as found previously by Bennett and co-workers (Davis et al., 1993) and Zhang et al, figure 1.3.3.7 B [112].

Cross reactivity and Pan NF disease:

From the experiments above, NF155, NF186, and NF140 share some but not all subunits in their extracellular domains.[113] Patients with antibodies against epitopes involving the third fibronectin-like domain, recognise NF155 and not NF186 and NF140. However, in Pan NF positivity, patients have strong IgG reactivity against all 3 neurofascin isoforms, indicating that at least 1 target epitope is in a domain that is shared by all 3 neurofascin isoforms. Because all the domains of NF140 are also present in NF155 and NF186, a response to NF140 is sufficient to explain this response to NF155 and NF186 and hence PanNF disease described later in this section[114, 115].

Paranodes: Contactin, Caspr, NF155 and paranodal organisation:

The integrity and function, of paranodes depend on septate-like junctions, comprising (NF155) on myelin loops and heterodimers of contactin1 (CNTN1) and (Caspr) on the axolemma. Binding of NF155 antibodies induces detachment of terminal myelin loops, nodal lengthening, increased periaxonal space and displacement of Kv channels at the paranode.

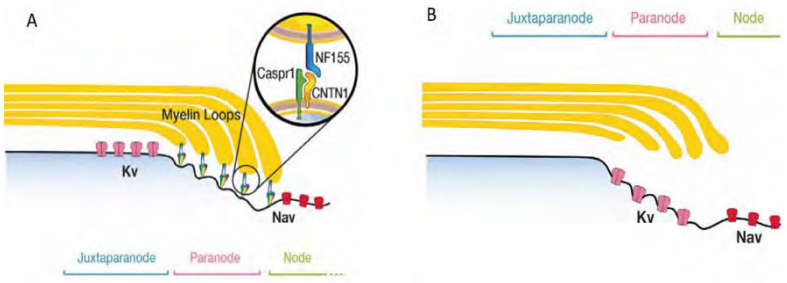


Figure 1.3.3 8: (A): Normal paranodal junction: neurofascin 155 (NF155), contactin1 (CNTN1), contactin-associated protein-1 (Caspr1). (B) Binding of NF155 antibodies induces detachment of terminal myelin loops, nodal lengthening, increased periaxonal space and dislocation of Kv channels at paranode.

Adapted from Uncini et al, JPNS 2023[105]**.**

Structure of contactin

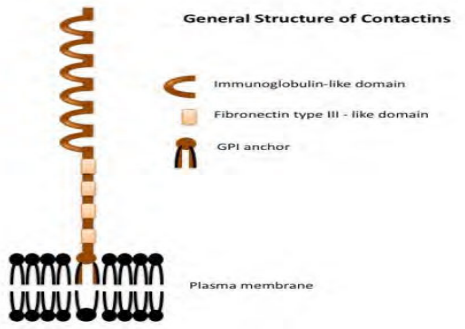


Figure 1.3.3.9: Schematic representation of Contactin1

Contactins are a group of cell adhesion molecules that are mainly expressed in the brain and play a pivotal role in the organization of axonal domains. Contactins comprise a family of six members. Their absence leads to malformed axons and impaired nerve conduction. Contactin mediated protein complex formation is critical for the organization of the axon in early central nervous system development

Contactin is indispensable for early interactions between axons and glia. It bolsters the paranodal junction formation by establishing a complex with the transmembrane protein Caspr1. In the axolemma, it interacts with glial neurofascin155 to establish the axon-glial contact with NF155. The role of contactin in paranodal junction formation was shown by knocking out the CNS CNTN1 gene in mice. As a result, paranodal junctions were disrupted due to mislocalization of the shaker-type potassium Kv1.2 channels typically delineating juxtaparanodal regions. (Fig 1.3.3.8). This suggests that contactin is needed to position Kv1.2 and thus to contribute to the paranodal outward current and thereby proper action potential repolarization during action potential conduction.

CNTN1 IgG4 antibodies are detected in a small proportion of patients with CIDP-like clinical features, who again share a relatively uniform phenotype [116]. They potentially induce their pathological effects via a comparable mechanism to that proposed for NF155 antibodies, however the key difference is that CNTN1 IgG4 has a direct blocking effect on the CNTN1/Caspr interactions with NF155.

Structure of contactin-associated protein-1 :

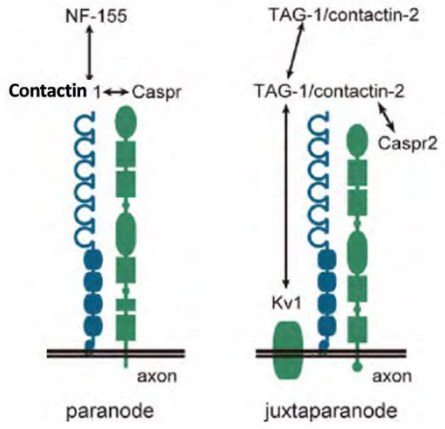


Fig 1.3.3 10: Schematic representation of Contactin-associated protein-1

The formation and stability of myelinated axons depend on members of the contactin-associated protein (Caspr) family, comprising Caspr1–5. The Caspr family are transmembrane proteins with similar structures, though each exhibits functional specificity. CaspR1 is a 190-kDa neuronal transmembrane protein encoded on chromosome 17q21 and is highly concentrated at paranodes . As a member of the neurexin superfamily, Caspr1 contains a large extramembrane domain, a single transmembrane region, and a short intracellular region.

Caspr1, is primarily located at the paranodes. It forms a complex with contactin1 and NF155, acts as a barrier between the nodes of Ranvier and internodes and indirectly involved in the propagation of action potentials and mediation of signal transport. Caspr2, functions to stabilize resting potential by forming a complex with K+ channels. The best established clinical syndromes associated with Caspr2 antibodies are Marvans syndrome,neuromyotonia and limbic encephalitis[117, 118]. Little is known regarding the precise functions of Caspr3, Caspr4, and Caspr5 proteins. Evidence suggests that Caspr3 is involved in motor control and learning. A lack of Caspr4 and Caspr5 may be a risk factor for autism spectrum disorders.

Structure of the Juxta-paranode

Antigenic targets at the juxtaparanode are also now emerging, but their associated peripheral neuropathies are less well characterised. Normal function depends on the stability of the VGKC complex, in which VGKCs colocalise with CNTN2 and Caspr2 in myelinated peripheral neurons. Pathogenic IgG antibodies have now been shown to bind associated proteins such as LGI1 and Caspr2, rather than the ion channels themselves, example LGI4 recently reported in elderly patients initially diagnosed with subacute CIDP[119].

Table 1.3.3.1: Nodal, juxta-paranodal, and paranodal antibodies associated with clinical syndromes [108]

Nodal	Paranodal	Juxta-paranodal
Neurofascin 186	Contactin1	Contactin-associated protein 2
Gliomedin	Contactin associated protein 1	Contactin 2/TAG 1
Gangliosides • GM1a/b, GD1a, GalNac-GD1a • GM1, GM1b, GD1a • GD1b and/or • other disialosyl gangliosides	Gangliosides • GT1a, GQ1b • GQ1b, GT1a	LGI4
	Neurofascin 155	

Ganglioside antibodies

Gangliosides are structurally and functionally polymorphic sialic acid containing glycosphingolipids that are widely distributed in the human body. Gangliosides are present in all mammalian cells but are enriched in peripheral neural membranes where they form 10-20% of the membrane lipid composition[120]. They are targets for autoimmunity and act as receptors for microbes, such as influenza viruses, and toxins, such as the cholera toxin. Ganglioside antibodies are important especially in acquired demyelinating immune-mediated neuropathies, like Guillain–Barré syndrome (GBS) and its variant, Miller–Fisher syndrome (MFS).

Gangliosides interact with external ligands and as a result, affect cell functions dependent on external cues [121]. Gangliosides are classified by their synthesis pathways and corresponding chemical structures. A schematic view of the gangliosides and their synthesis pathway is presented in figure1.3.3.11. Ganglioside antibodies may also be nodal, paranodal, internodal or axonal and are associated with various clinical syndromes (table 1.3.3.1 and table 1.3.3.2).

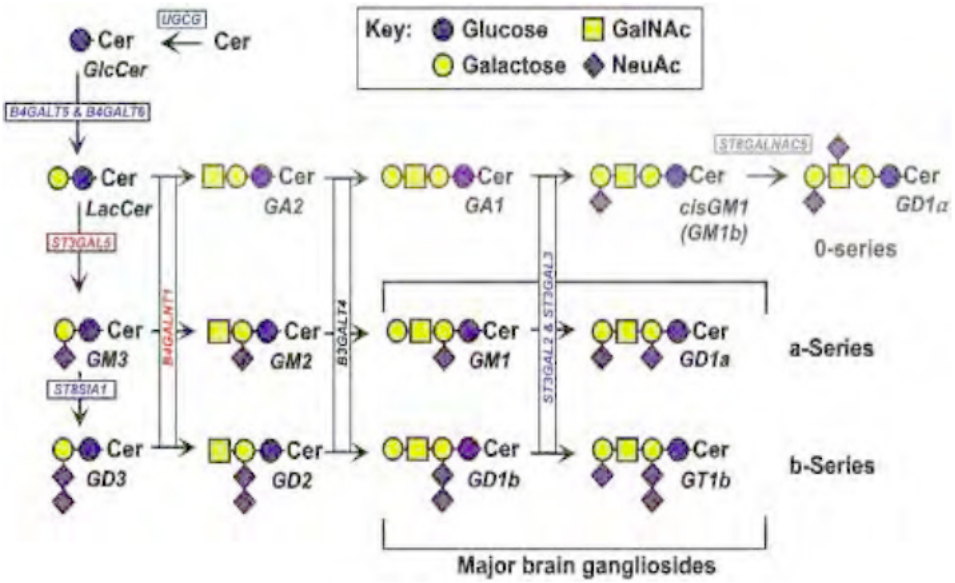


Figure 1.3.3.11. Gangliosides are composed of a sialylated oligosaccharide chain attached to a ceramide core. Autoantibodies against are found against the highlighted a and b series. In the nomenclature, M(ono), D(i), T(ri) and Q(uad) refer to the number of sialic acids.
Adapted from Li & Schnaar 2018 [122]

Table:1.3.3.2 Clinical Syndromes associated with specific gangliosides [123]

Antibody	Isotype	Clinical Syndrome	References
GM1, GD1b, asialo-GM1	IgM>IgG	Multifocal Motor Neuropathy	123, 124
SGPG, SGLPG	IgM	Chronic sensory motor demyelinating neuropathy	125
GD1b, GD2, GD3,GT1b,GQ1b, GALOP	IgM	Chronic sensory ataxic neuropathy	123, 124, 126
GQ1b, GT1a	IgG	Miller Fisher Syndrome	126
GM1, GM1b, GD1a, GalNAc-GD1a	IgG	Acute motor axonal neuropathy	123, 124
Sulphatide	IgM	Chronic sensory neuropathy	124

Clinical features of autoimmune nodopathies:

As this is an emerging topic, the clinical phenotypes and treatment responses described are not absolute as numbers are small and there is lack of widespread antibody testing across varying population groups. Given more widespread testing and IgG subtyping, the clinical presentation among different population groups may differ. Current literature is mainly from European and Asian population groups and is described according to 4 main antibodies and respective IgG subclasses. NF155 antibodies, specifically of the IgG4 subtype, are associated with a fairly homogenous phenotype, specifically being more aggressive disease , distal > proximal, motor predominant and associated with sensory ataxia, tremor, and good response to rituximab but not IVIg [127-129]. Further defining features of NF155 antibody-positive patients noted by Ogata et al are a younger age at onset, with a significantly higher CSF protein, potentially reflecting prominent spinal root involvement[130]. There are reports of NF155-positive patients also displaying optic neuritis and CNS demyelination with a good response to IVIg mainly from the East[131].

CNTN1 IgG4 antibodies are detected in a lower proportion of patients, who again share a relatively uniform clinical phenotype, characterised by older age, aggressive disease onset, motor predominance, early axonal loss and poor IVIg responsiveness and better response to escalation therapy example Rituximab[116] CNTN1 is a common target for autoantibody-mediated peripheral neuropathy and 1-2% of idiopathic membranous glomerular nephritis (MGN) cases, presenting typically with nephrotic syndrome.Fehmi et al reported, CNTN1 IgG4 antibodies, in peripheral nerves and renal glomeruli in 12 biopsy proven MGN [132]. Greater awareness of concomitant idiopathic MGM and peripheral nerve dysfunction in the setting of CNTN1 IgG4 disease should alert the clinician to earlier diagnosis and implementation of effective treatment for both entities[132].

Neuropathic pain is a prominent feature in patients with Caspr antibodies. The resolution of pain and functional recovery following rituximab treatment supports an antibody mediated process.
In addition, the observed pathological features are not those of a small fibre neuropathy, implying that the symptom of neuropathic pain may be a direct consequence of paranodal antibody binding[133].

NF186 is less well characterised. In a recent study patients presented with acute or subacute onset, asymmetric weakness or numbness, distal weakness [134]. Sensory ataxia, tremor and central nervous system demyelination were rarely observed. Nerve conduction studies revealed demyelination. Most patients were CST responsive.

More recently a novel juxtparanodal antibody, LGI4 was described by Zhang et al in an elderly cohort of four patients with a subacute motor sensory neuropathy resembling GBS[119].
Pan-NF disease is characterised by severe disease of acute onset (resembling AIDP) and high mortality and a good response to Rituximab if recognised and treated early[114]. However this has been described in a very small cohort of 8 patients, 4 of whom demised[114].
Clinical manifestation of disease severity and response to therapy may depend not only on the immunopathogenic antibody but the subclass as well. IgG4 subclass NF155 and CNTN1 antibodies are best characterised thus far, and predict for a severe chronic phenotype. IgG4 has a compact structure due to trans heavy chain CH1eCH2 domain interaction, resulting in no accessibility for complement to the CH2 domain which fixes complement. Thus, IgG4 cannot activate complement as it is unable to bind C1q. In addition, IgG4 does not internalize the target antigens [135] . Hence avoiding complement inhibitors such as Eculizumab in this category of IgG4 mediated disease is useful. NF186 IgG1 subtypes are less specific and have been reported in MND, MS, and HIV dementia.
Other subtypes include pan NF IgG 1 and IgG 3 which have been described as severe and acute disease, resembling GBS or AIDP, despite not being of the IgG4 subtype [114, 115].
Table 1.3.3.3 is a summary of the clinical features of autoimmune nodopathies and table 1. 2.3.4: is a summary of the clinical features of specific nodal-paranodal antibodies
.

Table 1.3.3.3 : Summary of the clinical features of Autoimmune Nodopathies [136]

Clinical and Electrophysiological features of a Nodal-paranodal Neuropathy	
Clinical symptoms and signs	Electrophysiological
Respiratory insufficiency	Decreased CV
Acute/Subacute	Prolonged DML
Severe nadir disability	Prolonged F waves
Predominant distal motor weakness	Conduction blocks
Severe sensory ataxia/neuropathic pain	Temporal dispersion
Tremor	Decreased CMAPS
Cranial Neuropathies	Early axonal loss
ANS Dysfunction	
Associated disorders	
Nephrotic Syndrome	
IgG paraprotein	
Lymphoproliferative disorders	
Treatment Response	
No/transient response to IVIG or PE	
Good response to Rituximab /or corticosteroids	
Radiology	Histology
Nerve Root/Plexus enhancement and/or	Axonal Degeneration
Root enlargement	Nodal widening, paranodal myelin detachment
	No overt inflammation or segmental demyelination

CV=conduction velocity, DML=distal motor latency, CMAP=compound muscle action potentials

Table 1.3.3.4: Clinical features of specific Nodal-paranodal antibodies[136]

Typical Features of specific Nodal-paranodal antibodies				
Characteristic	NF155	PanNF	CNTN1	Caspr 1 or CNTN1/Caspr complex
Neuropathy	Severe non-length dependent neuropathy			
Age	51.5	57.4	60	57.5
Male: Female	1.6:1	3.5:1	3.3:1	2.3:1
Initial AIDP diagnosis				
Ataxia				
Tremor				
Pain				
Cranial nerve palsies				
Respiratory Failure				
ANS failure				
Nerve Root abnormalities				
Associated disorders				
Nephrotic Syndrome MGN (CNTN 1) FSGN(PanNF)				
Lymphoproliferative				
CCPD				

CV=conduction velocity, DML=distal motor latency, CMAP=compound muscle action potentials

Key	Absent	<25%	25-50%	50-75%	75%-100%

1.3.4 Motor Neuron Syndrome in HIV

Epidemiology:

Motor neurone disease affects about two in every 100,000 people each year in the United Kingdom and about 5000 people are living with the disease at any given time [137, 138]. The condition is more common in males and people of European descent [139]. Limited information is available regarding the epidemiology of MND among black South Africans. Quansah et al reported that genetic differences between African and non -African subgroups and among different African populations in SA may account for the scant prevalence of MND among the black African population [140, 141]. In 1989, Cosnett et al retrospectively analysed 86 patients with MND in KZN [142]. The reported incidence of MND per 100 0000 among the different race groups were as follows; 0.88, 1.4, 2.7 among blacks, Indians and whites respectively. There was a male preponderance amongst all race groups, which accords with world experience. However, among the black population neurological manifestations occurred 2 decades earlier compared to Indians and whites. Possible explanations for the above is that black patients have a higher prevalence of polio and post-polio syndrome, HIV and HTLV1, and exposure to mechanical trauma at an earlier age, which may have played a role [142].

The more recent Western Cape study [143], reported the highest incidence in the European ancestry group (2.62; 95% CI 2.49–2.75), the lowest in the African ancestry group (0.56, 95% CI 0.0–1.23), and an age and sex adjusted incident rate in between these two in the mixed ancestry group (1.09, 95% CI 0.80–1.37). The above findings are most likely related to the complex genetic ancestry among people of mixed ancestry in the western cape with 17-29% receiving Europe proportional ancestry compared to those of African descent who have received little or no European genetic admixture. One possible explanation reported by the authors is that the frequencies of the C9orf72 expansion mutation in the European and mixed ancestry group (8–9%) were comparable to other populations of European origin, however the expansion mutation was not found amongst a small sample of African ancestry.[143] Other interesting findings is the earlier peak of the disease and less bulbar presentation among the African ancestry in this study.

It is uncertain as to whether concomitant HIV infection or HIV-infected MNS was excluded in all the above studies as HIV infection and therefore HIV-infected MNS is more common among young people of African ancestry as reported in chapter 3. However, in this subcategory they were more likely to have a bulbar onset presentation, which was not reported in the above studies.

Clinical features and spectrum of disease:

The clinical features of MND are those of progressive neurological deterioration involving the motor neurons in the cerebrum, brainstem and spinal cord. Although the clinical presentation and progression of MND varies considerably, the course is progressive, and over 70% of patients die within 5 years of presentation and 10% may survive for more than 10 years [139].

The disease phenotype is often classified by the site of onset. Sixty five percent (65%) of patients present with limb symptoms, 30% present with bulbar dysfunction and 5% have respiratory-onset disease. Initial symptoms of weight loss and isolated emotional lability have also been reported. As motor neurons are affected segmentally in patients with ALS, the initial clinical presentation depends on which part of the neuro-axis the disease affects. Symptom onset is usually asymmetrical. Neurological examination generally reveals a combination of upper motor neuron and lower motor neuron features. Extraocular and sphincter muscles are spared in patients with ALS, although subtle changes in eye movements have been reported. Sensory examination is usually normal, although 10% of patients may describe minor sensory symptoms.

Table 1.3.4.1: MND phenotypes [139]

Phenotype	Clinical features	Comments	Median survival
Amyotrophic Lateral Sclerosis	Multiple spinal segments affected with UMN and LMN signs	Most common phenotype	3-5 years
Primary Lateral Sclerosis (PLS)	UMN signs only	70% develop ALS in 3-5 years	>20yrs for those who do not progress to ALS
Progressive Muscular Atrophy (PMA)	LMN signs only	Variable evolution to ALS	Typically 8-10 years, subset survive > 20 years
Progressive Bulbar Palsy (PBP)	Speech and swallowing initially affected	Aspiration pneumonia is the usual cause of death	2-3 years
Flail Arm Syndrome	Symmetric wasting and weakness of the arms	Upper motor neuron signs in lower limbs	Variant of ALS, median survival of 5 years

PLS=Primary lateral Sclerosis, PMA=Progressive Muscular Atrophy, PBP=Progressive bulbar Palsy

Electrodiagnostic testing in MND [144]

The World Federation of Neurology (El Escorial) criteria classifies MND into mimics, co-existing and variant disorders. "MND mimics" includes diseases, which secondarily affects the motor neurons, such as spondylotic myelopathy, exogenous toxins, lymphoma, metastatic and paraneoplastic disease, monoclonal and dysimmune gammopathies, vasculitis, multifocal motor neuropathy (MMN), CIDP,

and infections(polio, herpes zoster, HIV and coxsackie viruses). Other degenerative conditions, such as Friedreich's ataxia and hereditary spastic paraparesis can also mimic a motor neuron presentation[145]. "Coexisting disorders" occur in patients who have pre-existing neurologic disease superimposed on ALS, such as diabetic radiculoplexoneuropathy or ulnar neuropathy. "Variant disorders" refer to diseases that affect another component of the nervous system in addition to the motor system. Therefore, electro-diagnostic testing, clinical progression of the disease and exclusion of other disease mimics is particularly important for identifying and distinguishing the coexisting diseases and mimic disorders from true MND. Electro-diagnostic studies also help to quantify the lower motor neuron damage making them an integral part of clinical and therapeutic trials.

Specific criteria for the diagnosis of ALS are the principles of the Revised El Escorial criteria combined with the Awaji modifications [144] Nerve conduction study and needle electromyography are the most important diagnostic testing for MND. The former is used primarily to help rule out other disorders, and the latter to establish evidence for widespread active denervation and chronic re-innervation. The above criteria have a high specificity but lower sensitivity especially for atypical ALS example bulbar onset disease. Therefore, more recently the novel Gold Coast Criteria was introduced which has higher diagnostic sensitivity especially for atypical phenotypes [146]. Unfortunately, the above was not available at the time of the study, and hence some cases may have been missed reflecting a type 2 statistical error. However, the criteria states that disease mimics must be excluded and this includes HIV. Therefore MND, meeting the EL Escorial diagnostic criteria, in the setting of HIV was re-termed MNS. Chapter 2 discusses the available literature of HIV-infected MNS in other regions of the world and chapter 10 compares HIV-infected MNS to HIV-uninfected MND in terms of clinical, electro diagnostic, pathophysiology and response to therapy.

Pathophysiology

MND is familial in 5% of cases, and shows a Mendelian pattern of inheritance.[137] The clinical phenotype of familial ALS is similar to that of the sporadic form of the disease. At least 15 genes have been associated with the various types of familial MND, and variants in these genes account for 30% of these cases[137]. In common with other neurodegenerative diseases, neuronal loss accompanied by insoluble protein inclusions are core pathological features of ALS. Our knowledge of major aggregate owes much to proteomics: the identification of TDP-43 and CSF chitinase as the major component of inclusions in over 95% of ALS cases (except those with genetic ALS due to SOD1 or FUS mutation) and 50% of FTD cases was achieved using liquid chromatography-tandem mass spectrometry (LC-MS/MS) of urea-soluble brain fractions[147]. However, sporadic MND is a complex disease, in which genetic and environmental factors combine to increase the risk of developing the condition.[138] It is speculated that several environmental factors contribute to neuronal degeneration. This includes neurotoxins such as sterol glucosides and β-methylamino-l-alanine (BMAA) which are found in cycad flour and agricultural chemicals such as pyrethroid insecticides and formaldehyde. Excessive physical activity, repeated head

injury and exposure to viruses have also been implicated in MND [137, 138, 148] .The possibility that MND may be caused by a virus is one that has been entertained for many years. Most of the speculation originates from the longstanding observation that both MND and poliomyelitis cause selective destruction of the anterior horn cells in the spinal cord. Both diseases affect motor neurones in the brain stem and cerebral cortex, and years after an attack of acute paralytic poliomyelitis, a minority of individuals develop a syndrome of progressive muscular weakness and wasting (Post-Polio Syndrome) which has similarities to MND. Viruses implicated in MND include retroviruses such as HIV, HTLV1, endogenous retroviruses (HERV-K) and enterovirus such as polio, coxsackie, and echo virus[149]. Epigenetic modifications provide a plausible link between the environment and alterations in gene expression that might lead to disease phenotypes[138, 150].

HERV K and MND or MNS

Human endogenous retroviruses (HERVs) constitute approximately 6% of the human genome and are referred to as "junk DNA". These retroviral sequences are probably remnants of infections that occurred millions of years ago, resulting in the integration of provirus genomes into the DNA of germline cells[151]. HERV proviruses may have accumulated nonsense mutations that have rendered them defective. However, endogenous retroviral sequences may get expressed during select pathological circumstances such as immunosuppression [152]. Involvement of retroviruses in the pathophysiology of ALS have been suspected due to the consistent finding of reverse transcriptase viral particles in serum of patients with ALS at levels comparable to those of HIV-infected patients[153-156]. HIV-infected patients may develop MNS which responds to treatment with ART as published in the current attached article for review and other articles[154, 157, 158]. This suggests either HIV itself or HERV-K maybe implicated in the pathogenesis of HIV associated MNS.

Bowen et al reported that MNS is a rare manifestation of HIV infection. In a prior literature review, 29 cases had been identified [159]. In a study of 1,700 patients with HIV infection, the prevalence of HIV-associated ALS was estimated at 3.5 cases/1,000 patients in the pre-ART era which is greater than the prevalence of ALS in the general population (6 cases per 100,000) .[137] The clinical presentation differs in HIV-infected MNS (see published manuscript 2).The mechanism by which HIV infection results in MNS is unclear. HIV infects infiltrating macrophages, microglia, and astrocytes and not motor neurons. Hence the effect is likely indirect or, possibly immune mediated. It has been previously suspected that patients with sporadic ALS activate HERV-K in cortical neurons and anterior horn cells.[154] Expression of HERV-K or its envelope protein in neurons in cell culture experiments causes degeneration of motor neurons producing a phenotype that is indistinguishable from ALS [160-162]. A postulate is that HIV-tat protein released extracellularly from HIV-infected cells is taken up by uninfected cells and activates HERV-K [159]. It is therefore a plausible hypothesis that controlling HIV infection within the CNS would indirectly control HERV-K activation in neurons and result in clinical improvement. In 2 patients

reported by Bowen et al in whom HERV-K levels were measured in the plasma, high levels of activation were detected at the onset of neurologic symptoms, and in one patient in whom HERV-K was measured repeatedly, the levels gradually fell and became undetectable following ART therapy. This paralleled clinical recovery. However, it remains unknown whether ART can directly control HERV-K expression. HIV associated MNS, although rare, may be a treatable complication of HIV infection especially if there is optimization of ART CNS penetration. Monitoring of HERV-K levels in the CSF of HIV-infected patients with MNS may be necessary in a future prospective study since optimal control of HIV alone may not be sufficient for controlling HERV-K replication if the association between HERV-K and MND is true.

Treatment and management of MND

Currently there is no curative therapy available for MND and no treatment will significantly alter disease progression. Treatment remains largely supportive[163]. Previously, the only medication licensed for treatment of MND was Riluzole which is a synthetic benzothiazole with glutamine antagonist activity [164]. A Cochrane review that pooled data from 3 clinical trials, shows that the drug may extend life by 2-3 months and increases the chance of an additional year of survival by only 9%. Recently the randomized, double-blind, multicentre, placebo-controlled CENTAUR trial demonstrated the safety and efficacy of sodium phenylbutyrate-taurursodiol (PB-TURSO) in persons with ALS (PALS), leading to its conditional approval in Canada in June 2022 and full approval in the USA in September 2022.

Various clinical trial drugs are being tested in new studies which include Tofersen (gene silencing therapy in SOD 1 mutations), low dose IL2 therapy (MIROCALS Trial), Edaravone (ADORE Trial: anti-oxidant therapy) , Memantine and trazadone (MND-SMART Trial). Results will be available once trials are completed.

Whether the above will be effective in HIV-associated MNS is uncertain. In patients with a HIV-associated motor neurone syndrome, treating the viral infection with ARVs reverses the clinical scenario as demonstrated in the attached article, arguing for a viral or immune aetiology[163].

1.3.5 : HIV-infected MG:

MG is an autoimmune disorder caused by antibodies targeting the neuromuscular junction. These antibodies bind to the postsynaptic end plate resulting in destruction of post synaptic protein, impaired signal transduction and muscle weakness. The weakness maybe focal or generalised and commonly affects ocular-bulbar and proximal muscles. Respiratory muscles are less commonly affected. In most populations, the prevalence of MG is 150–300 per 1,000,000 individuals, with an annual incidence of more than 10 in 1,000,000 people. [165]

The most common antibodies detected in MG are antibodies against acetylcholine receptors (AChR) which account for 85% of antibodies detected. The AChR antibodies belong to the IgG1 and IgG3 subclasses, and can therefore activate complement resulting in AChR loss and destruction of the architecture of the post synaptic membrane. Additionally they are bivalent and can cross-link receptors leading to the endocytosis and destruction. The most widely used method for this antibody detection is radioimmunoprecipitation assay.

MuSK antibodies occur in 6% of patients with MG, and they are primarily of the IgG4 subclass. They do not activate complement and are functionally monovalent. Their pathogenicity stems from inhibition of interactions between MuSK, collagen Q or LRP4, thereby reducing AChR clustering. Heckmann et al described a high percentage of MuSK positive MG patients of African ancestry, however the HIV status was not reported [166]. Clinical features of MuSK MG include acute onset and rapidly progressive bulbo-respiratory failure. Most of the patients reported thus far respond well to maintenance immunotherapy. MuSK is also detected via radioimmunoprecipitation and the titre corelates with disease severity[167].

Approximately 10% of patients are AChR and MuSK antibody negative. Some of these patients have LRP4 antibodies which are IgG1 subclass and hence activate complement. They are also responsible for AChR clustering. Additional, less common antibodies of interest are directed against agrin, titin, KV1.4, ryanodine receptors, collagen Q, and cortactin and striational antibodies[99, 167] Their pathogenicity, specificity for MG, diagnostic and prognostic value are less well characterised[167].

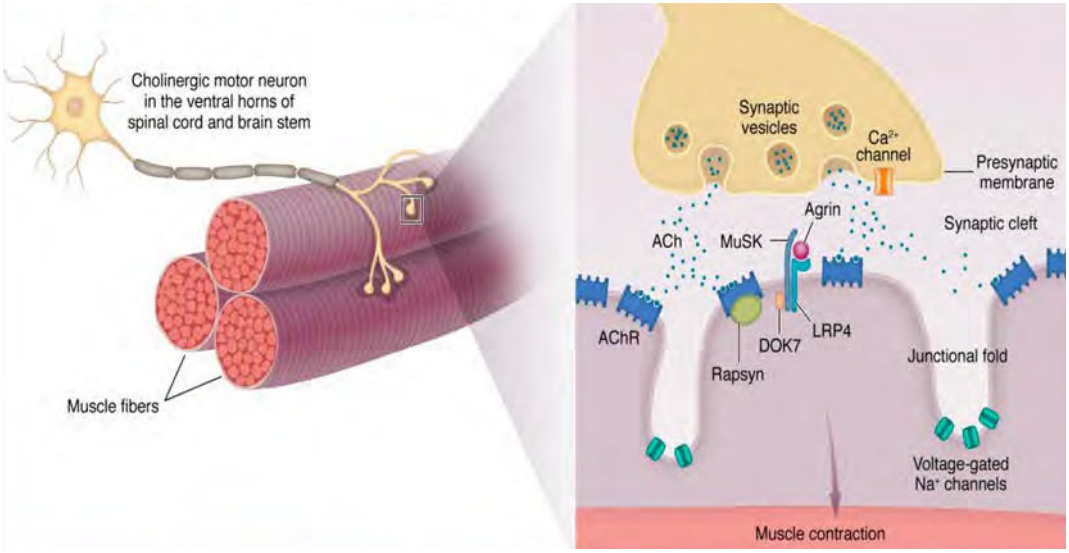


Figure 1.3.5.1: Neuromuscular Junction, Structural mechanisms of the agrin-LRP4-MuSK signalling pathway in neuromuscular junction differentiation:
Adapted:Henry J. Kaminski, 2024, Journal of Clinical Investigations

Treatment of the above condition should be tailored to the individual patient and guided by the MG severity and IgG subclasses. Treatment includes symptomatic drug therapy, immunosuppressive drug therapy, IVIG, PLEX , thymectomy and supportive therapy [168]. Thus far there is limited literature regarding immunosuppressive therapy in HIV-associated MG or the role of thymectomy in HIV-infected MG.

The above background knowledge of HIV immunology and the various neuromuscular disorders, enabled the broad aims of this project to emerge which included:

1. A scoping review of the available published worldwide literature (Chapter 2)
2. Comparative studies of the clinical, laboratory and treatment outcomes in the respective subcategories (Chapter 3)
3. To test for known nodal-paranodal antibodies in HIV-infected immune mediated radiculoneuropathies and in CCPD using live cell based assays (CBA, Chapter 3)
4. To screen for novel antibodies and to establish pathogenicity of the antibodies using myelin co-culture screens (Chapter 3)
5. To identify novel antibodies using immunoprecipitation followed by Mass Spectrometry (Chapter 3)

Chapter 2:

Scoping review of chronic immune mediated HIV-associated neuromuscular syndromes

Literature regarding neuromuscular disease in HIV is limited. A scoping review of the available worldwide literature with respect to HIV associated CIDP, MG, MNS, PM LSP and autoimmune nodopathies was undertaken. This enabled us to systematically identify and synthesize an existing and emerging body of literature on the above topic with respect to clinical and demographic features of the diseases, working definitions, treatment protocols, conceptual boundaries and knowledge gaps.

Methods

The study was approved by the UKZN Biomedical Research Ethics Committee. A PRISMA-ScR format was used by creating a structured format for identifying the research question and analysing the literature. A comprehensive set of databases and websites were investigated which included PUBMED, WOS, EBSCOHOST, SCOPUS.

Research question:

The research questions for this scoping review are as follows:

1. What is the incidence of HIV-associated neuromuscular syndromes namely; CIDP, MNS, MG, PM LSP, nodopathies in other parts of the world?
2. What are the clinical, demographic and laboratory differences between HIV-infected and HIV-uninfected patients?
3. What treatment was administered in the above conditions in other parts of the world, especially in those who are immunocompromised or have clinical AIDS?
4. What are the treatment outcomes in terms of clinical response to therapy?
5. What are the documented adverse effects of therapy especially in the immunocompromised category?
6. What are the drug-drug interactions especially between ARVs and immunotherapy?.
7. Can we define pathophysiology of the disease

Research objectives:

To review the available current literature for the above diseases especially with respect to 1) epidemiology 2) response to therapy (immunomodulatory and ARVs) as well as 3) safety and efficacy of therapy.

Eligibility criteria, data sources and search method:

The eligibility criteria were published articles which included case reports and case series, abstracts from congresses, poster presentations and chapters published in textbooks. In our **first level** screening, all eligible literature regardless of language were assessed. Only human studies and English language studies were included, unless an available English translated version was available. No limits were set for the published articles in terms of regions of study, or year of publication. Animal studies, and reports from social media platforms were excluded.

We searched for evidence using electronic databases which included PubMed, EBSCOhost, Scopus, and World of science. The following keywords were used singularly and or in combination HIV, Human immunodeficiency syndrome, AIDS, acquired immunodeficiency syndrome, CIDP, chronic inflammatory demyelinating polyneuropathy, radiculopathy, ventral root, pure motor lumbosacral polyradiculopathy, motor neuron disease, motor neuron syndrome, ALS, amyotrophic lateral sclerosis, myasthenia gravis, myopathy, myositis, polymyositis, dermatomyositis, inclusion body myositis, autoimmune nodopathies, pan-neurofascin, NF155, NF186, contactin, Caspr nodal-paranodal-juxtaparanodal.

In our **second level** of screening, abstracts and/or full text articles that were in English were included. Articles and abstracts that were not in English or were not translated to English, review articles, HIV-uninfected patients, neuromuscular disease related to drugs/toxins, infections example TB, and malignancy were excluded.

Table 2. 1: Inclusion and Exclusion criteria

	Inclusion criteria	**Rationale**
1	Study type	Articles published to date
		Any geographic location
		English language translation or abstract in English
2	Publication type	Published articles, case series, case reports, and abstracts from conferences, poster presentations.
		Only Human studies
3	Context	Literature discussing HIV associated CIDP, MND/S/ALS, myositis, PM LSP and MG
	Exclusion criteria	
1	Study type	Other non-English translation of the article
2	Publication type	review articles
		Social media reports
		Non-human studies
3	Context	Studies discussing immune mediated neuromuscular disease in HIV-uninfected patients, drugs/toxins or other infections or malignancies causing neuromuscular disease in HIV example drug induced myopathies, neuropathies in HIV, polyradiculopathies due to lymphoma

Fig 2.1: Flow chart of stages of article selection

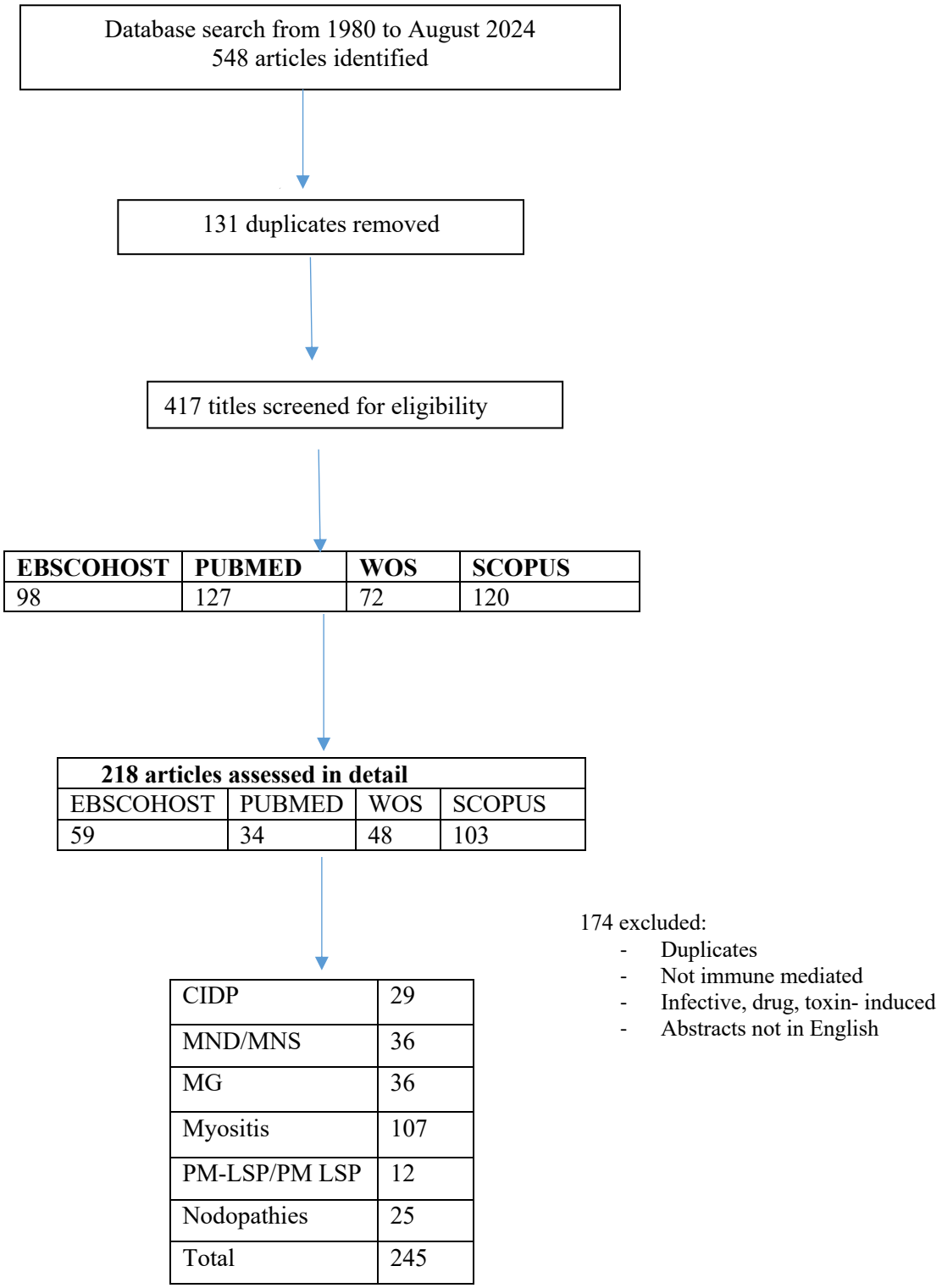


Table 2.2: Immune mediated neuromuscular disorders identified in each Database and assessed in detail

	EBSCOHOST (51)	PUBMED (27)	WOS (44)	SCOPUS (96)	Total articles assessed (245)	articles excluded	Reasons for exclusion	Articles included in the study
CIDP	10	6	8	5	29	17	11=review articles 4 = no cases of CIDP documented 2= Spanish/Japanese	12
Nodopathies	8	6	4	7	25	24	3= Review 21=No HIV infected cases reported	1
MND/ALS/MNS	11	7	11	11	36	9	5=review articles 4=Letter to editor (Cases already included) 2= patient was HIV-uninfected 2= HTLV1 infection	27
MG	7	4	13	12	36	8	7=review articles 2=No cases of HIV-infected patients with MG	28
PM LSP	4	3	2	3	12	11	3=due to infective aetiology 4=AIDP 3=sensory motor CIDP/lymphoma	1
Myositis/myopathy	21	8	11	65	107	19	12=Infection 10= drug induced 5 = review articles 2= German	88

Table 2.3: Spectrum of Muscle disease described in HIV-infected patients

	Number of articles/case reports	Number of cases	Treatment	Response to therapy
Inflammatory Myopathies			Prednisone, ART,IVIG	Good
PM	26	139		
DM	12	13		
IBM	10	42		
Necrotizing	3	12		
Non-specific myositis	18	72		
Rhabomyolysis	2	4	Self-limiting	
AZT induced mitochondrial cytopathy	12	86	withdrawal of drug	
Nemaline Rod	4	4	Nil	
HIV wasting syndrome	2	8	Nil	
Infective	15	42	Antibiotics, antiparasitic	Good

The spectrum of muscle disease in the context of HIV is broad. The above topic will be dealt with in a future study, focusing on the clinical features, histological changes and response to therapy of HIV-infected patients with muscle disease. The available literature, regarding CIDP, MNS, MG, and PM LSP are listed in the following tables:

Table 2.4: Charting the data of CIDP patients with HIV

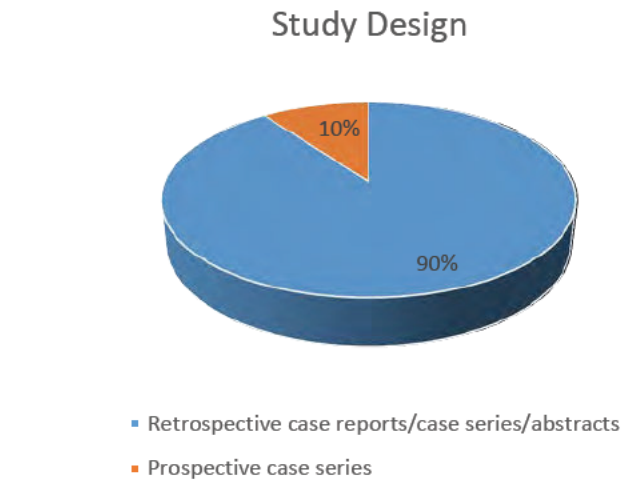
	Authors and year of Publication	Title	Journal	Authors location	Research Design	Number of cases	CD4 count	Treatment	Adverse Events	Outcome
1.	Difini,A *et al.*[169] *1987*	Neuromuscular manifestations of HIV-infections-electrophysiological and clinical features	Clinical Neurophysiology	USA, Miami	*Retrospective case series, Abstract, Congress*	8	ND	nil	ND	ND
2.	D Kiprov et al [170] 1988	Antibody: associated peripheral neuropathy, successful treatment with plasmapheresis	Journal of clinical apheresis	USA	Retrospective case series	4	ND	PE	ND	4 improved
3.	Gibbels E, et al [171] 1988	HIV associated CIDP with onion bulbs in the sural nerve biopsy, clinical pathological study	Acta Neuropathologica	Germany	Case report	1	normal	ND	ND	ND
4.	Cruz Martinez, J et al [172] 1990	CIDP as a first manifestation of HIV	EMG and clinical neurophysiology	USA	Retrospective case series	3	ND	Prednisone	Nil	2 patients improved 1 deteriorated
5.	F Ghika-Schmid et al[173] 1994	Range of neuromuscular involvement in 47 patients with HIV	French Med journal	France	*Retrospective case series*	4	ND	*Nil (Spontaneous improvement)*	*ND*	*improved*

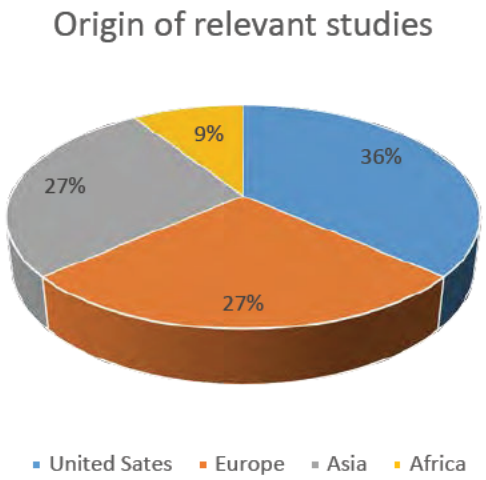
USA=United States of America, PE=Plasma Exchange, ND=Not documented

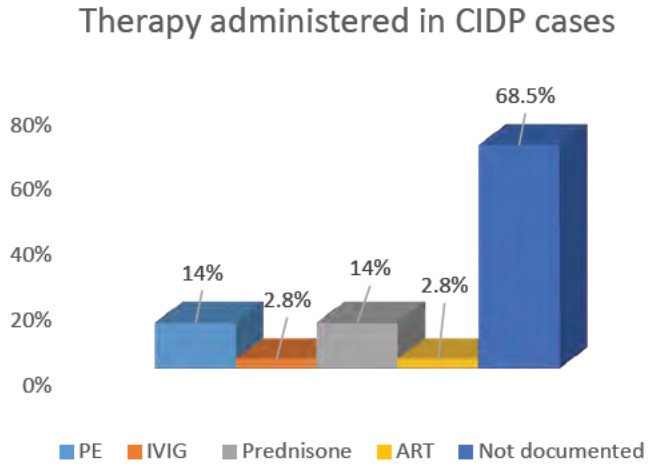
	Authors and year of Publication	Title	Journal	Author's location	Research design	Number of cases	CD4 count Cells/mm^3	Treatment	Adverse Events	Outcome
11.	Listyawan, Yudiyanta T et al [174] 2021	CIDP in HIV infected patient responding to plasmapheresis	Clinical Neurophysiology	Indonesia	Case Report	1	ND	PE	ND	Improved
6.	Rajabally Y, et al [175] 2000	CIDP caused by HIV infection in a patient with asymptomatic CMT 1a	Journal of PNS:	France	Case report	1	347	IV steroids	ND	improved
7.	Brew B. *et al.*[176] *2005*	HIV-related neuromuscular disease	Journal of neurological science	USA	Abstract of the 13th world neurology congress	1	ND	ND	ND	ND
8.	H.Y Jo, et al [177] 2009	CIDP associated with HIV infection	Clinical neurophysiology	Asian	Retrospective case report	1	ND	Prednisone & IVIG	ND	Improved
9.	Kume K et al 2013[178]	Successful treatment of HIV associated CIDP by early initiation of antiretroviral therapy	clinical neurology	Japan	Retrospective case report	1	466	ART	Not documented	Improved
10.	A Mochan, et al[179] 2016	CIDP in an HIV endemic population, A prospective case series from Johannesburg SA	Journal of neurological sciences:	South Africa	Prospective case series	10	87-747 Mean 364	ND	N/A	N/A
12	Cheng et al[180] 2022	IRIS presenting as CIDP	Medicine (Kaunas)	China	Case Report	1	109	PE and IVIG + ART	ND	Improved

USA=United States of America, PE=Plasma Exchange, ND=Not documented, N/A =not available

Figure 2.2 Summary of CIDP cases:







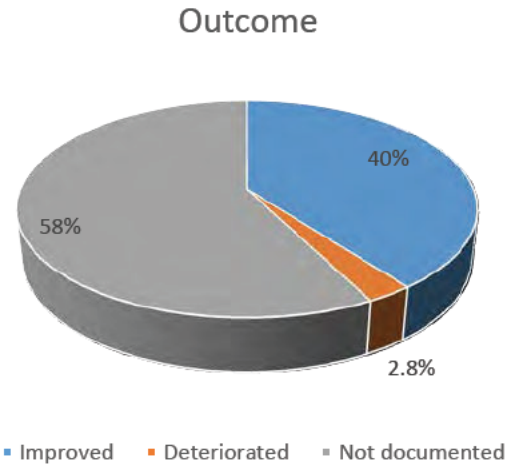


Table 2.5: Charting the data of MNS patients with HIV

	Authors	Title	Journal	Location	Research Design	Number of cases	CD4 count	Treatment	Adverse Events	Outcome
1.	Verma A, et al [181] 1990	HIV related neuromuscular syndrome simulating motor neuron disease	Neurology	USA	Case report	1	ND	ART	ND	Demised (Opportunistic infection)
2.	Gomez, CM et al [182] 1997	MND in HIV	Rev Neurology	Spain	Case report	1	ND	ND	ND	ND
3.	Galasso G et al [183] 1998	MND and HIV infection in a 30-year-old HIV positive heroin abuser, a causal relationship	Clinical neuropathology	Italy	Case report	1	340	Nil	Nil	demised
4.	Sastre-Garriga J, et al [184] 2000	LMN Disease in an HIV infected woman	Journal of neurology	Spain	Case report	1	540, 1000	NIL	ND	Demised
5.	McGowan, DJ, et al [185] 2001	An ALS like syndrome with new HIV infection and complete response to ARVs	neurology	USA	Case report	1	44	ART	ND	improved
6.	Moulinger A, et al[186] 2001	Reversible ALS like disorder in HIV infection	Neurology:	USA	Retrospective Case series	6	Mean 82	ART	ND	improved

USA=United States of America, ART=Antiretroviral Therapy, ND=Not documented

	Authors	Title	Journal	Location	Research Design	Number of cases	CD4 counts	Treatment	Adverse Events	Outcome
7.	Von Giessen, H J, et al [187] 2002	Reversible ALS-like disorder in HIV.	Neurology	USA	Case report	1	447	ART	ND	Complete recovery
8.	Zoccolella, et al [188] 2002	A case of concomitant ALS and HIV	European journal of neurology	Italy	Case report	1	360	NIL	ND	Demised
9.	D Pearl et al [189] 2003	LMN Syndrome and HIV	Sexually transmitted infections	UK	Case report	1	280	NIL	ND	Demised
10.	Calza, L et al [190] 2004	Transient reversal of HIV-associated MND following introduction of HAART	Journal of chemotherapy	Italy	Case report	1	636	Riuzole, ART	ND	Improved transiently
11.	Berger,JR et al [191] 2005	Brachial Amyotrophic Diplegia in a patient with HIV	Archives of neurology	USA	Case report	1	244	ART	IVIG	Deteriorated
12.	Alves L, et al [192] 2006	MND and HIV, 3 new cases	Neurology	Germany	Retrospective case report	3	Mean 42	ART	ND	ND

USA=United States of America,UK=United Kingdom, ART=Antiretroviral Therapy, IVIG= Intravenous Immunoglobulin, ND=Not documented

	Authors	Title	Journal	Location	Research Design	Number of cases	CD4 count cells/mm3	Treatment	Adverse Events	Outcome
13.	Ariatti, G A, et al [193] 2006	Progressive MND in 2 patients with HIV-infection, a causal relationship	Journal of PNS	Italy	Abstract	2	250,340	ND	ND	ND
14.	Verma A, et al [194] 2006	Primary lateral sclerosis with HIV infection: report of 2 cases and review of HIV-associated MND	Neuromuscular disorders	India	Case report	2	1	ART	ND	Improved
15.	Henning F, et al [195] 2008	Bibrachial amyotrophic diplegia associated with HIV	JNNP	South Africa	Case report	1	382	ART	ND	Deteriorated
16.	Nalini A, et al [196] 2009	Flail arm-like syndrome associated with HIV-1 infection	Annals of Indian Academy of neurology	India	Case report	1	142	ART	ND	stabilised
17.	Kulkantrakorn K, et al[197] 2010	ALS-like presentation in HIV positive patient	Clinical Neurophysiology	Thailand	Case report	1	98	ND	ND	ND
18.	V Almeida, et al [16] 2010	Pseudobulbar syndrome in 2 patients with HIV	ALS	Portugal	Retrospective report	1	473	ART	ND	Deteriorated

ART=Antiretroviral Therapy, ND=Not documented

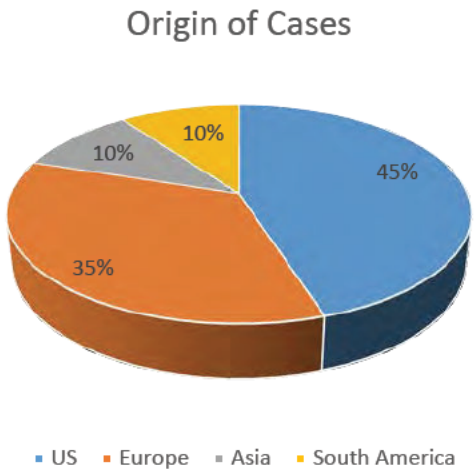
	Authors	Title	Journal	Location	Research Design	Number of cases	CD4 count	Treatment	Adverse Events	Outcome
19.	Cachia D, et al [198] 2012	Brachial amyotrophic diplegia in the setting of HIV	BMJ	USA	Case report	1	377	ART Riuzole	ND	Demised
19.	Orsini,M et al [199] 2012	MND and acquired axonal neuropathy association in HIV infection: case report and update	Current HIV Res	Brazil	Case report	1	300	ART	ND	Deteriorated
20.	Anand KS, et al [200] 2014	ALS-like presentation in HIV positive patient	Journal of the international association of AIDS CARE	India	Case report	1	98	ART	ND	ND
22.	Bowen LN, et al [159] 2016	HIV-associated MND: HERV K activation and response to ART	Neurology	USA	Case series	5	402, 4, 889, 446, 9	ART	ND	3 improved 2: stabilised
23.	Lorenzoni,P, et al[201] 2017	MND in patients with HIV infection	Clinical neurology and neurosurgery	Brazil	Case report	2	250,341	ART	ND	1 demised, 1 improved

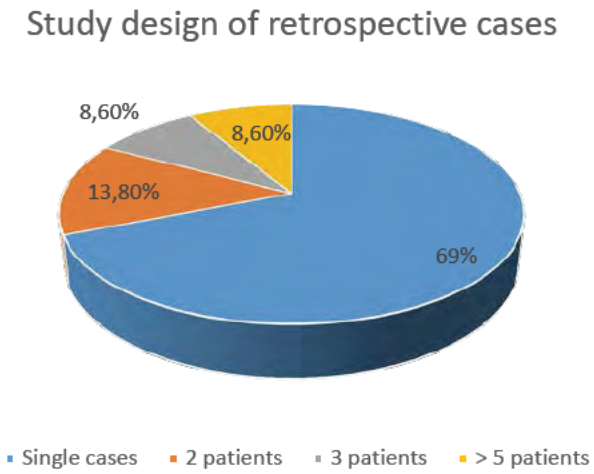
USA=United States of America, ART=Antiretroviral Therapy, ND=Not documented

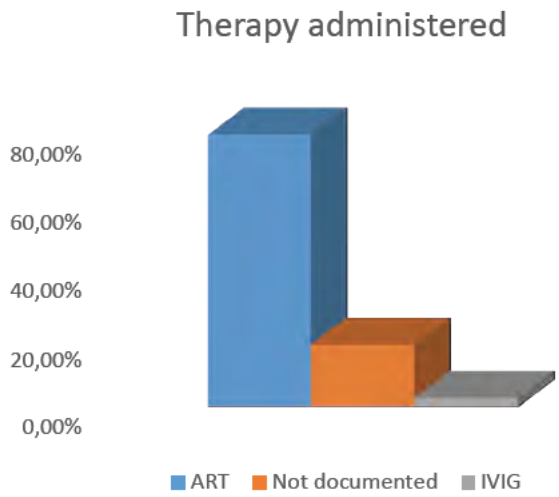
	Authors	Title	Journal	Location	Research Design	Number of cases	CD4 count	Treatment	Adverse Events	Outcome
24.	Suici-VI et al[202] 2019	HIV infection mimicking ALS, 3 year follow up	Journal of neurology	Romania	Case report	1	36	ART	Nil	improved
25.	Quevedo-Ramiez, AI et al [203] 2020	ALS after combined ARV treatment	ID Cases	Peru	Case report	1	800	ART	Nil	demised
26.	Satin ZA,et al [32] 2021	ALS like d/o in 3 HIV positive patients	Neurology	USA	Case Series	3	42-150, normal counts	ART	ND	3 improved
27	Sodeifian, F [204] 2022	Juvenile ALS in a HIV	Neurology Letters	Iran	Case Report	1	290	ART	ND	Improved

USA=United States of America, ART=Antiretroviral Therapy, ND=Not documented

Fig 2.3 Summary of the literature describing MNS in HIV







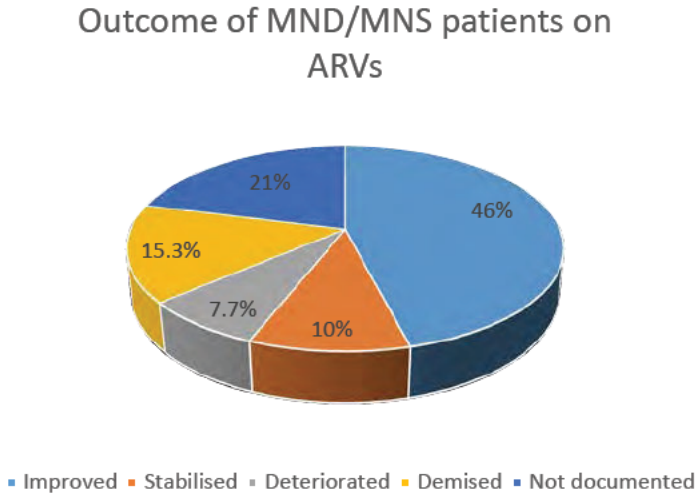


Table 2.6: Charting the data of MG patients with HIV

	Authors	Title	Journal	Location	Research Design	Number of cases	Antibodies	Treatment	Adverse Events	CD4 count	Outcome	Thymectomy CT Thymus
1.	Nath A, et al [205] 1990	Immune studies in HIV with MG, a case report	Neurology	USA	Case report	1	AChR Ab^{+}	Mestinon ART	ND	371	improved	ND CT Thymus (N)
2.	Martini L, et al [206] 1991	AIDP and MG : an exceptional associated	Rev Neurogique	France	Case report	1	AChR AB^{+}	Not documented	ND	1067	ND	ND CT Thymus (N)
3.	Vittecoq D, et al [207] 1992	Recovery from MG in a patient infected with HIV	Clinical infectious diseases	France	Case report	1	Mestinon ART	AChR AB-	ND	960	improved	ND
4.	Wullenweber et al [208] 1993	Myasthenia Gravis, AIDS and neurones	Nervenarzt	German	Case report	1	Mestinon ART	AChR AB^{+}	ND	ND	improved	ND
5.	Tiab M et al [209] 1993	Occurrence of MG during HIV infection, 2 cases	Annals of medicine	France	Case report	2	Article in French	Article in French	Article in French	Article in French	Article in French	Article in French
6.	Authier, F, et al [210] 1995	Transient MG during HIV infection	Muscle and nerve	USA	Case Report	1	AChR AB-	Mestinon ART	ND	340	improved	ND CT Thymus (N)

USA=United States of America, ART=Antiretroviral Therapy, AChR AB= Acetylcholine receptor antibody, ND=Not documented

	Authors	Title	Journal	Location	Study Design	Number of cases	Antibodies	Treatment	Adverse Events	CD4 count	Outcome	Thymectomy CT Thymus
7.	Strong J et al [211] 1998	Seronegative MG and HIV infection	Canadian journal of neurological sciences	US	Case report	1	AChR AB-	IVIG , Mestinon ART	NIL	250	improved	ND CT Thymus (N)
8.	Sadaat et al 1998	Ritonavir associated Myasthenia Gravis	Muscle and nerve	US	Case Report	1	AChR AB-	Mestinon ART	NIL	290	improved	ND CT Chest (N)
9.	Verma A, et al [212] 1995	MG associated with dual infection of HIV and HTLV1	Muscle and nerve	US	Case report	1	AChR AB- Antistriatal AB–	Mestinon ART	ND	871	improved	ND CT Thymus (N)
10.	Maradona JA, et al [213] 1995	MG and SLE in associated with HIV virus infection	Clinical infectious diseases	Spain	Case report	1	AChR AB^{+}	Prednisone (antifungal cover) ART	NIL	369	Improved	ND CT Thymus (N)
11.	Mural H, et al[214] 2001	MG associated with HIV infection	Japan	Japan	Case report	1	AChR AB-	Article in Japanese, Abstract in English	Article in Japanese, Abstract in English	Article in Japanese, Abstract in English	Article in Japanese, Abstract in English	Article in Japanese, Abstract in English
12.	Chiesa, et al [215] 2003	Efavirenz containing ART in an HIV-infected patient with MG	AIDS	Brazil	Case report	1	AChR AB^{+}	ART	ND	54	Cured	ND CT Thymus (N)

USA=United States of America, ART=Antiretroviral Therapy, AChR AB= Acetylcholine receptor antibody, ND=Not documented

	Authors	Title	Journal	Location	Study Design	Number of cases	Antibodies	Treatment	Adverse Events	CD4 count	Outcome	Thymectomy/ CT Thymus
13.	Patel VB, et al [216] 2004	Possible MG and LEMS in the same patient: Case report and review of the literature	African Journal of neurological sciences	SA	Case report	1	AChR Ab^{+}	Mestinon ART	NIL	854	Mildly improved	ND CT Thymus (N)
14.	Gorthi, S P, et al [217] 2005	HIV infection with MG	Journal of the association of physicians of India	India	Case report	1	AChR AB^{+}	Mestinon ART	Not documented	Not documented	improved	ND CT Thymus (N)
15.	Hayat G et al [218] 2007	Co-occurrence of MG and HIV infection with normal CD4 counts	Muscle and Nerve	USA	Case report	1	AChR AB-	Mestinon, ARVs	NIL	683	improved	ND CT Thymus (N)
16.	Kurokawa T, et al [219] 2008	Anti- MUSK antibody positive MG with HIV infection successfully treated with Cyclosporin	Clinical neurology and neurosurgery	Japan	Case report	1	AChR AB-	steroids, cyclosporine, Mestinon, ART	NIL	Not documented	Improved with cyclosporine, worse with ARVs	ND CT Thymus (N)
17.	Knopf L, et al [220] 2010	HIV and MG, case report and review of the literature	Journal of clinical neuromuscular disease	USA	Case report	1	AChR AB+	Mestinon Prednisone AZA	Nil	423	Improved	Thymectomy (Thymic hyperplasia)

USA=United States of America, ART=Antiretroviral Therapy, AChR AB= Acetylcholine receptor antibody, ND=Not documented

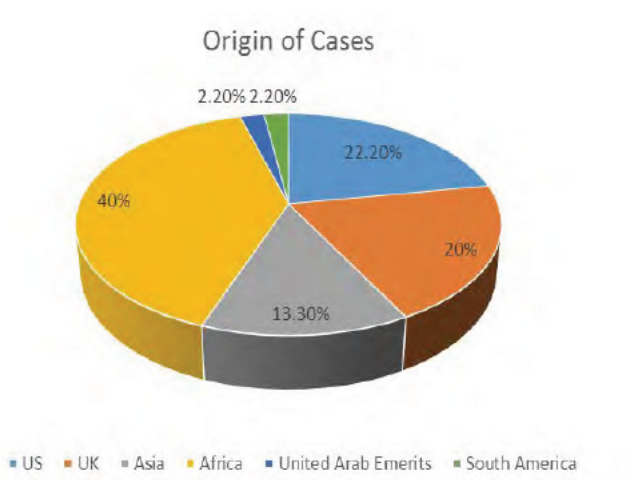
	Authors	Title	Journal	Location	Research Design	Number of cases	Treatment	Antibodies	Adverse Events	CD4	Outcome	Thymectomy
18.	Hung WL , et al [221] 2011	HIV associated MG and impacts of HAART, One case report, review of literature	Clinical neurology and neurosurgery	Thailand	Case report	1	AChR AB^{+}	Mestinon, ARVs	Nil	327	Worse on ARVS	ND CT: Thymic hyperplasia
19.	KuntzerT, et al [222] 2011	Rituximab is successful in an HIV positive pt with MUSK ab	Neurology	USA	Case report	1	MuSK^{+}	RTX, steroids, MMF, IVIG, PLEX	ND	244-575	Complete recovery	ND
20.	Ragunathan, K, et al [223] 2015	MUSK MG as a manifestation of immune restoration disease in a HIV-positive patient	Journal of Neurology	USA	Case report	1	Musk +	Prednisone and AZA & PE	NIL	520	improved	ND
21.	Virot E, et al [224] 2017	Autoimmune Dx in HIV	Medicine	France	Cross sectional study	2	AChR AB-	IVIG , Mestinon ART	Nil	695,275	improved	ND CT thymus normal
22.	Sherpa M et al [225] 2017	Comorbid HIV and MUSK MG: Case report and literature review	American Journal of case reports	USA	Case report	1	Musk^{+}	Mestinon, ART, AZA, IVIG	NIL	383	improved	ND (CT thymus N)
23.	Suthar, R et al [226] 2018	MG in an HIV positive girl	Indian journal of paediatrics	India	Case report	1	AChR AB-	Prednisone, Mestinon, ART	NIL	1289	improved	ND CT Thymus (N)

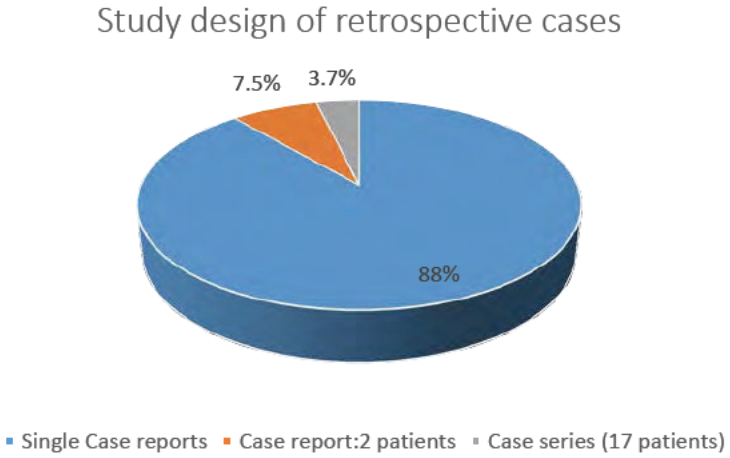
USA=United States of America, ART=Antiretroviral Therapy, AChR AB= Acetylcholine receptor antibody, ND=Not documented

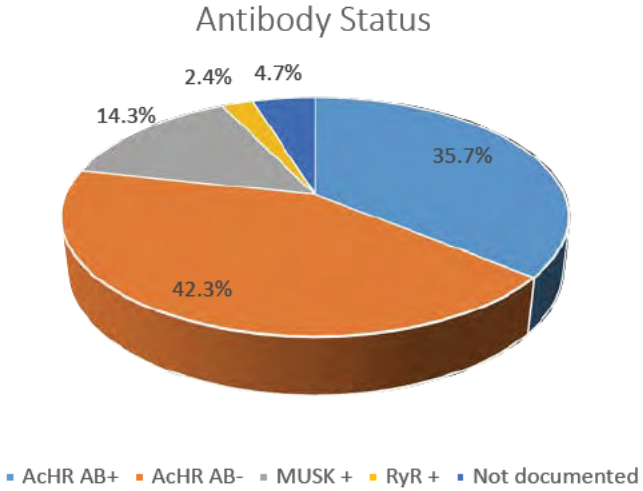
	Authors	Title	Journal	Location	Research Design	Number of cases	Treatment	Antibodies	Adverse Events	CD4	Outcome	Thymectomy
24.	Aglave, V, et al[227] 2019	A rare case of MUSK MG in HIV patient	journal of neurological sciences	Dubai	Case report	1	Musk +	Prednisone Mestinon, AZA, ARTs	ND	170	**Improved**	ND CT Thymus (N)
25.	Heckmann et al [228] 2020	Management issues in MG patients living with HIV; Case series and review of the literature	Frontiers in Neurology	South Africa	Case series	17	11: AChR Ab + 1: Musk Ab +	AZA: 9 Steroids:11 MTX:4 Cyclosporine:1 MMF: 1 Cyclophosphamide:1 RTX: 1 PE/IVIG:4	1 case of Herpes Zoster	See ref	10 : MMS 7: improved with mild symptoms	ND
26.	Wang Y, et al [229] 2020	Case report orbital cellulitis and MG	Frontiers in Immunology	China	Case report	1	Mestinon, tacrolimus, prednisone	AChR AB-RyR+	NIL	242	improved	ND CT thymus (N)
27.	Wirtz P, et al [230] 2020	Dysphagia and respiratory failure in an HIV patient: MUSK MG	Acta Neurologica Belgica	UK	Case report	1	IVIG and prednisone	AChR AB-MusK+	NIL	537	improved	ND
28	Santo, AD 2021	Atypical MG in HIV	JNS	Greece	Case report	1	IVIG and prednisone	AChR +	Nil	255	Improved	ND

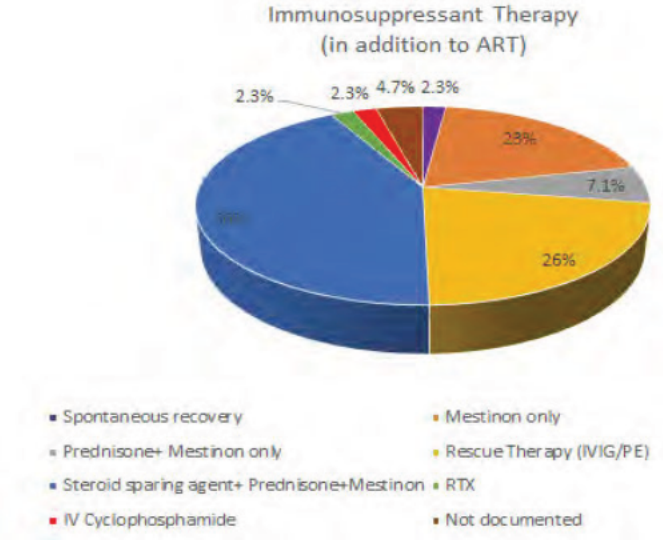
UK=United Kingdom, ART=Antiretroviral Therapy, AChR AB= Acetylcholine receptor antibody, MuSK= Muscle Specific Tyrosine Kinase, RyR= Ryanodine, ND=Not documented, AZA=Azathioprine, MTX=Methotrexate, MMF=Mycophenolate Mofetil, RTX=Rituximab, PE=plasma exchange, IVIG=intravenous immunoglobulin, MMS=minimal manifestation status

Fig 2.4 Summary of the literature describing MG in HIV









Results:

There were 11 published articles in the literature describing CIDP in HIV-infected patients. Ninety percent (90%) of the HIV-infected CIDP studies were retrospective case reports, abstracts from congresses or small case series (Table 2.3, Fig 2.1). There was one prospective case series from SA consisting of 10 patients. Fifty five percent (6/11) of articles were single case reports and 5 were small case series. In total there were 35 documented cases in the world describing CIDP in HIV. Thirty six percent of the cases originate from the US, 27% from the Europe and Asia and 9% from Africa. With respect to therapy and outcome; 38% of patients received either PE, IVIG, CST or ART, with a positive outcome in 40% of cases. In the large majority of cases, therapy and outcome was not documented.

There was only one reported case of a possible nodopathy in an HIV-infected patient with GBS described in a European cohort and another with NF155 antibodies in Japan [13, 231] . Response to IVIG and CST was documented in this case. There are no reported cases of nodal disease from Africa. There are also no case reports of CCPD in HIV and only 1 documented case from Tunisia in an HIV-uninfected patient.[232] The majority of the cases of HIV-uninfected nodopathies arise from Europe and the East.

There is only one case series of HIV associated pure motor lumbosacral polyradiculopathy consisting of 4 patients from South Africa described by Benatar et al in 2000 [6]. All 4 patients recovered spontaneously within two weeks.

Similarly, 69% of HIV-infected MNS cases were retrospective single case reports, and 8.6% were case series consisting of 2 or 3 cases respectively and 13% consists of ≥5 patients (Fig 2.2). The largest case series, reported by Moulinger A et al [17], consisted of 6 patients. Thus far 39 cases of HIV-infected MNS have been reported in the literature world-wide. Thirty-five percent of cases originate from Europe, 45% from the US and 10% from Asia and 10% from South America. Seventy nine percent of patients received ART, 2.5% IVIG and in 18% therapy was not documented. Outcome with ART was variable with 46% showing improvement, 10% stabilised, 7.7% deteriorated, 15.3% demised and 21% not documented (Fig 2.2)

With regard to HIV-infected MG, there were 42 documented cases, 23 of which were retrospective single case reports, 2 case series consisting of 2 patients each and a single retrospective case series consisting of 17 patients. Majority of the cases were from the South Africa, followed by US, Europe, Asia, United Arab Emirates and South America. Forty two percent were AChR-AB negative, 35.7% were positive for AChR antibodies,14.3% positive for MuSK antibodies, and 2.4% ryanodine antibodies. With regard to therapy, in addition to ART, the majority (50%) received combination

therapy (CST, Mestinon, steroid sparing agent), 26% rescue therapy with IVIG/PE, 23% mestinon only and 2.3% IV cyclophosphamide or Rituximab. One patient responded to ART without immunosuppressive therapy (Fig 2.3).

In majority of case reports of neuromuscular diseases in HIV, adverse effects of therapy was not documented, except 1 case of Herpes Zoster infection reported by Heckmann et al in the MG cohort[233].

Discussion

In summary, available literature in the various categories are restricted to case reports or small case series. There is little or no comparisons made between the HIV-infected and HIV-uninfected cohorts in the above cases with respect to demographics, clinical presentation, laboratory investigations and treatment response.

However by inference and comparing with literature from the HIV-uninfected cohorts we noted the following potential differences. Forty percent (40 %) of HIV-infected CIDP patients improved on treatment, whereas in the HIV-uninfected category, 40-45% are refractory to therapy. However, this figure may change as the outcome was not documented in 40% of patients. Approximately 50% of HIV-associated MNS patients, improved on ART, whereas in HIV-uninfected MND, there is no cure and usually no improvement as it is neurodegenerative. MuSK positivity was 14% and 26% required rescue therapy in the HIV-infected MG cohort, which is slightly higher than the HIV-uninfected cohorts. Antibody testing was not done in 37.5% of patients. In addition, the above the scoping review, confirms and highlights the following areas of deficiency in the literature:

a. Articles are limited mainly to case reports and small case series, hence the need for larger case series or case-controlled studies
b. The lack of comparative studies with HIV-uninfected patients in terms of clinical presentation, electrophysiology and laboratory investigations and response to therapy
c. The paucity of prospective studies in the pre-ART and post-ART era
d. The paucity of studies from Africa where the HIV burden is high
e. There are no published studies of autoimmune nodopathies from Africa and only 1 limited case report of nodal disease in HIV from Japan[13] This patients clinical characteristics and response to therapy may be suggestive of anti-NF155 antibody-positive neuropathy or CIDP. HIV-associated CIDP patients also respond well to corticosteroids and therefore it is possible that the positive antibody is an epiphenomenal in HIV or a false positive due to prior IVIg administration, as seen with other neuronal antibodies found in IVIG[234]
f. There are no studies or case reports of CCPD in HIV or from Africa

g. Use of specific and well validated clinical and electrodiagnostic criteria and functional scales is not mentioned in most case reports, hence not reliable comparisons between studies cannot be done.
h. The lack of objective documentation of response to therapy by using appropriate functional assessment scales and temporal response to immunomodulatory therapy and ARVS is not documented
i. Lack of immediate as well as long term safety and efficacy of immunotherapy in the HIV-infected patients
j. No long-term follow-up of patients
k. The pathogenesis of disease in HIV which may differ from the HIV-uninfected cohort, is not defined

In summary the scoping review highlights the rarity of documented cases, both in the pre-ART and in the post-ART era and from the African continent where the interplay of various genetic and environmental factors may impact on disease and where the incidence of HIV is the highest

Chapter 3: PHD thesis outline, thesis aims, broad study design and methods used for prospective work

Thesis aims and broad study design:

In view of the rarity of the above neuromuscular syndromes and paucity of reported HIV-associated cases especially in the post ART era, prospective work from a single neurology centre would require many years to recruit and follow-up adequate number of patients, possibly spanning a decade. Therefore retrospective chart reviews were preferred to generate adequate patient numbers. Furthermore the electronic data capturing system at our hospital facilitated easy data extraction. This included detailed data at presentation, long term follow-up data and treatment outcomes which enabled us to draw reliable conclusions with a high number of cases from the pre-ART era.

Prior to being included in the study alternate aetiological possibilities were excluded and established diagnostic criteria satisfied as detailed in the publications. As SA has a high burden of not only HIV but concomitant infections such as tuberculosis, viruses, fungal and spirochaetal disease, these potential mimics, including malignancy and other inflammatory disorders were vigorously excluded. Additionally known antibodies such as ganglioside antibodies, paraprotein and paraneoplastic antibodies and connective tissue diseases were excluded. Live cell based assays and myelin co-cultures for specific nodal-paranodal antibodies were performed on patients CSF and serum with CIDP, PM LSP, DRG, CCPD and 5 patients with MNS. Lactosylceremide antibodies were tested by ELISA in all patients with CCPD.

Radiological investigations included MRI spine, orbits and brain and PET imaging(where indicated) to help exclude inflammatory disorders such as neurosarcoidosis and malignancy .

The protocol overview, and immune and genetic laboratory methods are discussed later in this chapter.

Retrospective Studies:

Primary Objectives:

The primary objectives of the **4 retrospective studies** were to identify, describe and compare the above syndromes in HIV-infected patients to the HIV-uninfected category in terms of:

a) Demographic features of the disease (age, race, gender)
b) Clinical presentation of the disease in each category (motor vs sensory, proximal vs distal, bulbo-respiratory signs, ocular signs, central involvement)
c) Disease progression or recovery over time as assessed by functional scores at presentation and follow up at 3,6,12,18, 24 and 36 months respectively. This included the following:
    I. ODSS (Overall Disability Sum Score) and INCAT (Inflammatory Neuropathy Cause and Treatment Score) for CIDP at presentation and 3,6,12, 24,36 months follow-up
    II. mRS (Modified Rankin score) for PM LSP and autoimmune nodopathies
    III. ALSFRS Scores for MNS
    IV. MG QoL , MG ADL, MG MMT scale for MG
d) Electrodiagnostic, radiological and laboratory features example CSF protein counts in CIDP, PM LSP, auto-immune nodopathies and radiology in PM LSP, autoimmune nodopathies, CIDP, CCPD and MNS
e) Treatment outcomes in terms of death or recovery according to functional scales

Secondary Objectives

a) To report response to immunosuppressive or immunomodulatory therapy. This included the following categories:
b) Induction Therapy
    a. Corticosteroid therapy (Oral or IVI)
    b. Intravenous immunoglobulin
    c. Plasma exchange
c) Maintenance Therapy
    a. azathioprine, methotrexate , cyclophosphamide, tacrolimus, mycophenolate mofetil
    b. B cell depleting agents : rituximab
d) ART combinations
e) To document side effects to therapy and drug - drug interactions
f) To evaluate change in functional scores with varying CD4 counts and viral load.
g) To explore and speculate on the pathogenesis of disease in HIV.

Research Design and study site

This study was a detailed retrospective review of patient records with the initial or later confirmed diagnosis of MND/S, immune mediated inflammatory neuropathies, polyradiculopathy and MG. A prospective study was also performed and is discussed briefly in this chapter. All patients attended IALCH neuromuscular clinic and were identified using the respective ICD10 codes namely (G61.81, G12.2, G70) .

Context:

The neuromuscular unit at IALCH services the province of KZN which has a population of 19 million people and is the epicentre of HIV. As such, we diagnose a large number of patients with lower motor neuron syndromes which may be co-incidental or due to HIV infection itself.

Inclusion Criteria and exclusion criteria are discussed in each article:

Ethical approval:

Research Ethics Committee Reference No: BE272/15 was granted on the 3 November 2017 and ethical approval for the prospective study was granted in 2023 BE 5861/2023. Patient consent was not required for a retrospective chart review. Informed consent for blood and CSF extraction was obtained from all patients for prospective studies and secondary projects arising from the primary study. Informed consent was also obtained from the surviving sister for the case report to publish histology and radiology, ship serum and CSF to the University of Oxford, UK and for whole exome sequencing and whole genome sequencing performed at University College London, UK. A material transfer agreement was signed between UKZN and University of Oxford and UCL for the current and future studies.

To establish co-cultures consisting of neurons and Schwann cells (SCs), the study used SCs harvested from rat embryos from pregnant females, which were sacrificed solely for the purpose of obtaining tissue for research using a schedule 1 method of the UK Home Office Animals (Scientific Procedures) Act of 1986.

Methods:

Detailed methods for the retrospective studies are described in the 6 articles.

Statistical Planning:

Data was entered in Microsoft Excel and analysed using Intercooled Stata Version 13. Descriptive statistics such as frequencies, percentages, means, median, IQR and standard deviations were used to summarise the results. The median and IQR of functional scores were also calculated and compared.

Value of the Study

This study would help to improve our understanding of the above diseases in the context of HIV immunology in terms of:

1. Clinical presentation and progression of disease,
2. Pathogenesis of the disease
3. Response to immunosuppressive therapy
4. Impact of ART and immune reconstitution on disease progression and outcome
5. Establishment of protocols for the safe use of immunosuppressant therapy in HIV-infected patients.

Prospective studies:

The initial project was followed by a subsequent prospective study entitled:

a. Clinical relevance of nodal-paranodal antibodies in chronic HIV-infected immune mediated radiculoneuropathies.
b. Case report of CCPD in siblings : Immune mediated or genetic.

The detailed study design is discussed in the relevant published manuscripts .

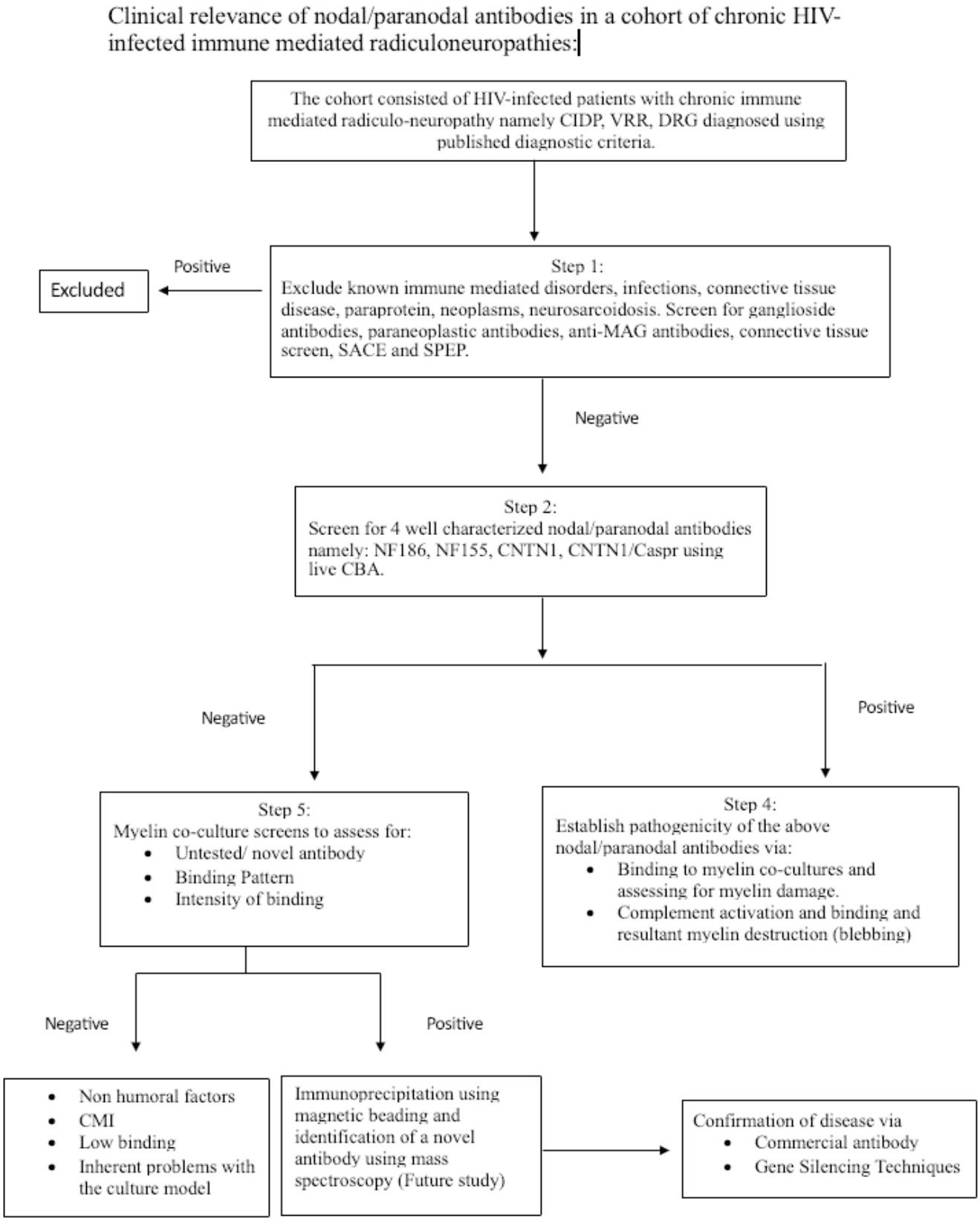
See figure 1.3.1: Algorithm for prospective manuscript

The main aim of this study was to :

1. Determine the prevalence of nodal-paranodal antibodies in the context of HIV immune mediated radiculoneuropathies
2. Determine the IgG subtypes in the context of HIV infection
3. To explore the potential relevance of these antibodies in the context of HIV.
4. Pathogenicity was assessed via the following laboratory measures :
    a. Binding of the relevant antibody to HPSC myelin co-cultures and subsequently measuring or quantifying antibody mediated myelin damage microscopically.
    b. Assessment of complement activation and complement mediated myelin damage by adding normal human saline (source of complement) to myelin co-cultures with and without antibodies and assessing for accelerated myelin damage compared to controls.
5. Explore the immune pathogenesis and genetic basis of CCPD

All patients included in the study tested negative for ganglioside antibodies . Traditional myelin antibodies example myelin basic protein and MP22 was found not to be pathogenic in peripheral nerve demyelination in previous studies and hence not screened for in our study cohort.[104]

Figure 3.1: Algorithm for Prospective study



Locally the above investigations are performed for research purposes only. Well established international research centers perform Live CBA for diagnostic purposes if clinically indicated.

Laboratory methods for the immune and genetic work

The immune and genetic tests used for experiments in the subsequent prospective work on HIV immune mediated radiculoneuropathies and CCPD include

a. Live cell based assay which was restricted to 4 clinically well characterised nodal-paranodal antibodies namely NF155, NF186, Contactin1, and Caspr
b. Myelinating co-cultures screens using human induced pluripotent stem-cells (hiPSC) derived from sensory neurons and rat Schwann cells and complement activation tests.
c. ELISA for lactosylceremide antibodies in the siblings with CCPD
d. Immunoprecipitation tests using magnetic beading for novel antibody detection
e. Mass Spectrometry to identify the target/novel protein
f. Whole Exome Sequencing and mitochondrial genetics

Due to a lack of a dedicated neuroscience facility in SA, the above immune and genetic work were performed in the UK:

1. Live CBA, myelin co-culture screens, LacC ELISA and immunoprecitation experiments were performed at the Nuffield Department of Clinical Neurosciences, John Radcliffe Hospital, Oxford, UK. In September and October 2023, I spent some time at the above research and clinical institute under the direct supervision of Associate Professor Simon Rinaldi. During this time, I gained experience with clinical assessments of patients with inflammatory neuropathies in particular autoimmune nodopathies. In addition, under the guidance of Professor Simon Rinaldi I was able to observe, understand, interpret and analyse the immune tests performed on our cohort of South African patients.
2. Mass spectrometry was performed at the Target Discovery Institute, Nuffield Department of Medicine at Oxford, by Georgina Berridge.
3. Genetic testing which included WES and mitochondrial genetics was performed at the Department of Neuromuscular disorders, University College London(UCL) , Queen Square, London , UK, by Professor Henry Houlden and his team.

The ELISA for ganglioside antibodies were performed locally in South Africa. This panel includes antibodies (IgG and/or IgM) to GM1,asialo-GM1,GM1b,GD1a,GD1a, GD2,GD3, GD1b, GT1a, GT1b , GQ1b, SGPG, SGLPG and sulphatide.

Antibody Detection:

Various laboratory tests are available for antibody detection. These include:

a. Enzyme-linked immunosorbent assay (ELISA)
b. Radioimmunoassay (RIA)
c. Live and fixed cell based assays (CBA)
d. HiPSC myelin co-culture screens (Novel antibodies)
e. Live and fixed tissue based assays (TBA)
    i. Teased nerve fibres
f. Ultrasensitive assay technology
    i. Chemiluminescence, electroluminescence, proximity extension assay(immune-PCR))

Each test has its advantages, disadvantages and differing sensitivity and specificity. Improving sensitivity and specificity of a test result may possibly be achieved by cross-validation with different test methods, inter-laboratory validation and the combined testing of serum and CSF samples or simply repeating the test. However, this may not apply in all cases example live CBA using CSF was less sensitive for nodal/paranodal antibodies compared to serum and using multiple tests led to worse sensitivity[235]. In general test results should always be interpreted in context with the clinical presentation. In case of an unexpected positive or negative result, re-testing of the sample or performing confirmatory tests might be considered as there may be subjectivity interpreting fluorescent microscopy and end point titres for tests such as live CBA and myelin co-cultures and poor inter-laboratory and intra-laboratory agreement across certain laboratories, hence impacting on interpretation of results (discussed in later chapters). ELISA has been used as the gold standard for the measurement of fluid biomarkers and antibodies. However, a major limitation is it requires large sample volumes (50–100 μL) per test and millions of analyte molecules to generate a detectable signal, which limits assay sensitivity[87]. The lowest limit of detection is seldom below nanograms/millilitre (ng/ ml) range, and the multitude of proteins present in blood or cerebrospinal fluid (CSF) at lower concentrations cannot be detected. Newer, ultrasensitive and multiplexing technologies are now available to measure disease biomarkers and other molecules. These are based on a similar mechanism underlying sandwich ELISA which is the formation of immune complexes of antibodies binding to the analyte(s) of interest[87]. However, sensitivity is significantly increased by novel antigen capture and display methods as well as amplification, imaging, and statistical signal handling methods. Examples include (single molecular array, chemiluminescence, electroluminescence, proximity extension assay(immune-PCR))[87]. Tests used in this study for antibody detection include live CBA and hiPSC myelin co-cultures to establish pathogenicity and test for novel antibodies.

Methods for Live Cell Based Assay and myelin co-cultures:

Courtesy of Nuffield Department of Clinical Neurosciences,
University of Oxford, United Kingdom

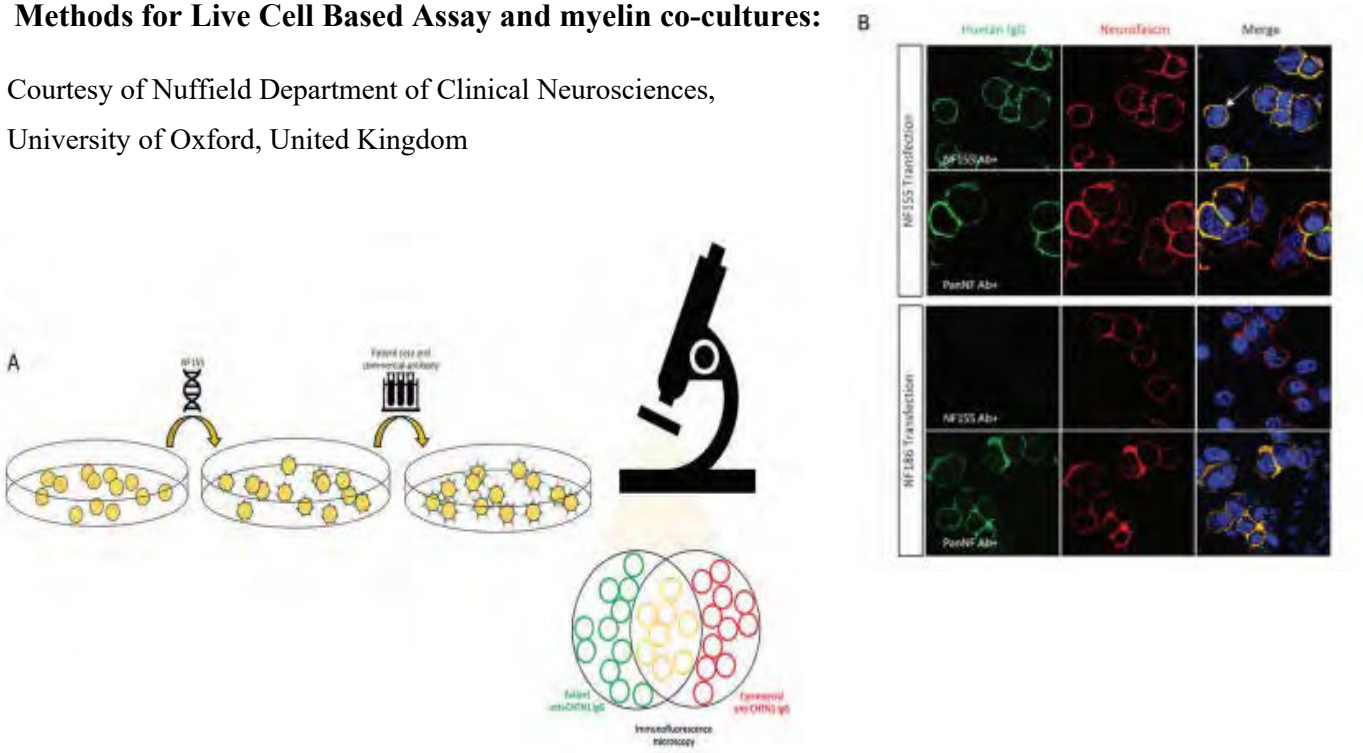


Fig 3.2 Fehmi J, et al. Pract Neurol 2021;21:284–291. doi:10.1 136/practneurol-2021-002960

(A) Illustration shows how HEK cells are transfected with plasmid vectors encoding the desired nodal/paranodal protein target, that is, NF155, or NF186 or CTN1/Caspr which is then expressed within the cell membrane. Subsequent exposure to patient sera allows paranodal/nodal Abs (green), if present, to bind to the target antigen. Concurrent exposure to a commercial Ab targeting the same protein (red) can both be visualised independently using commercial fluorescence markers and should co-localise if the patient is paranodal-nodal Ab positive (merge). (B) Fluorescence microscopy images (×63 magnification) taken using either NF155 Ab+ or pan-NF Ab+ patient sera incubated with NF155 or NF186 transfected HEK cells. Human IgG (green) from both patients bind to NF155 transfected cells and co-localises with the commercial NF Ab (red). However, only IgG from the patient with pan-NF Abs also binds to NF186 transfected cells, emphasising the importance of testing for reactivity to both antigens individually. DAPI (blue) identifies the cell nuclei. Abs, antibodies; CNTN1, contactin1; HEK, human embryonic kidney; IgG, immunoglobulin G; NF, neurofascin.

Live Nodal/Paranodal Cell-Based Assays

All sera were screened for IgG antibodies to neurofascin-155, neurofascin-186, contatctin-1 and Caspr1 using a live, cell-based assay (CBA), following previously described methods with slight modification [236]. In brief, HEK293T cells on poly-L-lysine coated 13mm coverslips at 80–90% confluence was transiently mono-transfected with human neurofascin-155 or human neurofascin-186 mammalian-expression vectors, or co-transfected with both human contactin1 and human Caspr1 at equimolar concentrations, using Jet-PEI transfection reagent. After 16 h, the cells were washed and replaced with Dulbecco's Modified Eagle Medium (DMEM) containing 10% foetal bovine serum (FBS). 24 hours later, sera and eluates were diluted 1:100 in DMEM + (Bovine serum albumin) BSA (1%) and incubated with the cells for 1 h at room temperature. Co-incubation with commercial chicken neurofascin primary antibody, was used to confirm successful transfection and to assess for co-localisation with any bound human IgG. Following serum incubation, cells were washed 3 times with DMEM + HEPES (20 mM) and fixed for 5 minutes in 4% PFA. Secondary antibody incubation was with goat anti-human and goat-anti- chicken IgG. To determine antibody subclass unconjugated mouse anti-human IgG subclass 1-4 antibodies was used at 1:100 followed by a fluorescently tagged tertiary antibody (goat-anti-mouse IgG). Positivity was assessed by an observer blinded to the clinical data using fluorescence microscopy. Considering the intensity of the membrane signal and co-localisation of the human IgG signal with the commercial antibody, the assay was scored on a 5-point scale as follows: 4+ very strong positive, 3+ strong positive, 2+ positive, 1+ negative (non-specific background or faint/poorly co-localised human IgG signal only), 0 no human IgG binding seen.

Myelinating Co-Cultures (Human induced pluripotent stem-cell derived) using AD2 cell line

Human-induced pluripotent stem cell (hiPSC) differentiation, and plating of sensory neurons

Myelinating co-cultures were prepared using human induced pluripotent stem cells (hiPSC)-derived sensory neurons and primary rat Schwann cells [237] . HiPSCs from control subjects was obtained via the StemBANCC consortium at the University of Oxford. In brief, hiPSCs are differentiated to sensory neurons using a combination of small-molecule mediated dual-SMAD inhibition and wnt activation. On day 11 of differentiation, sensory neuron precursors are seeded onto 13 mm diameter glass coverslips (approximately 20,000 cells per coverslip) or 96-well flat, glass-bottom imaging plates (Sensoplate Microplate, Greiner-Bio) (approximately 5000 neurons per well) previously coated with poly-D-lysine (PDL) (10 µg/mL) overnight and reduced growth-factor matrigel (Corning). Neurons were maintained in neurobasal media supplemented with N2, B27, Glutamax and anti-anti plus recombinant human β-NGF, GDNF and BDNF (all growth factors 25 ng/ml), supplemented with Rho-associated, coiled-coil containing protein kinase (ROCK) inhibitor on days 11–12, on days 11–

14 and cytosine arabinoside on days 12–14. Neurons were incubated at 37 ◦C in 5% CO2 for 4 weeks with twice-weekly medium changes prior to addition of Schwann cells for myelination.

Schwann cell harvesting and myelination of sensory neurons with rat Schwann cells

Primary Schwann cells were isolated from the sciatic nerves of rat pups. Mother and pups were killed by rising concentration of CO2 in accordance with Schedule 1 of the UK Home Office. Animals (Scientific Procedures) Act 1986. Sciatic nerves were rapidly dissected and digested in a mixture of collagenase (3mg/ml) (Worthington, Lorne Labs) and dispase II (3.5mg/mL) (Roche) for 1 h at 37 ◦C with frequent gentle agitation. Nerves were washed in DMEM + FBS (10%) and gently triturated using a fire-polished glass Pasteur pipette. Dissociated cells were seeded into tissue culture flasks overnight and expanded in Schwann cell expansion medium containing charcoal-stripped FBS (10%) (Sigma), Forskolin (4 µM), recombinant human NRG1-β1 EGF domain (80 ng/mL) (Cat. 396-HB, R&D Systems) and recombinant murine NGF (10 ng/ml) (Cat. 450-34, Peprotech) in DMEM/F12 (Gibco). Cells were serially treated with 5–10 µM Ara-C to eliminate fibroblasts. Expanded Schwann cells were added to the neuronal cultures (25,000 cells per coverslip or 5000 cell per 96-well) and allowed to proliferate and align with the axons for 1 week in basal media containing: (CS-FBS) (10%), insulin (5 mg/ml) (Sigma), holo-transferrin (100 mg/mL) (Sigma), rhNGF (25 ng/mL) (Peprotech) (Sigma), Selenium (25 ng/mL) (Sigma).
From this point on, cultures were maintained in 'myelination medium' containing: 5% CS-FBS, ascorbic acid (25 µg/mL), phenol-free matrigel (1:300) (Corning) and hrNGF (25 ng/mL) in 'complete' neurobasal medium. Myelinating cultures were matured for at least 4 weeks before being used in subsequent experiments.

Immunoassay for topographical human IgG binding

"Sera were assessed for topographical binding using mature myelinated co-cultures. These were incubated with patient sera diluted at 1:100 in N2 'complete' neurobasal media with 1% BSA and human nerve growth factor (NGF) (25 ng/ml) for 1 h at 37°C. If NHS (Source of complement) was added this was done for a further hour or 24 hours. Cultures were washed with PBS and fixed with 2% PFA for 30 min at room temperature. After washing sequentially with PBS and DMEM (including 20 mM HEPES) cultures were incubated with goat anti-human IgG AF488 (1:750) (A11013, ThermoFisher Scientific) in DMEM/HEPES/1% BSA for 1h at room temperature. Cultures were washed sequentially with DMEM/HEPES and PBS and then permeabilised with ice cold methanol (30-45 minutes on ice). Cultures were blocked with 5% normal goat serum (NGS) in PBS before incubation with primary antibodies chicken anti-Neurofilament 200 (1:10,000) (4680; Abcam) and rat anti-myelin basic protein (1:500) (Ab7349, Abcam) over night at 4°C. Secondary antibody incubation was with biotinylated goat anti-chicken IgY (1:500) (BA9010, Vector Laboratories), goat anti-rat IgG Alexa

Fluor 546 (1:1000, 1hr, RT) (A11081, Life Tech) followed by streptavidin pacific blue (1:500,45 mins, RT) (S11222, Life Technologies). Coverslips were mounted onto glass slides with Vector shield (H1000, Vector), and fluorescence images of IgG nodal labelling in myelinating cultures were acquired on a laser scanning confocal microscope (LSM 700, Zeiss) using the x63 or x20 objectives. 10-15 z-sections at 0.5 µm interval were exported as maximum projection images. Brightness and contrast were adjusted for presentation." (Fehmi et a l., 2023)

Topographical binding of human IgG to myelin co-cultures:

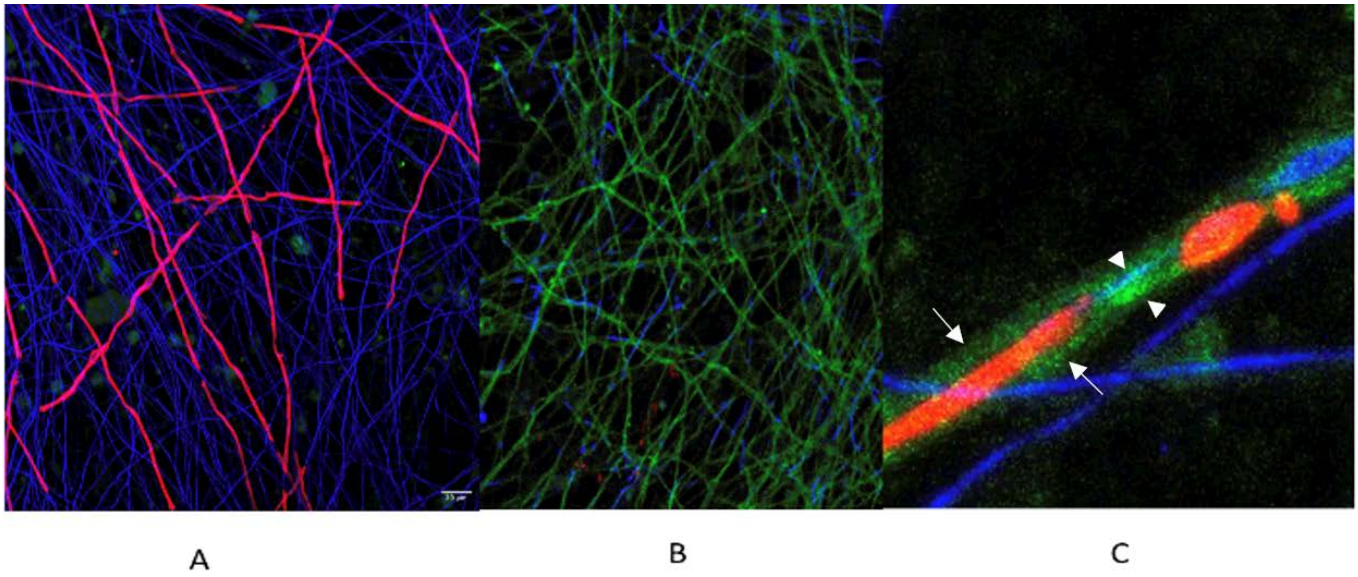


Fig 3.3 : Myelin basic protein (MBP, red) indicates myelin, and neurofilament (NF200, blue) indicates axons. Human IgG is green. Scale bar, 25µm (A and B), 5um (C)

The myelinating co-cultures used in the experiments contain "folds" of myelin across numerous internodes, a basal lamina, and normal nodes of Ranvier with the expected molecular architecture. This has been confirmed microscopically in previous studies[237].

Figure 3.3 (A) depicts the myelin sheath which is stained red with myelin basic protein and axons stain blue with neurofilament 200 stain. Three pattens of binding are depicted in Figure 8.1.2. Human IgG (green) from patients sera binds to myelinated co-cultures.
Note (A) is a negative control, (B), diffuse axonal pattern , (arrowheads, C) paranodal localisation and (arrows, C) binding to myelin sheath .

Lactosyl Ceremide (LacC) detected via ELISA:

Immunolon 2HB plates were used for LacC ELISA. Stock and working LacC solutions were sonicated for 3 minutes before use. Working solutions for ELISA were made by further dilution of stock glycolipids in methanol to 2μg/ml.
To create LacC complexes, 2 component LacC in a 1:1 ratio was mixed and sonicated for 3 minutes.
As a negative control, 100μl of methanol only was added to several wells per ELISA plate.
100μl of the LacC solution was added per well and allowed to air dry for overnight in the fume hood.
Plates were kept at 4 ºC for at least 1h prior to further use.
Plates were blocked with 200μl/well of 2% BSA/PBS for 1h at 4°C.
Serum samples were diluted as required in 1% BSA/PBS. 100μl of the diluted solution was then applied to each coated well of the ELISA plate. Incubation was for 2h at 4°C.
The primary solution was tipped and shaken off, and the plates plunged into cold PBS then emptied five times.
Next, 100μl of the appropriate secondary antibody (HRP conjugated), diluted 1:3000 in 1% BSA, was applied to the wells and incubated for 1h at 4ºC.
The plates then underwent the same wash protocol as for the primary antibody.
Detection was performed with 50μl/well of o-Phenylenediamine dihydrochloride solution.
O-Phenylenediamine (dihydrochloride) is a chromogenic substrate that is suitable for use in ELISA procedures that utilize horseradish peroxidase (HRP) conjugates. This substrate produces a soluble end product that is orange-brown in colour and can be read spectrophotometrically at 450 nm)

The reaction was terminated with 25μl of 4M H_2SO_4. Optical density at 492nm was detected by an automated plate reader.

Principles of immunoprecipitation using Magnetic Beads to identify neural antigen in myelin co-culture of patient 1:

Immunoprecipitation (IP) is a widely used method in many different research fields, aimed at isolation of the target antigen or its binding partner for downstream analysis. As protein–protein binding may involve transient and weak interactions, it is critical to use a method that offers rapid binding kinetics with minimal nonspecific binding. Dynabeads are magnetic beads which are nonporous, uniform, super-paramagnetic, monodispersed polystyrene beads that are widely used for IP. The coating provides a defined surface area for the adsorption or conjugation of various molecules, in particular antibodies. Bead uniformity and shape provide consistent physical and chemical properties and are instrumental in minimizing nonspecific binding (Figure 3.4). In addition, optimal binding kinetics and high reproducibility allow for rapid and efficient binding to the target. Dynabeads help ensure high antibody-binding capacity and accessibility, low nonspecific binding, and high yield. The chemical properties of the beads also eliminate the pre-clearing step and reduce the consumption of antibodies, making these magnetic beads ideal for IP.

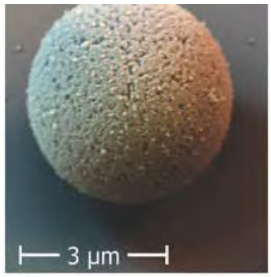


Fig 3.4: Scanning electron micrograph of Dynabead: Non-porous, uniform nature of the beads enable low non-specific binding, high reproducibility, low antibody consumption and fast binding kinetics. *Ault et al July 2010, Microscopy and Microanalysis 16(S2):630-631*

Step 1:

Preparation of Dynabeads: Covalent coupling of the candidate antibody to the magnetic bead which is pre-coated with protein G:

Protein G is the preferred coating in this experiment as it has high affinity for human IgG1-IgG4 antibodies. CSF or serum is then incubated with the magnetic dynabead coated with Protein G overnight. In the above case/s (Patient 1,30,49), patients CSF (patient 30) is preferred to serum, as it is more likely to contain a higher proportion of disease specific antibodies compared to serum which may have a multitude of non-specific antibodies especially in the HIV-infected population. Furthermore, we have already demonstrated moderate to strong pathogenetic binding of these antibodies in the CSF to myelin co-cultures. Additionally, western blot of patients CSF compared to controls can be performed prior to IP to demonstrate a significant band in the CSF to potentially confirm a dominant or high concentration of the candidate antibody in the CSF. However western blot is not preferred as it will detect all IgG antibodies and not specifically those that are disease relevant. Assumptions are as follows:

1. CSF contains a pathogenetic monoclonal Ab or a high concentration of the candidate antibody (CSF OBs +, T2 pattern)
2. The antibody binds strongly to the neural antigen in the myelin co-culture. This has been established in a previous experiment, figure 8.2.4.
3. The Ab has a high affinity to the Ag.
4. The Ab is likely IgG (IgG1-IgG4) and therefore beads are coated with Protein G, if IgM is the predominant antibody use goat anti human IgM to coat beads.
5. The Ab is specific to the candidate Ag and will not bind to multiple neural Ag's in the myelin co-culture.

The candidate antibody may be cross linked to the bead; however, this is often not required for magnetic beads as the antibody will bind irreversibly to the bead. This will facilitate easy separation of the Ag-Ab complex.

Coupling Volume:
5mg beads=500µL coupling
For Scaling
Coupling (µL) =100 x beads (mg)
250 µL C2
+ 250 µL (C1 + Ab)
= 500 µL coupling

Steps required:

a. Wash freeze-dried beads with a C1 buffer, vortex for a few seconds.
b. Add beads to patients CSF and incubate 3hours at 4 ^{0}C on a rotating mixer.
c. Wash again with C1 buffer to remove unbound antibodies.
d. Extract beads using a magnet and resuspend at 10mg/ml.

Step 2:

Extract neural antigens from co-culture and suspend in a solution.

Serum and CSF will be added to hiPSC axonal cultures, and independent Schwann cell cultures to confirm binding to non-myelinating Schwann cells versus axons.

A) M-PER mammalian protein/lipid/CHO extraction.

The complete cell lysis reagent contains a mild, nondenaturing detergent that dissolves cell membranes to extract and solubilize total protein or lipid or CHO from most cellular compartments. Extraction is accomplished in 5 minutes and requires little or no additional mechanical disruption. M-PER reagent is formulated for minimal interference with downstream biological applications. The reagent has been validated for use with several cell lines, including primary, suspension and adherent cell types; the resulting cell lysates are compatible with many downstream assays including immunoassays, enzyme assays and a variety of common reporter assays.

OR

B) Myelin Co-culture Lysate

Myelin co-culture tissue is homogenized in radioimmunoprecipitation lysis and extraction buffer . Place plates on ice and leave for 15minutes. Detach cells by pipetting/scraping. Incubate for 30minutes at 4 °C with gentle mixing and inversion.

Once fully homogenised centrifuge at 14,000 g for 10minutes at 4°C. Only Ag-Ab fractions of the supernatant will be collected, add a protease and phosphatase inhibitor (inhibits proteolysis dephosphorylation and denaturation) and store at −80°C for Step 3.

Step 3:

Incubation of dynabeads with antigenic extracts from myelin co-culture

Add dynabeads containing primary antibody to the solution/lysate in Step 2 containing myelin antigen and incubate up to 4hrs or overnight (under rotatory agitation).

This is followed by centrifugation of the tubes, remove the supernatant and wash the beads in lysis buffer three times (each time centrifuging at 4°C and removing the supernatant), to remove unbound protein

Using a magnet remove dynabeads containing candidate antibody bound to neural /myelin Ag of interest.

As antibodies are cross linked to beads, Ag can easily be separated from the Ab by washing them off using a low pH, iso-osmolar buffer example 0.1M glycine at pH 2.5

Methods to separate beads from Ag-Ab complexes if Ab are not cross linked include:

1. Glycine Buffer
2. SDS buffer
3. Urea buffer

Step 4:

Identification of Candidate antigen:

Western Blot or Mass Spectroscopy of candidate Ag that has been removed from the beads can be compared to control samples

Proteins are selected as candidate antigens when they fulfilled any of these criteria:

Protein score > 100

peptide sequence coverage >5% or two or more peptides identified with the absence of the same criteria in the control sample

Step 5:

Confirmation that Ab/Ag detected is disease relevant via the following methods:

1. Generation of an Ag specific assay or pre-adsorption against a commercial antibody
2. Genetic strategy using siRNA

siRNA:

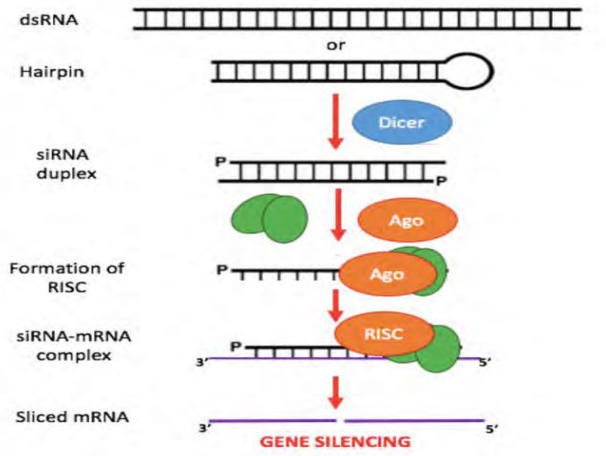
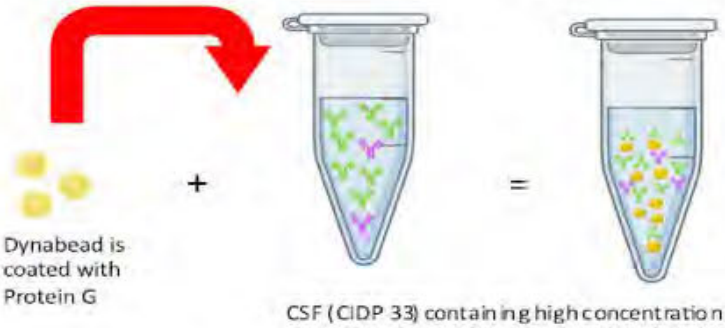


Fig 3.5 The mechanism by which natural siRNA causes gene silencing through repression of translation occurs as follows:

Long dsRNA (which can come from hairpin, complementary RNAs, and RNA-dependent RNA polymerases) is cleaved by an endo-ribonuclease called “Dicer” which cuts the long dsRNA to form short interfering RNA or siRNA; this is what enables the molecules to form the RNA-Induced Silencing Complex (RISC). Once siRNA enters the cell it gets incorporated into other proteins to form the RISC. Once the siRNA is part of the RISC complex, the siRNA is unwound to form single stranded siRNA. The strand that is thermodynamically less stable due to its base pairing at the 5′end is chosen to remain part of the RISC-complex. The single stranded siRNA which is part of the RISC complex now can scan and find a complementary mRNA. Once the single stranded siRNA (part of the RISC complex) binds to its target mRNA, it induces mRNA cleavage. The mRNA is now cut and recognized as abnormal by the cell. This causes degradation of the mRNA and in turn no translation of the mRNA into amino acids and then proteins. This results in silencing the gene that encodes for that mRNA.

IMMUNOPRECIPITATION OF CANDIDATE ANTIGEN USING DYNABEADS

STEP 1: CSF is incubated with Protein G coated dynbeads to elute candidate antibody.



Dynabead is coated with Protein G

CSF (CIDP 33) containing high concentration of monoclonal candidate immunoglobulin and lower concentrations of non-specific immunoglobulin is incubated with dynabeads coated with protein G.

Candidate immunoglobulin binds in high concentrations to dynabeads compared to other immunoglobulins and is cross linked to the dynabead. The dynabead is removed using a magnet.

Dynabead containing candidate antibody cross linked to the magnetic bead.

= Dynabead

= Candidate immunoglobulin (UNKNOWN)

= Other non- specific immunoglobulins

EXTRACTION AND IMMUNOPRECIPITATION OF MYELIN ANTIGEN

Step 2: Extract myelin membrane protein from the co-culture and incubate in a solution with dynabeads containing candidate antibody.

Myelin membrane antigens are extracted from myelin co-culture.

Solution containing extracted myelin antigens.

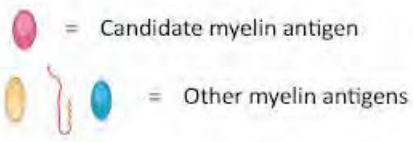
Dynabeads containing candidate antibody is added to the solution and incubated overnight.

Myelin antigens bind to candidate antibody cross-linked to dynabeads.

Myelin antigens that bind to candidate immunoglobulin is extracted from the solution using a magnet.

Step 3: Separation of antigen from beads

Dynabeads containing candidate Ag bound to immunoglobulin.



= Candidate myelin antigen

= Other myelin antigens

Step 4: Gene Sequencing and Western Blot of candidate antigen and **step 5** confirmation tests.

OR

Transfer DRY beads to TDI promptly for mass

Alternate Method used in patient 1:

Bind the serum IgG to live culture cells, then lyse the myelin co-culture with bound Ag-Ab neural complexes, incubate with dynabeads , and use a magnet to pull down whole complexes. This may limit IgG binding to intracellular targets.

Culture preparation

Sensory neurons differentiated from IPSCs seeded onto matrigel coated 10cm dishes.
4 dishes created from 1/6 well differentiation.
After 2 weeks, seeded with rat derived primary Schwann cells.
1 week in Schwann cell basal medium
6-8 weeks in myelination medium

IP

Wash cells with PBS x 3 (pre-warmed)
Incubate with patient sera containing candidate antibody (1:100)
Dilute in 4-8ml N2 media + 1% BSA + NGF (1:4000)
Incubate at 37ºC for 60mins
Remove (and reserve as control) supernatant
Wash cells – 3x PBS (at 4°C)

Lysate

Lyse by adding 495ul of cold lysis buffer (RIPA) + PIC (5ul)
Put plates on ice, leave 15 minutes
Detach cells by pipetting / scraping
Incubate 30mins at 4ºC with gentle mixing / inversion
Ensure fully homogenised by repeat pipetting
Centrifuge 5mins 10k, Keep supernatant (Ag+Ab bound fraction) and discard pellet (should not contain Ab)

Volume at this stage = 500 (+500) = 500-1000ul

Magnetic beading

Prepare dynabeads by rolling – 10 minutes – and washing x3 in PBS using magnetic rack.

(Protein G for IgG, Protein A for IgM)

Add lysates to beads (volume – try 50ul with 500ul lysate) and incubate 3hr at 4ºC on a rotating mixer.

Wash x3 200 µl RIPA PBS using magnetic rack.
Re-suspend in 100 µl RIPA and transfer supernatant to fresh tube.
Remove dry magnetic beads containing the Ag-Ab complexes.

Elution:

Step 1

Use 40µl 0.1M Glycine (pH 2.6) for elution.

Incubate with rotation for 2 minutes RT. Remove beads with magnet and transfer eluate to fresh tube.

Repeat and collect 4 fractions .

Step 2.

Add 40uL pre-heated glyto 90ºC 1% LSB.

Gently pipette to re-suspend the magnetic bead-Ab-Ag complex.

Heat for 10 min at 85ºC. (To detach the Ag-Ab complexes from the beads)

Place the tube on the magnet and transfer the supernatant/elute to fresh tube

This will enable one to transfer Ag-Ab complexes detached from the beads to the Target Discovery Institute for mass spectrometry, beads remain behind attached to magnet. Transfer DRY beads to TDI promptly for mass spectroscopy

Principles of Mass Spectrometry

Mass spectrometry is an analytical technique that can provide both qualitative (structure) and quantitative (molecular mass or concentration) information on analyte molecules after their conversion to ions. The molecules of interest are first introduced into the ionisation source of the mass spectrometer, where they are ionised to acquire positive or negative charges.

Principles of Mass Spectrometry:

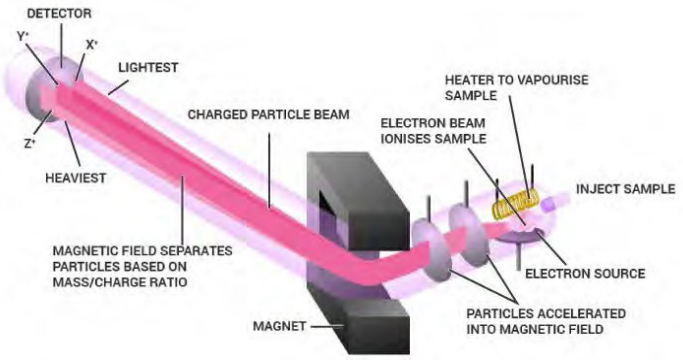


Figure 3.6: Mass Spectrometer adapted from webpage https ://byjus.com/chemistry/mass-spectrometry

This ionized beam is then passed through a series of electric or magnetic fields depending on the type of the sample and its properties. These charged and deflected ions are incident onto a detector which is capable of distinguishing the charged particles falling onto it. Based on the mass spectrum produced by the charged ions, one can identify the atoms and molecules constituting the sample by comparing them with known masses or through a characteristic fragmentation pattern. The computer displays the signals graphically as a mass spectrum showing the relative abundance of the signals according to their m/z ratio as depicted in the mass spectrograph below.

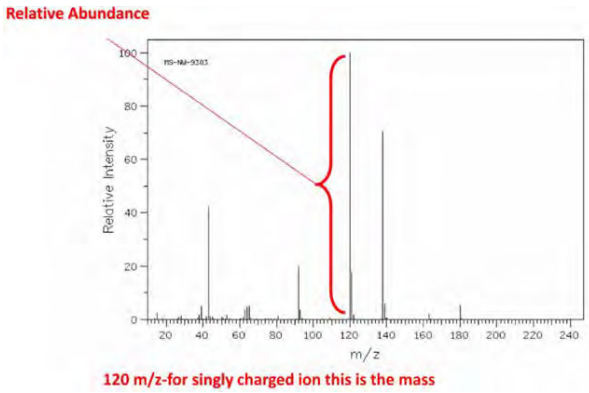


Figure 3.7: Example of a mass spectrograph

4 main parts of a mass spectrometer

1. **Ionizer –** The bombarding of the sample is done by the electrons. These electrons move between cathode and anode. When the sample passes through the electron stream between the cathode and anode, electrons with high energy displace electrons out of the sample and form ions.

   Types of Ionization

   These include gas phase methods (electron ionization (EI), chemical ionization (CI), direct analysis in real time (DART) and inductively coupled plasma (ICP)), desorption methods (matrix assisted laser desorption ionization (MALDI), fast atom bombardment (FAB), thermal ionization sources, plasma ionization sources and liquid metal ion sources (LMIS)) and spray methods (electrospray ionization (ESI) and desorption electrospray ionization (DESI)). MALDI and electrospray are the 2 soft ionization techniques is often used for peptides, proteins, and DNA

2. **Accelerator –** The ions placed between a set of charged parallel plates are attracted to one plate and repel from the other plate. The acceleration speed can be controlled by adjusting the charge on the plates.
3. **Deflector –** Magnetic field deflects ions based on its charge and mass. If an ion is heavy or has two or more positive charges, then it is least deflected. If an ion is light or has one positive charge, then it is deflected the most.
4. **Detector –** The ions with correct charge and mass move to the detector. The ratio of mass to charge is analysed through the ion that hits the detector.

Types of Mass Spectrometers:

1. Quadrupole Mass Spectrometer
   Is a stalwart in the field. Ideal for routine high throughput applications. Its name derives from its 4 parallel rods, which, when oscillated at varying voltages, filter ions based on their specific mass-to-charge ratios. This technique advantageously combines the two different mass analysers. The combination of rapid analysing speed, high compound fragmentation efficiency, and high mass resolution capability has made it an important analytical technique.

2. Time-of-flight mass spectrometer : operates on a different principle. TOF in mass spectrometry is a technique in which the full mass spectrum is acquired as snapshots instead of sequentially stepping by the series of m/z values while it is acquiring the data. It separates ions based on their 'flight time' through a field-free region after being accelerated by an electric field of known strength. The time it takes for an ion to reach the detector is dependent on its mass-to-charge ratio,

allowing for high resolution and accuracy. These attributes make it a favoured choice in complex applications like proteomics or metabolomics.

3. TIMS: capture ions within a confined space using electric or magnetic fields. By adjusting the voltage at a specific rate, ions are ejected based on their mass-to-charge ratio, providing a mass spectrum. What sets the ion trap apart is its ability to perform multiple rounds of fragmentation, enabling a detailed examination of complex molecules. This high sensitivity and detailed output make it an ideal choice for intricate tasks like peptide sequencing and structural elucidation of organic compounds.

4. Liquid Chromatography/MS
   Liquid chromatography (LC) coupled to tandem mass spectrometry, called LC-MS/MS (sometimes abbreviated simply as LC-MS), is a powerful technique for the analysis of peptides and proteins. This methodology combines efficient separations of biological materials and sensitive identification of the individual components by mass spectrometry. Complicated mixtures containing hundreds of proteins can be analysed directly even when concentration levels of different proteins vary by orders of magnitude. LC-MS/MS can be used alone or in combination with 1-D or 2-D electrophoresis, immunoprecipitation, or other protein purification techniques.

Identification of protein targets using Mass Spectrometry:

Any human protein can now be identified directly from genome databases based on minimal data derived by mass spectrometry. As in genomics, increased automation of sample handling, analysis, and the interpretation of results have generated an avalanche of qualitative and quantitative proteomic data. Protein-protein interactions can be analysed directly by precipitation of a tagged bait followed by mass spectrometric identification of its binding partners.

Analysis of proteins and proteomes by Mass Spectrometry:

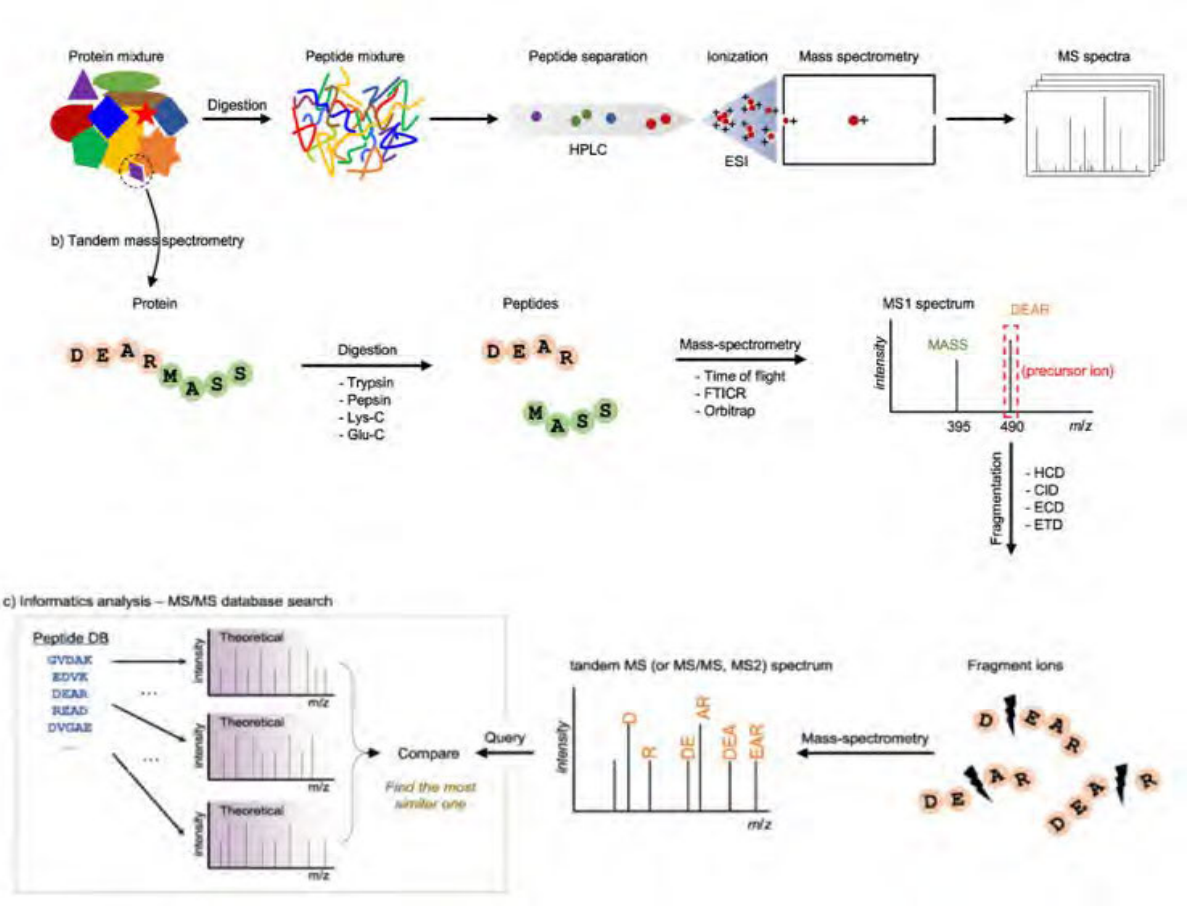


Fig 3.8. Conventional MS-based proteomics experiments for peptide and protein identification. a) Overview of MS-based proteomics. A protein mixture from a biological source is digested into peptides (usually by trypsin). The peptides are separated by one or more steps of high-performance liquid chromatography (HPLC) column and are ionized by electrospray ionization (ESI) at the end of the column. The resulting peptides enter the mass spectrometer and the peptides eluting at the time point are recorded in a mass spectrum (MS1). The peptides can also be ionized using matrix-assisted laser desorption/ionization (MALDI), where the peptides are ionized out of a dry, crystalline matrix via laser pulses. b) Besides a mass list of peptides in MS1 spectra, some prioritized peptides (precursor ions) are fragmented by energetic collision with gas, and the products are recorded in the tandem or MS/MS spectrum. (This figure is the conceptual illustration for a single protein. All peptides from a protein mixture shown in a) are analysed together in single MS run). c) Peptides are most commonly identified using a database search approach, where an experimental MS/MS spectrum is compared with theoretical spectra predicted for peptides from a protein sequence database.

Peptide sequencing by tandem mass spectroscopy

The sequence of peptides can be determined by interpreting the data resulting from fragmenting the peptides in tandem mass spectrometers. In this technique, one peptide species out of a mixture is selected in the first mass spectrometer and is then dissociated by collision with an inert gas, such as argon or nitrogen. The resulting fragments are separated in the second part of the tandem mass spectrometer, producing the tandem mass spectrum, or MS/MS spectrum. With newer instruments, multiple collisions impart energy onto the molecule until it fragments. (This is low-energy fragmentation, in which any single hit is not sufficient to break the peptide bonds. In high-energy fragmentation, the molecules have higher velocity, and a single hit can break bonds)

Advanced TIMS-TOF MS technologies such as trapped ion mobility spectrometry (TIMS) and parallel accumulation serial fragmentation (PASEF) have now allowed for rapid and efficient data acquisition with higher sensitivity and increased coverage of the proteome.

Protein identification by database searching.

A key advance in biological mass spectrometry was the development of algorithms for the identification of proteins by mass spectrometric data matched to a database, originally using a set of peptide masses, and now increasingly using the fragmentation spectra of the individual peptides. Obtaining the complete sequence of a peptide from the tandem mass spectrum was time consuming at best and often impossible. With the availability of the complete sequence of an increasing number of model species, the peptide sequencing problem, formerly a holy grail in biological mass spectrometry, is reduced to a database correlation, enabling automation and the scaling up of proteomics experiments.

<u>Peptide Mass Fingerprinting</u>

In this method, a “mass fingerprint” is obtained of a protein enzymatically degraded with a sequence-specific protease such as trypsin. This set of masses, typically obtained by MALDI-TOF, is then compared to the theoretically expected tryptic peptide masses for each entry in the database.

The proteins can be ranked according, to the number of peptides matches. More sophisticated scoring algorithms take the mass accuracy, and the percentage of the protein sequence covered into account and attempt to calculate a level of confidence for the match. Generally, peptide mass fingerprinting is used for the rapid identification of a single protein component.

Method for Whole exome sequencing:

(Courtesy of the Henry Houlden Laboratory , UCL, Queens Square , United Kingdom)

Genomic DNA was extracted from peripheral blood samples according to standard procedures of phenol chloroform extraction. WES on each proband was performed as described elsewhere (Mencacci et al., 2016) in Macrogen, Korea. Briefly, target enrichment was performed with 2 µg genomic DNA using the SureSelectXT Human All Exon Kit version 6 (Agilent Technologies, Santa Clara, CA, USA) to generate barcoded whole-exome sequencing libraries. Libraries were sequenced on the HiSeqX platform (Illumina, San Diego, CA, USA) with 50x coverage. Quality assessment of the sequence reads was performed by generating QC statistics with FastQC http://www.bioinformatics.bbsrc.ac.uk/projects/fastqc

The bioinformatics filtering strategy included screening for exonic and donor/acceptor splicing variants. In accordance with the pedigree and phenotype, priority was given to rare variants (<0.01% in public databases, including 1,000 Genomes project, NHLBI Exome Variant Server, Complete Genomics 69, and Exome Aggregation Consortium [ExAC v0.2]) that were fitting a recessive (homozygous or compound heterozygous) or a de novo model and/or variants in genes previously linked to central or peripheral demyelination. Mitochondrial genetics was also performed

Thesis Outline

Introduction, background literature, literature review and study design

Chapter 1: Introduction and background literature

- 1.1 Introduction
- 1.2 History of HIV and HIV immunology
- 1.3 Background Literature of CIDP, VRR, autoimmune nodopathies, MNS/MND and MG

Chapter 2: Scoping review of neuromuscular syndromes in HIV

Chapter 3: Thesis aims, broad study design and methodology.

Chapter 4:

A comparison of clinical, electro-diagnostic, laboratory and treatment outcome differences in a cohort of HIV-infected and HIV-uninfected patients with Myasthenia Gravis .

Aim: To determine the clinical features, response to immunosuppressants and antibody profile of HIV-associated MG

Hypothesis: HIV-infected MG present with severe bulbar -respiratory failure and are likely Musk antibody positive due to clinical phenotype.

Chapter 5:

A comparative study of motor neuron syndrome in a cohort of HIV-infected and HIV-uninfected patients.

Aim: To determine the clinical features and response to therapy of HIV-infected patients with MNS and compare the above to HIV-uninfected MND

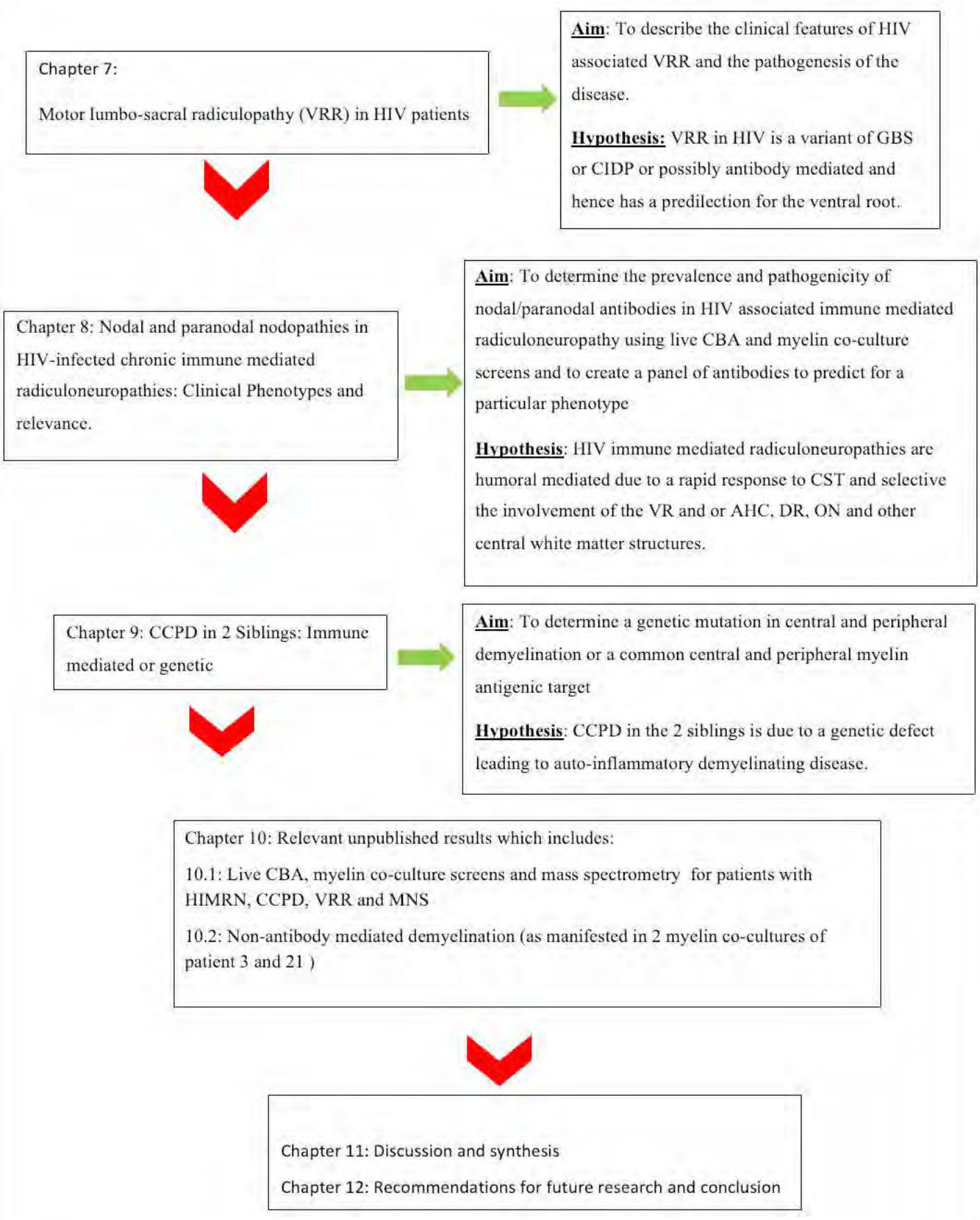
Hypothesis: HIV-associated MNS present with severe symptoms, however, show a prompt recovery with ART suggesting a viral or immune mediated aetiology.

Chapter 6:

A comparison of clinical, electro-diagnostic, laboratory and treatment outcome differences in a cohort of HIV-infected and HIV-uninfected patients with chronic inflammatory demyelinating polyneuropathy

Aim: To characterize and compare HIV-infected CIDP patients to HIV-uninfected patients in terms of clinical presentation, electrophysiology and response to therapy

Hypothesis: HIV-infected CIDP patients have a different clinical presentation and response to therapy compared to the HIV-uninfected cohort and hence a different pathogenesis.


Chapter 7:
Motor lumbo-sacral radiculopathy (VRR) in HIV patients
Aim: To describe the clinical features of HIV associated VRR and the pathogenesis of the disease.
Hypothesis: VRR in HIV is a variant of GBS or CIDP or possibly antibody mediated and hence has a predilection for the ventral root.
Chapter 8: Nodal and paranodal nodopathies in HIV-infected chronic immune mediated radiculoneuropathies: Clinical Phenotypes and relevance.
Aim: To determine the prevalence and pathogenicity of nodal/paranodal antibodies in HIV associated immune mediated radiculoneuropathy using live CBA and myelin co-culture screens and to create a panel of antibodies to predict for a particular phenotype
Hypothesis: HIV immune mediated radiculoneuropathies are humoral mediated due to a rapid response to CST and selective the involvement of the VR and or AHC, DR, ON and other central white matter structures.
Chapter 9: CCPD in 2 Siblings: Immune mediated or genetic
Aim: To determine a genetic mutation in central and peripheral demyelination or a common central and peripheral myelin antigenic target
Hypothesis: CCPD in the 2 siblings is due to a genetic defect leading to auto-inflammatory demyelinating disease.
Chapter 10: Relevant unpublished results which includes:
10.1: Live CBA, myelin co-culture screens and mass spectrometry for patients with HIMRN, CCPD, VRR and MNS
10.2: Non-antibody mediated demyelination (as manifested in 2 myelin co-cultures of patient 3 and 21)
Chapter 11: Discussion and synthesis
Chapter 12: Recommendations for future research and conclusion

Chapter 4

Frontiers in Neurology

A comparative study of MG in a cohort of HIV-infected and HIV-uninfected patients

Kaminie Moodley[1], FCN; Pierre LA Bill[1], FCN; Vinod Bhagu Patel[1], PhD

Affiliation

1. Department of Neurology, University of KwaZulu-Natal, Durban, South Africa

Given the intriguing findings of the scoping review, and previous studies describing MuSK antibodies in the African cohort of MG patients, we retrospectively reviewed MG in HIV-infected patients[166] with view to detecting the proportion of patients who are AchR negative and prospectively testing for other antibodies in HIV-infected MG patients. We speculated that these antibodies are likely influenced by concomitant HIV infection or genetic factors.

Additionally the use of potent immunosuppressant therapy like cyclophosphamide, which is frequently used in our centre for patients in crisis or refractory disease, is poorly described in HIV, except for scant literature from haem-oncology units. Additional modalities of treatment such as IVIG, PE, or B cell depleting therapy is also poorly described in HIV.

The article describes the clinical, electro-diagnostic, serology and treatment outcome differences in these 2 cohorts of patients with the view of prospective studies. These studies will focus on MuSK, agrin, LRP4 or novel antibodies and their IgG subclasses to determine whether there is an IgG1 predominance, as in other HIV related autoimmune diseases such as nodopathies discussed in chapter 7. MuSK antibodies are usually of the IgG4 subclass, therefore it is interesting to prospectively test for antibody subclasses to determine if genetic factors or HIV alone influences IgG subclass switching during sero-conversion.

A comparison of clinical, electro-diagnostic, laboratory and treatment outcome differences in a cohort of HIV-infected and HIV-uninfected patients with Myasthenia Gravis

Kaminie Moodley[1], FCN; Pierre LA Bill[1], FCN; Vinod Bhagu Patel[1], PhD

Introduction and background

Myasthenia Gravis (MG) is an autoimmune disease of the neuromuscular junction, with a reported global prevalence rates of 150-250 cases per million individuals and an estimated annual incidence of 8-10 cases per million person years [238, 239]. This incidence, including South Africa, is similar worldwide[240].

In South Africa, there are eight million people living with human immunodeficiency virus (HIV)[241]. The coexistence of HIV infection with MG occurs uncommonly. The aetiological association between MG and HIV is uncertain. HIV may induce MG, MG may occur coincidentally in an HIV-infected patient or vice versa. However the management of MG in the setting HIV-infection is uncertain. Data is limited to a small case series from South Africa and case reports[9, 242-246].

The use of immunosuppressant drugs including azathioprine and corticosteroids in HIV-infected patients with other neuromuscular diseases has been previously described [247-249] . However the use of rescue therapy, with intravenous immunoglobulin (IVIG), plasma exchange (PLEX) or pulse IV(intravenous) cyclophosphamide for poorly controlled MG in the setting of HIV has been described in 3 cases[246]. The use of these agents, especially IV cyclophosphamide, is of concern in patients who are HIV-infected as they are at risk for opportunistic infections especially those with low CD4 counts. In South Africa tuberculosis (TB) is endemic and the prevalence has doubled in the HIV era[250]. Other opportunistic infections such as candidiasis, aspergillosis, herpes simplex, herpes zoster and toxoplasmosis are additional infective risks.

We aimed to describe the clinical and demographic features of HIV-infected patients with MG, and their response to immunosuppressant therapy in particular PE/IVIG and IV cyclophosphamide.

Methods:

The study was a retrospective chart review of a cohort of patients with MG from the neuromuscular clinic at Inkosi Albert Luthuli Central Hospital (IALCH) in Durban between 2003 and 2019. The study was approved by the University of KwaZulu-Natal (UKZN) Biomedical Research Ethics Committee (ethics number: BE 272/15). This unit provides service to approximately 11 million people. An estimated 19% of the population is HIV positive in the 15-49 years age category and the province of Kwa-Zulu Natal (KZN) has 40% of the HIV burden in SA [241].

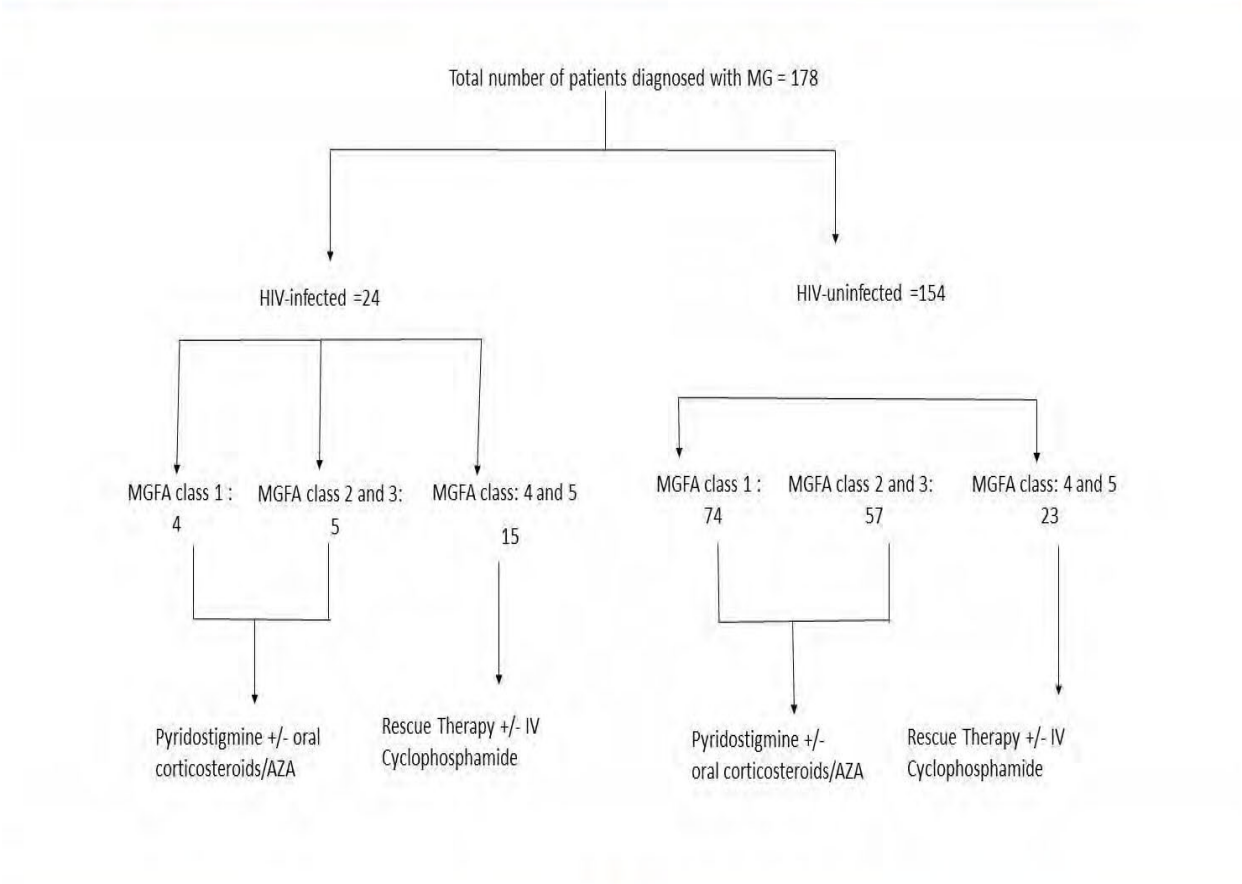
Patients fulfilling the clinical criteria for MG (fatigable, fluctuating weakness) with one or more positive confirmatory tests for MG were included in the study. Confirmatory tests included a positive acetylcholine receptor antibody (AChR-Ab) test, repetitive nerve stimulation study (RNS) showing a decrement of >10% and positive ice pack, neostigmine or edrophonium tests.

Patients were excluded if they did not meet the clinical criteria for MG, or the HIV status was unknown. Data extracted included demographic features, duration, onset and course of the disease, clinical presentation, antiretroviral therapy (ART) in the HIV-infected category. Response to therapy (number of exacerbations and time to minimal manifestation status -MMS), functional recovery scored as Manual Muscle Testing (MMT), MG quality of life (MG-QOL 15) scale, and MG activity of daily living (ADL) was recorded pre-treatment and post treatment at 3 to 6 monthly intervals [251-253]. Adverse events to treatment including opportunistic infections were extracted. Electrophysiological data (RNS), blood tests including AChR-Ab, CD4 counts and viral loads where available, CT chest and histology of the thymus if available were included in the analysis. The tests for MuSK, agrin, LRP4 antibodies are not routinely available in South Africa.

Our current management protocol for mild to moderate MG (including ocular MG), regardless of HIV status is anticholinesterase inhibitors (pyridostigmine) combined with corticosteroids and/or azathioprine as first line therapy. Other immunosuppressant therapy (including IVIG, rituximab, and cyclophosphamide) are added if patients were refractory to or developed side effects to first line therapy. Patients with myasthenic crisis received PLEX or IVIG. Cyclophosphamide was reserved for patients in crisis, refractory to PLEX or IVIG. Four-6 doses of intravenous cyclophosphamide are given at two weekly or monthly intervals at a dose of 500-1000 mg combined with 2-mercaptoethane.Patients were adequately counselled regarding side effects of treatment and informed consent was obtained. The dose and frequency was adjusted at the discretion of the clinician depending on the clinical response and adverse events such as leukopenia, thrombocytopenia or anaemia (neutrophil count of < 1000 cells/µl, lymphocyte count < 500 cells/µl, platelet count < 50cells/µl or haemoglobin <8g/dl respectively) or complicating infections. All patients were screened for existing infection with a full blood count and differential white blood count, urine microscopy and culture and chest X- ray. They were screened for hepatitis B and C prior to being prescribed

methotrexate or rituximab. Maintenance therapy, if required, included pyridostigmine, corticosteroids, used individually or in combination depending on individual patient requirements.
The cohort was divided into two categories of HIV-infected MG and HIV-uninfected MG. Within each category patients were further classified according to severity at presentation (see consort diagram). Data at 1 month, 6 months, 1 year, 3 year and 5 year time points were reviewed.

Consort Diagram for MG



The definitions as per Myasthenia Gravis Foundation of America (MGFA) post-interventional classification [168, 254, 255] used in this study are a) minimal manifestation status (MMS), b) exacerbation**,** c) crisis and d) refractory [168]. In this article combination rescue therapy refers to PE/IVIG with IV cyclophosphamide.

Data Availability:

Anonymized data will be shared by request from any qualified investigator.

Statistics:

Characteristics associated with HIV-infected and HIV-uninfected MG patients were compared using Chi Square tests for categorical variables. Functional scores of ADL, QOL and MMT were initially categorized as positive (> 0) and negative (0). Fisher's exact test was used to compare HIV-infected and HIV-uninfected patients at each time point. Overall functional scores were summarized by medians (IQR) and Wilcoxon rank-sum test used for comparisons at each time point. Because of small numbers geometric means were used for patients receiving combination therapy (PLEX/IVIG + IVI Cyclophosphamide) and t-tests used for comparison. A random effects model including HIV status and time point adjustments for repeated measures was then used to examine trends over time between the two groups. A z-test was used to compare groups and time points. To compare the rate of change, the model was run separately for each group and the beta coefficient for time compared. A Kaplan-Meier (KM) curve was subsequently used to determine if time to remission differed between groups. Follow-up time was used to censor patients not obtaining MMS and truncated at 60 months. A log rank test was used to compare the MMS curves between groups. Chi-squared tests were used in order to determine if confounders such as demographic factors and clinical characteristics (age, race, gender, antibody status, thymic pathology) associated with HIV status influenced outcome. These factors were examined in the group receiving cyclophosphamide and only race was found to be significantly different. Medians of MGADL, MGQOL and MMT score were compared between HIV-infected and HIV-uninfected patients receiving IV Cyclophosphamide at diagnosis, 3 years and 5 years using Wilcoxon rank-sum test. Since all HIV-infected patients receiving cyclophosphamide were black African, the analysis was repeated for black patients only. Stata/IC V1.5 was used for statistical analysis.

Results:

One hundred and seventy eight (178) patients fulfilled the clinical criteria for MG of which 116 (65%) were females. The demographic characteristics of the cohort, including gender, age, and ethnic distribution are detailed in Table 4.1. Twenty-four (13.4%) were HIV-infected. Notable findings in the HIV infected myasthenia gravis group of patients was a younger median age, and a predominance of black females. HIV infection preceded the development of myasthenia gravis in the majority of HIV infected patients. One patient (4%) became HIV-infected several months after MG was diagnosed. Viral loads and CD4 counts are listed in table 4.1. Thirteen (54.2%) of the HIV-infected cohort were on efavirenz, tenofovir, emtricitabine before the diagnosis of MG. Six patients on ART had undetectable viral loads. Ten (41.8%) patients were diagnosed with HIV-infection at the time of MG and were later commenced on ART. Six (60%) patients received ART while in ICU and 4 (40%) a month after discharge.

Sixty two (40%) HIV-uninfected patients had T2 Diabetes Mellitus (T2 DM) and 10 had other autoimmune disorders whereas only one HIV-infected patient had Lambert-Eaton myasthenic syndrome (LEMS). The diagnosis of combined LEMS and MG was based on clinical findings, positive high frequency repetitive stimulation studies and positive AChR-Ab and voltage gated calcium channel antibody [256].

Table 4.1: Demographic characteristics of HIV-infected and HIV-uninfected patients with MG

	HIV-infected (n=24)	HIV-uninfected (n=154)	P value
Age (median, IQR))	34(27.5-41)	47.11 (32-65)	0.001
Female	19(79.2)	97(63)	0.12
Race (%)			<0.001
Black	23(95.8)	67(43.5)	
Indian	1 (4.2)	65 (42.2)	
White	0 (0)	20 (13)	
Mixed Ancestry	0 (0)	2(1.3)	
Autoimmune disease			<0.001
Diabetes Mellitus		62 (40)	
RA		2 (1.3)	
LEMS	1 (4)	0 (0)	
Dermatomyositis		2 (1.3)	
Hashimoto's thyroiditis		6 (3.9)	
Time of diagnosis of MG in relation to HIV infection (%)			
After HIV	13 (54.2)		
At the same time as HIV	10 (41.6)		
Before HIV	1 (4.2)		
CD4 count at diagnosis (cells/µl) (median, IQR)	390 (186-461)		
Viral Load at diagnosis (copies/ml) (median, IQR)	17222 (9154-98100))		

LEMS=Lambert Eaton Myasthenic Syndrome, RA=Rheumatoid arthritis, HIV=human immunodeficiency syndrome, MG=myasthenia gravis

Clinical findings at diagnosis are listed in Table 9.2. Bulbar, respiratory, and limb muscle weakness was more prevalent HIV-infected MG group. Signs of ocular muscle weakness were more common in the HIV-uninfected MG cohort. The HIV- infected MG cohort were more severely affected, with 12/24 patients (50%) requiring ventilation at presentation and exacerbations, including MG crisis were commoner.

Table 4.2: Clinical Profile of HIV-infected and HIV-uninfected patients with MG

	HIV-infected (n=24)	HIV-uninfected (154)	P Value
Clinical Presentation			<0.001
Ocular muscle weakness	20 (83.3)	118 (76.6)	0.306
Bulbar muscle weakness	12 (50)	48 (31.1)	<0.001
Respiratory muscle weakness	14 (60.9)	26(16.9)	<0.001
Skeletal muscle weakness	21 (87.5)	80 (51%)	<0.001
MGFA Grade at presentation (%)			
1	4(16.7)	74(48.1)	0.001
2a/2b	3 (12.5)	35(22.7)	
3a/3b	2 (8.2)	22(14.2)	
4b	3 (12.5)	9 (5.8)	
5	12 (50)	14 (9.1)	<0.001
Number of exacerbations during follow up			0.02
<5	6 (25)	85 (55.6)	
5-10	10 (41.7)	65 (42.2)	
>10	8 (33.3)	4 (16.2)	
≥ 1 crises	6 (25)	7 (4.5)	

MG=Myasthenia Gravis, MGFA=Myasthenia Gravis foundation of America

Investigations, treatment and outcome are listed in table 6.3. Fifteen (62%) of the HIV-infected cohort were AChR-Ab positive compared to 124 (80.5%) of the HIV-uninfected cohort, p = 0.047. There were no significant differences between the two cohorts with respect to the radiological and histological findings of the thymus.

In the HIV-infected MG cohort on PE/IVIG and IV cyclophosphamide, the median CD4 count at diagnosis was 110 cells/µl (IQR 96-193cells/ µl) and 88 cells/µl (IQR 68-108 cells/ µl) 2 weeks after cyclophosphamide. The median viral load at diagnosis was 47481 copies/ml (IQR 45545-119292 copies/ml). HIV viral loads were not repeated after cyclophosphamide unless indicated.

With regard to treatment, 13/24 (54%) of the HIV-infected MG group required rescue therapy using either PE or IVG combined with pulse IV cyclophosphamide compared to 17/154 (11%) in the HIV-uninfected cohort. Maintenance therapy was not significantly different between the two groups.

At 5 years, after correcting for baseline severity of disease, 8 (33%) of the HIV-infected group remained refractory to treatment and 16 (64 %) obtained MMS compared to 10 (6.5%) and 144 (93 %) in the HIV-uninfected cohort respectively

Table 4.3: Investigations, treatment and clinical status at follow up in the HIV-infected and HIV-uninfected cohort

	HIV-infected (24)	HIV-uninfected (154)	P Value
Investigations			
Positive AChR Ab Test (%)	15(62)	124 (80.5)	0.047
Positive Ice Pack Test (%)	18 (75)	136 (87)	0.11
Positive Edrophonium Test (%)	24(100)	115 (74.6)	0.12
Decremental response of >10% on RNS Test (%)	20 (83)	82 (52)	0.005
CT Thymus			0.47
Normal	7 (29)	65 (42)	
Hyperplasia	12 (50)	62 (40)	
Mass Lesion	5 (21)	27 (18)	
Thymectomy	20 (83)	97 (63)	0.27
Histology			
Thymic Hyperplasia	15 (75)	73 (75)	
Thymoma	3 (15)	23 (23)	
Thymolipoma	1 (5)	1 (1)	
Thymic Carcinoma	1 (5)	0 (0)	
Treatment			
Rescue /Induction Therapy			
IVIG only	1 (4.2)	2 (1.2)	
PE only	1 (4.2)	3 (2)	
PE or IVIG + IV Cyclophosphamide	13 (54)	17 (11)	0.001
IV Neostigmine	13 (54)	21 (14)	
Maintenance Therapy			
AZA/Oral Pyridostigmine and prednisone	20 (84)	118 (77)	
Rituximab	0 (0)	1 (0.6)	
Oral Pyridostigmine only	4 (16)	36 (24)	
Side effects of Immunosuppressive Therapy			
Opportunistic infections	0 (0)	0 (0)	
Haemorrhagic Cystitis	0 (0)	2 (11.7)	
Clinical status at 5 years follow up			
Minimal manifestation status (0-3)	16 (64)	144 (93)	<0.001
Refractory Disease	8 (33)	10 (6.5)	<0.001

AChR Ab =Acetylcholine receptor antibodies, RNS=Repetitive nerve stimulation, IVIG=intravenous immunoglobulin, PE=plasma exchange, IVI=intravenous, AZA=Azathioprine

Figure 4.1: Functional scores and time to minimal manifestation status for the HIV-infected and HIV-uninfected cohort

Fig 4.1A:

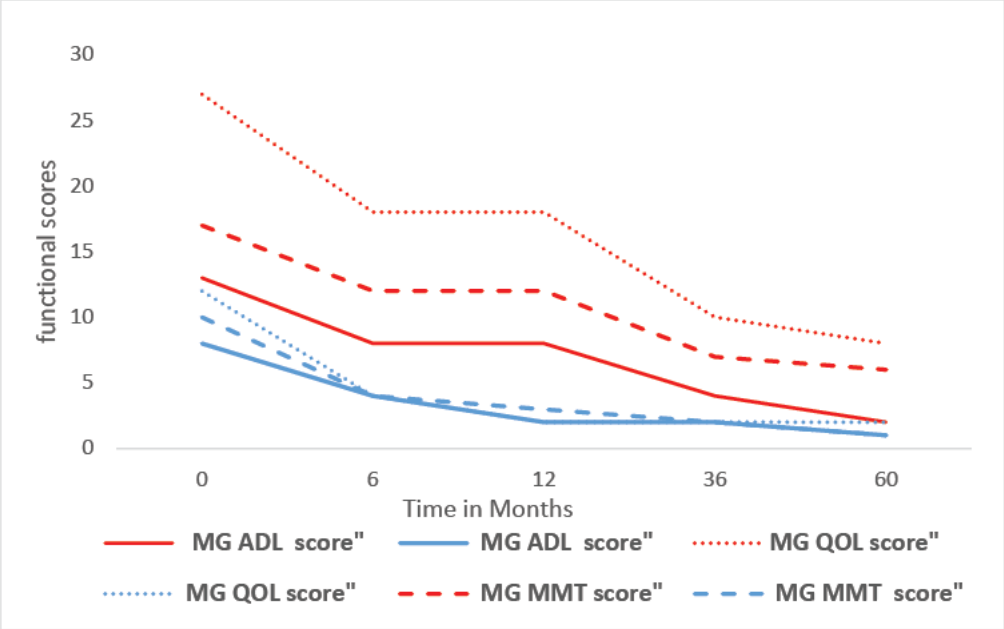
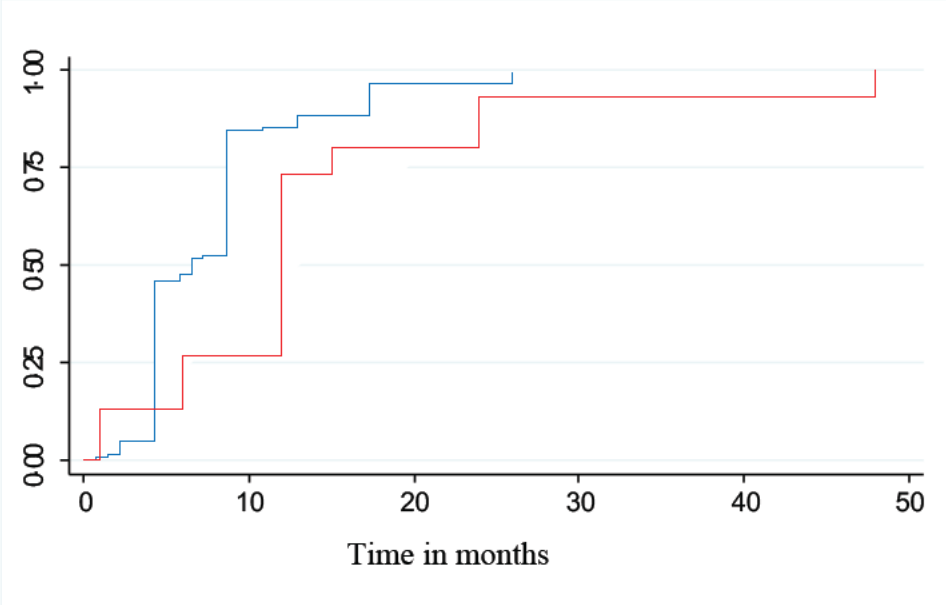


Fig 4.1B



A=Functional scores over time, B= Kaplan Meier Curve of Time to Remission,

MMT=Manual Muscle Testing, ADL=Activity of daily living, QOL=Quality of life, ---- = HIV-uninfected, ---- = HIV-infected

Figure 4.1 shows functional scores and time to MMS over 60 months in the entire HIV-infected and HIV-uninfected cohort. The median MGADL, MMT, MGQOL (Figure 4.1A) functional scores are significantly higher in the HIV-infected cohort, p= <0.001 compared to the HIV-uninfected cohort. The above scores decrease significantly over time with no statistical difference in the rate of decrease between cohorts. Figure 4.1B shows the KM curve for the entire cohort. One hundred and sixty (89.8%) of 178 patients obtained MMS over a median of 72 months (IQR 48-126 months). Within a median time of 9 months (95% CI: 6 – 12 months), 144 of 155 (93.6%) HIV-uninfected patients obtained remission, while 16/23 (64%) of the HIV-infected group obtained remission in a median time of 12 months (95% CI 6-15 months). The probability of obtaining remission was significantly greater in HIV-uninfected cohort compared to HIV-infected cohort (OR 7.25; 95% CI: 2.5-21.0), p < 0.001. The Kaplan-Meier curves of time to remission differed significantly by HIV status, p = 0.0007, (log rank test of equality of time to remission). This indicates that the time to obtaining remission was significantly shorter for HIV-uninfected patients than HIV-infected patients.

Figure 4.2 shows the functional scores and time to MMS in the HIV-infected MG and the HIV-uninfected MG receiving PLEX/IVIG and cyclophosphamide. The mean MMT, MG-QOL, MGADL (Fig 4.2A) scores are significantly higher in HIV-infected MG compared to HIV-uninfected MG. Over 5 years, the scores decrease significantly, the rate of decrease is significantly greater in the HIV-uninfected MG compared to HIV-infected MG (p = 0.0014, 0.0018, 0.001 respectively). Figure 4.2B reflects the KM curve for those who received IVIG or PLEX with cyclophosphamide. HIV-uninfected patients (median follow-up 72 months, IQR: 48-192 months) had a similar follow up time to HIV-infected patients (median follow up 60 months, IQR 12-72months), p = 0.08. Of the 30 patients on the above combination therapy, 13/17 (76.5%) in the HIV-uninfected category obtained MMS compared to 5/13 (38.7%) in the HIV-infected category (OR 4.3, 95%; CI: 0.7-30.6) but this did not reach statistical significance, p=0.13. The median time to MMS for the 13 HIV-uninfected patients was 15 months (IQR 12-36 months) and for the 5 HIV-infected patients was 24 months. This was not statistically significant (p=0.68).

Figure 4.2: Functional scores and Kaplan-Meier time to minimal manifestation status for the HIV-infected (13 patients) and HIV-uninfected (17 patients) cohort receiving PLEX/IVIG and IVI Cyclophosphamide

Fig 4.2A

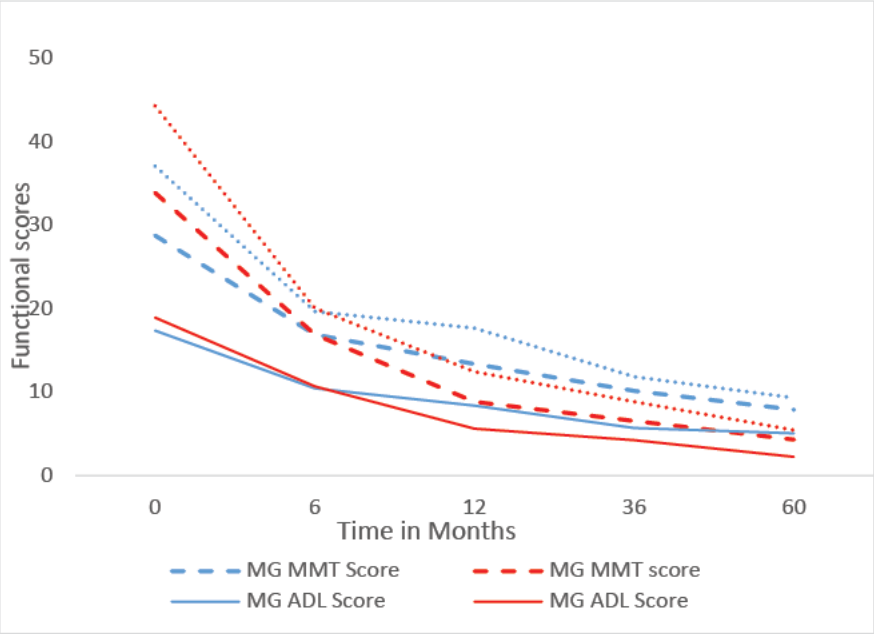
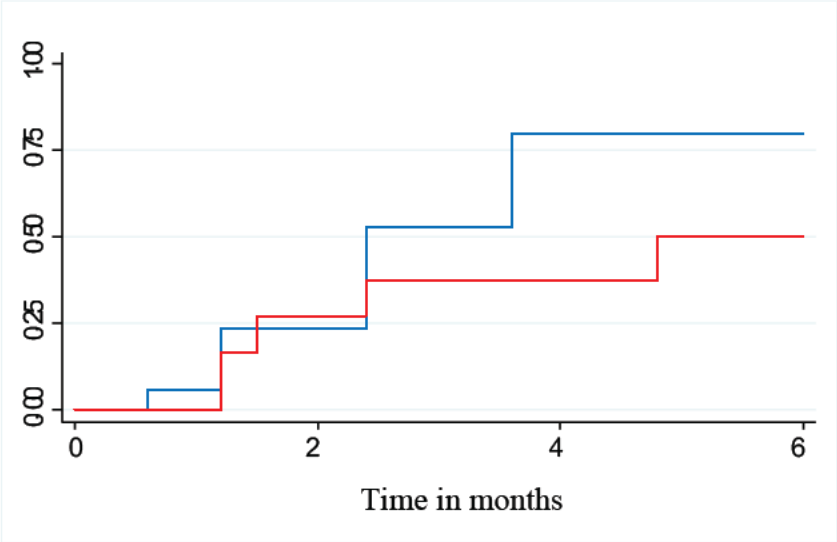


Fig: 4.2B



A=Functional scores over time, B= Kaplan Meier Curve of time to remission, MMT=Manual Muscle Testing, ADL=Activity of daily living, QOL=Quality of life ----- = HIV-infected,
----- = HIV-uninfected

Confounding factors such as age, gender, thymic pathology and antibody status were similar in both cohorts receiving IVI cyclophosphamide except for race. Since all HIV-infected patients receiving cyclophosphamide were black African, the analysis was restricted to black patients only. However, there were no significant differences in outcome in the black only groups.
No adverse events were documented in patients receiving combination rescue therapy (PLEX or IVIG + IVI cyclophosphamide) in particular there were no opportunistic infections in the HIV-infected category. In the HIV-uninfected category 2 patients experienced haemorrhagic cystitis.

Discussion

In the above study, significant differences are that a) HIV-infected MG patients were more likely to be young, black females. This is consistent as MG is commoner among young females and that HIV is more prevalent among young black South African females, b) HIV-infected MG presented with more severe generalised disease as evidenced by higher MGFA grades and functional scores (MMT, MGQOL, MGADL) and were more likely to present with bulbar-respiratory failure requiring ventilation. This category of patients were managed following the treatment protocol for HIV-uninfected MG patients which includes, respiratory-bulbar support, rescue therapy using either IVIG or PLEX and IV cyclophosphamide if required, followed by maintenance therapy. The use of a neostigmine infusion in patients admitted to ICU or a high care setting was useful for symptomatic control of the disease, c) the rate of functional improvement was significantly lower, time to MMS was longer and response to therapy (number of exacerbation or crises) higher in the HIV-infected cohort compared to the HIV-uninfected cohort. Time to MMS and side effect profile for the group on combination rescue therapy (IVI cyclophosphamide/PLEX/IVIG) was not statistically different in both categories. Patients with milder disease were managed with oral immunosuppressant therapy (AZA and corticosteroids and pyridostigmine) with similar outcomes in both categories.

The safe use of IV cyclophosphamide is not well described in the setting of HIV-infected MG. Clinical guidelines on safe and effective prescription of ARTs with concomitant cytotoxic immunosuppressive agents is limited, except in the setting of AIDS-related B cell lymphoma [257, 258] Bone marrow reserve, drug-drug interactions with ART, prophylaxis for mycobacterium, fungal and viral infections and the use of granulocyte-colony stimulating factor when complicated by bone marrow suppression are all considerations in the management of HIV-infected patients with MG. Standard treatment protocols from haem-oncology centres treating AIDS related B cell-lymphoma may lend guidance. This study suggests that using the protocol listed in the methods sections of the article, regardless of the CD4 count or viral load may be safe, provided patients are carefully monitored. None of the patients received prophylaxis for opportunistic infections or granulocyte stimulating factor. As expected, CD4 counts decreased following cyclophosphamide administration with no documented clinical infection. If severe neutropenia was observed, IV cyclophosphamide was

withheld until the neutropenia recovered spontaneously. The use of prophylactic drugs for opportunistic infections or granulocyte stimulating factor may be of value in this setting.
Various autoimmune diseases occur between acquisition of HIV infection and clinical AIDS. These include systemic lupus erythematosus, rheumatoid arthritis, idiopathic thrombocytopenic purpura and neurological conditions such as AIDP, CIDP and inflammatory myositis [259]. The mechanism resulting in autoimmune disease in HIV infected individuals are poorly understood, although B lymphocyte dysfunction and molecular mimicry between HIV proteins and autoantigen may play a role.

MG, including MuSK related MG, occurring during immune reconstitution has been reported[243, 260, 261]. Ten patients in our cohort who were commenced on ARTs after the diagnosis of MG showed transient worsening of their MG in the initial stages of commencing ARVs. This is supported by similar findings reported by Heckman et al[246]. This worsening may coincide with the first wave of immune reconstitution. Several immune factors may act in synergy during immune restoration resulting in exacerbations. Factors include pathogenic T cell response derived from the memory T cell pool, a Th1-type CD4 and CD8 response, loss of function of T regulatory cells and overproduction of IL21 and IL6.This may result in expansion of the autoreactive repertoire and immune aggression [262, 263]. Loss of central tolerance, which is pathognomonic of MG, may occur in HIV. Tropism of HIV to the thymus and antigenic mimicry between normal thymic components and core p17 and p24 protein of HIV have been described [264]. McCune reported that the receptors for HIV are present on 90% of all thymocytes and intrathymic macrophages. Although theoretically thymic atrophy is expected in HIV, 75% of the HIV-infected cohort had thymic hyperplasia, 5% thymoma, thymolipoma and thymic carcinoma. One may speculate that with HIV infection, chronic peripheral T cell lymphopenia (median CD4 count 190) may result in thymic rebound and upregulation of thymopoeisis.
In the above cohort, the HIV-infected MG patients with severe bulbar-respiratory failure were more likely to be AChR-Ab negative or have a very low titre of AChR-Ab. This may support an anti-MuSK pathogenesis, or an unknown antibody compared to those with antibodies to AChR, agrin or LRP4 [265-267]. There are 6 reported cases of HIV-associated MuSK antibody MG, documented in the scoping review[233, 261, 268-270] . MuSK Ab+ve MG in HIV-uninfected patients are treatment refractory with severe bulbar respiratory failure and are usually of the IgG4 subtype [266, 271]. The association between MuSK and the low-density lipoprotein receptor-related protein 4 (LRP4) results in a tetrameric complex on the postsynaptic membrane. This complex is phosphorylated by agrin and is essential for AChR clustering [272]. African patients with bulbo-respiratory dysfunction are likely to be MuSK positive[166].Whether this bulbo-respiratory phenotype is influenced by HIV or genetic factors alone is unknown as the HIV-status was not reported in this study[166]. As in nodopathies (Chapter 8), MuSK antibodies in HIV are possibly IgG1 due to impaired class switching. However, this requires further testing with prospective basic science studies and long term follow-up studies screening for the

above antibodies namely agrin, LRP4 and MuSK and their IgG subclass to draw reliable conclusions in HIV-infected patients at different stages of HIV[271]. Future testing for MuSK antibodies or identifying a new antibody will be useful.

Specific antibody testing when using a broad immunosuppressant such as cyclophosphamide is irrelevant until more specific therapies are available. Future studies, which include antibody panels and targeted use of monoclonal antibodies like Rituximab in HIV will be useful. One HIV-uninfected patient with refractory MG received Rituximab with good results. The use of Rituximab in HIV-infected MG is limited to case reports [246, 270] The use of B-cell depleting agents, PLEX and other immunosuppression in HIV MG is not well described. The use of Rituximab in HIV-infected MG is limited to case reports. [246, 270] Kuntzer described successful use of Rituximab in a patient with immune reconstitution bulbar onset MuSK MG[270]. Our study is the first to document the safe use of IV cyclophosphamide in the setting of HIV-infected MG. Clinical guidelines on safe and effective prescription of ARTs with concomitant cytotoxic immunosuppressive agents is limited, except in the setting of AIDS-related B cell lymphoma [257, 258]

Limitations of the study is that it is a retrospective, hospital based study and therefore patients may have been missed and results may not be generalizable to the community. One can only draw broad general conclusions regarding the outcome and side effect profile in the 2 groups due to the discrepancy in numbers which makes statistical comparison difficult. More symmetrical sample sizes with randomised controlled studies are needed to measure reliable response to therapy and properly assess the safety profile of IVI cyclophosphamide in HIV-infected patients with respect to infections and bone marrow suppression. However, this is not possible due to the low frequency of myasthenia gravis in the context of HIV. No infections were documented despite regular follow-up and screening. This may reflect a type 2 statistical error as it is possible that patients with minor infections were not reported or were managed elsewhere. No confounding factors were found to be significant between the HIV-infected and HIV-uninfected groups except for race. Hence the analysis of outcome was adjusted for race only and multivariate analysis was deemed unnecessary.

Conclusion

HIV-infected MG patients present with more severe bulbar-respiratory signs requiring supportive care in ICU. This study suggests that immunosuppressive drugs, including IV cyclophosphamide, may be safe and efficacious in HIV-infected MG. Prospective studies of MuSK or new antibodies, immune function studies, and possibly thymic histology maybe valuable to explain the pathogenesis of MG in HIV and perhaps segregate alternate therapeutic avenues. This will allow for the establishment of treatment guidelines of MG in HIV.

Author Contributions:

KM developed the concept, collected and analysed the data and generated the manuscript, VBP and PB peer reviewed the manuscript. All authors approved the final manuscript.

CHAPTER 5

Journal of Neurological Science

A comparative study of motor neuron disease in a cohort of HIV-infected and HIV-uninfected patients

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12.1 SUMMARY

Given the encouraging results of the previous publication, we undertook a retrospective study HIV-associated MNS. To assess the above in a meaningful way we compared MND in HIV-uninfected patients to HIV-infected patients who met the clinical and electrophysiological El-Escorial criteria for MND. The viral theory of MND has been rejuvenated in the last 5 years. It is now recognised that enteroviruses similar to polio viruses can persist and induce immune-mediated diseases which may occur long after the viral infection has cleared. Human retro-viruses such as HIV and HTLV1 has been implicated in a number of cases of MNS (see scoping review and background literature). Human endogenous retroviruses (HERVs) are ancient microbial remnants that are integrated into our chromosomes during repeated infections that occurred over several million years of our evolution. They are usually said to be “harmless, junk DNA.” However in 2011, Avindra Nath et al reported that HERV-K is highly concentrated in the brain tissue of patients who died of ALS [162]. Similarly Bowen et al speculated that patients with a HIV-infected MNS may also have high levels of CSF HERV-K[159]. This led us to explore the concept of HIV-infected MNS and to speculate on a common viral pathogenesis in both HIV-associated MNS and MND.

However, an immune mediated pathogenesis, as in PM LSP, which is also a pure motor syndrome described in the subsequent chapter remains a possibility. This may be related to antibodies targeting anterior horn cells in the spinal cord or robust CTL responses in advanced HIV. Therefore, a small cohort of patients with HIV-infected MNS were also tested for nodal/paranodal and ganglioside antibodies and their serum added to sensory neuron co-cultures to further explore potential immune mediated causes. The results are included in the prospective studies in chapter 8. The findings of our study and its limitations are outlined in the published manuscript

Introduction and background:

Several human immunodeficiency virus (HIV) associated neuromuscular syndromes have been described namely symmetrical polyneuropathies [3], inflammatory demyelinating polyneuropathies [247], inflammatory myopathies [273], neuromuscular junction disorders such as myasthenia gravis [242] and pure motor syndromes such as motor neuron syndrome (MNS) and pure motor lumbo-sacral radiculopathy (PMLR) [6, 139].

In HIV-uninfected patients, motor neuron disease (MND) is a progressive neurodegenerative disease with mortality exceeding 90% within 5 years [139]. The clinical presentation of MND is diverse and includes amyotrophic lateral sclerosis (ALS), primary lateral sclerosis (PLS), progressive bulbar palsy (PBP) and progressive muscular atrophy (PMA)[140]. Various genetic mutations have been described in sporadic and familial MND which include C9orf72, TARDBK, SOD1, TBK1, NEK1 [137, 148] . MND may be an oligogenic disease and in addition to "causative" genes various "other genes" have been reported to modify the phenotype[137, 138]. Environmental factors such insecticides, mechanical trauma, smoking, viruses and epigenetic factors may have a role in MND pathogenesis[137, 138]. Various viruses including the human endogenous retrovirus type-K (HERV-K) and other retroviruses have been implicated in MND[154, 155, 157, 158, 160, 274, 275].

MNS is an uncommon neurological complication of HIV-infection limited to few reported cases. [159, 186, 276, 277] HERV-K may have an important aetiological association in both HIV-infected MNS and MND [154, 155, 157-160, 186, 274-278] . Therefore combined genetic and epidemiological studies are required to better understand the viral or immune basis of HIV-infected MNS. The combination of environmental and epigenetic factors may be pivotal in the development of MND. In this article, we describe and compare the outcomes of 35 HIV-infected patients with MNS and 101 HIV-uninfected patients with MND.

Methods:

This study is a retrospective chart review, using ICD-10 codes for MND/S from 2003 to 2017. The study was carried out at the Department of Neurology, Inkosi Albert Luthuli Central Hospital (IALCH), Durban, South Africa.

Standard Protocol Approvals:

Ethical approval was obtained from the Biomedical Research Ethics Committee at the University of KwaZulu-Natal (KZN), ethics number BE272/15. Although not required as the study was a retrospective chart review, telephonic consent was obtained from patients who were alive and contactable.

Inclusion criteria were patients with pure motor syndromes with known HIV status, who fulfilled clinical and electrophysiological EL Escorial criteria for motor neuron disease and included those who had a progressive pure LMN presentation not attributable to any other aetiologies and therefore most likely represented PMA [144].

Exclusion criteria were unknown HIV status, pure motor neuropathies attributable to other aetiologies such as multifocal motor neuropathy with conduction blocks, spinal muscular atrophy, infective or inflammatory conditions such as viral infections (HTLV1, HSV, CMV, enteroviruses, and echo viruses), tuberculosis, syphilis, malignancies (myeloma), paraneoplastic radiculopathies, connective tissue diseases and endocrine disorders such as insulinoma and hyperparathyroidism. Patients with HIV-associated ventral root radiculopathies,(criteria for PM LSP discussed in chapter 5 and 7) were excluded from the study. From our local experience and according to Benatar et al HIV associated PM LSP may represent a distinct clinical entity [5]. These patients present with ventral root enhancement on MRI and respond to corticosteroid therapy making MNS/MND less likely [6]

All patients (MND/S) were screened for the above diseases using blood tests (including ganglioside antibodies, paraneoplastic antibodies, insulin levels, parathyroid hormone), CSF (CMV, HSV, HTLV1, enteroviruses, echoviruses PCR, gene expert for TB, FTA) , genetic testing for spinal muscular atrophy where clinically indicated, electrophysiological tests and appropriate radiological investigations. The above blood, CSF, electrophysiological and radiological investigations are standard of care routine investigations done on all patients with suspected MND/S and not done for research purposes only. This is because our neurology unit has a high burden of HIV and CNS tuberculosis and exclusion of mimics of MND is imperative especially infective/inflammatory disorders.

All patients had electrophysiological tests done. This included nerve conduction studies of all four limbs and EMGs of at least three of the following regions that is lumbo-sacral, thoracic, cervical and bulbar. All HIV-infected patients had contrast administered during MRI spine and brain to exclude infective/inflammatory lesions. HIV-uninfected patients had un-contrasted MRI imaging of the brain and spine. Contrast was administered at the discretion of the radiologist if any suspicious lesion was identified.

Information was extracted from patient records and longitudinal information was obtained by contacting surviving patients or their relatives. Information extracted included demographic features, onset, duration and progression of disease, medical co-morbidities clinical features regarding limb weakness, fasciculations, bulbar symptoms, sensory symptoms, ALSFRS-R[279] at diagnosis and 6 monthly intervals up to 18 months (or longer if available) , CD4 counts, viral load, CSF, blood results , MRI and response to therapy, which included antiretroviral therapy (ART) or corticosteroids.

HIV-infected patients with MNS were compared to HIV-uninfected patients with MND for differences in demographic data, onset and progression of disease, CSF findings, electrophysiological and radiological findings and response to therapy (ART or corticosteroids).

The primary aim of the study was to determine if there is a difference in survival outcomes between HIV-infected MNS patients and HIV-uninfected MND patients and to determine the effect of ART on disease progression. This was done by assessing rate of disease progression at six-monthly intervals up to 18 months using the ALSFRS-R scores and correlating these scores with CD4 counts and viral

loads at 6 monthly intervals. Secondary aims included demographic, clinical, radiological and CSF differences between the two categories.

Data Availability:

Anonymized data will be shared by request from any qualified investigator.

Statistical Analysis:

Data was entered in Microsoft Excel and analysed using Prism Software. Descriptive statistics such as percentages, inter-quartile ranges (IQR), medians and p-values were used to summarise the results in the HIV-infected and HIV-uninfected categories. A subgroup analysis was also performed among black patients with MND and MNS. Tests used to calculate the above included: Fisher Exact Test for categorical variables, Mann-Whitney U Test for continuous variables, Spearman Test for correlation between CD4 counts, viral loads and functional scores. A p-value of < 0.05 was regarded as being significant.

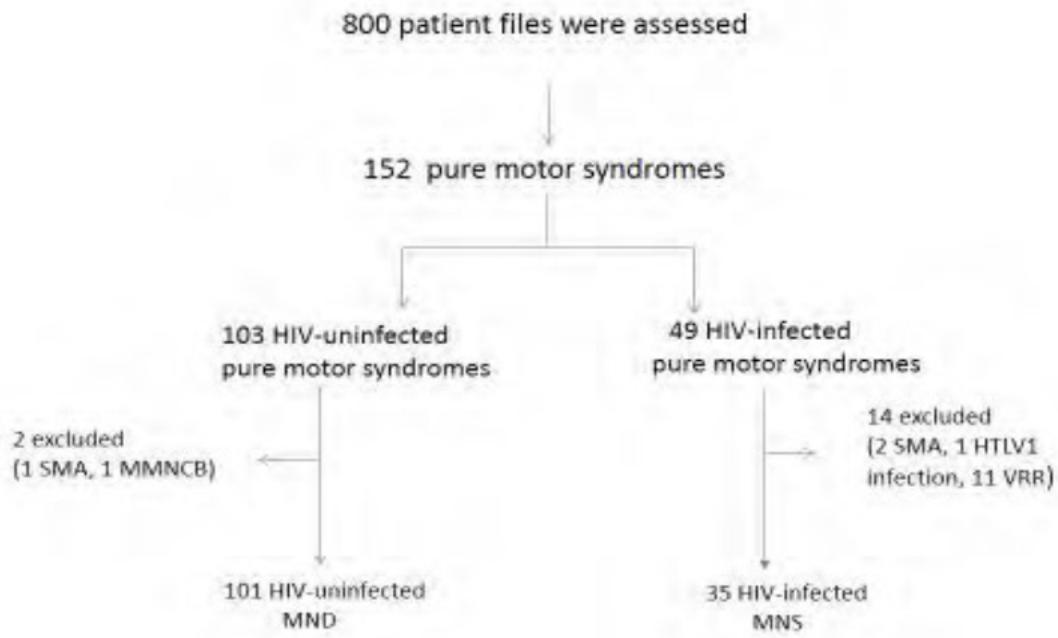
Indirect standardization was used to compare the study population to KwaZulu-Natal and adjust for differing age distributions and race in the HIV-infected and HIV-uninfected population. The age and race specific MND/S prevalence rates/100 000 were estimated from the mid-year population of KwaZulu Natal 2017 [42, 280, 281] . A standardized mortality ratio (SMR) was calculated from the sum of expected number of deaths in each stratum using the total KZN population. The Kaplan-Meier curve expressed survival in both categories up to 120 months.

Results:

Demographic features:

One hundred and fifty two patient charts were reviewed. Sixteen patients were excluded from the study, 14 in the HIV-infected category and 2 in the HIV-uninfected category. In the HIV-infected category, one patient had HTLV1 infection, two had genetically proven spinal muscular atrophy (SMA) and 11 patients had steroid responsive ventral root radiculopathies. In the HIV-uninfected category, one patient had genetically proven spinal muscular atrophy and one patient had multifocal motor neuropathy with conduction block with positive GM1 antibodies. (figure 5.1).

Figure 5.1. Consort Diagram describing data analysis



HIV= Human Immunodeficiency Virus, SMA=Spinal Muscular atrophy, MMNCB=Multifocal motor neuropathy with conduction blocks, HTLV1=Human T cell lymphocytic virus type 1, PM LSP=pure motor lumbar-sacral polyradiculopathy, MND=Motor Neuron Disease, MNS=Motor Neuron Syndrome

category was 66 (IQR 57-74) and in the HIV-infected category 41 (IQR 33-45). Thirty three (94%) of the HIV-infected patients were less than 50 years of age whereas 91 (91%) of the HIV-uninfected patients were over 50 years at the time of presentation. Racial categories in the HIV-infected category included 33 (94%) Black African, 1(3%) White and 1(3%) Indian, whereas in the HIV-uninfected category 39 (39%) were Black African, 21(21%) White and 41(41%) Indian. This equates to MND race adjusted prevalence rates/100000 in KZN of 0.44 among Black Africans, 4.1 among Indians and 4.4 among Whites. The MNS race adjusted prevalence rates/100000 in KZN were 2.4, 10 and 40 for Black Africans, Indians and Whites respectively[280, 281]. Age standardization in the MNS category showed highest prevalence rates/100 000 between the age groups 40-44 years, 45-49years which was 2.8 and 3.77 respectively whereas in the MND category the age adjusted prevalence rates/100000 over the age of 65yrs ranged from 8-12[280-282]. Similar age categories were compared for the Black African sub-analysis only. See table 5.3.Comparisons between the other 2 race groups were not done as numbers were too small for any valuable comparison.

Clinical Presentation:

All patients in this study had sporadic MND. The median time from onset of motor symptoms to presentation was 15 months in the MND group and five months in the MNS group. In 10 HIV-infected patients, MNS was the presenting illness of HIV. Seventeen (49%) of the HIV-infected category presented with a combination of quadriparesis and bulbar-respiratory symptoms compared to 18 (18%) of the HIV-uninfected category. This correlated to the lower median ALSFRS-R score (median 28) in the HIV-infected category compared to HIV-uninfected category (median 44). Twenty seven patients (78%) in the HIV-infected category presented with ALS, 8(22%) with progressive bulbar palsy, and 0 (0%) with PLS or PMA. In the HIV-uninfected category 52 patients (52%) presented with ALS, 36 (36%) with PBP, 23 (23%) with PMA. Nineteen (55%) of HIV-infected MNS had symmetrical signs at onset, and 16 (16%) in the MND category

Electrophysiology:

The electrophysiological findings are summarised in table 5.2 (Online supplementary data). All patients had preserved sensory nerve action potentials (SNAPS). Fifteen(15%) of HIV-uninfected patients and eleven(32%) of HIV- infected patients had sural sensory amplitudes 50%-75% below the lower limit of normal, adjusted for age normative values. Seventy five percent (76/101) of HIV-uninfected patients and 100% (35/35) of HIV-infected patients had fasciculations on EMG. Thirty-nine (89%) HIV-infected patients had thoracic para-spinal denervation and 29(82%) had tongue fasciculations.

Table 5.1: Significant demographic, clinical, electrophysiological and CSF differences between Black African patients with MND and MNS (pV<0.05)

	HIV-infected MNS (N=33)	HIV-uninfected MND (N=39)
	N (%)	N (%)
Age		
Median (IQR)	41 (33-44)	63 (54-74)
< 50 years	31 (84%) *1.66	6 (15%) * 0.36
50-64 years	2 (12%) *0.89	15 (38%) *1.94
65-90 years	0 (0%) * 0	18 (46%) *3.9
Sex		
Male	18 (31)	21 (54)
Median time from onset of disease to presentation (months)		
Months (IQR)	5 (2-6)	22 (12-25)
Clinical Presentation at Diagnosis		
Quadriparesis + Respiratory Bulbar symptoms	16 (67)	8 (33)
ALS scores at presentation		
Median (IQR)	26 (24-28)	43 (41-44)
Symmetry at onset	11 (33)	4 (10)
Medical comorbidities		
T2 Diabetes Mellitus	1 (3)	7 (18%)
Hypertension	1 (3)	5 (13%)
Electrophysiology		
Fasciculations	33 (100)	23 (58)
Thoracic Paraspinal denervation	30 (91)	20 (51)
Tongue fasciculations	27 (81)	10 (25)
CSF		
Lymphocytes (cells/ul)	8 (4-12)	0 (0-0)
Polymorphocytes (cells/ul)	0 (0-2)	0 (0-0)
Protein (g/dl)	0.78 (0.4-0.9)	0.38 (0.3-0.4)
Glucose (mmol/L)	3.9 (3.4-4.2)	4.1 (3.7-4.8)
Time to death (months)		
Median (IQR)	8 (6-11)	20 (22-28)
Alive at 10 years	16 (49)	0 (0)
Cause of death		
Related to MND/S	15 (45)	28(71)
Unrelated or unknown	2 (6)	11(28)

*=Prevalence rates/100000 in KZN adjusted according to race and HIV status

Other categories with PV>0.05 not in the table include: Gender, Subtypes of MND and Radiology

Table 5.2: NCS and EMG findings in HIV-infected MNS and HIV-uninfected (For supplementary file, online publication)

	HIV-uninfected n=101(%)	HIV-infected N=35(%)	P Value
Normal SNAPS in all 4 limbs	86 (85)	24 (68)	
Reduced SNAPs in LL	15 (15)	11 (32)	
Reduced CMAP (<50% expected value)	90 (89)	35(100)	
fasciculations	76 (75)	35 (100)	
Thoracic paraspinal denervation	64 (63)	31 (89)	<0.05
Tongue Fasciculations	51 (50)	29 (82)	<0.001

Radiology:

Brain MRI imaging changes included Wallerian degeneration in 48% (48/101) of the HIV-uninfected category and 57% (20/35) of the HIV-infected category. (Figure 5.2, image 1, table 5.1). None of the MNS patients had root enhancement on MRI spine or signal change on MRI brain or spine to support a vacuolar myelopathy or HIV associated dementia.

Figure 5.2. Radiological Features of HIV-infected patients MND (image 1) (Supplementary online publication)

Image 1: MRI (T2 Flair) of Wallerian degeneration (arrows) in HIV-infected patient with MND

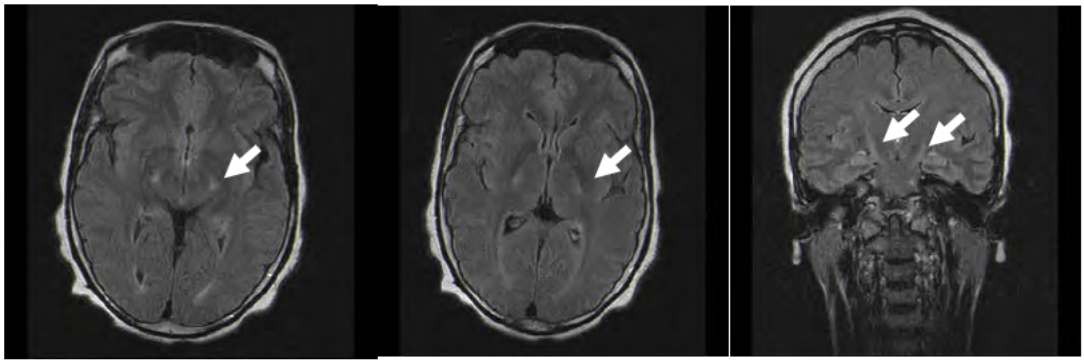


Image 1a: Axial Image 1b: Axial Image 1c: Coronal

CSF:

CSF lymphocyte counts and protein levels were elevated in the HIV-infected category (P<0.001). CSF was normal in the HIV-uninfected patients (Table 6.1).

Survival Outcome:

The Kaplan-Meier curve (Figure 5.3) demonstrates survival in both categories of patients where longitudinal data was available. This included 65% (26/35) in the HIV-infected population and 85% (86/101) in the HIV-uninfected population. Rapid mortality occurred in both groups within 12 months; 35% (8/26) in the HIV-infected category and 20% (12/86) in the HIV-uninfected category. Thereafter the HIV-uninfected patients continue to die, with the majority of deaths occurring within 5 years and three patients with progressive muscle atrophy surviving beyond 10 years.

In the HIV-infected category, if patients survived beyond 12 months, the likelihood of long- term survival is higher compared to the HIV-uninfected category. Seventeen of the ARV treated HIV-infected MND patients survived more than 10 years. HIV-uninfected patients are more likely to die of MND than the ARV treated HIV-infected patients (96% vs 39%, P<0.001, OR 37.5, 95% CI: 10.7-131.5). However, there is also a shorter time to death in the HIV-infected category especially in the first 12 months when ARV naïve or immune reconstitution is still in process (median time 8 months; IQR 8-10) compared to the HIV-uninfected category (median time 18 months; IQR 12-26). The standardized mortality ratio was 0.54 (95% CI 0.43-0.65), which implies that mortality is 50% lower in the HIV-infected category compared to the HIV-uninfected category which was statistically significant.

The cause of death in 83% (15/18) of the HIV-infected category was due to MNS, 17% (3/18) were unknown or unrelated. One HIV-infected patient died of an aspiration pneumonia despite immune

reconstitution. In the HIV-uninfected category 81% (80/101) of deaths were due to MND and 19% (21/101) were unrelated or unknown.

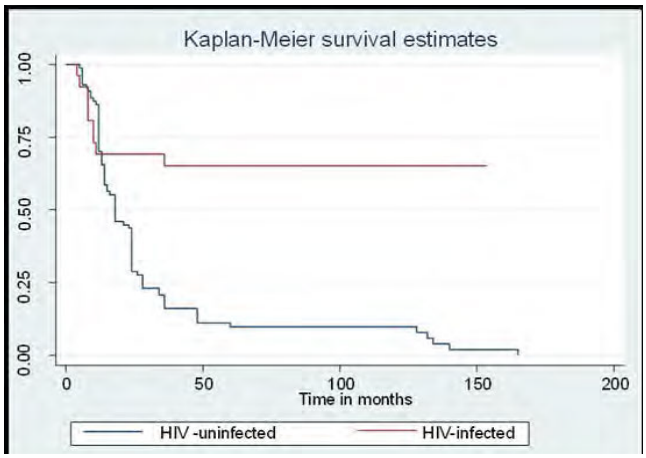
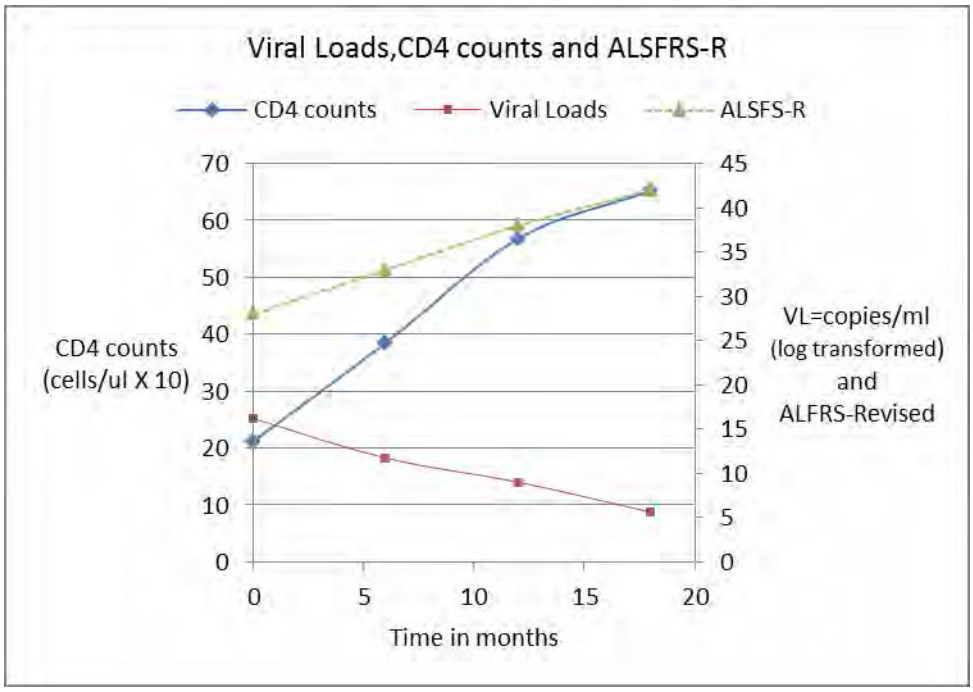


Figure 5. 3: Kaplan –Meier survival estimates in the HIV-infected and HIV-uninfected categories over Time.

Relationship between CD4 count, viral load and functional scores

Figure 5.4 shows a relationship between CD4 counts, viral loads and ALSFRS-RS scores respectively. This suggests that there may be a correlation between immune reconstitution and functional recovery. This may imply a viral or immunological pathogenesis given the benefit of ART. All HIV-infected patients were initially ARV naïve and commenced on ARVs (regimen 1a: Stavudine, lamivudine, efavirenz) after the diagnosis of MNS. This resulted in immune reconstitution (rising CD4 counts and decreasing viral loads), which correlated with clinical recovery reflected by increasing ALSFRS- RS in all except one HIV-infected patient. Five patients (14%) were treated with IVI methylprednisone at a dose of 1g daily IVI with no clinical response.

Figure 5.4: Relationship between Viral Loads, CD4 counts and ALS FRS-R Scores over time.



Significant demographic, clinical, electrophysiological and CSF differences between Black African patients with MND and MNS.

Table 5.3 shows a subgroup analysis between Black African patients with MND and MNS. Significant differences are that Black African patients with MNS are younger (median age of 41, IQR 33-44), present earlier (median 5 months, IQR,2-6), have more severe disease and lower ALSFRS scores (median of 26,IQR 24-28) compared to Black African patients with MND who are older (median age 63, IQR 54-74), present later (median of 22 months IQR 12-25),and have less severe disease (median ALSFRS Score 43 , IQR 41-44). MNS patients were more likely to present with symmetrical signs and a combination of quadriparesis and respiratory-bulbar symptoms. A greater number of Black African MNS patients had fasciculations, thoracic paraspinal denervation and tongue fasciculations compared to Black African MND patients. The CSF also showed higher lymphocyte and protein counts compared to the black MND subcategory. Sixteen Black African MNS patients survived with ART and those who did die, died earlier in the disease if not timeously commenced on ART. Even after indirect age standardization between HIV-infected and HIV-uninfected Black African patients in KZN, MNS was still more prevalent among younger patients in the MNS category (1.66/100000 in age category <50yrs) compared to the Black African MND subgroup where prevalence/100000 was 3.9 in age category 65-90yrs. See comparisons for similar age categories in table 5.4

Table 5. 3: Significant (PV<0.05) demographic, clinical, electrophysiological and CSF differences between Black African patients with MND and MNS

	HIV-uninfected MND (N=39)	HIV-infected MNS (N=33)
	N (%)	N (%)
Age		
Median (IQR)	63 (54-74)	41 (33-44)
< 50yrs	6 (15%) * 0.36	31 (84%) *1.66
50-64 years	15 (38%) *1.94	2 (12%) *0.89
65-90 years	18 (46%) *3.9	0 (0%) * 0
Median time from onset of disease to presentation (months)		
Months (IQR)	22 (12-25)	5 (2-6)
Clinical Presentation at Diagnosis		
Quadriparesis + Respiratory Bulbar symptoms	8 (33)	16 (67)
ALSFRS scores at presentation		
Median (IQR)	43 (41-44)	26 (24-28)
Symmetry at onset	4 (10)	11 (33)
Medical comorbidities		
T2 Diabetes Mellitus	7 (18%)	1 (3)
Hypertension	5 (13%)	1 (3)
Electrophysiology		
fasciculations	23 (58)	33 (100)
Thoracic paraspinal denervation	20 (51)	30 (91)
Tongue fasciculations	10 (25)	27 (81)
CSF		
Lymphocytes (cells/ul)	0 (0-0)	8 (4-12)
Polymorphs (cells/ul)	0 (0-0)	0 (0-2)
Protein (g/dl)	0.38 (0.3-0.4)	0.78 (0.4-0.9)
Glucose (mmol/L)	4.1 (3.7-4.8)	3.9 (3.4-4.2)
Time to death (months)		
Median (IQR)	20 (22-28)	8 (6-11)
Alive at 10 years	0 (0)	16 (49)
Cause of death		
Related to MND/S	28(71)	15 (45)
Unrelated or unknown	11(28)	2 (6)

*=Prevalence rates/100000 in KZN adjusted according to race and HIV status, Other categories with PV>0.05 include: Sex, Subtypes of MND and Radiology

Discussion:

Despite HIV-infected MNS being regarded as a potentially reversible motor neuron syndrome of viral or immune mediated origin, both MND and MNS meet the clinical and electrophysiological revised EL-Escorial criteria for MND and were therefore compared in this study. Both MND and MNS may both potentially have similar reversible aetiopathogeneses, with a possible response to ARTs in both categories. However, no studies using ART in the HIV-uninfected category has been done to date, as MND is considered purely neurodegenerative, even though viruses may play a potential role in the multi-step pathogenesis of the disease. Important differences in the MNS group include the following: majority were young Black African, greater severity of disease at presentation, recovery with ART, and reactive CSF.

Younger age and racial predilection may represent an artefact of the study population as most HIV-infected patients in South Africa are young Black African.[42, 280, 281] Ninety four percent (33/39) of the HIV-infected MNS and 39% (39/101), of the HIV-uninfected MND patients were Black African, which equates to prevalence rates among HIV-infected and HIV-uninfected Black Africans in KZN of 2.4/100000 and 0.44/100000 respectively.[280, 281] Cosnett et al reported a prevalence rate of 0.88/100000 among Black African patients with MND (HIV status unknown) in 1989 in KZN. [142] The peak age of presentation was the 4th decade compared to Indian and European patients who presented two decades later. According to Cosnett, the earlier presentation among Black African patients, which is consistent with the MNS cohort, may suggest a genomic difference between the race groups, environmental factor exposure or reflect a referral bias when standardized for age and race. Cosnett et al, reported high rates of poliovirus and HTLV1 and mechanical trauma among Black African patients which maybe a contributing factor.

In the subgroup analysis of black patients only, MNS occurs in younger black patients, presents earlier and with more aggressive disease (both clinically and electrophysiologically, table 4.3) compared to black MND patients, thereby eliminating race as a confounder. This therefore suggests that HIV infection maybe a key factor in the aetiopathogenesis of MNS and not genomic differences between race groups

Conclusions regarding racial differences in the other race groups would be unreliable given the small number of Indian and European patients who were HIV-infected. However after age standardization and adjustment for race the study shows that MND is still more prevalent in elderly Europeans ranging from 8-12/100000 compared to prevalence rates of 3.9/100000 among black MND patients between age groups 65-90 years. In the MNS category when adjusted for age, the disease is still more

prevalent in the 3rd and 4th decade and equates to prevalence rates of 1.66/100000 in black patients <50 years of age.

Comparisons with other studies from Sub-Saharan Africa was not possible as the results are highly variable and reliability regarding the diagnosis is questionable. [141]. However the general consensus was that African patients with MND present earlier than Europeans and progress more rapidly which may be explained by concomitant viral infections or genetic factors. [141] The age adjusted prevalence rates/100000 for MND and MNS in KZN was 3 and 1.64 respectively which is compatible with rates in prospective studies in other parts of the world [280, 281, 283]. Note that this differs from the age-adjusted results as this prevalence refers to the entire cohort as opposed to the five-year age segments. The race adjusted prevalence rates for the Black African patients with MND in KZN was lower (0.44/100000) which is also consistent with the belief that MND is uncommon among Black African Africans[283]. However when adjusting for age the prevalence rates of MND among black Africans increases to 3.9/100000 in the age category 65-90yrs. The higher prevalence rates of 1.66/100000 among Black Africans in the MNS group in age <50yrs compared to 0.36/100000 in the same age category in the black African MND group, supports the hypothesis that HIV-infection is a confounder (table 4.3).

Greater disease severity at presentation is suggested by lower ALSFRS-RS, greater symmetry of signs at presentation, and greater denervation on EMG, which included the thoracic paraspinal and the tongue muscles. This “advanced stage of ALS” is consistent with the findings reported by Cosnett et al among Black African patients who presented with advanced ALS rather than the benign variety. Many patients in this cohort may have had co-existent HIV-infection or HERV-K infection. Immunodeficiency, genetic factors and possibly higher CSF HERV-K viral loads may contribute to faster progression of disease in the MNS category. One may also postulate that HIV promotes the expression of HERV-K. There have been no studies to correlate CSF HERV-K viral load with clinical manifestation.

Functional recovery corresponded with immune reconstitution after ART therapy in the MNS category of patients. If patients survived more than 12 months after ART, they had reversal of disease with time. Seventeen patients survived longer than 10 years after ART in the MNS category. Reasons for longer survival comparing those that demised versus those that survived in the MNS category include early presentation, less disability at presentation, early commencement of ART and quick immune reconstitution. A multivariate analysis between the two categories was not attempted as the numbers were too small to draw reliable conclusions.

The inflammatory CSF changes are compatible with changes that occur with HIV [284]. As in previous studies, the HIV population had a significant lymphocytosis and raised CSF protein levels compared with the HIV-uninfected MND group. This is probably due to HIV CSF viral replication or may

represent replication of other retroviruses not routinely tested for example HERV-K. More studies are therefore required to study CSF changes in the HIV-infected MNS patients compared with HIV-infected without MNS.

Although HIV-infected MNS and MND are thought to be separate disease entities, MNS being infective, inflammatory or autoimmune and MND neurodegenerative, both categories of patients have similar clinical presentation and there is supporting evidence for a retrovirus contribution to disease in both categories [154, 275, 278, 285, 286] .Therefore the MNS category of patients is an important model to investigate the retroviral postulate in MND. Supporting literature by Westarp et al suggests that HIV-uninfected patients with sporadic MND had high circulating immune complexes and antibodies against the human spuma retrovirus compared to controls. These levels decreased after ART supporting the contention that sporadic MND maybe retrovirus induced[275, 278]. Previous studies have identified reverse transcriptase in patients with HIV-uninfected MND at levels comparable to HIV-infected patients further supporting the above contention[155, 157, 274] Post-mortem brain tissue from a number of patients with ALS had significantly higher expression of HERV-K compared to controls [286]. Li W et al also suggested that HERV- K is activated in a subpopulation of patients with sporadic MND and that its envelope protein may contribute to neurodegeneration[154]. The HERV-K virus was present in cortical and spinal neurons of MND patients but not healthy controls[154, 285]. Douville et al reported high levels of HERV-K *pol* transcripts with a specific pattern of expression including intact open reading frames which where highest in the cortical motor neurons and not detected in Parkinson's disease or accidental-death controls. The HERV-K expression strongly correlated with TDP-43, a multifunctional protein known to be dysregulated in MND[160, 162]. Transgenic mice expressing the envelope protein developed progressive motor dysfunction accompanied by selective loss of volume in the motor cortex [154, 285].

Similarly, Bowen et al described five HIV positive patients with MNS[159]. Three of these had detectable levels of plasma HERV-K, which became undetectable with ART therapy and corresponded to clinical recovery. The remaining two patients showed slow progression of disease. Alternatively, one may consider an immune pathogenesis for MNS because of T-regulatory cell reduction, while ART results in restoration of T-regulatory cells inducing immune tolerance and promoting recovery[287]. More recently HERV protein have become important targets for autoimmune disease and malignancy due to complex interactions between HERV and the immune system[288]. Larger prospective studies are required to better understand the role of HERV-K in MND or HIV MNS and autoimmunity. The above viral hypothesis is consistent with the "multistep hypothesis" of MND which states that genetic mutations alone cannot fully explain MND and that various environmental factors are likely to trigger molecular steps [137, 138]. The identification of reduced number of steps in patients with genetic mutations compared to those without mutations strongly

supports the idea that MND is a multistep process and the viral aetiology may provide a clue for uncovering the pathogenesis of MND[138]. The value of ART in HIV-uninfected MND is uncertain. However, a pilot trial of ART in early HIV-uninfected MND may prove illuminating.

Limitations of the study include comparison of two disease entities with similar clinical manifestation but possibly different aetiopathogenesis. However, the possibility that MND may have a viral contribution still exists. Other limitations include retrospective design, referral and race bias, erroneous coding, exact aetiology of death not obtainable at follow up in some patients.

Conclusion:

This study suggests that HIV-infected patients with MNS are more functionally disabled at presentation and die within the first year if untreated. ART therapy results in improved functional recovery with possible reversal of the disease process, which supports a viral or immune pathogenesis. Future prospective studies are required to evaluate the pathogenesis of HIV-associated MNS. This may extend clues to the "multi-step" pathogenesis of MND. Lastly active support for patients with MNS in the long term is warranted as survival and improvement is possible

Author Contributions:

KM developed the concept, collected and analysed the data and generated the manuscript, VBP and PB peer reviewed the manuscript. All authors approved the final manuscript.

CHAPTER 6

Neuroimmunology and Neuroinflammation

A comparison of clinical, electro-diagnostic, laboratory and treatment outcome differences in a cohort of HIV-infected and HIV-uninfected patients with Chronic Inflammatory Demyelinating Polyneuropathy

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Among HIV-infected patients, distal sensory peripheral neuropathy is the most frequent and occurs in the advanced stages of HIV. It accounts for 30% of symptomatic HIV associated peripheral neuropathies and occurs in 50% of patients living with HIV[289]. The above may be due to HIV itself or due to non-nucleoside reverse transcriptase inhibitors such as didanosine, zalcitabine, and stavudine in 15% to 30% of patients receiving each of these drugs[290]. Other less common peripheral nerve presentations include diffuse infiltrative lymphocytosis, mononeuritis multiplex, vasculitis of the peripheral nerve and inflammatory demyelinating polyneuropathy (AIDP and CIDP)[291]. Among HIV-uninfected patients at our clinic with chronic immune mediated neuropathies, CIDP accounts for majority of patients with peripheral nerve dysfunction. In HIV-infected patients with CIDP, the clinical presentation appeared benign and patients were CST responsive relative to those who were HIV-uninfected. This prompted us to further evaluate and compare the clinical presentation, laboratory findings, electrophysiology, response to therapy and to speculate about the pathogenesis of CIDP in the HIV-infected cohort relative to the HIV-uninfected cohort and whether this cohort of patients had humoral related disease or not. For the sake of brevity, and to avoid redundancy, interesting aspects of the study, comparison with existing published data, and associated limitations are outlined in the discussion section of the published manuscript.

Introduction and background:

Chronic Inflammatory Demyelinating Polyneuropathy (CIDP) is an acquired demyelinating immune mediated neuropathy. It is the most common treatable immune mediated chronic neuropathy worldwide with a prevalence ranging from 1-9 cases per 100 000. [9, 75-77, 292, 293]
CIDP is considered an autoimmune disorder in which an aberrant immune response is directed towards components of the myelin sheath. However the exact immunopathogenesis of the disease remains unknown. The response to immunosuppressive treatment is variable.[89, 90] Presently there is no biomarker to predict response to therapy.[77]

CIDP is known to occur in the setting of HIV, as are other immune mediated neurological disorders including a wide spectrum of neuropathies[83, 294, 12, 4, 5, 291, 293, 295, 296]. In HIV, it is commoner than acute inflammatory demyelinating polyneuropathy (AIDP). [2, 4, 291, 297, 298] Despite the above, the clinical presentation, primary treatment outcomes, electrophysiological and histological findings of CIDP in the setting of HIV is limited to case series and case reports.[299-305] Treatment recommendations include intravenous immunoglobulin (IVIG) or plasma exchange to limit the risk of infections with corticosteroid use. [2, 89, 297, 306, 307] Cost and co-existence of HIV associated renal disease (HIVAN) limits IVIG use and plasma exchange when treating CIDP.[308, 309] Locally, we manage HIV-infected CIDP patients with corticosteroid therapy unless contraindicated.
Our experience suggests that HIV-infected patients with CIDP have a benign course, with few or no relapses and respond rapidly to corticosteroid monotherapy. This suggests that the immune mechanisms in HIV-infected patients may aid recovery. The correlation between CD4 counts, viral load, and recovery is unknown.
We compared CIDP in HIV-infected and HIV-uninfected patients and describe differences between the two categories.

Methods:

The study was a retrospective chart review of a cohort of patients with idiopathic immune mediated CIDP from the neuromuscular clinics at Inkosi Albert Luthuli Central Hospital (IALCH) in Durban and Greys Hospital in Pietermaritzburg from 2003 to 2014. The two units are the only neuromuscular units in the province and provide a neurological service to approximately 11 million people. South Africa has the highest prevalence of HIV in the world, with an estimate of 6.3 million people living with HIV in 2014. Kwa-Zulu Natal (KZN) comprises of 40% of the HIV burden in SA .[310] The study was approved by the University of KZN Biomedical Research Ethics Committee (Ethics reference number: BE 272/15).

Patients fulfilling the clinical, electro-diagnostic and CSF criteria of the European Federation of Neurological Sciences/Peripheral nerve society (EFNS/PNS) for CIDP, were included in the study[311,

[312]. Patients were excluded if they did not meet the diagnostic criteria for CIDP, if their clinical presentation was suggestive of AIDP, if secondary causes were identified or their HIV status was unknown. Data extracted included age, sex, race, duration, onset and course of the disease, clinical presentation, antiretroviral therapy (ART) in HIV-infected patients, response to therapy categorised as time to respond, number of relapses and time to remission, degree of functional recovery scored as Overall Disability Sum Score (ODSS) and the Inflammatory Neuropathy Cause and Treatment (INCAT) scale prior to and after treatment at 3-6 monthly intervals up to 18 months follow up, side effects to treatment, electrophysiological and CSF results, CD4 counts and viral loads[313, 314].

The cohort was divided into two categories; namely HIV- infected and HIV-uninfected. Within each category patients were further classified as being corticosteroid responsive, IVIG responsive or requiring combination therapy (Figure 6. 1). Definitions for the above terms including remission, lack of efficacy as well as the management protocol followed by the neurology units at IALCH and Greys Hospital are included in the web data.

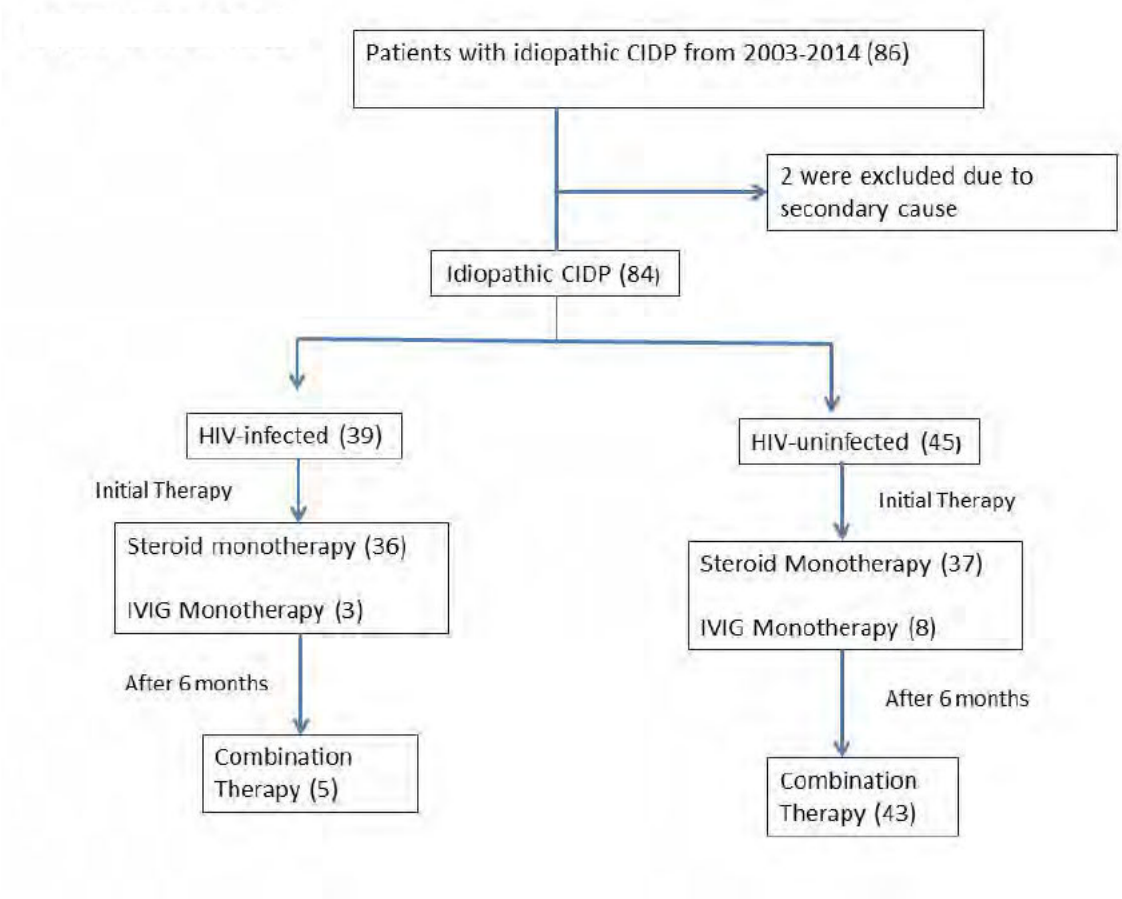


Figure 6.1: CIDP Trial Profile

Statistical Method:

Data was entered in Microsoft Excel and analysed using Prism Software. Descriptive Statistics such as percentages, inter-quartile ranges (IQR), medians, P values were used to summarise the results in the HIV-infected and HIV-uninfected categories. Tests used to calculate the above included Fisher Exact Test for categorical variables, Mann Whitney U Test for continuous variables, Spearman Test for correlation between CD4 counts, viral loads and functional scores. A p value of < 0.05 was regarded as being significant.

Results:

Eighty six patients fulfilled the criteria for definite CIDP; two were excluded due to the presence of an IgM paraprotein. There were 44 men and 40 females. The median age was 39.5 years, (IQR 27-66). The ethnic distribution was 56 Black Africans (66.67%), 22 Indians (26%) and six Whites (7.1%).

The associated aetiologies in table 6.1 show that 10 of the 84 (11.9%) patients had Type 2 Diabetes Mellitus (T2 DM), two had pulmonary tuberculosis many months after the diagnosis of CIDP probably due to long term immunosuppression from corticosteroid use and two were known to suffer from epilepsy. In the remaining patients no secondary cause was identified. There was no difference in the clinical characteristics between T2 DM patients and the HIV-uninfected patients without T2 DM (Table 6.6). These patients were included in the cohort.

Thirty nine of the 84 patients (46.5%) were HIV-infected and 45 were HIV-uninfected (53.5%). Of the 39 HIV-infected patients, 38 were Black Africans (97.5%) and 1 was Indian (2.5%). Sixty one percent were female. Median age was 37 years (IQR 30-42years). Compared to the HIV-uninfected patients, the HIV- infected patients were younger and had a female preponderance with p values of 0.0033 and 0.028 respectively. The median CD4 count was 384 cells/mm^3 (IQR 126-423 cells/mm^3), median viral load (VL) was 440 copies/ml, (IQR 0-34650 copies/ml). Twenty-three (59%) patients were ART naïve at the time of CIDP diagnosis and 16(41%) were on ARTs. Ten (62%) of the patients who were on ARTs received treatment for a median duration of 6.5 weeks (IQR 4-8). Patient 16 had treatment failure requiring second line therapy. Five patients (31%) received ARTs for more than five years (table 6.3)

As shown in table 6.1, 87.2% of the HIV-infected and 46.7% of the HIV-uninfected patients presented with a monophasic progressive course ($p= <0.0001$). Median duration of illness among the HIV-infected and HIV-uninfected patients was 6.7 months (IQR 2-9 months) and 12.5 months (IQR 2-23 months) respectively ($P =0.16$). Relapses were 12.8% among HIV-infected patients compared to

53.3% among HIV-uninfected patients (P=<0.0001). Four (10%) HIV-infected patients and nine (20%) HIV-uninfected patients had an acute presentation (P=0.31). The majority of patients in both categories (89.7% vs 80%) had a slowly progressive onset over several months (P =0.21). Clinical signs were symmetrical in 74% and 60% of HIV-infected and HIV-uninfected patients respectively (p =0.245). Forty two percent of HIV-uninfected patients and 17% of HIV- infected patients had distal weakness (p =0.001). In both categories the majority of patients presented with a combined sensory motor presentation (100% vs 93%). There was no difference in other clinical signs such as cranial nerve involvement, truncal weakness, tremor, or thickened nerves. No patients presented with respiratory, bulbar or autonomic signs or symptoms.

CSF analysis (table 6.1), revealed a difference in lymphocyte counts between the 2 categories, with a median cell count of 5.75cells/µl, IQR (0-7.2cells/µl) among HIV-infected patients and a median cell count of 0 cells/µl IQR (0-2cells/µl) among the HIV-uninfected patients (P =0.0002). Protein levels, polymorph counts and glucose levels were not different. T2 DM was associated with raised protein levels in 9/10 patients. Albumino-cytological dissociation was present in 60% of HIV-uninfected patients and 25% of the HIV-infected patients

Table 6. 1: Baseline Demographic features of the HIV-infected and HIV-uninfected CIDP categories

Demographic Features	HIV-infected (n=39)	HIV-uninfected (n=45)	P Value
Gender			0.028
Male	15 (38%)	29 (64%)	
Female	24(61%)	16(36%)	
Race			
Black African	38(97%)	18 (40%)	<0.0001
White	0 (0%)	6(13.3%)	<0.0001
Indian	1(2.6%)	21(46%)	<0.0001
Median Age (IQR)	37 (30-42)	53(29-66)	0.0033
Onset of Disease			
Slow onset/Progressive	35(89.7%)	36(80%)	0.208
Acute	4 (10.3%)	9(19.5%)	0.301
Tempo of Disease			
Slowly progressive	34(87.2%)	21(46.7%)	<0.0001
Relapsing remitting	5(12.8%)	24(53.3%)	<0.0001
Median duration of Disease (months)/IQR	6.7 (IQR 2-9)	12.5 (IQR 2-23)	<0.161
Distribution of weakness			
Symmetrical	29(74.3%)	27 (60%)	0.245
Distal	7 (17%)	19 (42%)	0.001
Denervation on EMG			
Proximal Muscles	4 (10%)	15 (33%)	<0.0001
Distal Muscles	1(0.02%)	19 (42%)	<0.0001
Clinical Presentation			
Combined motor and sensory	39 (100%)	42 (93%)	NS
Other illnesses			
DM	2(5.1%)	8(17.7%)	0.097
Diagnosis			
Clinical definite CIDP	39(100%)	45(100%)	
First Line Therapy			
Corticosteroid	36(92.3%)	35(77.7%)	0.168
IVIG	3(7.7%)	10(22.2%)	0.087
CSF (median)			
Protein (mg/dl)	1.38 (0.88-2.2)	1.1 (0.21-2.3)	0.45
Lymphocyte (cells/uL)	5.75(0-7.2)	0(0-2)	0.0002
Polymorphs (cells/uL)	0.28 (0-3)	0.45(0-4)	0.81
Glucose (mmol/L)	3.4(2.3-5.1)	3.8(3.1-6.8)	0.98

IQR=Interquartile range, DM=Diabetes Mellitus, CIDP=Chronic inflammatory demyelinating polyneuropathy, CSF=cerebrospinal fluid

Treatment, treatment outcomes and side effects to therapy are described in table 6.2, figure 6.2A and Figure 6.2B. Three HIV-infected patients (2 with T2 DM, 1 hypertensive) received IVIG as first line therapy and responded within 3 months. There were no relapses or side effects.

Figure 6.2A and 6.2B: Response to first line therapy (corticosteroids or IVIG) in the HIV-infected and HIV-uninfected categories

Fig 6.2A: Time to respond to first line

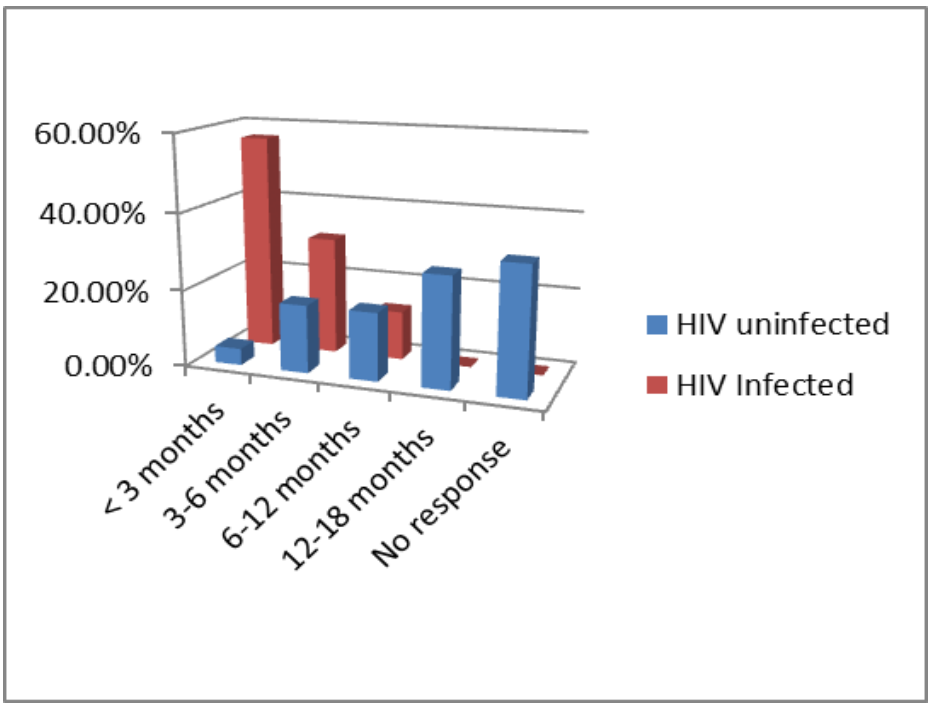
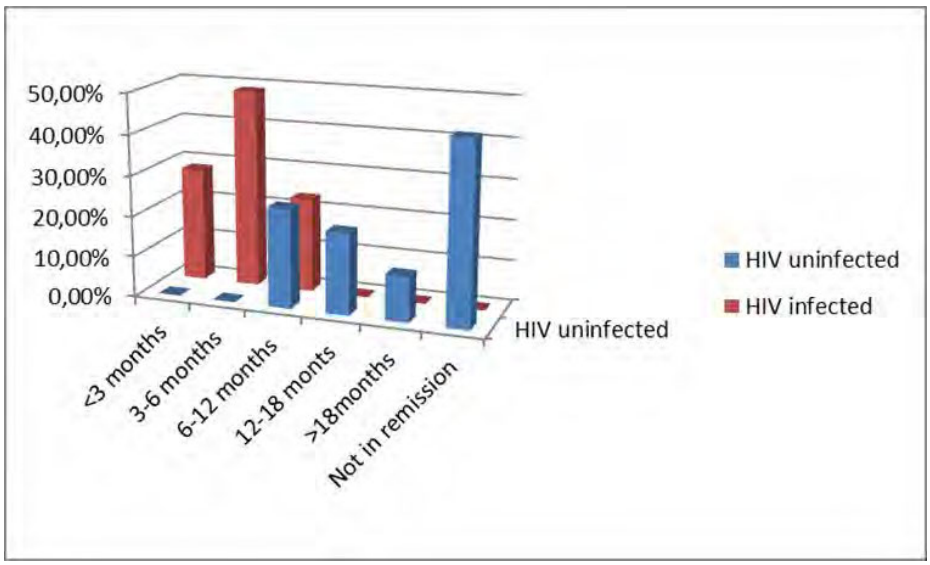


Figure 6.2B: Time to Remission in the HIV-infected and HIV-uninfected categories



Eighty six percent of the HIV-infected patients were corticosteroid responsive. Fifty six percent responded within the first three months, 30.7 % in 3-6 months and 12.8% in the 6-12 month period (Figure 6.2B). All HIV-infected patients showed a corticosteroid response by 12 months. Seventy six percent of the corticosteroid responsive HIV-infected patients were in remission within the first six months (Fig 6.2B) and 24% were in remission by 12 months. Seventy-seven percent of the HIV-infected patients were treatment free at remission, four patients were on less than 5mg prednisone /day and five patients were on azathioprine (AZA) and less than 5mg prednisone/day. Eight of the HIV-infected patients developed an increased BMI during corticosteroid therapy compared to baseline. No other side effects were documented.

The functional assessment scores (INCAT and ODSS) scores improved significantly from high scores at presentation (patients being quadriplegic) to being almost fully functional by 6-12 months and normal by 18 months.

Ninety two percent of patients had no relapses in the 18 month follow up period. Only three patients had less than five relapses during follow up (Table 6.2).

Twenty seven percent of the HIV-uninfected patients were corticosteroid responsive. Of the eight patients with T2 DM on IVIG, only 25% responded within three months. Ninety five percent of the HIV-uninfected patients required combination therapy (Table 6.2). Twenty-two percent of patients responded to first line therapy by six months, 17.8% by 12 months, 28.9 % at 12-18 months and 33.3% showed no response by 18 months (Fig 6.2A).Twenty four percent went into remission by 6-12

months, 20% by 12-18 months and 11% after 18 months. Forty four percent were not in remission by 18 months follow up (Fig 6.2B). Various combinations of therapy listed in table 6.2 were used to aid remission in the above patients.

The HIV un-infected patients experienced more side effects to corticosteroids compared to the HIV-infected patients ($p < 0.0001$). This included an increased BMI, development of a metabolic syndrome, osteoporosis, avascular necrosis of the hip, skin changes, infection (Pulmonary Tuberculosis) and gastrointestinal disturbances. Two patients had side effects to IVIG; one patient with T2 DM developed renal failure and another had a transfusion reaction (table 6.2). Treatment had to be discontinued. Despite therapy the functional assessment scores in the above category showed minimal improvement after 18 months.

Among the HIV- infected patients, improving CD4 counts and decreasing HIV viral loads at 18 months did not correlate with functional recovery. Electrophysiological studies showed no significant difference between, distal motor latencies, conduction velocities, conduction blocks, temporal dispersion and F-waves between the two categories (Table 6.4). However, there was a significant difference in the degree and distribution of denervation on EMG in the two categories. Only 12.8% of HIV-infected patients showed denervation in mainly proximal muscles whereas 75% of HIV-uninfected patients showed denervation in mainly distal muscles (table 6.1).

Table 6.2: Response to therapy, relapses, treatment at remission and side effects to therapy in the HIV-infected and HIV-uninfected category

Response to therapy	HIV-infected	HIV-uninfected	P Value
Corticosteroid Responsive	31/36 (86%)	10/37 (27%)	<0.0001
IVIG Responsive	3/3 (100%)	8/45 (17.7%)	0.2
Combination therapy after 6 months	5/39(12.8%)	43/45 (95.5%)	<0.0001
Number of Relapses in 18 months			
0 relapses	36(92.3%)	22(48.9%)	<0.0001
< 5 relapses	3(7.7%)	9(20%)	
> 5 relapses	0	14(31%)	
Treatment at Remission			
No treatment	30 (76.9%)	0.00	<0.0001
< 5mg corticosteroids only	4 (10.25%)	0.00	
Corticosteroids + Corticosteroid sparing agent	5(12.8%)	24(53.3%)	
Corticosteroid sparing agent only	0	2(4.4%)	
3 monthly IVIG	0	4(8.8%)	
Combination of corticosteroids, IVIG and corticosteroid sparing agent	0	13(28.9%)	
Rituximab	0	2(4.4%)	
Steroid Sparing agents			
Azathioprine	5(12.8%)	39(86.7%)	
Mycophenolate Mofetil	0	6 (13.3%)	
Methotrexate	0	2(4.4%)	
Cyclophosphamide	0	2(4.4%)	
IVIG	0	19(42.2%)	
Rituximab	0	2(4.4%)	
Side Effects of corticosteroids			<0.0001
Increase in BMI	8(19.1%)	33(73.3%)	
Hypertension/Diabetes Mellitus	0	6(13.3%)	
Osteoporosis	0	6(13.3%)	
Skin changes	0	4(8.9%)	
Avascular Necrosis of the hip	0	4(8.9%)	
Infection	0	2(4.4%) PTB	
GIT	0	16 (35.6%)	
Side Effects of IVIG			0.2
Transfusion reaction	0	1(2.2%)	
Renal failure	0	1(2.2%)	

IVIG=Intravenous Immunoglobulin, GIT=Gastrointestinal, PTB=Pulmonary Tuberculosis

Table 6.3: Relationship between CD4 counts, viral loads and onset of CIDP

	Duration on ARVs before diagnosis of CIDP (weeks)	Baseline CD4 counts (cells/ul) at the time of diagnosis of HIV	Baseline Viral loads at the time of HIV diagnosis(copies/ml) (copies/ml)	CD4 counts at time of CIDP diagnosis (cells/ul)	Viral load at the time of CIDP diagnosis (copies/ml)
Patient 1	4	5	1820175	50	1001068
Patient 2	12	40	971475	95	150275
Patient 3	8	2	1203860	128	450500
Patient 4	3	90	1004805	179	798560
Patient 5	6	115	703805	260	306780
Patient 6	7	101	98360	299	16040
Patient 7	4	126	58818	363	25428
Patient 8	8	260	690785	520	90320
Patient 9	3	100	499980	188	300350
Patient 10	9	122	981478	420	198239
Patient 11	244	95	1204707	610	undetectable
Patient 12	300	126	75666	960	undetectable
Patient 13	260	235	58560	766	undetectable
Patient 14	336	12	1950276	1226	undetectable
Patient 15	320	187	880765	812	undetectable
Patient 16	24	140	586866	100	1110000
Patient 16: Treatment failure, now on second line ARVs					

Table 6.5: Characteristics of patients requiring combination therapy in the HIV-infected category

Patient	Duration of Disease (months)	On ARVs at presentation	Viral Load at presentation	CD4 cct at presentation	Denervation on EMG
1	12	Yes	undetectable	610	2+
2	24	Yes	undetectable	960	2+
3	9	Yes	undetectable	766	1+
4	10	yes	undetectable	1226	2+
5	12	Yes	undetecable	812	3+

Table 6.4: Electrophysiology

Nerve	Median Motor					Ulnar Motor					Tibial Motor					Peroneal Motor				
HIV Status	HIV- infected		HIV -uninfected			HIV-infected		HIV-uninfected			HIV-infected		HIV-uninfected			HIV-infected		HIV-uninfected		
	Median	IQR	Median	IQR	P Value	Median	IQR	Median	IQR	P Value	Median	IQR	Median	IQR	P Value	Median	IQR	Median	IQR	P Value
DML(ms)	7.5	6.6-8.8	7.4	6.9-9.9	0.74	6.8	5.7-8.75	6.6	5.8-9.35	0.99	10.5	9.05-11.85	10.2	8.67-14.25	0.5	8.3	9-12.5	9	8-12.5	0.45
CV(m/sec)	27	21.99-28	21.45	15.9-26	0.09	20	19-27.8	23	18.5-28	0.23	21	17-28	22	13-26.6	0.64	18	15.3-26	19.1	19.15-25.05	0.06
Distal CMAP(mV)	6.5	3.55-7	4.6	2.9-7.6	0.66	5.8	3.8-6.8	5.35	3.2-6.9	0.88	6.2	4.05-7.8	4.2	2.8-6.5	0.48	2.7	1.8-4.7	4.5	3.6-6.5	0.018
Proximal CMAP(mV)	2.2	1.35-3.5	2.25	1.5-3.7	0.97	1.8	1.3-3.2	2.05	1.1-2.9	0.44	2.6	2.48-3.9	2.1	1.45-3	0.46	1.45	0.77-3.2	3.1	1.35-3.4	0.09
% Conduction Block	50.5	43-57	48.9	18.8-65	0.98	61.9	43.9-71.4	52.7	15.5-67	0.57	56.9	14.6-63	48.9	20.5-69	0.95	36	22-42	48	20.5-62	0.088
Distal Duration (ms)	15.5	3.6-30	16.6	10.2-18	0.74	13.6	10.2-18	14.6	11.7-16	0.4	20	12.3-28	12.9	10-17.7	0.062	17.1	13.8-28	12.5	10.7-18.8	0.012
Proximal Duration(ms) (P-D %)	20.1	14.5-23	22	16.6-31	0.013	18.1	12.9-22	18.9	16.2-19.5	0.64	26	13.25-34	19.2	12.7-24	0.228	23	17.4-31	20.9	13.2-23	<0.011
% change in Duration	22.77	15.8-55	20.3	16.9-56	0.21	19	14-74	29.59	8.6-62	0.2	22	12.8-29	24.7	4.4-61	0.11	30.3	23-38	19.1	16.5-33	0.73
Distal Area (mVms)	25.6	17.4-35	22.7	8.2-22	0.002	24	16.2-36	20.9	14.3-33	0.1	15	8.9-21.7	18	6.75-32.6	0.92	12.55	7.8-22	22.1	8.2-18.5	0.54
Proximal Area(mVms)	16.2	10.45-22	12.25	1.5-3.3	0.21	14	8.6-22	12.75	7.17-21.33	0.72	11.4	5.1-16	8.9	4.15-22	0.79	9.55	3.33-18.1	11.5	3.89-19.8	0.58
% change in area (D-P%)	*34.81	*42.31-*5.9	*25	*50- *12.53	0.66	*45	*61-*32	*35.5	*55.5-*12.6	0.21	*18.9	*57-*8.2	*39.8	*71-*8.9	0.87	*19.6	*41.5-*5.3	*22.8	*32.3-*10.5	0.21
F wave (ms)	43	39.9-52	45	39.9-65	0.3	43	36.5-48	42	36-54.4	0.66	65	62-68	69	62-72	0.5	62	52-68	66.5	56-76	0.43
	* indicates a negative value			DML=Distal motor latency			CV=Conduction velocity			CMAP=	Compound muscle actioon potential									
Sensory Nerve Conduction Studies																				
	Median SNAP					Ulnar SNAP					Sural SNAP									
	HIV- infected		HIV-uninfected			HIV- infected		HIV -uninfected			HIV infected		HIV -uninfected							
	Median	IQR	Median	IQR	P Value	Median	IQR	Median	IQR	P Value	Median	IQR	Median	IQR	P Value					
SNAP Amplitude(uV)	0	0-0	0	0-0	0.19	0	0-0	0	0-0	0.98	0	0-0	0	0-0	0.84					
Distal Latency (msec)	1.17(0-5.3)		1.5(0-4.8)		0.48	0.82(0-4.4)		0.74(0-4.2)		0.75	0.49(0-5.6)		0.45(0-4.1)		0.92					
	SNAP=Sensory nerve action potential																			

Table 6.6: Characteristics of the 10 CIDP patients with Type 2 Diabetes Mellitus

Race	HBA1c	HIV status	CSF Lymphocyte count	CSF Protein	IVIg Responsive	Time to respond months	Number of relapses in 18 months	Treatment on remission
I	8.6	uninfected	0	0.73	N	>12	>5	3 monthly IVIG
I	6.5	infected	22	1.38	Y	<3	0	AZA
I	6.8	uninfected	2	0.64	N	>12	>5	3 monthly IVIG
B	5.9	infected	12	0.98	Y	3-6	<5	AZA+ IVIG + MMF
I	8.9	uninfected	0	0.32	N	>18	>5	AZA+IVIG+ Rituximab
I	6.9	uninfected	0	0.98	N	>18	>5	AZA+IVIG+ MMF
I	6.5	uninfected	0	3.75	Y	6-12	<5	AZA+ IVIG + MMF
I	8.3	uninfected	2	1.9	N	>12	>5	3 monthly IVIG
I	6.2	uninfected	0	2.1	N	>12	>5	AZA+IVIG
B	5.8	uninfected	0	1.49	Y	>12	>5	3 monthly IVIG

M=Male, F=Female, I=Indian=Black,IVIg=intravenous immunoglobulin=azathioprine

Discussion:

In this study HIV-infected patients showed a rapid response to corticosteroid immunotherapy with minimal relapses. In contrast, the HIV-uninfected patients were refractory to treatment. The largest study is a prospective case series consisting of 23 patients (10 HIV-infected and 13 HIV-uninfected).[299] This study reported no clinical or electrophysiological differences between the two categories and no comparisons were made regarding treatment. Currently there are no comparative studies in the literature regarding treatment outcomes between HIV-infected and HIV-uninfected patients with CIDP. The current study showed significant differences in treatment outcomes, age, gender and disease progression.

CIDP usually shows a male predominance ranging from 1.31:1 to 2.8:1 in HIV-uninfected patients.[299, 315] Among the HIV-infected patients the male: female ratio was reversed. This reversal in the gender ratio in HIV-infected patients was reported in one other study.[299]

The median age of onset among HIV-uninfected patients compares well to previous reported epidemiological studies.[9, 293, 316] The median age of onset among HIV-infected patients was significantly younger. The female preponderance and younger age of onset of HIV-infected patients with CIDP is an artefact of the sample and unlikely related to gender or age susceptibility as 60% of HIV-infected people in South Africa are young black females (statistics South Africa: 2015).

Many HIV-infected patients presented with a monophasic slowly progressive course, whereas the HIV-uninfected patients presented with a relapsing remitting course. This may relate to possible but undefined differences in the underlying immune mechanisms in the two categories.

The mean CSF protein levels were slightly higher among the HIV-infected patients, although not significant. This is in contrast to the findings of a recent study, but compatible with a previous case series consisting of seven patients, six of whom had raised protein levels.[296, 299] High CSF protein levels reflect an immune mediated disruption of the blood nerve barrier at the level of the spinal roots which correlates with proximal; possibly root involvement among the HIV-infected patients. However, the difference in protein levels did not reach statistical significance possibly due to the short duration of the disease, small numbers and unregulated immunity among the HIV-infected patients. As in previous studies the HIV-infected patients had higher CSF lymphocyte counts which is most likely due to CSF viraemia rather than immunological changes that occur with CIDP. This CSF finding is consistent with other studies.[317, 318] Despite 59% of HIV-infected patients being on ARTs, a significant number still had ongoing CSF lymphocytosis. Many of these patients had a suppressed plasma viral load. This may represent inadequate penetration of ART into the CSF space or CSF HIV resistance resulting in ongoing CSF viral replication. [319]

The response to corticosteroid monotherapy among HIV-infected patients was clearly demonstrated within three months. Most of the HIV-infected patients were in remission by six months. This short duration of corticosteroid therapy seems to be a safe and cost-effective option in HIV-infected patients. Current literature states that IVIG or plasma exchange is the preferred choice due to the risk of infection with corticosteroid therapy.[8, 20,22, 27, 29] However in this study the risk of infection was negligible mainly due to the prompt response to corticosteroid monotherapy and hence the short duration of treatment. In this category the risk of infection was zero compared to the HIV-uninfected group who received longer duration corticosteroid therapy often combined with other immunosuppressants. In the HIV-uninfected group, 2(4.4%) of patients developed pulmonary tuberculosis most likely due to prolonged immunosuppression on combination immunosuppressive therapy.

This category of patients on prolonged high dose immunosuppression, in our unit are given INH for tuberculosis prophylaxis with pyridoxine cover due to the high risk of pulmonary tuberculosis in our environment. Nevertheless, breakthrough infections occurred in 4.4% of patients. The HIV-infected patients were not given prophylaxis. The only significant side effect documented among the HIV-infected patients was an increase in body mass index (BMI). All patients had BMI documented at baseline and thereafter three monthly while on corticosteroids. Only HIV-uninfected patients had bone density scans as they were on long term corticosteroids. This was not necessary among the HIV-infected patients as their duration of treatment was short.

The varying response to corticosteroid monotherapy in the two categories of patients suggests that the immunopathogenesis may be different. Autoantibodies targeting non-compact myelin which includes the node and flanking regions, compact myelin protein such as P0, PMP22 and Schwann cells may

select for a relatively benign course, and hence one may postulate that the above is a steroid-responsive antibody mediated neuropathy. The humoral mediated pathogenesis is explored and discussed in chapter 1. Nodal/paranodal antibodies were positive in <10% of the cohort. Ganglioside antibodies were negative in the entire cohort of patients including PM LSP and MNS which is in contrast to other regions of the world where ganglioside antibodies are more frequently reported example Korea[320]. This may be due to inter-laboratory variability of testing, population differences, or an untested ganglioside antibody [320]. The positive myelin culture screens in 3 HIV-infected patients argues for a possible novel antibody mediated process which will be explored in a future study using novel IP techniques and mass spectrometry as described in chapter 3. Support for antibodies in the pathogenesis was demonstrated by a good response to plasma exchange.[305] A decline in In HIV T-regulatory CD4 cells potentiates the emergence of autoimmune phenomena.[16, 321] ARTs result in immune reconstitution and up-regulation of the total number of CD4 T-regulatory cells which may contribute to remission. There is a single case report of CIDP resolving with ARTs alone.[301] In our study ARTs may have expedited recovery by restoring immune function.
In patients diagnosed as HIV-infected at CIDP diagnosis, ARTs and corticosteroids were commenced simultaneously. No deterioration occurred in this category. Concomitant use of corticosteroids may have potentially curtailed the immune reconstitution inflammatory syndrome (IRIS). This maybe a potential avenue for future therapy, in patients being initiated on ARTs.The 10 patients who had low baseline CD4 counts and high viral loads (Table 6.3, Patients 1-10) may have had CIDP as an IRIS phenomenon as they were commenced on ARVs for a short period. Patient 16 who, developed immunological and virological failure may have had an IRIS response when commencing second line therapy. Presently there are no markers to predict who will develop this complication.

Five patients on long term ARTs (table 6.3, patient 11-patient 15) had complete immune reconstitution and behaved as HIV-uninfected patients with prolonged refractory disease and poor response to steroids. Furthermore the long duration of disease prior to presentation and presence of denervation on EMG indicating axonal damage (Table 6.4 web data, table 6.1) may account for a poor response to corticosteroids.

Although AIDP was a potential diagnosis this was discounted as all patients met the ENFS/PNS clinical and electrodiagnostic criteria for CIDP, progressed beyond 12 weeks, had typical electrodiagnostic findings for demyelination (table 6.4), had no prior flu-like illness or diarrheal illness, had an insidious onset, had no bulbar, respiratory or autonomic symptoms, and none showed spontaneous recovery during the initial three months of progression. The speculation that recovery may have occurred despite corticosteroid therapy, can only be answered in a prospective study comparing placebo with corticosteroids. Nonetheless patients with suspected AIDP and spontaneous recovery during the initial three months were excluded from the study. Furthermore, the four HIV-

infected patients who presented acutely, all progressed beyond 12 weeks and responded to corticosteroid therapy within four weeks making AIDP very unlikely. The 9 HIV-uninfected patients, who presented acutely, showed a poor response to corticosteroid monotherapy in the first six months and required combination immunosuppressive therapy.

During the above study period we have seen approximately 100-120 HIV- infected patients with AIDP at our neurology institute who behaved differently from our HIV-infected CIDP cohort. All patients presented acutely and 70% had a preceding flu or diarrheal illness. Approximately 30% patients required ICU admission for bulbar /respiratory involvement or autonomic instability. Spontaneous recovery was seen in about 5% of these patients. All patients were treated with IVIG and not steroids. Therefore, we concluded that our cohort of patients had CIDP rather than AIDP and were steroid responsive rather than manifesting spontaneous recovery.

Isolated case reports of nerve histopathology in HIV-associated CIDP, demonstrates a macrophage predominant infiltrate and segmental macrophage induced demyelination along the length of the nerve , similar to HIV-uninfected CIDP patients[322]. No viral particles have been reported on light microscopy [322]. However, viral particles may be present intracellularly within CD4+ cells in nerve tissue example dendritic cells, macrophages and T cells. DNA or RNA isolation, PCR and gene sequencing may be useful techniques to identify HIV intracellular viral antigens. A novel HIV-exposed culture system of sensory neurons, macrophages and Schwann cells expressing human CD4 and CCR5, may be useful to further demonstrate pathogenicity, as demonstrated in a previous study of HIV distal sensory neuropathy[10]. Future prospective studies using hiPSC derived myelin culture models expressing, CD4 and CCR5 and HIV-infected macrophages, may prove useful to explore the direct neuropathogenic effects of HIV on myelin cultures.

Therefore, although HIV-infected patients satisfy the ENFS criteria for CIDP , the quick response to CST and the relatively benign monophasic course of CIDP in HIV-infected individuals is different. Revision of diagnostic criteria for CIDP in HIV-infected individuals may be required in future as the pathophysiology and histology of the disease in this cohort may differ. Further prospective studies may help define therapy and possibly refine diagnostic criteria for HIV associated CIDP. These prospective studies include; clinical confirmation of CST response and effect of ART on disease progression, PCR to identify HIV viral particles in peripheral nerve myelin, measure of ART penetration into peripheral nerve tissue and lastly the development of disease specific hiPSC myelin cultures model to detect novel neural antigen. The above will unravel some of the immune mechanisms responsible for demyelination in this cohort of patients.

Limitations of the study are that it is a retrospective study; numbers are small and have tertiary care setting bias. Other limitations are that many patients may have been missed as a result of not being referred or misdiagnosed.

Conclusion

This study suggests that treated HIV-infected CIDP patients have a short duration of disease, a benign course and are highly steroid responsive compared to their HIV-uninfected counterparts. Although we satisfy the ENFS criteria for CIDP, the quick response to corticosteroids and the relatively benign monophasic course makes AIDP or a nodopathy a possibility. Perhaps the course and progression of CIDP in HIV-infected individuals is different and revision of criteria for the diagnosis of CIDP in HIV-infected individuals is required. This study also shows that steroids are a cost-effective and safe option in HIV-infected CIDP patients especially in a resource limited setting. Further prospective studies confirming a rapid corticosteroid response in HIV-infected CIDP patients as well as unravelling the immune mechanisms responsible for CIDP in these patients is required to define future therapy.

Author Contributions:

KM developed the concept, collected and analysed the data and generated the manuscript, VBP and PB peer reviewed the manuscript. All authors approved the final manuscript.

CHAPTER 7

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Pure motor lumbo-sacral polyradiculopathy in HIV patients

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During our recruitment of HIV-infected CIDP patients, we identified a unique cohort of HIV-infected patients with a "***pure motor" lower motor neuron syndrome*** who improved following CST. Considerations included pure motor AIDP or an AIDP variant, pure motor CIDP, multifocal motor neuropathy, or a lower motor neuron variant of HIV associated MNS which is described in chapter 2. Pure motor syndromes with a predilection for the lumbo-sacral roots have also been described in paraneoplastic syndromes, or drug induced syndromes example intrathecal methotrexate in patients with haematological malignancies, although rare[323, 324]. Pure motor immune mediated neuropathies occur with MMN, AMAN, and motor predominant CIDP[323, 325] . GM1 and NF186 antibodies are described in pure motor syndromes that are inflammatory or immune mediated in nature [326]. Although not included in this published manuscript, subsequent testing for ganglioside antibodies and nodal-paranodal antibodies were negative in 10/11(91%) patients with PM LSP except for 1 patient who tested positive for NF186 antibodies. Similarly 5 patients with HIV-infected MNS were also negative for ganglioside and GM1 antibodies (Chapter 10). The above cases provide an interesting disease model for exploring the susceptibility or predilection of the ventral root to immune "attack" or due to HIV itself. PM LSP usually manifests in advanced AIDS (median CD4 count <200 cells/ul) suggesting that in addition to immune dysregulation and immune suppression, a high circulating viral load, inflammatory cytokines, complement activation, or CTL responses may be necessary to unmask antigenic epitopes.

We undertook a descriptive study of this unusual cohort of HIV-infected patients with PM LSP and in this published manuscript we discuss the clinical features, electrophysiology, radiological and CSF findings as well as response to therapy. In Chapter 8 and 10, we investigate possible pathophysiology of the above disorder by screening for ganglioside and 4 nodal-paranodal antibodies in this cohort. There is only 1 other case series from SA describing a similar cohort as the prevalence of this disorder has decreased with ART.[6]

Motor lumbosacral radiculopathy in HIV-infected patients

Introduction:

Progressive lumbosacral polyradiculopathy is a well described complication of late HIV infection and is usually caused by opportunistic infections such as cytomegalovirus (CMV), herpes simplex virus (HSV), varicella zoster virus (VZV), syphilis, tuberculosis (TB), cryptococcus and less commonly lymphoma, paraneoplastic polyradiculopathy ,chronic inflammatory demyelinating polyradiculopathy (CIDP), or diffuse infiltrative lymphocytosis (DILS). [4, 295, 327] Infective aetiologies, lymphoma and paraneoplastic polyradiculopathy are usually subacute and are progressive unless treated.[4, 327-331]
In 2000, Benatar et al described 4 HIV-infected patients who presented with acute or subacute weakness with spontaneous recovery. [6] Infective and inflammatory aetiologies were excluded. This entity was described as a "unique" clinical entity in the setting of HIV or a "variant of Guillain- Barre Syndrome (GBS) ".[6] Since 2000, there were no further documented cases in the literature.
Between 2010 and 2015 we retrospectively identified a similar cohort of 11 HIV-infected patients who presented with a pure motor lumbosacral radiculopathy. In this article, we add to the current literature regarding this unusual group of patients by describing the clinical presentation, demographic features, electro diagnostic, radiological, cerebrospinal fluid (CSF) findings and response to therapy.

Methods:

Patients were identified between 2010 and 2015 in the Department of Neurology at Inkosi Albert Luthuli Central Hospital, which is a 1000 bed tertiary hospital in Durban, Kwa Zulu Natal, South Africa. The Department of neurology sees approximately 8000 patients/year.
The inclusion criteria for patient selection were HIV-infected patients older than 18 years with lower motor neuron weakness involving exclusively the lower limbs, normal sensation, preserved sensory nerve action potentials (SNAPs) and lumbosacral root enhancement on MRI. Exclusion criteria were: abnormal sensation on clinical examination, upper limb or truncal involvement, upper motor neuron signs, sensory nerve action potential on nerve conduction studies which were less than 70% normal values , compressive or intra-spinal lesions accounting for the weakness, polyradiculopathies due to infective or malignant aetiology, clinical features of DILS or raised creatinine kinase levels with EMG or histological features of a myopathy, and electrolyte abnormalities for example hypokalaemia accounting for weakness and areflexia.
Data extracted from patient records included; clinical findings, laboratory results, electrodiagnostic findings (nerve conduction and needle electromyography), magnetic resonance imaging (MRI) of the thoracolumbar and lumbosacral spine, duration of therapy and response to therapy.
Tests to exclude infective causes of a polyradiculopathy included; CSF polymerase chain reaction (PCR) for VZV, CMV,HSV,EBV ; CSF Ziehl-Neelson (ZN) stain, culture and gene expert (where

available) for TB; CSF Venereal Disease Research Laboratory (VDRL), fluorescent treponemal antibody absorption (FTA-ABS) for syphilis; CSF cytology for malignancy (lymphoma); CSF cryptococcal antigen, India ink stain and cryptococcal culture; chest radiograph for pulmonary tuberculosis (TB) ; MRI spine for structural and inflammatory/infective lesions.
Patients were followed up and scored according to the modified Rankin Scale (mRS), to assess for relapses and response to therapy at 3 monthly intervals for 6 months and thereafter 6 monthly up to 18 months.

Results:

Clinical Features, CSF, Electrophysiological and MRI findings:

Eleven patients met the inclusion criteria. There were 6 females. The median age was 29, IQR (23-41) years. All patients were of black African ancestry. The mean duration of symptom progression (continuous and not stepwise) was 6.5, IQR (3-7.5) months. No patients had preceding flu-like illness, sensory complaints, or upper limb symptoms. On examination they had flaccid, symmetrical areflexic paraparesis with normal assessment of mental state, cranial nerves and upper limbs. Sensory testing to all modalities were normal and sphincters were normal.
CD4 counts are listed in table 7.1, median CD4 count of 327 cells/ul, IQR (146-457). None of the patients were on ART at the time of diagnosis. However, all patients were referred to ART clinics for monitoring or initiation of ARTs according to the South African ART guidelines applicable during the study period. Blood investigations which included routine tests such as full blood count, urea and electrolytes, autoimmune screen (anti-nuclear factor, anti-neutrophil cytoplasmic antibodies), creatinine kinase, rapid plasma reagin test, vitamin B12 /folate, glucose and serum protein electrophoresis did not reveal any abnormalities.
The CSF median polymorphocyte count and lymphocyte counts were 0 cells/ul, IQR 0-2 and 16 cells/ul, IQR 1-18 cells/ul respectively. The CSF median glucose and protein was 3.1 mmol/L, IQR 2.8-3.4 mmol/L and 1.02g/dl, IQR 0.98-3.4 g/dl respectively (Table 7.1) The CSF tested negative for viruses (CMV, HSV, HTLV1, EBV and VZV), TB, syphilis and cryptococcus. CSF cytology was negative. Five patients had negative ganglioside antibodies; these antibodies were not tested for in the other 6 patients.

Table 7.1: Demographic, laboratory, electrophysiological and radiological features of PM LSP

No.	Age	CD4 count at diagnosis	mRS Scores at presentation	Duration of progression of symptoms	CSF				Electrophysiological Findings		MRI	Time to recovery	mRS at 18 months	Relapses within 18 months
	years	cells/ul		months	P	L	Glu	Protein	Preserved SNAPS	Para-spinal denervation	RE	months		
1.	27	657	4	3	0	32	3.2	0.98	Y	Y	Y	3	0	Nil
2.	22	155	4	2	0	0	3.7	1.02	Y	Y	Y	2	0	Nil
3.	42	480	5	4	0	16	2.3	3.68	Y	Y	Y	3	0	Nil
4.	29	149	5	3.5	0	4	3.1	1.83	Y	Y	Y	4	0	Nil
5.	21	265	5	2	2	17	3.3	1.02	Y	Y	Y	5	0	Nil
6.	24	450	4	4	1	18	2.8	1.69	Y	Y	Y	4	0	Nil
7.	18	380	4	4	0	24	3.6	2.61	Y	Y	Y	3	0	Nil
8.	51	140	4	2	0	0	3.4	0.77	Y	Y	Y	2	0	Nil
9.	32	124	4	5	1	12	4.2	1.52	Y	Y	Y	3	0	Nil
10.	40	112	5	4	2	9	4.1	0.89	Y	Y	Y	5	0	Nil
11.	43	389	4	2	0	5	3.2	1.34	Y	Y	Y	4	1	Nil

RE= Root enhancement/ P=Polymorphocyte count (cells/ul)/ L=Lymphocyte count (cells/ul) / Glu= glucose (mmol/L)/Prot=Protein (g/dl)/ RE=Root enhancement

Table 7.2: Electrophysiological findings of patients with Motor lumbosacral radiculopathy in HIV-infected patients: Motor Studies

	Pt: 1	Pt: 2	Pt: 3	Pt: 4	Pt: 5	Pt: 6	Pt: 7	Pt: 8	Pt: 9	Pt: 10	Pt: 11
	R/L	R/L	R/L	R/L	R/L	R/L	R/L	R/L	R/L	R/L	R/L
Peroneal											
Amplitude	2.2/2.4	4.2/3.8	1.9/2.3	4.4/3.8	2.6/1.8	1.8/2.6	4.1/4.4	5.2/4.8	0.9/1.1	3.4/3.6	5.2/5.1
DML (ms)	5.2/4.8	4.8/5.1	6.2/6.1	5.9/5.8	5.4/5.6	6.3/6.2	5.8/5.9	5.5/4.9	6.9/7.1	4.6/4.2	4.4/4.2
CV (m/s)	44/46	46/48	41/43	40/41	44/46	41/43	44/45	42/43	38/41	42/44	38/40
F Response	58/61	62/68	59/58	73/75	78/68	59/58	59.5/61	abs/64	abs/abs	62/67	73/75
F Estimate	51/50	49/50	52/53	58/54	51/53	52/53	54/55	48/49	abs/abs	57/58	61/62
Tibial											
Amplitude	3.1/3.5	2.8/2.6	1.8/2.1	4.2/4.4	3.2/3.8	2.8/2.6	3.9/4.1	6.7/7.2	1.2/2.1	2.8/2.6	6.1/6.8
DML (ms)	7/6.6	5.8/5.6	6.8/6.6	4.8/4.9	5.9/6.1	5.8/5.6	4.3/4.5	5.3/5.5	6.8/6.9	6.8/6.6	5.9/6.1
CV (m/s)	48/49	40/41	42/44	44/45	51/48	41/40	45/46	43/42	38/39	41/40	43/44
F Response	62/65	70/68	64/74	64/62	65/68	69/70	abs/70	65/72	abs/71	69/70	64/62
F Estimate	52/52	54/56	48/49	54/55	51/52	54/56	55/abs	53/54	58/abs	54/56	56/54
Median											
Amplitude	8.2/8.5	10.2/10	n/a	n/a	n/a	12/12.8	8.6/8.1	10.9/11.8	n/a	11.1/10.9	6.1/6.2
DML (ms)	3.2/3.3	4.1/4.2	n/a	n/a	n/a	4.1/3.8	3.9/3.8	4.4/4.5	n/a	4.1/4.2	3.9/3.8
CV (m/s)	58/52	58/56	n/a	n/a	n/a	55/56	48/45	44/45	n/a	51/50	49/51
F Response	28/29	31/29	n/a	n/a	n/a	24/28	24/26	28/32	n/a	31/33	29/28
F Estimate	31/30	30/31	n/a	n/a	n/a	28/28	32/30	33/34	n/a	35/32	32/34
Ulnar											
Amplitude	6.9/6.3	5.8/5.2	n/a	n/a	n/a	8.8/9.1	7.9/6.1	9.8/9.1	n/a	8.6/9.1	9.4/9.2
DML (ms)	2.6/2.1	3.3/3.1	n/a	n/a	n/a	2.9/2.6	2.6/2.1	3.1/2.7	n/a	2.9/2.6	3.2/2.8
CV (m/s)	50/48	58/56	n/a	n/a	n/a	54/56	52/49	46/48	n/a	52/54	52/56
F Response	31/29	26/24	n/a	n/a	n/a	28/25.5	31/29	31/30	n/a	28/25.5	31/30
F Estimate	30/30	26/27	n/a	n/a	n/a	30/31	33/32	34/35	n/a	30/31	34/35

Table 7.3: Electrophysiological Findings in Patients with Motor lumbosacral radiculopathy in HIV-infected patients: Sensory Studies

SNAP Amplitudes (uV, R/L)	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9	Patient 10	Patient 11
Sural	9.8/10.5	12.5/12.3	11.2/12.3	10.3/9.5	11.5/11.6	12.2/12.9	14.1/13.9	9.5/9.3	16/15.8	12.2/13.1	15.1/15
Superficial Peroneal	3.5/4	6.7/6	5.6/5	4.8/5	6.6/6	7.3/7.5	6.5/6.1	7.1/7.4	6.8/6.5	7.1/7.7	7.8/7
Median	45/58	68/62	not done	not done	not done	85/76	58/56	115/98	not done	45/48	85/96
Ulnar	24/28	45/44	not done	not done	not done	48/53	77/76	65/64	not done	32/38	38/27

Table 7.4: Needle EMG Findings

	Spontaneous Activity				MUP			Recruitment Pattern
Needle EMG Findings	IA	Fib	PSW	fasciculations	Amplitude	Dur	PPP	
Lumbar Paraspinals (L4,L5)	2+ (3+ in patient 11, 7)	1+	2+	0	normal	normal	3+	Reduced (single unit recruitment in patient 11)
Gluteus Medius	1+	2+	1+	0	normal	normal	2+	Reduced (Single unit in pat 1,9,11)
Quadriceps	1+	1+	1+	0	normal	normal	2+	Reduced (single unit recruitment in patient 11)
Tibialis Anterior	1+	1+	1+	0	normal	normal	1+	Reduced
Gastrocnemius	1+	1+	1+	0	normal	normal	1+	Reduced

fasciculations /##: Duration/ PPP: Positive Sharp Waves*: Fibrillation Potentials/ **: Positive Sharp Waves/#:

Motor and sensory electrophysiological tests are listed in table 7. 2 and table 7. 3 respectively. Normal values for our electrophysiology laboratory are listed in the appendix. The compound muscle action potential (CMAP) of the tibial and peroneal nerves were reduced in amplitude with median CMAP of 3.6mV, IQR (2.2-4.2) and 3.5mV, IQR (2.6-4.2) respectively. The distal motor latency (DML) and conduction velocity (CV) were within normal range for both the tibial and peroneal nerves. The F responses were either absent or prolonged, median 62ms, IQR (59-70.5) and 68ms IQR (64-70) for the peroneal and tibial nerves respectively compared to the respective F estimates of 53ms, IQR (50-55) and 54ms, IQR (52-55). There were no conduction blocks or temporal dispersion. The sural and superficial peroneal sensory nerve action potential (SNAP) amplitudes were present in all patients, although marginally reduced most likely due to coexistent HIV peripheral neuropathy. The median sural and superficial peroneal SNAP was 12.5uV, IQR (10-13) and 6.5uV, IQR (5.7-7.1) respectively which is greater than 80% the expected lower limit of normal (see supplementary data). The peak sensory latencies for both nerves were normal: median 4.1ms, IQR (3.9-4.2) and 3.1ms, IQR (2.27-3.3) for the sural and superficial peroneal respectively. The upper limb motor and sensory nerve conduction tests were done in 7/11 (63%) and were normal (table 7.2 and 7.3).

Needle EMG findings are listed in table 7.5.

Muscles examined included the lumbar paraspinals (lower and mid lumbar), gluteus medius, quadriceps, tibialis anterior and gastrocnemius. These muscles demonstrated neurogenic changes as evidenced by increased insertional activity, positive sharp waves, fibrillation potentials, and reduced or single unit recruitment of polyphasic motor unit potentials with greater involvement of proximal rather than distal muscles.

All 11 patients had MRI with gadolinium, of the thoracolumbar and lumbosacral spine. Imaging revealed root enhancement of the lumbosacral ventral roots in all patients (Figure 7.1). There were no identifiable structural abnormalities and no thoracic root enhancement.

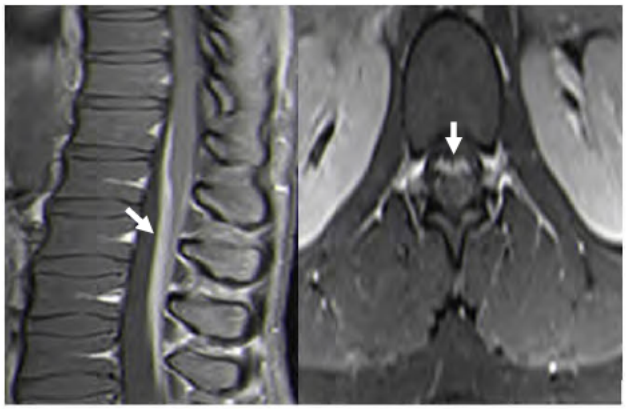

Figure 7.1: Post contrast sagittal and axial Lumbosacral spine images showing ventral root enhancement (arrows)

All patients were treated with corticosteroids (prednisone) at an initial dose of 1.5mg/kg/day at diagnosis for 4-6 weeks or longer if needed. Thereafter corticosteroid therapy was tapered and stopped according to side effects or response to therapy. This was done at the discretion of the attending neurologist. Sixty three percent (64%), 7/11, showed maximum recovery within the first 4 weeks of treatment and recovered fully by 3 months. In this category corticosteroids were tapered over 6-8 weeks and stopped by 3 months. Twenty (36 %), 4/11 received initial corticosteroid doses for periods longer than 4 weeks as they had taken longer to respond. Eighteen percent (2/11) recovered fully by 4 months and the other eighteen (18%), 2/11 by 5 months respectively. In this category of "slower responders", corticosteroid therapy was stopped by 6 months. All patients had no residual clinical deficit except patient 11 who despite demonstrating a good response to corticosteroid therapy by 4 months, had minimal residual deficit at 18 months follow up with a mRS of 1. The median time for recovery in all categories was 3.4 months, IQR (1.8- 5.6).

There were no relapses during the 18 month follow up. Within the period of corticosteroid therapy there were no documented side effects and no patients required corticosteroid sparing immunosuppressive agents or long-term corticosteroids therapy. Six patients had CD4 counts <350 cells/µl and qualified for ART according to ART guidelines at that time. HIV titres were not documented. Three patients were commenced on ART at 4 months after the diagnosis. These 3 patients had recovered prior to ART commencement. The other 3 patients were commenced on ART 6 months after presentation. At 18 months follow up 7 patients were on ART.

Discussion:

The 11 patients presented in this article represent an unusual cohort of HIV-infected patients with a subacute motor lumbosacral radiculopathy. Sphincter function and upper limbs were normal in all patients. The MRI showed gadolinium enhancement confined to the lumbar ventral roots. In other infective aetiologies such as syphilis, TB or viral infections both dorsal and ventral roots are involved, enhancement maybe nodular and patchy with coexistent myelitis, intramedullary granulomas, subdural collections, or discitis [332-334]

This clinical scenario of symmetrical ascending weakness, areflexia and high CSF protein is suggestive of Guillain Barre Syndrome (GBS).[52, 303] More recently the boundaries of GBS have expanded and variations include a paraparetic GBS where the upper limbs and cranial nerves are spared.[335] A further variant is associated with HIV seroconversion.[336] [36]. These patients typically have a CSF pleocytosis as seen in our cohort, which may reflect HIV viral replication in the CSF space rather than immunological changes that occur with the subacute motor lumbosacral radiculopathy [337, 338]. The axonal variant of GBS, associated with a high rate of preceding *Campylobacter jejuni* infection may present as a pure motor axonopathy [339]. Our patients may meet some of the criteria for a

“variant GBS “ [339]. Benatar et al described 4 patients with similar clinical findings. They described these patients as a possible variant of GBS or a distinct clinical entity [6]. However, the unusual features include duration of progression, limitation of signs to the lower limbs, CSF pleocytosis and response to corticosteroid therapy, known not to be of benefit in GBS [340, 341] .

The above cohort may therefore be consistent with a proximal motor variant of CIDP involving demyelination of the ventral roots rather than GBS. Evidence for the above includes progression > 8 weeks in all cases, prolonged or absent F responses with normal DMLs, ventral root gadolinium enhancement on MRI, raised CSF protein and rapid response to corticosteroid therapy with no relapses. Denervation on needle EMG suggests secondary axonal loss.

Moodley et al described CIDP in the setting of HIV. In that particular cohort of patients demyelination was distal rather than proximal, patients had sensory and motor symptoms rather than exclusively motor manifestations, and both upper and lower limbs were involved [247, 299]The rapid response to corticosteroid therapy and the predilection for ventral roots may suggest an antibody mediated process that targets the ventral roots only. The production of these antibodies may be a transient phenomenon during HIV infection as none of the patients relapsed during the 18 month follow up despite stopping corticosteroid therapy by 6 months or less. Alternately, immune reconstitution with ART may have prevented relapses by re-establishing tolerance, by increasing the number of functional T-regulatory cells and, hence maintaining remission. Therefore diseases in HIV may recover with immune reconstitution example HIV associated CIDP, motor neuron syndrome or even myasthenia gravis despite their being insufficient literature to support the above [22, 248]. In HIV variable or unexpected patterns can occur and therefore some disease may be exacerbated, and others improve.

The wide range of CD4 counts may also support an immune-mediated process, which is independent of the stage of HIV. The high CD4 counts in some patients were not explained by concomitant DILS as the patients had no clinical features of DILS[4, 342].

The above clinical presentation is also unlikely to be MNS. The response to corticosteroid therapy differentiates the above disease entity from a lower motor neuron variant of MNS which does not respond to corticosteroid therapy. Furthermore, none of the PM LSP/PM LSP patients had bulbar-respiratory failure which was common among the HIV-infected MNS group.

Seven of the 11 patients were on ARTs at 18-month follow-up. However, only 3 patients started ART at 4 months after presentation. These 3 patients had recovered prior to commencing ART on corticosteroid therapy alone. By 6 months all patients had recovered. Hence recovery was likely corticosteroid induced, as no patients showed spontaneous recovery before corticosteroid therapy. However, it is likely that ART induced immune reconstitution may have prevented relapses as all patients on ART at 18 months follow up had CD4 counts above 350 cells/ul.

Since the SA Government roll-out programme in 2017 which commences all HIV-infected patients on ART at the time of diagnosis irrespective of CD4 counts, we rarely encounter the above group of

patients. This may support an immune basis for the disease as tolerance is re-established with ART. However, a decrease in opportunistic infections and lymphoproliferative disorders may also influence the outcome.

The interest of the above study was selective involvement of the ventral root and a pure motor phenotype. Pure ventral root radiculopathies, are uncommon and poorly described. It has been described, in a small case series and individual case reports in the context of HIV and paraneoplastic neuropathies [247, 249, 343] Currently, there is no antibody panel that predicts for a pure motor phenotype except antibodies against GM1 and NF186 in multifocal motor neuropathy (MMN) and AMAN respectively[136 344]. NFI86 and GM1 antibodies, that were tested after publication of this manuscript were negative in our cohort of patients and the results thereof are in Chapter 10. Others include GM1b,GD1a, GalNAc-GD1a, asialo-GM1 although this clinical- pathological corelation is not absolute [344]. Despite research, the exact mechanism by which GM1 antibodies produce a pure motor phenotype is not understood. Similar principals discussed in previous MMN studies, may however apply to the PM LSP disease model as described below . Recent studies using hiPSC-derived motor and sensory neurons, confirm that both motor and sensory neurons express GM1 antibodies, however GM1 is expressed in abundance in motor nerves[345, 346]. Other factors include enhancement or inhibition of epitope availability by cis interacting gangliosides [347], discrepancies in the pathogenicity of different GM1 antibodies, greater susceptibility of motor nerves to complement mediated cytotoxicity [348], novel antigens (GM1 was negative in all PM LSP patients) , dual antigenic targets, co- factors such as HIV itself may be required to enhance or block epitope sites and lastly region variations in protective mechanisms, or its localised failure may provide explanations for the predilection of GM1 or an "untested" antibody for the ventral root despite wide expression of the antigen. Future research is required using various techniques, such as immunohistochemistry, western blotting, mass spectroscopy, genetic techniques and proteomic analyses, to identify and characterize these antigens in different experimental models. Prospective work using ventral root or motor neuron cultures vs sensory neuron cultures may be useful to expand this concept further. An algorithm for a future protocol defining antigenicity of the ventral root compared to the dorsal root is provided in chapter 12.

Limitations of this study include its retrospective design, small patient numbers due to rarity of the disease, lack of a control arm, not controlling for patients on ART and lack of follow-up of patients with corresponding CD4+ counts and viral loads.

Conclusion:

Studies are required to understand the pathogenesis of this disease, to identify the possible antigenic targets. This may help refine therapy in HIV uninfected patients with pure motor radiculopathy example paraneoplastic PM LSP [329-331]

Author Contributions:

KM developed the concept, collected and analysed the data and generated the manuscript, VBP and PB peer reviewed the manuscript. All authors approved the final manuscript

CHAPTER 8

Autoimmune Nodopathies

Acquired neuropathies have a range of causes, some resulting in life threatening neurological deficits, including paralysis, autonomic dysfunction, and bulbo-respiratory failure. GBS and CIDP are the commonest immune-mediated neuropathies. Within these 2 groups, diverse subtypes are increasingly recognised, some with atypical features and poor response to conventional therapies as discussed in chapter 1. Detection of these subtypes is of clinical significance, as treatment may differ. Intricate regions of the peripheral nerve, which include the node and flanking regions, are currently topical, as immune "attack" in this region may result in potentially treatable neuropathies termed 'autoimmune nodopathies' discussed in the background literature in chapter 1.

Despite the entity of nodopathies being uncommon, and our patients not meeting all the described clinical criteria, we postulated that some of the HIV-infected patients with demyelinating inflammatory neuropathies may have a possible nodo-paranodopathy, despite the quick response to CST, which is not uncommon in some autoimmune nodopathies. Clinical features suggestive of a nodopathy included acute onset, some with distal > proximal involvement, cranial nerve dysfunction, central signs and marked sensory ataxia. Various authors have reported steroid responsive nodopathies such as Querol et al and Miura et al relating to contactin1 antibodies[349, 350] and Kadoya et al to neurofascin 155 antibodies [351]. Clearly, not all nodopathies are refractory to conventional therapy and may differ in different population groups, with different co-morbidities, genetic constitution and different immunoglobulin subtypes. [349-352] The rapid recovery of patients following CST, with no relapses during the follow up period may argue for an antibody mediated nodal block or may support a cell mediated demyelinating disorder. The selective involvement of the ventral root in PM LSP or dorsal root also argues for an antibody mediated process. Other factors that may contribute to rapid recovery is that the immune attack in HIV is transient and lacks strength, or immune tolerance is restored with ART and hence does not allow for a relapse. Furthermore the prevalence of nodal disease in the HIV-infected cohorts and among the African population is unknown (Chapter 2, scoping review). Therefore screening for nodopathies in this prospective cohort of HIMRN patients consisting of (CIDP, DRG,PM LSP) will fill important knowledge gaps in the existing literature. The postulate was that HIV IMRN is a transient steroid responsive nodo-paranodopathy due to autoantibodies generated during HIV infection binding to nodal sites.

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Nodal-paranodal antibodies in HIV-immune mediated radiculo-neuropathies: Clinical phenotypes and relevance.

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Abstract

Background

Nodal-paranodal antibodies are occasionally found in patients initially diagnosed with chronic inflammatory demyelinating polyneuropathy (CIDP) or Guillain-Barré syndrome (GBS). The frequency of nodal-paranodal antibodies in HIV-infected patients with chronic immune mediated radiculo-neuropathies has not been previously described.

Methods:

HIV-infected patients who met the inclusion criteria for chronic immune-mediated radiculo-neuropathies (IMRN) were screened for IgG antibodies directed against nodal (neurofascin (NF)186) and paranodal (NF155, contactin1 (CNTN1) and contactin-associated protein, Caspr1) cell adhesion molecules, using a live, cell-based assay.

To delineate pathogenicity, binding of human IgG to myelinated co-cultures, derived from hiPSC (human induced pluripotent stem cell) sensory neurons and primary rat Schwann cells, was assessed by incubation with patients' sera positive for nodal or paranodal antibodies. Normal human serum was also added as a source of complement to assess for complement activation as a mechanism for myelin injury.

Results:

Twenty-four HIV-infected patients with IMRN were included in the study, 15 met the EFNS/PNS clinical and diagnostic criteria for CIDP, 4 had ventral root radiculopathies (PM LSP) and 5 had dorsal root ganglionopathies (DRG). Five patients with CIDP had combined central and peripheral demyelination. Three patients (12.7 %) tested positive for Neurofascin IgG1 antibodies in the

following categories: 1 patient with PM LSP was NF186 positive and 2 patients were NF155 positive with DRG and mixed sensory motor CIDP with optic neuritis respectively.

Conclusion:

The frequency of the above nodal-paranodal antibodies is similar among IMRN regardless of HIV status. However, interpretation of the results in the context of HIV is challenging and there is uncertainty as to whether these antibodies are pathogenetic or not, especially at low titres. Larger prospective immune studies are required to establish pathogenicity of the antibodies in the context of HIV, and to establish a panel of antibodies to predict for a particular clinical phenotype, namely pure motor, pure sensory, combined central and peripheral demyelination, mixed sensory motor CIDP and CIDP with optic neuritis.

Introduction:

The immunology of chronic inflammatory demyelinating polyneuropathy (CIDP) is poorly defined. Most neurologists conclude that CIDP is an auto-immune disease mediated by cellular toxicity or antibodies to neural antigens within compact myelin, Schwann cells, and non-compact myelin at the nodal-paranodal-juxtaparanodal region [77, 353].This is supported by the response to immunomodulatory therapy (intravenous immunoglobulin (IVIg), plasma exchange (PE), corticosteroids (CST) and monoclonal antibodies (Rituximab)), and peripheral nerve histology.[77] Studies have focused on cytokines, cellular infiltrates and searches for antibodies without any consistent results except in cases of nodal- paranodal disease, where antibodies against neurofascin(NF), contactin1(CNTN), Caspr have been best characterised [236, 349, 351, 354]. This has been described mainly in the Western and Asian literature in HIV-uninfected patients, accounting for less than 10% of CIDP cases either due to the rarity of the disease, genetic predisposition, lack of widespread testing or due to unidentified antigens [231, 350, 351, 355, 356].

NF186 is a cell adhesion molecule which is critical for the formation and maintenance of the node of Ranvier. Genetic ablation of NF186 results in loss of NrCAM, an axonal adhesion molecule that binds to ankyrin and gliomedin. This leads to dispersion of sodium channels and conduction failure[357]. NF155, on myelin loops, and heterodimers of CNTN1 and Caspr on the axolemma form septate like junctions which maintain the structural integrity of the paranode. These septate like junctions are absent in mice that lack CNTN1, Caspr or NF155. This results in mislocalisation of juxtaparanodal voltage-gated potassium (Kv1.2) channels and hence disruption of saltatory conduction due to failure of repolarization of the action potential.[104] There is emerging evidence that nodal-paranodal neuropathies may be pathologically distinct diseases without the segmental macrophage-induced demyelination seen in CIDP and instead is characterised by focal paranodal myelin detachment and lengthening of the node on electron microscopy [136,103, 358] .

A previous study describing CIDP and pure motor lumbosacral polyradiculopathy (PM LSP) in HIV-infected patients, suggested that the above cohorts are highly corticosteroid (CST) responsive with shorter duration of disease, compared to HIV-uninfected counterparts.[247, 359] Steroid responsive nodo-paranodopathies have been reported by Querol et al and Miura et al with CNTN1 antibodies[349, 350] and Kadoya et al with NF155 antibodies [351]. Davies et al described one HIV-infected patient with GBS with a strong binding of IgG to axons on cultures, however no antigenic target was reported [231]. This rapid recovery of HIV-infected CIDP and PM LSP patients, with no relapses, may argue for an antibody mediated nodal block. We postulated that chronic immune mediated radiculo-neuropathies in HIV-infected patients are a steroid responsive nodo-paranodopathy due to autoantibodies generated during HIV infection binding to nodal sites. As the term HIV-associated immune mediated radiculo-neuropathies is rather cumbersome, it will be abbreviated to HIMRN for the rest of the manuscript. This study tests for nodal and paranodal antibodies targeting NF, CNTN1, Caspr, or CNTN1/Caspr complex in chronic HIMRN with different phenotypes and speculates about the pathogenesis of immune mediated demyelination in the setting of HIV. There is currently no available literature describing nodal-paranodal-juxtaparanodal antibodies in HIMRN, their clinical relevance in the setting of HIV, or the differences in nodal-paranodal antibody expression across the different phenotypes.

Methods:

Patient Recruitment:

The study was a prospective descriptive study of a cohort of HIMRN, above 12 years of age, which included CIDP, ventral root radiculopathies (PM LSP) and dorsal root ganglionopathies (DRG) seen at Inkosi Albert Luthuli Central Hospital (IALCH), a quaternary referral centre in Durban, South Africa between January 2015, and December 2020. South Africa has the highest prevalence of HIV in the world, with an estimate of 7.3 million people living with HIV in 2017. Kwa-Zulu Natal (KZN) suffers 40% of the HIV burden in South Africa. The study was approved by the University of KZN Biomedical Research Ethics Committee (Ethics reference number: BE 272/15).

Inclusion Criteria:

HIV-infected patients, older than 12 years, with chronic HIMRN were included in the study. Patients were diagnosed with HIV prior to or at the time of diagnosis of the HIMRN. All patients were required to have peripheral nerve dysfunction with or without central disease. Additionally, they needed to fulfil the clinical, electrodiagnostic, and CSF criteria of the European Federation of Neurologic Sciences (ENFS)/ Peripheral Nerve Society (PNS) for CIDP, criteria for PM LSP and DRG. For the purpose of the study, PM LSP was defined as: a) pure motor presentation, b)

progressive disease beyond 3 months c) ventral root enhancement on MRI[249] . DRG was described as an immune mediated pure sensory non-length dependent clinical presentation of longer than 3 months, absent sensory nerve action potentials on electrophysiology, normal motor studies with or without abnormal blink responses or dorsal root enhancement on MRI[360].

Exclusion Criteria:

Patients were excluded if they did not meet the diagnostic criteria for CIDP, PM LSP, or DRG, if the clinical presentation was suggestive of GBS, or if secondary causes were identified such as drugs, toxins, paraneoplastic antibodies or connective tissue disease or their HIV status was negative or unknown. Patients with known immune mediated radiculo-neuropathies, who later acquired HIV were also excluded from the study, as their disease was not related to HIV.

DILS, mononeuritis multiplex were excluded as these were not antibody mediated.

Investigations:

Bloods and CSF:

All patients were screened for HIV with an HIV ELISA test, baseline CD4 counts and HIV viral load. Other tests included connective tissue screen, ganglioside antibodies (GQ1b, GD1a, GD1b, GT1b, GM1, GM2, and GM3), paraneoplastic antibodies and serum paraprotein. In addition, CSF IgG index and oligoclonal bands (OBs) were evaluated in some patients.

Patients with optic nerve involvement, in addition to the above were evaluated for aquaporin 4(AQP4), myelin oligodendrocyte glycoprotein (MOG) antibodies and raised serum angiotensin converting enzyme levels for neuromyelitis optica and neurosarcoidosis respectively. Further tests were conducted to exclude infective or neoplastic causes of a polyradiculopathy as highlighted in the Appendix , Table A. 2.

Immune Tests:

Live CBA:

All patients, prior to commencing therapy, had 10 ml blood and CSF extracted and stored in 1ml aliquots at -80 degrees. These samples were tested for IgG antibodies directed against nodal (NF186) and paranodal (NF155, CNTN1 and Caspr1) cell adhesion molecules, using a live, cell-based assay, as previously described [114, 132, 231].In brief, human embryonic kidney 293T (HEK) cells were transiently transfected with plasmid constructs containing the human cDNA sequences for either CNTN1 and Caspr1, NF155, or NF186. After 24 hours, the cells were washed and incubated with patient sera for 1 hour at room temperature. Fluorescently tagged secondary and tertiary antibodies against human IgG or human IgG subclasses 1-4 were used to visualise cell membrane binding by an investigator blind to the sample identity.

Myelin co-cultures and complement activation:

Sera (heat inactivated to abolish complement activity and diluted to 1:50) from patients positive for nodal-paranodal antibodies using live CBA, were assessed for topographical IgG binding using

myelinated co-cultures. Co-cultures were generated using sensory neurons derived from human induced pluripotent stem cells (hiPSC) and primary rat Schwann cells, according to a previously published protocol [237].

For immunolabelling, live co-cultures were incubated with patient sera for one and 24 hours respectively at 37 ° C, with or without the addition of normal human serum (NHS), at a dilution of 1:5, as a source of complement. They were then fixed prior to labelling with secondary fluorescent antibodies against human IgG. Subsequent permeabilization allowed immunostaining of the axons and myelin with primary and secondary antibodies. Serum containing antibodies to known antigens, as well as normal human serum, were run as positive and negative controls, respectively. Serum samples were blinded by an independent investigator. Confocal images were acquired with a x20 lens, and cultures assessed for evidence of focal IgG binding and complement induced injury.

Myelin, axons, human IgG were immunostained to assess for myelin or axonal damage and topographical binding of human IgG to neural tissue.

Electrophysiology and Radiology:

Standard motor and sensory nerve conduction studies were performed on all patients (eight motor nerves including f-wave latencies, four sensory nerves). Electromyography was also performed to assess for axonal loss (Table A1).

Patients with suspected optic neuritis (ON) had optic coherence tomography (OCT), visual evoked responses (VEP) and MRI brain and orbits performed. Patients with DRG had blink responses, MRI brain and spine and whole-body positron emission tomography to exclude occult malignancy. Patients with PM LSP had an MRI spine. All patients had a chest radiograph to exclude pulmonary tuberculosis.

Treatment Protocols:

Patients were treated with standard therapy for CIDP which included CST and/or IVIG [77, 92].

If they presented acutely and were clinically suspected of having GBS they were initially treated with IVIG. Patients who had acute onset disease and progressed beyond 4 weeks, were managed as CIDP with CST.[247] All patients with PM LSP and DRG were treated with steroids, in line with previous experience [359]. Escalation therapy included IVIG, Azathioprine (AZA), plasma exchange (PLEX), and Rituximab. This was added at the discretion of the physician, guided by adverse events and response to initial therapy. This is based on standard treatment guidelines used by our unit and international guidelines[92].

Statistical Analysis:

Data was entered in Microsoft Excel and analysed using Prism Software. Descriptive statistics such as percentages, interquartile ranges (IQR), medians, mean and range were used to summarize the results.

Results:

Fifty-eight patients were initially recruited for the study. Of these, 30 were excluded as they were HIV-uninfected, one had a history of n-hexane use, two declined HIV testing, and one was diagnosed with tuberculosis polyradiculopathy on CSF polymerase chain reaction (PCR).

Demographics and clinical features at presentation:

Twenty-four HIMRN patients were assessed. Table 8.1 highlights the clinical and demographic features. All participants were of black African ancestry, 13 (54%) were female, median age of 38.5 years (IQR 22-43 years). The cohort consisted of the following clinical categories; 4 (16,7%) had PM LSP, 5 (21%) had DRG, and 15 (62.3%) had mixed motor and sensory (MSM) CIDP. Among the patients with MSM-CIDP, 4 (27%) patients had optic neuropathy (ON), and 1(6.67%) patient had combined central and peripheral demyelination (CCPD). All patients with ON had poor visual acuity on the Snellen chart, 3 (75%) had swollen discs and 1(25%) had optic atrophy (figure 8.1). The patient with CCPD had subclinical central nervous system involvement. She was investigated with further imaging because of a positive family history of CCPD (figure 8.2). Two (40%) patients with DRG had clinical trigeminal nerve involvement. Thirteen (54%) of patients presented acutely, 8 (33%) presented with autonomic dysfunction, and 10 (41%) with ataxia, 6 (25%) with pain. The median Modified Rankin Scale (mRS) scores at presentation were 4.

Relevant blood and CSF findings:

The mean CD4 count was 202 cells/µl, IQR 155-212 cells/µl and viral load was 40313 copies/ml (0-90666 copies/ml), (Table A3) Sixteen (66%) of patients had a polyclonal gammopathy, three patients had positive antinuclear antibody (ANA) titres of 1:160, speckled pattern, 2 were positive for Ro antibodies and Ma2 antibodies respectively (supplementary file, table 2). These patients were still included in the study as the clinical presentation was not related to positive antibody tests. CSF analysis revealed an elevated protein in all patients, median of 1.21g/L (IQR: 0.98 g/L-1.67g/L) and a CSF lymphocytosis, median of 8 cells/ul, IQR (6-16 cells/ul).

Serum and CSF oligoclonal bands were tested for in 11/24 (48%) patients. Six patients with MSM-CIDP had comparable bands in both serum and CSF, 4 patients (3 PM LSP, 1 CCPD) had bands in the CSF only and 1 patient with PM LSP had differing bands in the CSF compared to serum (type 3 pattern). The IgG index was elevated in 6/11 (4 PM LSP, 1 CCPD, 1 mixed motor sensory CIDP). The median IgG index was 0.8, IQR (0.5-0.9), consistent with intrathecal IgG synthesis (table A3).

Paranodal antibodies:

Twenty-one (87.5%) patients in this cohort tested negative for IgG antibodies against paranodal/glial isoform Neurofascin (NF)155, and nodal/axonal isoform NF140 and NF186, Contactin1 and Caspr-1. Patient 3 (MSM-CIDP with optic neuritis) and patient 16 (DRG) tested positive for NF155 (IgG1, titre 1:100), and patient 21 (PM LSP) tested positive for NF186 (IgG 1, titre: 1:400), (Table A2, Table 8.1, and figure 8.4)

Myelin co-cultures and complement activation:

In the above 3 patients who tested positive for nodal-paranodal antibodies using live CBA, there was no binding of human IgG to myelin or axons at 1 hour and 24 hrs respectively. There was also no complement activation or accelerated myelin damage after the addition of complement.
Figure 8.5.

Nerve Conduction studies (NCS) and optical coherence tomography (OCT):

All 15 patients with MSM-CIDP met the electrodiagnostic criteria for CIDP.The four patients with PM LSP, had preserved SNAPs and severe axonal changes including denervation in the lumbar paraspinal muscles supporting the diagnosis of a radiculopathy. The five patients with DRG had absent SNAPs and normal motor studies, 2(50%) had abnormal blink responses. Three patients with ON had abnormal VEP; 1 had absent visual evoked responses, 4 had abnormal OCTs. (Table A4)

Radiology:

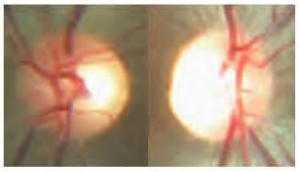
The MRI spine showed ventral root enhancement in all 4 patients with PM LSP (figure 8.2). Patient 16 who tested positive for NF155 antibodies showed possible dorsal root enhancement. There was no enhancement of the trigeminal nerve or ganglion in the 2 patients with clinical trigeminal neuropathy. Patient 2 had extensive symmetrical cerebral white matter disease, including the splenium and genu of the corpus callosum (Figure 8.3) and 2 patients had optic nerve enhancement on MRI orbits, one of whom was patient 3 with NF155 positivity.

Treatment and response to therapy:

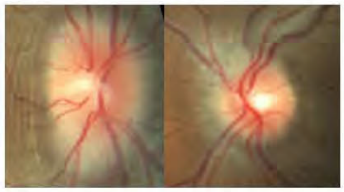
Twelve (80%) of patients with MSM-CIDP responded well to CST and 3 (20%) required escalation therapy (patient 2, 3, 6). All patients with PM LSP responded well to CST monotherapy, including patient 21 who was NF186 positive. Those with DRG responded poorly and required combination therapy (CST, AZA, IVIG). Patient 17 had received plasma exchange and patient 16 (NF:155 positivity) has had poor treatment response and is awaiting escalation therapy with Rituximab. Intravenous methylprednisolone was administered to those with suspected optic neuritis (patients 3,6,11), and responded well to steroid therapy, however patient 3 (NF155 positive) required AZA in addition to CST due to frequent relapses. The patient with CCPD was treated with IVIG initially as she was thought to have GBS, and thereafter maintained on CST and AZA with good response. IVIG was considered in patients 13 and 14 as they had concomitant DM. The mRS scores at 6 months, highlighted in table 8.1, show significant improvement in most categories except DRG. The impact of antiretroviral therapy (ART) on recovery was not assessed in this article. Eight (30%) of patients were on ART at the time of presentation, the rest were ART naïve.

Figure 8.1: Fundal Images (Patient 3, 6 and 11 showed swollen discs and patient 5 optic atrophy)

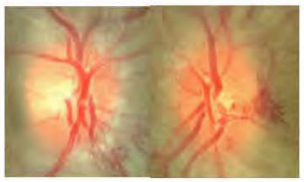
Patient: 3



Patient: 5



Patient: 6



Patient: 11

Table 8.1: Clinical and demographic features of the HIV-infected cohort with immune mediated radiculo-neuropathy

	Clinical							Investigations and Treatment						MRS Scores	
	Clinical Diagnosis	Onset	Motor or Sensory	CN palsy	Autonomic	Pain	Ataxia	CD4 count	NCS (Overall impression)	MRI Spine/brain	CSF Protein	(+) nodal-paranodal antibodies		Nadir MRS	MRS at 6 months
P1	CIDP	Acute	M & S	X	✓	✓	✓	122	Demyelinating		1.24g/L		CST	4	2
P2	CIDP	Progressive	M & S	X	X	X	X	335	Demyelinating	White matter Dx	1.28g/L		IVIG, CST+AZA	4	2
P3	CIDP	Progressive	M & S	✓ ON	✓	X	✓	189	Demyelinating	Normal	1.22g/L	NF:155 1:200 (IgG1)	CST+AZA methylprednisone	4	0
P4	CIDP	Progressive	M & S	X	✓	X	✓	288	Demyelinating	ND	1.06g/L		CST	3	1
P5	CIDP	Acute Onset, progressive	M & S	✓ ON	✓	X	✓	356	Demyelinating	Normal	1.41g/L		CST	3	2
P6	CIDP	Acute	M & S	✓ ON	X	✓	X	123	Demyelinating	Optic nerve enhancement	1.12g/L		IVIG, IVI methyl prednisone, CST, AZA	4	3
P7	CIDP	Progressive	M & S	X	✓	✓	✓	366	Demyelinating	ND	1.76g/L		CST	4	1
P8	CIDP	Progressive	M & S	X	X	✓	X	168	Demyelinating	ND	0.99g/L		CST	4	3
P9	CIDP	Acute	M & S	X	X	X	X	432	Demyelinating	ND	0.88g/L		CST	5	2
P10	CIDP	Acute	M & S	X	X	X	X	132	Demyelinating	ND	1.72g/L		CST	5	1

Table 8.1: Clinical and demographic features of the HIV-infected cohort with immune mediated radiculo-neuropathy

	Clinical							Investigations and Treatment						MRS Scores	
	Clinical Diagnosis	Onset	Motor or Sensory	CN palsy	Autonomic	Pain	Ataxia	CD4 count	NCS (Overall impression)	MRI Spine/brain	CSF Protein	(+) nodal-paranodal antibodies		Nadir MRS	MRS at 6 months
P11	CIDP	Acute	M & S	✓ ON	X	X	X	312	Demyelinating	Optic nerve enhancement	1.15g/L		CST, IV Methylprednisone	3	0
P12	CIDP	Progressive	M & S	X	X	X	X	196	Demyelinating	ND	0.77g/L		CST	4	1
P13	CIDP	Acute	M & S	X	X	X	X	122	Demyelinating	ND	1.08g/L		CST	4	1
P14	CIDP	Acute	M & S	X	X	X	X	166	Demyelinating	ND	1.33g/L		IVIG+CST	5	2
P15	CIDP	Acute onset Progressive	M & S	X	X	X	X	196	Demyelinating	ND	0.77g/L		IVIG + CST	4	1
P16	DRG	Acute	Sensory only	✓ TN	✓	X	✓✓✓	277	Absent SNAPs Normal motors studies	Vague DRE	0.95g/L	NF:155 1:200 IgG1	CST/AZA/IVIG ? RTX	4	5
P17	DRG	Acute	Sensory only	X	X	X	✓✓✓	180	Absent SNAPs, normal CMAPs	No DRE	0.88g/L		PE+IVIG+CST	3	4
P18	DRG	Progressive	Sensory Only	X	✓	X	✓✓	223	Absent SNAPs Abnormal blink	No DRE	0.61g/L		CST+AZA+IVIG	5	4
P19	DRG	Progressive	Sensory Only	X	✓	X	✓✓	214	Absent SNAPS Abnormal Blink	Vague DRE	2.13g/L		CST+AZA+IVIG	4	4

Table 8.1: Clinical and demographic features of the HIV-infected cohort with immune mediated radiculo-neuropathy

	Clinical							Investigations and Treatment						MRS Scores	
	Clinical Diagnosis	Onset	Motor or Sensory	CN palsy	Autonomic	Pain	Ataxia	CD4 count	NCS (Overall impression)	MRI Spine/brain	CSF Protein	(+) nodal-paranodal antibodies		Nadir MRS	MRS at 6 months
P20	DRG	Acute onset Progressive	Sensory only	✓ TN	X	X	✓✓✓	122	Absent SNAPs Normal motors studies	No cranial nerve enhancement	1.21g/L		IVIG+CST	4	3
P21	PM LSP	Progressive	Motor Only	X	X	X	X	210	Normal SNAPS, Axonal	VRE	2.34g/L	NF186 1:400 (IgG1)	CST	5	1
P22	PM LSP	Acute	Motor only	X	X	X	X	112	Axonal, normal SNAPs	VRE	2.23g/L		CST	4	1
P23	PM LSP	Progressive	Motor only	X	X	X	X	208	Normal SNAPs Reduced CMAPs	VRE	1.66g/L		CST	4	2
P24	PM LSP	Progressive	Motor only	X	X	X	X	212	Axonal, Normal SNAPS	VRE	2.56g/L		CST	5	2

CST=corticosteroid therapy, IVIG=intravenous immunoglobulin, AZA-azathioprine, M&S=motor and sensory, MRS=Modified Rankin Scale, ON=Optic nerve, TN=Trigeminal nerve, PMLSP=Pure motor lumbar-sacral polyradiculopathy. DRG=Dorsal Root ganglionopathy, NF=Neurofascin, ND=not done

Fig: 8.2: Patient 21 and 23 : Post contrast sagittal and axial MRI images showing Ventral Root enhancement

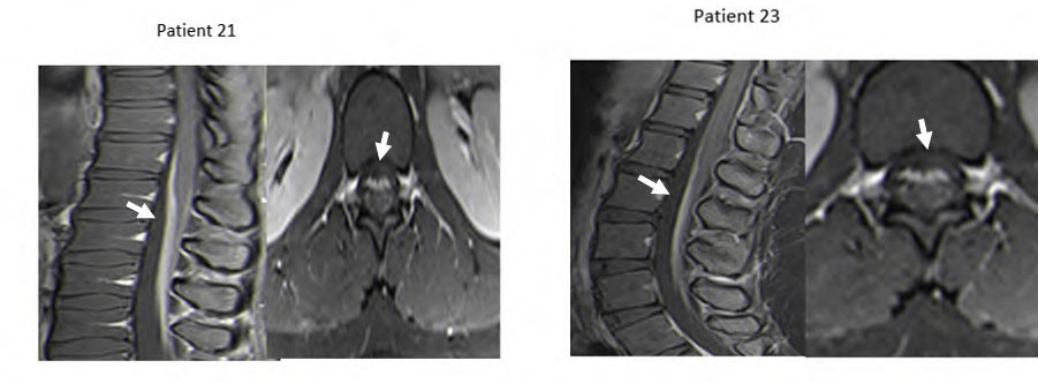
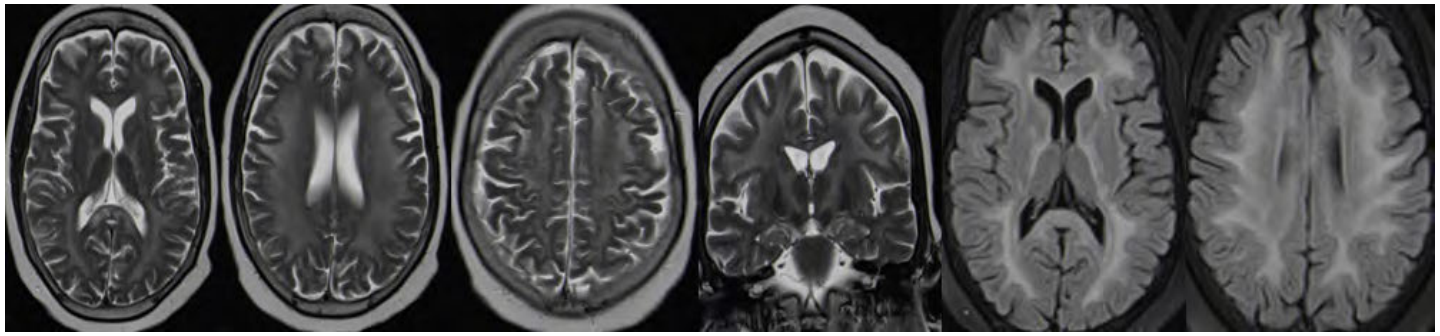


Figure 8.3: Patient 2: T2 and flair axial and coronal MRI brain scans



Diffuse symmetrical involvement of the white matter including the corpus callosum

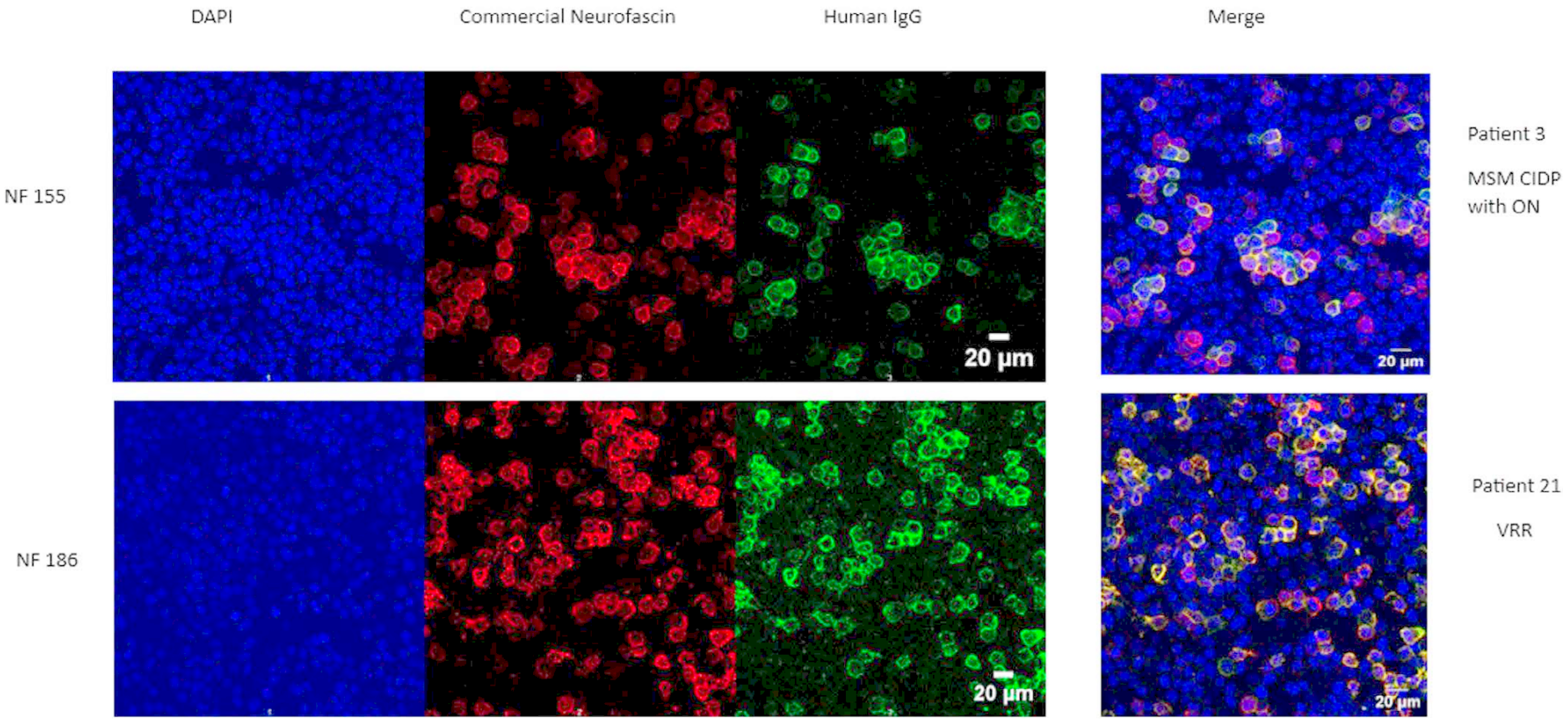
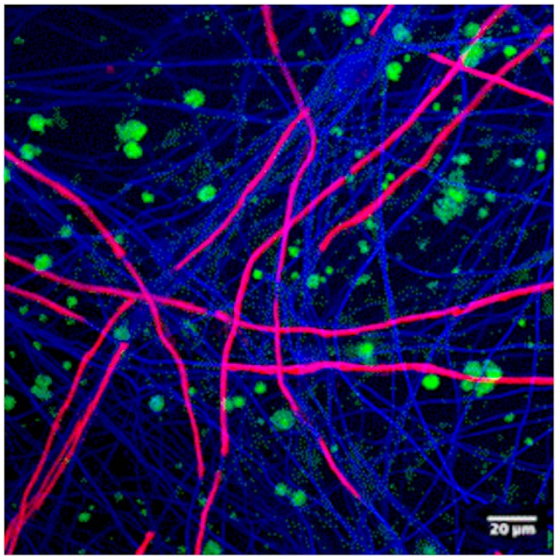


Fig 8. 4: Cell-based assay using fluorescence microscopy. HEK cells are transfected with plasmid vectors encoding the different isoforms of neurofascin, which are expressed as proteins within the cell membrane. Incubation with patient 3 and patient 21 sera allows human neurofascin antibodies (green), if present, to bind to their target antigen. Co-localization (yellow fluorescence) with a commercial neurofascin antibody (red), confirms NF155 and NF186 as the target antigen in patient 3 and 21 respectively.



There is no binding of human IgG to neural structures (myelin and axons) up to 24 hrs after adding sera of patient 3,16 and 21 and 20% normal human serum as a source of complement. There is also no myelin injury demonstrated during this time frame.
Green=Human IgG, Red =myelin, Blue=axons

Figure 8.5: Myelinated Co-cultures: Axons derived from Hi PSC and myelin from rat Schwann cells.

Discussion:

The main findings of this study are that 3 (12.5%) of chronic HIMRN were positive for nodal/paranodal antibodies. This is comparable to the frequency of nodal-paranodal antibodies reported in HIV-uninfected immune mediated neuropathy cohorts [361]. The clinical profiles of the seropositive patients are MSM-CIDP with ON (NF155 positivity), DRG (NF155 positivity) and PM LSP (NF186 positivity). All 3 patients had purely IgG1 subclass of antibodies. None of the patients tested positive for antibodies against CNTN1 or contactin1/Caspr1.

Thus far, the best characterised clinical profile relates to NF155 IgG4 antibodies [127]. The other well described category is pan- neurofascin antibodies (cross reactivity with both the nodal/axonal NF186 and NF140 isoforms, and paranodal/glial NF155 isoform antibodies) reported by Fehmi et al and more recently Appeltshauser et al [114, 115]. Clinical features of the NF155 IgG4 category include acute onset with chronic progression, distal motor predominance, sensory ataxia, tremor, central involvement, and refractoriness to treatment especially IVIG and steroids. Patients with pan-neurofascin antibodies differ in that they present with life threatening, acute onset GBS-like symptoms of bulbar-respiratory failure, autonomic instability, fulminant and progressive quadriparesis and cranial nerve involvement. They often require prolonged ventilation and are also refractory to conventional therapy.

Patients with CNTN1 IgG4 (chapter 10, Fig 10.2B) antibodies have a relatively uniform clinical phenotype, characterised by older age, aggressive disease onset, motor predominance, early axonal loss, and poor response to IVIg [116, 349]. They may also present with a membranous glomerular nephritis and nephrotic syndrome due to deposition of CNTN1-containing immune complexes in renal glomeruli [132]. However, these are less common. Patients with Caspr antibodies present with prominent neuropathic pain[133] .

NF186 antibodies are less well described and currently do not define a clinically distinct cohort. However, a recent publication by Liu et al, described NF186 nodopathies (IgG subclass not defined) in a small cohort of 13 patients [134]. The main features included acute or subacute onset, progressive or relapsing course, asymmetrical proximal and distal weakness, and CST or IVIG responsiveness. Dysautonomia, sensory ataxia and cranial nerve involvement were infrequent. Devaux et al reported NF186 antibodies in 12 % of GBS patients, the IgG subclass was not specified[362]. Burnor et al reported 4 patients with NF186 antibodies(IgM) and 1 patient with idiopathic neuropathy (IgG1) [113]. IgG2 neurofascin antibodies have been recently described in a paediatric cohort and majority were IVIG responsive[363]. Cortese et al reported 5 patients with non-IgG4 subtype NF antibodies who demonstrated clinical features indistinguishable from seronegative CIDP patients, including good response to IVIG[364]. NF antibodies have been reported in other diseases such as multiple sclerosis, HIV encephalopathy, motor neuron disease and paraneoplastic syndromes [104, 365, 366]. NF antibodies especially of the IgG1 subtype, therefore, appear to be less specific and less likely to define a clinical profile of patients[367].

The current literature, suggests that predominantly IgG4 subclass of antibodies and pan-NF antibodies define a distinct population, characterised by acute onset and poor response to conventional therapy [127, 128, 367-369]. IgG4 differs from other IgG subclasses in that it does not activate complement, less likely to internalize the target antigen, bocks protein-protein interaction and develops later in the disease [127, 357, 361].Longitudinal studies suggest that IgG1 and IgG3 occur in acute monophasic disease and IgG4 in chronic relapsing disease. [370] The IgG3 subclass sequentially switches to IgG1, then 2 and 4. [371] Autoantibodies gain antigen affinity with each switch, with IgG4 showing the highest affinity to its target [372, 373]. The trigger for IgG switching, may depend on HLA alleles, chronic antigenic exposure, or a multitude of immunological mechanisms as in HIV [374].

The favourable response to CST in PM LSP and MSM-CIDP with ON and in HIV associated CIDP reported in previous studies may be consistent with an IgG1 or non-IgG4 subclass[247, 358]. Many of these patients especially the PM LSP and HIV-associated CIDP reported in a previous study also displayed short duration monophasic illness consistent with IgG1 disease. In this study the clinical significance of the IgG1 antibodies in patient 3,16 and 21 remains uncertain as patients required additional immunosuppression except for patient 21, with PM LSP who responded well to CST monotherapy. Patient 16 (DRG) has shown treatment failure requiring Rituximab treatment and patient 3 (MSM CIDP with ON) required azathioprine in addition to CST. Furthermore, all patients had chronic progressive disease, except for patient 21 with PM LSP.

The literature is limited regarding nodal-paranodal antibodies in DRG or PM LSP even in the HIV-uninfected population. Querol et al reported 4 patients with moderate to intense IgG reactivity against DRG, and 3 patients with mild reactivity against motor neurons [361]. However, no target antigen was identified. Contactin1 is highly expressed in the DRG and antibodies to contactin1 may therefore pathophysiologically explain the marked sensory ataxia in these patients [375]. Recent antibodies described in DRG include anti-AGO1 antibodies associated with Sjogren's syndrome [376 377]. Antibodies associated with ON are aquaporin-4, MOG, CRMP, glial fibrillary acidic protein (GFAP), GQ1B, and more recently NF155 antibodies [378-380]. The IgG4 subclass is usually described in NF155 optic neuritis, therefore relevance of an IgG1 subclass in patient 3, again, is uncertain but may still be relevant depending on the stage of disease. Screening for novel antibodies using optic nerve cultures may prove useful in the future. With respect to CCPD (patient 2), targeting antibodies common to both compartments, which include NF155, MPZ, MBP or novel antibodies maybe a clue to the immunopathogenesis of this disease.[119, 381-384]

Despite most of the cohort testing negative for paranodal antibodies, many factors favour an antibody mediated attack. This includes CSF-restricted oligoclonal bands, favourable response to PLEX or IVIG in some patients in this cohort and in other case studies [231], and selective involvement of the ventral root, dorsal root and ON. The node and flanking regions, consists of a multitude of ion channels and related proteins, that may be pathogenetic targets. [108] Additional targets include antigens

within central or peripheral compact myelin, for example MBP, MP0, which were not analysed in this study. Comprehensive panels that include all available antigenic targets are required to elicit the pathogenetic antigen-antibody combination. The pathogenetic antibody for HIMRN may be an unidentified or untested target. Newer targets are constantly being identified such as the recently described, LG14 juxta-nodal antigen in a Japanese cohort [119].

In addition, to constitutive immune responses in CIDP, consideration must be given to the influence of the HIV pathogenetic process on tolerance. This is rather expansive and beyond the scope of this article. However, some of the factors in HIV that may influence tolerance include the qualitative or quantitative dysfunction of subsets of T-reg cells including CD25+ Foxp3 Treg cells [17, 385], downregulation of IL10, TGF-β and activation-induced cytidine deaminase (AID) which may promote IgG class switching [386]. CD8+ T cell proliferation and secretion of pro-inflammatory cytokines (IL1, IL6, TNF α, IFN-γ)[387] leads to premature thymic involution and decrease in autoimmune regulator gene (AIRE) expression. [70] Other factors include non-specific B cell hyper-stimulation resulting in low affinity, polyreactive antibodies as in patients 2,6,10,14 who tested positive for autoimmune antibodies without clinical disease, polyclonal gammopathy in 16 patients and oligoclonal bands in both serum and CSF. Exclusive oligoclonal bands in the CSF may be seen in HIV, opportunistic infections, CIDP and PM LSP [388, 389]. Characterization of the subtypes of intrathecal IgG synthesis and identifying neural antigen-specific CSF IgG may refine the immunopathogenesis of HIMRN especially in the PM LSP and CCPD category.

Despite, no comparative studies between Europe and Africa, unpublished data show that low-titre IgG1 subclass, NF155 and NF186 antibodies, are different in our cohort compared to the rest of the world. Currently in HIV-uninfected European cohorts, only IgG4 and PanNF antibodies define a distinct disease entity. IgG1subclass NF155 or NF186 monospecific antibodies, as seen in our HIV cohort are not well understood, and difficult to interpret in the HIV context [127, 367, 370] . The emergence of an IgG1 subclass clone is likely due to immune dysregulation in HIV, or other undefined genetic or immune factors which impair immunoglobulin class switching. [374] Studies show that in HIV infection there is early and profound depletion of mucosal memory $CD4^+$ Th cells, later T follicular cells, and dysregulation of cytokines such as IL4,IL5,IL10,IFN gamma and TGF beta which plays an important role in the regulation of immunoglobulin class switching[390, 391]. Other factors, include regulatory sequences such as super-enhancer, C-alpha gene and T-box transcription factor (T-bet) which is modified in HIV and other chronic viral infections[392,393,394].

HIV disrupts the ability of the humoral immune response to produce neutralizing antibodies or form effective immune memory, preventing viral clearance [395]. Changes in the microenvironment shifts production of B cells to short-lived plasma cells early in the response. Polyclonal B cells are recruited

into both the plasma cell and germinal centre compartments, inhibiting the formation of a targeted, high-affinity response[396]. Finally, memory B cells shift toward an "atypical" phenotype, which may further impair the production of effective high affinity antibodies, in addition to impaired class switching [347]. Low antigenic affinity antibodies may therefore influence binding to myelin co-cultures resulting in a negative outcome as in most of our cohort. Other reasons for no binding are discussed in detailed in the manuscript.

Future laboratory experiments are required to understand the profound impact of HIV on T cells, germinal centre reactions, peripheral and central B cell tolerance checkpoint mechanisms and somatic hypermutations required for immunoglobulin class switching. Such experiments may offer a possible explanation for IgG1 antibodies in our cohort and may provide indirect clues to understanding disease severity, chronicity and risk factors for a relapse even in the HIV-uninfected categories based on IgG subtypes.

Teasing out the complexity of the immune responses in HIMRN requires a systems approach where one needs to examine cross-sectionally and longitudinally many and all aspects of the immune response as immune responses vary with time. Furthermore, cytokine effects vary depending on the environment in which they function, being different not only in the tissue within which they are effective, but also the combination in which they exist and the stage of the immune response. This may be relevant especially in the pro-inflammatory context of HIV.

The lack of binding of human IgG to myelin co-cultures, may support an exclusive cell mediated response. However, this alone, does not exclude an antibody mediated pathogenesis. Possible considerations for lack of binding include low antibody titres, low antigenic affinity IgG1 antibodies which may switch to higher affinity IgG subclass over time, antigenic targets in myelin co-cultures being structurally different to antigens present in chronic HIV-infected neural tissue, presence of different tissue cytokine patterns in pathological nerve tissue or lastly, an unknown concomitant co-factor required to enable binding of circulating antibodies. This is difficult to mimic invitro.

This study has several limitations which includes the following: small sample size, especially in the subcategories of PM LSP, DRG, ON, CCPD, lack of control patients, limited testing for other known or unidentified nodal-paranodal antibodies, use of primary rat, hiPSC myelin co-cultures which may be antigenically different from chronically diseased or HIV-infected nerve tissue, not controlling for different stages of HIV infection or effects of ART, limited longitudinal follow up of patients and laboratory error.

Nevertheless, the above serves as a platform for future larger studies using myelin derived from hiPSC, incubated with patients' serum containing 'live' HIV virus and other co-factors. This will create an 'inflammatory' environment, which may alter the expression or configuration of antigens enabling binding. Additionally, human myelin cultures will overcome species differences in antigen expression and be more reflective of the disease model in its 'live, native form'.

Other potential studies include use of human VR, DRG, oligodendrocytes, or even ON cultures to define antigenicity of these sites regardless of HIV status or techniques such as gene sequencing, PCR, or immunoblot of protein lysates derived from different neural tissue. Ideally, but not always practical or ethical, is the use of human nerve tissue from HIV-infected patients, to assess for binding of human IgG to neural antigen as different isoforms of antigens may exist in this cohort due to chronic viral exposure and chronic inflammation.

Conclusion

The study, although small, is the first study from Africa. It highlights the fact that nodal-paranodal antibodies occur at a similar frequency in HIV-infected and HIV-uninfected IMRN and is comparable to European counterparts.

However, interpretation of results in the context of HIV infection, especially with IgG1 subtypes and low antibody titres is challenging as many antibodies occur as an epiphenomenon in HIV and may therefore be non-specific and non-pathogenetic.

Larger prospective studies are required to better define the relevance of these antibodies in the context of HIV and to determine if they define a specific clinical entity.

The study provides direction for future research, in terms of the importance of developing panels of antibodies or other biomarkers that select for specific phenotypes, that is pure sensory, pure motor, optic neuritis and CCPD.

It also highlights the importance of testing for IgG subclasses and establishing pathogenicity of the antibodies by using a myelin culture model that is more reflective of the HIV-infected cohort. This will ultimately provide direction for the establishment of targeted immunotherapy. Lastly, a prospective systematic study of the loss of immune tolerance will have relevance, not just to CIDP and HIV, but across the spectrum of autoimmune disease in general. This study serves as a platform to further explore these aspects of autoimmune disease**.**

Authors Contributions: KM designed and conceived the study. All patient data and samples were contributed by KM. Patient samples analysed by VM and JF using live CBA and myelinated co-cultures. KM wrote the original manuscript. SR revised the work critically for important intellectual content and supervised the laboratory work. VBP, AAM and PLAB edited and reviewed the manuscript. All authors approved the final manuscript.

CHAPTER 9:

Immune mediated or genetic? Combined central and peripheral demyelination in 2 Siblings of African Origin

The finding of 2 siblings in the 4th published manuscript with CCPD prompted us to explore the immunopathogenesis and genetics of CCPD in greater detail. We also considered the impact or HIV on pre-exiting autoimmunity as one sibling had contracted HIV during her illness and literature is scant regarding the impact of HIV on pre-existing autoimmunity.
CCPD is a heterogeneous entity which spans almost the entire spectrum of demyelinating disorders ranging from MS, ADEM, NMO, MOGAD, CIDP, AIDP and its variants such as MFS. The exact immunological trigger which results in demyelination in both central and peripheral compartments of the nervous system is unknown. This may occur due to loss of tolerance occurring simultaneously or sequentially in the respective compartments, due to breakdown of the blood-brain-barrier or may reflect disease chronicity and/or severity. CNS and PNS myelin are produced by oligodendrocytes and Schwann cells respectively. They are embryologically and structurally different in terms of their quantitative glycolipid, glycoprotein composition and ultrastructural features of the nodal-paranodal-juxtaparanodal region[397, 398]. As in CIDP and MS, the exact immunopathogenesis of CCPD is speculative and both cell mediated and humoral mechanisms may occur synergistically or independently[399]. There are several recognised common antigenic targets to both CNS and PNS myelin which include MOG, MAG, GQ1B, NF155, MBP, PMP 22, MP-0, lactosylceremide and those that remain to be identified which may be significant in the humoral arm of CCPD[400-406].

Genomic data from the African continent is lacking for most diseases and is virtually non-existent for rare diseases such as CIDP and its variants which include CCPD. This genomic information is likely different compared to European countries and is required to fill the gap in the worlds genetic data bases which consists of predominantly European phenotypes. African populations are likely to be genetically diverse compared to non-Africans due to extensive population substructure, less linkage disequilibrium (LD) among loci and several genetic adaptations in response to diverse climates, diet and exposure to chronic infections such as HIV[407]. Large genome wide association studies are required for CIDP, which should include the African continent. This will enable the discovery of more mutations and establishment of scoring systems example CADD scores specific for African people. To date, almost no genes have been described in CCPD. Limited genes have been described in CIDP across all continents, not just Africa. The limited data that exists arises largely from the West and Japan. This includes *SH2D2A*, and *the M3 allele of alpha-1 antitrypsin* or *perforin* which may play a

role in demyelination [408-410]. The following HLA associations have been described which include *HLA-DR2, HLA-DR3 ,HLA-DQ2* and a strong association of *HLA-DRB15* with anti-NF155 CIDP[411, 412 413]. Blum et al, described several plausible candidate genes in GBS and CIDP mainly in association with autoimmunity [414]. Novel inborn errors in both coding and non-coding genes (which act as regulators for the coding genes) that participate in tolerance include TYK2 gene in SLE, PTPN22 in diabetes mellitus, CTLA4 gene coding for inhibitory T cell signals [386, 414, 415]. Genetic variations in *PRF1,* the promoter region of *FCGR2B,* and single nucleotide polymorphisms of TAG-1 has been associated with IVIG responsiveness in a cohort of Japanese patients with CIDP [416, 417]

The above novel cases of CCPD among 2 African sisters, provide a platform to explore the confluence of genetic, immune, and environmental factors in the context of autoimmune disease, where pregnancy and exposure to other chronic infections such as HIV or toxins are potential triggers. These cases provide the opportunity to learn and explore the entity of functional genetics and highlights the importance of genome wide association studies in CIDP which includes patients of African origin. It also allows one to speculate about the impact that HIV has on pre-existing autoimmunity.

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Immune mediated or genetic? Combined central and peripheral demyelination in 2 Siblings of African Origin

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Introduction

Chronic inflammatory demyelinating polyneuropathy is the commonest chronic autoimmune neuropathy, with diverse clinical presentations[77]. It is now recognised that in some patients the demyelinating process may also affect the brain, cranial nerves, and spinal cord. In these cases, the lesions may be similar to those seen in acute disseminated encephalomyelitis (ADEM), myelin oligodendrocyte antibody associated disorders (MOGAD), neuromyelitis optica (NMO) and multiple Sclerosis (MS) [400, 418-420]. However, larger prospective studies are required to better characterize and compare these lesions. Ogata et. al reported 13 of 150 patients with symptoms related to peripheral neuropathy in a cohort of MS patients, 4 of whom had a demyelinating neuropathy [382]. Similarly, patients with ADEM and MOGAD may have a concomitant acute inflammatory demyelinating polyneuropathy (AIDP)[400, 421]. The above has been described using many terms such as combined central and peripheral demyelination (CCPD), CIDP with CNS involvement and CIDP with multifocal CNS demyelination[232, 382, 422-424]. CCPD is an uncommon occurrence, possibly due to subclinical central involvement or the rarity of the disease[382, 418]. The unravelling of the immunopathology of the disease is intriguing and current literature supports an antibody mediated process targeting myelin antigens common to the CNS and PNS, for example neurofascin (NF)155, myelin basic protein (MBP), myelin associated glycoprotein (MAG), GQ1b and MOG [383, 424-426].
We report on two sisters who had CCPD. These cases allow us to speculate on the immuno-genetic pathogenesis of CIDP as well as consider novel antigens and how HIV may impact on pre-existent autoimmune diseases.

Case 1

The first patient is a female in her late twenties, whose illness began one-month post-partum. She presented with asymmetrical lower limb weakness and paraesthesia which progressed over 3 weeks to

include the upper limbs. She was severely disabled being unable to walk, write or hold objects in her hands, complicated two months later by bilateral poor vision. Her bedside cognition was normal. Ophthalmological examination confirmed impaired visual acuity (RE: counting fingers LE: 20/100), bilateral central scotomas, poorly reactive pupils, and bilateral swollen discs (figure 9.2) She clinically had thickened ulnar, common peroneal and sural nerves. Her distal power was MRC grade 4- at the ankles, fingers, wrists, and knees, 4 at the hips, elbows, and the shoulder joints. She had a glove and stocking sensory impairment to pin prick and light touch up to the knees and elbows respectively while joint position sense was impaired at the toes and fingers. Optical coherence tomography confirmed thickened retinal nerve fibre layer bilaterally, RE:293um, LE:191um, and prolonged P100 latencies on visual evoked potential testing RE: P138 ms, LE: P128 ms. (Fig A1) Having excluded other diseases such as infections, paraproteinemia, connective tissue disease, malignancy, and toxin exposure, and after confirming demyelination on electrophysiology (Table A5.1 and A 5.2), she was managed as having CIDP with a 5-day course of intravenous immunoglobulin (IVIG) combined with intravenous (IV) methylprednisone. Despite her vision improving to near normal over the following weeks, her quadriparesis improved minimally over the subsequent 8 months while maintained on azathioprine (AZA) at 150mg daily and 30mg oral prednisone. She was therefore commenced on mycophenolate mofetil (MMF) at a dose 1.5g BD and improved over the subsequent 2 years such that she was able to walk with support, hold objects and feed herself. One year later she relapsed, with progressive quadriparesis and visual loss, having defaulted treatment for 6 months. Examination confirmed poor visual acuity (RE: nil light perception, LE: counting fingers), bilateral optic atrophy, paraplegia in the lower limbs, MRC grade 1-2 power in the UL and marked sensory impairment for joint position sense, light touch and pin prick up to the knees in the lower limbs and elbows in the upper limbs. An MRI brain was done at this stage (figure 9.1). Despite a repeat course of IVIG and IV methylprednisone she demised from respiratory failure due to diaphragmatic weakness from a phrenic nerve neuropathy. Post-mortem histology confirmed demyelination of the cerebral white matter and peripheral nerves (figure 9.3).

Case 2:

The younger sister, in her early twenties, with the same biological parents, presented with sudden onset facial weakness, two weeks post-partum. The weakness progressed to involve both upper limbs and lower limbs within two weeks. Clinically she had normal cognition and ophthalmological examination, bifacial weakness, quadriparesis with distal power of MRC grade 4- in both the upper and lower limbs, areflexia and glove and stocking sensory impairment for pin prick, light touch, and joint position sense. Despite normal visual acuity, the visual evoked potential, P100 latencies were bilaterally prolonged: RE: 118ms, LE: 120ms, and OCT of the RNFL was normal.

Her NCS were consistent with a demyelinating polyneuropathy (Supplementary file, table 1). Initially she was managed as having an acute inflammatory demyelinating polyneuropathy (AIDP) with IVIG, and later commenced on steroids as she continued to progress beyond 8 weeks. She was started on corticosteroid therapy (CST): 60mg/d which was gradually tapered to 15mg/d as maintenance therapy combined with AZA 100mg daily for the subsequent 4 months.

Although initially HIV-uninfected, 2 years later, she tested HIV positive with a CD4 count of 333 cells/mm^3 and an unknown viral load. She was commenced on anti-retroviral therapy (ART). Her weakness and sensory symptoms improved during the subsequent 2 years while maintained on 10mg/d prednisone and 100mg AZA until September 2022. She made a full recovery allowing discontinuation of her immunosuppressive therapy. Her current CD4 count is 1348 cells/ul with an undetectable HIV viral load.

She lived with her sister. Both had no exposure to toxins, or recreational drugs. They were unaware of similar clinical presentations in the neighbourhood or among family members.

Patient 1 was HIV uninfected. Serological investigations in both sisters were negative for antibodies against gangliosides,MOG, aquaporin 4, paraneoplastic and paranodal antigens which included Contactin1,Caspr1, neurofascin(NF)-155, NF186 and NF-140. Glycolipid lactosylceramide antibodies were also negative in patient 1.

Serum angiotension converting enzyme was normal , no paraprotein was identified and the connective tisue screen was negative.

The cerebralspinal fluid examination in patient 1 had no cells, protein 0.97g/l, glucose 3.5 mmol/l ,(serum glucose of 5,2mmol/l), and patient 2 had 8 lymphocytes per µl, protein of 1.28g/l, glucose 3.8mmol/l (serum glucose of 6.1 mmol/l). Type 2 pattern oligoclonal bands were positive in the CSF of both patients. Patient 1 and 2 had an IgG index of 0.81 and 0.92 respectively. Tuberculosis, syphilis, viral infections such as HTLV1 (human T-cell lymphocytic virus), HSV (Herpes simplex virus), CMV(Cytomegalovirus), HZ (Herpes Zoster), JCV (John Cunningham virus) were excluded, (Tables A 5.1 and A.5.2)

MRI brain was consistent with demyelination in both patients, figures 9.1A and 9.1B. In patient 2, repeat NCS showed complete recovery and repeat brain imaging showed almost complete resolution of the white matter lesions. (figure 9.1, image 3). A primary, biochemically defined inherited leukodystrophy was unlikely (Leucocyte arylsulphase A activity and very long chain fatty acids were within normal range for both patients, (Table A5.1). Whole exome sequencing, carried out as previously described[427], revealed 2 potential mutations in the *PPFIA4 gene* and the *CHCHD10 gene* in the exome SYNS-10336/L14048 of patient 2. Although not previously linked with the phenotype present in these patients, both identified variants were predicted to be functionally deleterious by their corresponding in-silico scores. Further segregation analysis as well as functional assessment would be necessary to assess their contribution to the patient's phenotype. No mutations were identified in

genes linked to leukodystrophy, mitochondrial cytopathy, metabolic disorders or inherited neuropathy.

Table 9.1

Differential diagnosis of CCPD
Acquired
Immune mediated: ▪ CIDP and its variants ▪ GBS and its variants (Bickerstaff's encephalitis) ▪ Neurosarcoidosis ▪ *Less commonly: MS, NMO, MOGAD
Malignancy: ▪ Paraneoplastic disorders ▪ Neurolymphomatosis
Drugs and toxins: ▪ Toluene, N-hexane ▪ Immune checkpoint inhibitors ▪ Tacrolimus ▪ Anti-TNF therapy
Vitamin E deficiency (malabsorption syndromes)
Infections: ▪ Leprosy ▪ HTLV-1
#Inherited disorders:
Mitochondrial disorders: ▪ NARP ▪ Pyruvate dehydrogenase deficiency syndromes ▪ MNGIE
Storage diseases: ▪ Refsum's disease ▪ Sulfatide lipidosis
Neuropathies: ▪ CMT1X (GJB1 mutation)
Leukodystrophy: ▪ Metachromatic leukodystrophy, ▪ Krabbe leukodystrophy
Others: ▪ Abetalipoproteinemia ▪ Famililial amyloidosis
*Genetic-autoinflammatory disorders

Table 9.2

Associated antibodies
▪ Neurofascin-155 ▪ Lactosylceremide ▪ GQ1B ganglioside ▪ Myelin-oligodendrocyte glycoprotein ▪ Aquaporin-4 ▪ Collapsin-response mediator protein 5 (CV2/CRMP5) ▪ ##Myelin-associated glycoprotein

CCPD = Combined central and peripheral demyelination, GBS=Guillain–Barré syndrome, CIDP=chronic inflammatory demyelinating polyneuropathy, MS=multiple sclerosis, NMO=neuromyelitis optica, MOGAD= myelin oligodendrocyte glycoprotein associated disease. NARP=neuropathy, ataxia, retinitis pigmentosa, MNGIE=mitochondrial neurogastrointestinal encephalopathy syndrome

* = The association is uncommon and there is probably a common peripheral or central myelin antigenic target

= Progressive signs and symptoms, present early in life, usually not corticosteroid responsive

* = Signs of systemic disease (fever, skin rash, polyarthralgia) and multisystem involvement

= rare association

Fig 9.1: MRI images: T2 axial, coronal and T2 FLAIR images

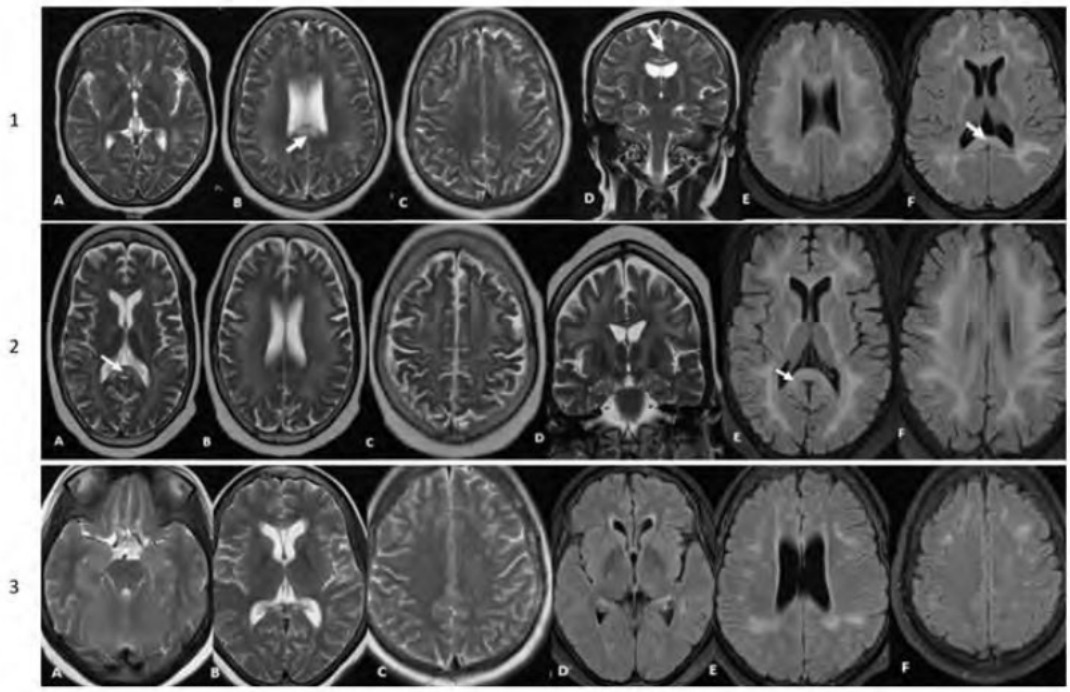


Image 1 (Patient 1 during a relapse) and Image 2 (Patient 2: pre-treatment): Diffuse T2 (A-D) and T2 FLAIR (E, F) symmetrical white matter hyperintensities involving the bilateral hemispheres and the splenium and genu of the corpus callosum(arrows).Image 3: Post-treatment images of patient 2: T2 (A, B, C) and T2 FLAIR (D, E, F) demonstrating significant resolution of previous white matter hyperintensities seen in image 2.

Fig 9.2 : Fundal images showing bilateral swollen discs

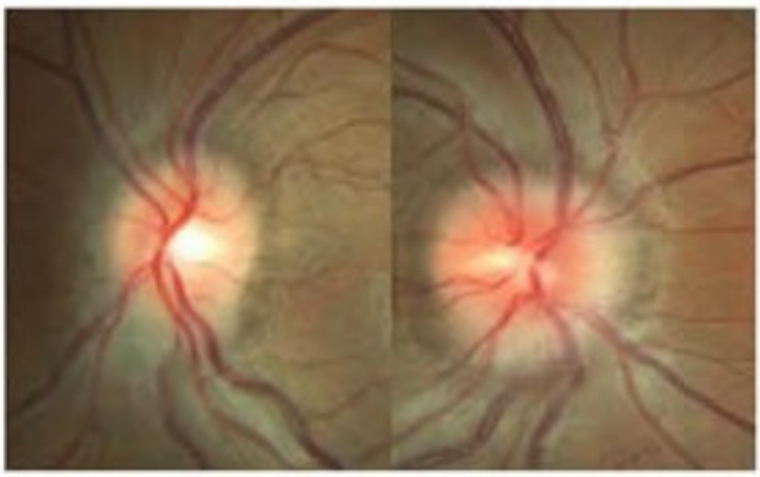
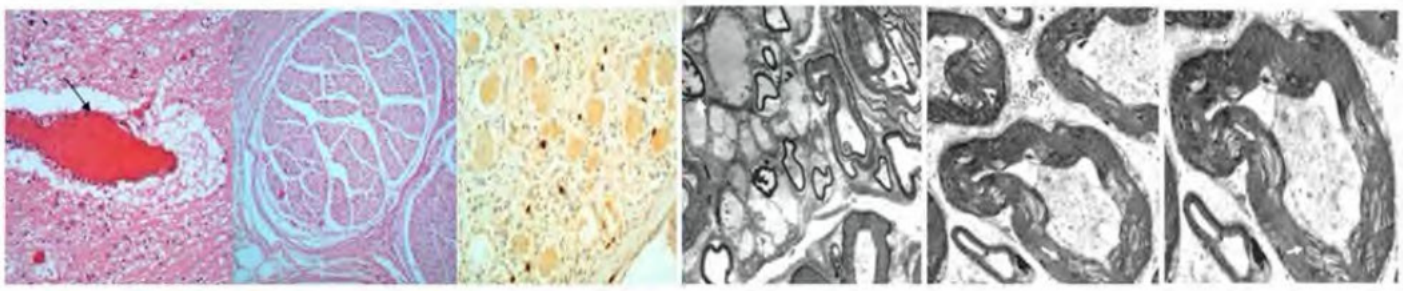


Figure 9.3: Postmortem histology of brain and peripheral nerve



A: Brain | B: Phrenic nerve | C: CD3 and CD68 positivity | D: Femoral nerve | E: Femoral nerve | F: Higher magnification (Femoral Nerve)

A and B: H&E staining of sections of the brain and phrenic nerve showing patchy spongiosis indicative of demyelination, perivascular lymphocytic infiltrates (arrow) and evidence of perivascular demyelination. C: Dual immunohistochemical staining demonstrating infiltration of the phrenic nerve by T lymphocytes (CD3) and macrophages (CD 68). Electron microscopy demonstrating thinning of myelin sheaths relative to axons (Fig D), marked splitting and separation of myelin lamellae (E,F: arrows).

Discussion:

We propose that the above case highlights the following novel features a) combined central and peripheral demyelination b) genetic predisposition of 2 sisters of African descent to CIDP c) exposure to foreign antigen as a trigger during pregnancy d) and e) impact of HIV on autoimmune disease.

Acquired and inherited differential diagnoses for CCPD include CIDP,AIDP, drugs and toxins and inherited metabolic disorders such as metachromatic leukodystrophy, Refsums disease, mitochondrial disease, and inherited genetic disorders example Charcot Marie Tooth disease with GJB1 mutations and others listed in table 9. 1.

Evidence for CNS involvement in CIDP was reported in the late 1980s by Mendell et al [428]. There has been one reported case of tumefactive central demyelination in MADSAM [429]. Kira et al reported CNS lesions in 8 % of patients positive for NF155 antibodies[425]. Cases of CCPD with NF155 antibodies have been reported mainly from Japan and fewer from Europe [382, 402, 430, 431]. Hou et al reported CCPD with aquaporin 4 and MAG antibodies[423]. More recently, glycolipid lactosylceramide antibodies were described in 2 patients from Japan, with 10 previous cases documented in the literature[432].Other antibodies described in CCPD include GQ1b antibodies, one case reported in Saudi Arabia, MOG antibodies and more recently paraneoplastic antibodies [418, 433, 434](table 9. 2).

Subclinical visual pathway involvement has been documented using optical coherence tomography (OCT) and visual evoked potentials (VEP) in various other cohorts of patients with CIDP [435, 436].

CCPD has also been reported in larger case series from Europe with no identifiable antibodies[437]. To our knowledge, our cases are the first reported from Africa

Unusual findings in our patients were that of extensive symmetrical white matter involvement and significant involvement of the genu and splenium of the corpus callosum in both patients. This may be seen with toxins such as benzene or toluene, which was not the case in our patients. Although, genetic leukodystrophies and mitochondrial disease are possible, [421, 438] the presence of CSF-restricted oligoclonal bands, temporal dispersion and conduction blocks on nerve conduction, the dramatic response to corticosteroids with resolution of the lesions on MRI, normalisation of the nerve conduction studies (patient 2), relapse of disease when stopping steroids (patient 1), and pregnancy being the common precipitating factor, makes an acquired immune-mediated aetiology most likely, although genetic testing will be the gold standard to differentiate acquired disorders from inherited. Despite negative live cell based assays for known nodal-paranodal antibodies (NF186, NF155, Contactin1, Caspr1) , a nodo-paranodopathy due to a novel or untested antibody, targeting both peripheral and central myelin, or cell mediated dysfunction remains a possibility[439].

CNS and PNS myelin are embryologically and structurally different in terms of their quantitative glycolipid, glycoprotein composition and ultrastructural features [397, 398]. As in CIDP and MS, the exact immunopathogenesis of CCPD is speculative as both cell mediated and humoral mechanisms may occur synergistically [399]. There are several recognised common antigenic targets to both CNS and PNS myelin (Listed in table 9.2) and those that remain to be identified in prospective studies[400-406]. Future studies using induced human pluripotent oligodendrocyte myelin stem cell culture screens or animal models may help identify a yet unidentified or untested target.

Distinguishing acute onset CIDP (A-CIDP) from AIDP is often challenging especially at the onset of clinical presentation . Sixteen percent of initially diagnosed CIDP patients present acutely and are often distinguished from AIDP by not having autonomic dysregulation, bifacial weakness or requiring mechanical ventilation and other factors discussed in chapter 1 [80, 82]. However, there is emerging evidence that patients initially diagnosed with A-CIDP and some patients with AIDP are nodo-paranodopathies which is a pathologically distinct entity [103, 104, 358]. Examples of the above include nodo-paranodopathies associated with NF-155 (IgG4 subtype) and pan-neurofascin disease[114, 127].

Whole exome sequencing revealed 2 potential mutations in the *PPFIA4 gene* and the *CHCHD10 gene* in the exome SYNS-10336/L14048 of patient 2. The first mutation is a close interactor of KIF1A which is linked to mutations in neuronal microtubule (MT) motor protein. KIF1A is associated with a recessive axonal neuropathy and KIF1A neurological disorder (KAND)[440]. The PPFIA4 gene, despite a high combined annotation dependent depletion (CADD) score of 32 (based on European data), is usually not linked to neurological disease[441]. The *CHCHD10 mutation* is associated with amyotrophic

lateral sclerosis and less frequently, mitochondrial disorders [442, 443]. The CCPD in the 2 patients may be consistent with a mitochondrial disorder. Central and peripheral demyelination is a described feature in mitochondrial disease such as NARP (mitochondrial disorder associated with neuropathy, ataxia, retinitis pigmentosa or LHON (Leber's hereditary optic neuropathy)[444, 445]. However, the steroid responsive nature of the disease in patient 2 is an unusual feature for mitochondrial dysfunction, although episodic relapses and improvement has been described. Known mutations for mitochondrial diseases, demyelinating CMT and inherited leukodystrophies have been excluded in this patient. The 2 described mutations are likely incidental.

Genomic data from the African continent is lacking for most diseases and is virtually non-existent for rare diseases such as CIDP and CCPD. Wild type genomic information is possibly different from European populations and is required to fill the gap in the worlds genetic data bases. Large genome wide association studies are required for CIDP, which should include the African continent. This will enable the discovery of more mutations and establishment of CADD scores specific for African people.

To date, almost no genes have been described in CCPD. Limited genes have been described in CIDP across all continents, not just Africa. The limited data that exists arises largely from the West and Japan. This includes *SH2D2A*, and *the M3 allele of alpha-1 antitrypsin* or *perforin* which may play a role in demyelination [408-410]. The following HLA associations have been described which include *HLA-DR2, HLA-DR3 ,HLA-DQ2* and a strong association of *HLA-DRB15* with anti-NF155 autoimmune nodopathies[411, 412 413]. Blum et al, described several plausible candidate genes in GBS and CIDP mainly in association with autoimmunity [414]. Novel inborn errors in both coding and non-coding genes (which act as regulators for the coding genes) that participate in tolerance include TYK2 gene in SLE, PTPN22 in diabetes mellitus, CTLA4 gene coding for inhibitory T cell signals [386, 414, 415]. Genetic variations in *PRF1,* the promoter region of *FCGR2B,* and single nucleotide polymorphisms of TAG-1 has been associated with IVIG responsiveness in a cohort of Japanese patients with CIDP [416, 417].

Whole genome sequencing, which includes the non-coding introns may be useful in our patients to identify candidate genes that may be implicated in tolerance and autoimmunity. The genetic confirmation of Lawrence Moon Biedl syndrome in the son of patient 2, was most likely co-incidental. We are awaiting WES in the second sister and later WGS in both patients which may lend clues to genetic mutations that have been missed or not identified.

There are case reports of genetic neuropathies such as *CMT 1A, HNPP, CMT 1B, CMT X, CMT 4C*, co-existent with CIDP or have a CIDP-like presentation. These have been associated with pathogenic

variants in genes such as *SH3TC2 in CMT 4C, MPZ in CMT 1B, PMP 22 in HNPP, MARS 1 in CMT 2U* and with other inherited disorders such as metachromatic leukodystrophy and mitochondrial disorders such as mitochondrial neurogastrointestinal encephalopathy (MNGIE)[446-451]. Munch et al reported a case of severe AIDP with a duplication at chromosome 17p11.2-12, a known genetic cause of CMT1A[452]. The postulate is that environmental triggers may initiate an immune attack in genetically defective myelin, or the putative capacity of the Schwann cell to act as a myelin antigen presenting cell, as shown in mutant mice models with chronic myelin damage, which may trigger inflammation[453, 454]. Comparing defects in myelin protein from CIDP patients to wild type myelin may provide clues to latent inherited myelin defect. Differentiating CIDP-like hereditary neuropathy from true inflammatory CIDP is often challenging and genetic testing remains the gold standard, although acute onset, response to immunotherapy, and temporal dispersion and conduction blocks may lend clues.

Pregnancy was the precipitating event in both patients. It is well known that maternal T-cell responses are specifically altered during pregnancy to accommodate the developing foetus. These maternal T cells acquire a transient state of tolerance for foetal H2 antigens that may lead to autoimmunity [455, 456]. Other factors that may result in autoimmunity during and post pregnancy is microchimerism, where there is persistence of a small population of cells or DNA in the mother that is derived from a genetically distinct foetus. This bidirectional trafficking of maternal and foetal cells can persist for decades [457]. McCombe et al reported worsening of CIDP in 5 pregnant women and the onset of the disease in 4 patients during post-partum, due to cross reactivity between foetal and maternal neural antigens [458]. Immune changes post-partum, may have also resulted in potential immune reconstitution inflammatory syndrome similar to HIV-infected patients commencing ART[459].

The co-existence of HIV infection in case 2 may have resulted in a dampened immune response, possible switching of IgG4 to IgG1, and hence a better response to therapy and less aggressive disease. Previous studies show that CIDP in the setting of HIV is less aggressive and more steroid responsive compared to HIV-uninfected counterparts [247]. Furthermore in the chapter 7, antibodies were commonly IgG1 in HIV-infected patients.
The above 2 cases add to the current literature as they are the first cases of 2 siblings with CCPD from Africa, with a common precipitating factor namely pregnancy. The above cases highlight the possibility that genetic factors may predispose to demyelination and autoimmunity, the role of pregnancy in autoimmunity and the importance of identifying antigens common to both the CNS and PNS. Limitations include not performing whole genome sequencing and not using a human derived myelin culture model of oligodendrocytes and Schwann cells in their native environment to screen for novel antigens. Identification of the causative genes in autoimmunity and tolerance may require whole genome sequencing or long-read sequencing technologies to cover for non-coding and

structural genetic variations. Moreover, screening additional family members , for example the elder sister which was not done in the above cases, as well as carrying our Sanger validation studies in the whole family is important for shedding more genetic information on the identified variants.
Nevertheless, prospective studies with larger patient numbers will include genome wide association studies in CIDP and screening for “novel” or “untested” antibodies.

Conclusion:

The above novel cases of CCPD among 2 African sisters, provide a platform to explore the confluence of genetic, immune, and environmental factors in the context of autoimmune disease, where pregnancy and exposure to other infections or toxins are potential triggers.
The cases highlight the need for building patient cohorts with similar clinical manifestations and carrying out multicentre genome wide association studies. This will allow for better understanding of single nucleotide polymorphisms in different populations, and in complex diseases such as immune mediated demyelination which is likely due to an interplay between multiple genes and various environmental factors such as pregnancy.
However, in the context of this uncommon disorder, antibodies against NF155, lactosylceremide, AQP-4, GQ1B, MAG, MOG, CV2/CRMP5 should be excluded, despite majority of reported cases being antibody negative. Future discovery of novel pathogenetic antibodies will direct therapy example BCDT if anti-NF155 positive.

Contributorship: KM wrote the original draft of the manuscript. SR,VBP,PLAB,AAM contributed to subsequent drafts and revisions. SR contributed with the live CBA and hiPSC myelin co-culture screens. SE and HH contributed with WES analysis. All authors approved the final version

CHAPTER 10:

Relevant unpublished results of live CBA, myelin co-cultures and mass spectrometry performed on the patients from published manuscripts 2,3,4,5 after publication:

In total 76 HIV-infected patients were screened for nodal/paranodal and ganglioside antibodies using live CBA and ELISA respectively. This cohort included 5 patients with HIV-infected MNS (manuscript 2) and 11 patients with HIV associated PM LSP (manuscript 4) and 10 patients with DRG, 8 (CCPD + ON) and 24 (MSM DN) patients (manuscript 5) + 18 additional patients recruited after publication of the 4th published manuscript) as testing was done in stages.

A summary of these results and clinical features are included in table 10.1:

Six serum samples (6/76 (9.2%)) were positive for the nodal/paranodal antibodies.
Three (3/6 (50%)) were of the NF155 IgG1 subtype, with low antibody titres.
Two patients (2/6 (33%)) were of the NF186 IgG1 subtype
One patient (1/6 (17 %)) was CNTN1 IgG4 positive (IgG1,2,4).
None of the patients were PAN NF positive.
Four (4/6 (66%)) of antibody positive patients had a mixed sensory motor demyelinating neuropathy (2 was NF155 positive, 1 was NF 186 positive and 1 was CNTN1 positive), 1 (16.6%) patient had a PM LSP was NF186 positive, and 1(16.6%) patient with DRG was NF155 positive. Fifteen patients with pure motor syndromes (PM LSP+MNS) were negative for both NF186 and GM1 antibodies. All patients were negative for ganglioside antibodies, paraneoplastic antibodies and other antibodies included in table A1.

Patients with CCPD (includes CIDP with ON) tested negative for ganglioside, lactosylceramide and nodal/paranodal antibodies. One patient with the initial diagnosis of CIDP with ON was NF155 positive.

Three (4%) of the above patients (Fig 10.4), showed moderate to strong binding to myelin co-cultures. All 3 patients had the initial diagnosis of MSM CIDP

Table 10.1 Summary of positive results of Live CBA and myelin co-culture screens not included in published manuscript.

	MSM DN	PM LSP	MNS	DRG	CCPD	CIDP
Total number of patients tested (76)	42	11	5	10	4	4
Total number of nodal/paranodal +ve	3	1	0	1	0	1
Positive myelin co-culture screens (3)	3					

	Clinical Phenotype	NF186	NF155	CNTN-1	CNTN/CaspR	IgG subtype	CD4 count (cells/mm^3)
Patient 0	MSM DN	-ve	1:400	-ve	-ve	IgG 1	148
Patient 3	MSM DN +ON	-ve	1:200	-ve	-ve	IgG1	189
Patient 16	DRG	-ve	1:200	-ve	-ve	IgG1	277
Patient 21	PM LSP	1:400	-ve	-ve	-ve	IgG1	210
Patient 66	MSM DN + NS	-ve	-ve	1:6400	-ve	IgG1,2,4	105
Patient 71	MSM DN	1:400	-ve	-ve	-ve	IgG1	236

PM LSP=ventral root radiculopathy, DRG=Dorsal root ganglionopathy, MSM=mixed sensory motor , DN=demyelinating neuropathy, NS=nephrotic

Figure: 10.1 Live CBA for patient 0: NF 155 IgG1 positive (1:400)

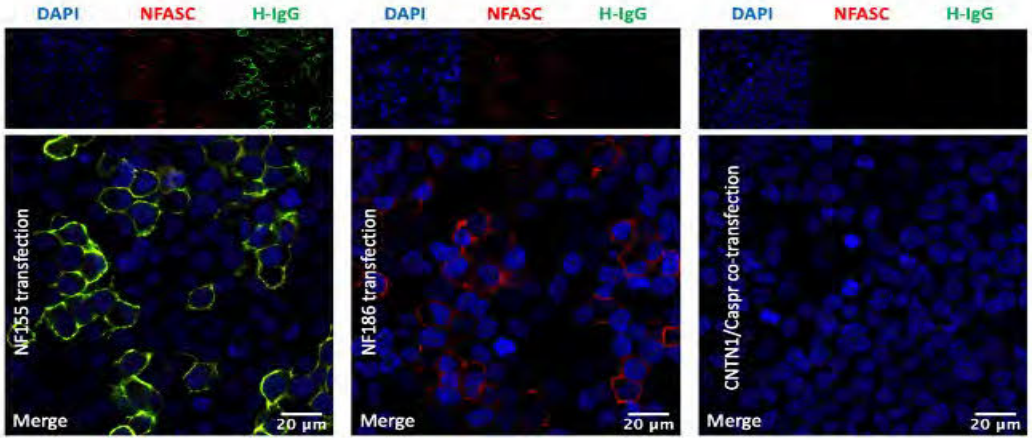


Figure 10.2: Live CBA for patient 66: CNTN1 (IgG1,2,4) positive (1:6400)

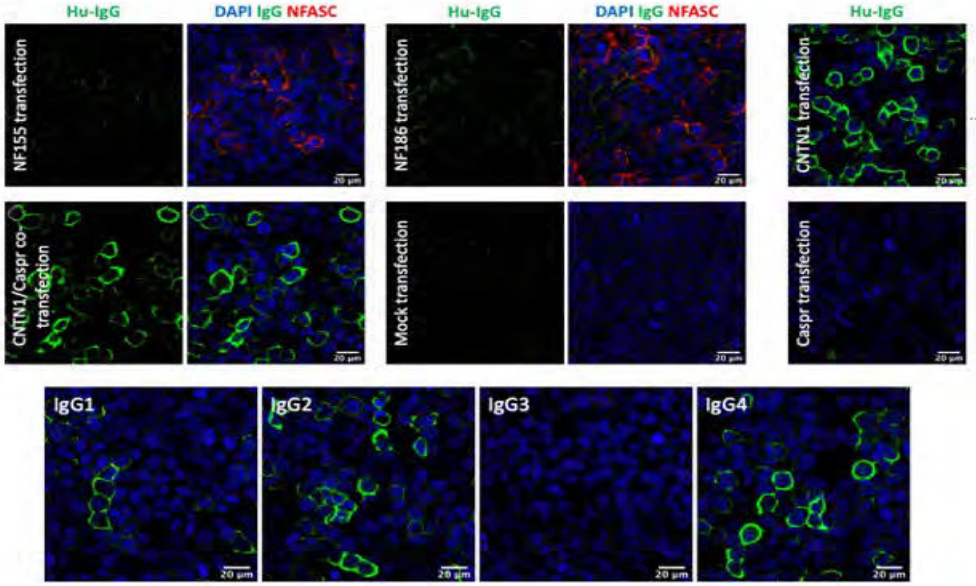
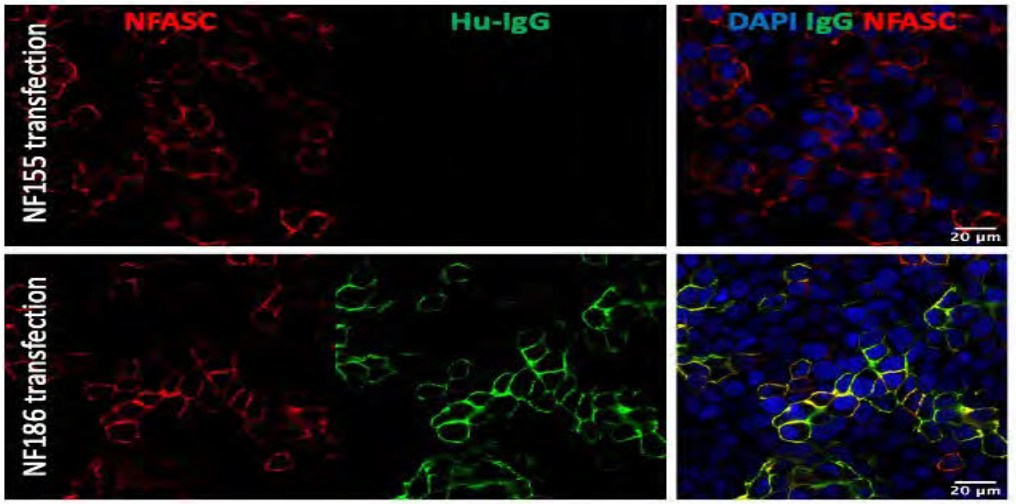


Fig 10.3: Live CBA for patient 71: NF 186 IgG1 positive (1:400)



Cell-based assay using fluorescence microscopy. HEK cells are transfected with plasmid vectors encoding the different isoforms of neurofascin, CNTN1, Caspr and CNTN1/Caspr complex which are expressed as proteins within the cell membrane. Incubation with patient 0,66 & 71 serum allows human anti-NF155(green), CNTN1(green) and NF186 (green) antibodies, to bind to their target antigen. Co-localization (yellow/green fluorescence) with a commercial NF155 (red), NF186 (red) and CNTN1 (green) stain confirms NF155, CNTN1 and NF186 as the target antigen respectively (Fig 10.1, fig 10.2 and fig 10.3). Note: In fig 10.2 Caspr,(green),CNTN1/Caspr complex are negative . IgG subtyping demonstrates positive IgG4>IgG2>IgG1. Background HEK reactivity in Fig 10.2 is commonly seen in CNTN1+ cases due to endogenous CNTN1 expression by HEK cells.

Figure 10.4 Positive myelinating co-cultures (Patient 1,30, and 49):

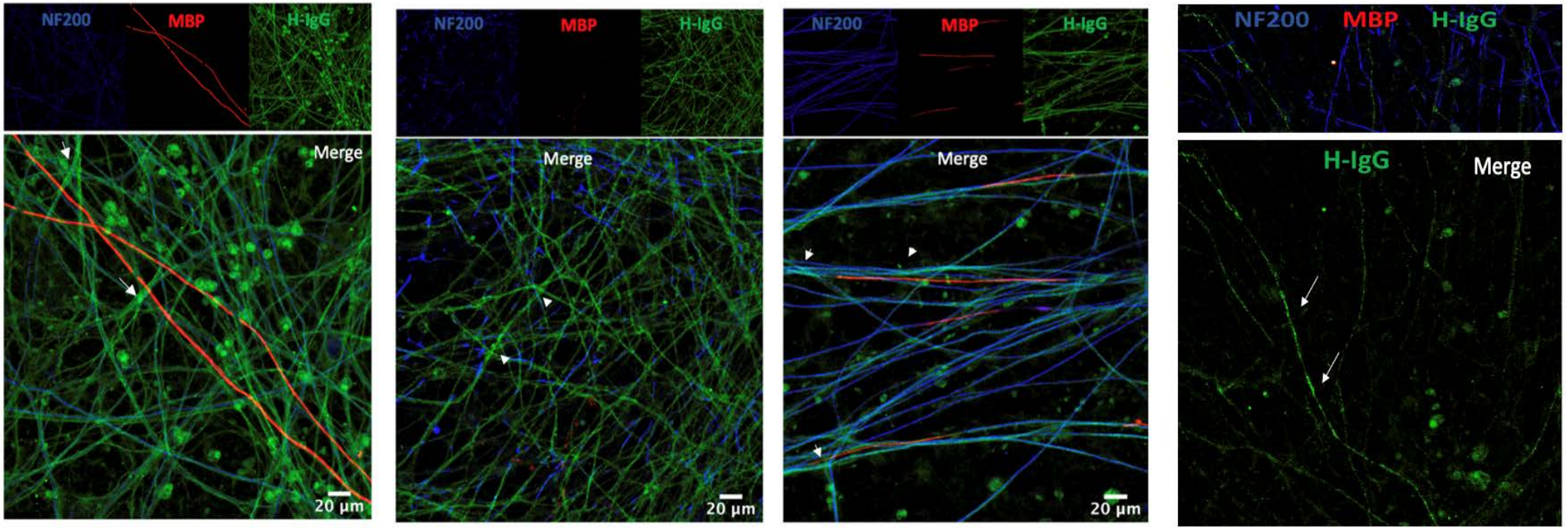


Figure A and B shows human IgG from serum of patient 1 and serum and CSF of patient 30, binding to non-myelinating Schwann cells in myelin-co cultures (arrow-heads). In figure C, human IgG from serum of patient 49 binds to occasional axons. Fig A and B show moderate to intense binding along non myelinating Schwann cells as it wraps itself around the axon. Microscopically this may appear similar to axonal binding. To differentiate the binding site (axon vs Schwann cell) serum will be added to cultures containing axons only or Schwann cells only. The antigenic target is unknown in all 4 cultures and immunoprecipitation experiments using magnetic beads will be carried out in prospective study to identify this unknown Ab-Ag complex using the method described in chapter 3. Results of mass spectrometry for Patient 1 is described below.

Results of IP using Magnetic beads (see methods section) and Mass spectrometry for patient 1/ CIDP-1

Aim

To identify the target of neuronal binding of human IgG found in sera of CIDP 1 (Patient 1, Fig 10.4A).

Hypothesis and a brief description of the experiment:

The mixed sensory motor demyelinating peripheral neuropathy in CIDP-1 is caused by human IgG antibodies binding to a single or possibly more than one Schwann cell antigenic target.

This is based on the binding pattern seen on myelin co- cultures (Figure : 10.4 A) . It is presumed that there is a single antigenic target which explains the Schwann cell IgG binding pattern in the cultures, and that antibodies binding this target are the cause of the associated demyelinating peripheral neuropathy. The relevant Ag-Ab complex from the myelin co-culture screen incubated with serum of CIDP 1 was immunoprecipitated using magnetic beading immunoprecipitation protocol in chapter 3.

The relevant peptides/proteins were identified on mass spectrometry using the following criteria

1. Most or highly enriched protein detected on mass spectrograph and not necessarily a unique hit.
2. The target antigen should be a potential neuronal or glial membrane protein , in the case of CIDP 1, more specifically a Schwann cell target in the neural tissue data base

Relative enrichment, measured as fold change, was identified by plotting relative enrichment and p values for significance compared to comparators (target positive control or blank) on scatter plots/volcano plots and Venn diagram (Fig 10.5,fig 10.6,fig 10.7) for unique hits.

Results of CIDP 1 was primarily compared against positive and negative controls using the 2 sample student –test combined with permutation FDR set at 5 % (Bio 046 vs CIDP 1, Blank vs CIDP1). Pairwise comparisons against other samples (SR1192, IMA) was performed secondarily to identify potential unique hits.

Confirmation of the target will be done prospectively using an antigen specific assay (generally transient transfection to produce over-expression of the protein of interest in HEK cells) or other techniques such as gene silencing techniques discussed in section chapter 3.

Patients:

CIDP1: binds to non-myelinating Schwann cells.

Controls:

1. Positive Control: Bio-146 (binds human CNTN1 on paranodes)
2. Negative control: Blank 1,2,3 -no sera added, consists of cells only

Cultures:

Myelin co-cultures were prepared according to established controls described in chapter 3.

Table 10.2: Description of CIDP-1 vs Positive and Negative controls

Sample name	Medium volume	Cells	Treatment – human patient serum diluted 1:100 in cell medium (N2Complate* + 1% BSA + human NGF (nerve growth factos), 25 ng/ mL)	Binding
Blank 1 Blank 2 Blank 3	Dry beads	Human iPSC-derived neurons and rat Schwann cells	No – just medium added to cells	Non-specific binding to beads
Bio146_1 Bio146_2 Bio146_3	Dry beads	Human iPSC-derived neurons and rat Schwann cells	Yes (positive control – CNTN1 expected to be detected)	Axon
CIDP1_1 CIDP1_2 CIDP1_3	Dry beads	Human iPSC-derived neurons and rat Schwann cells	Yes (unknown)	Non-myelinating rat Schwann cells

N2Complete – Neurobasal medium (Life Technologies, 12348017) supplemented with B27 (Life Technologies, 12587010), N2 (Life Technologies, 17502048), Glutamax (Invitrogen, 35050038) and Antibiotic-Antimycotic (Invitrogen, Cat 15240062)

Results of the mass spectrometry analysis for CIDP1 vs control (Using TIMS-TOF mass spectrometry, electrospray ionization)

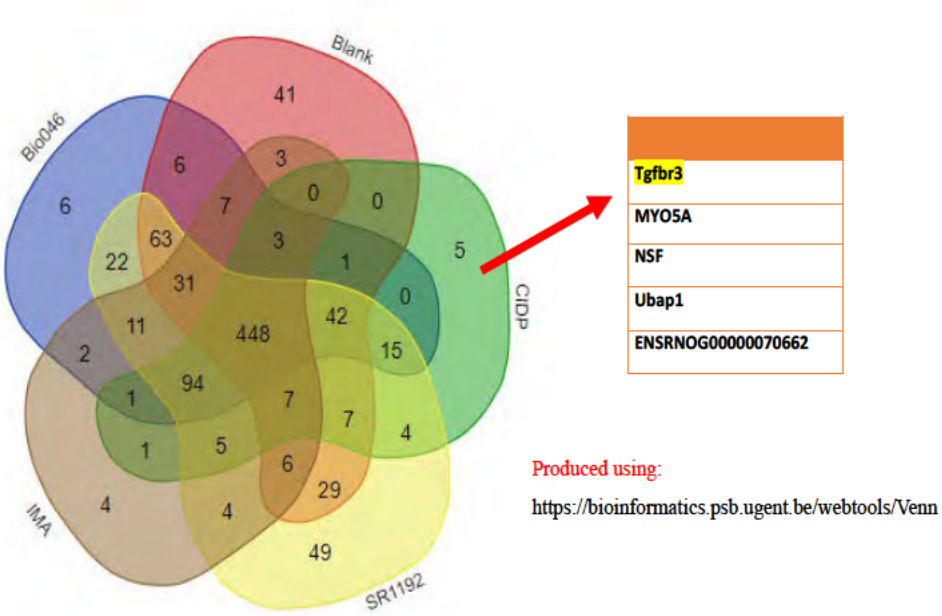


Figure 10.5: Venn diagram shows proteins exclusively in at least 2 of the 3 biological triplicates of each condition. The 5 proteins unique to CIDP-1 are listed in the table above. Tgfbr3 is the only protein reported to be present on Schwann cells.

Volcano plot comparing Bio046 (positive control) vs CIDP 1

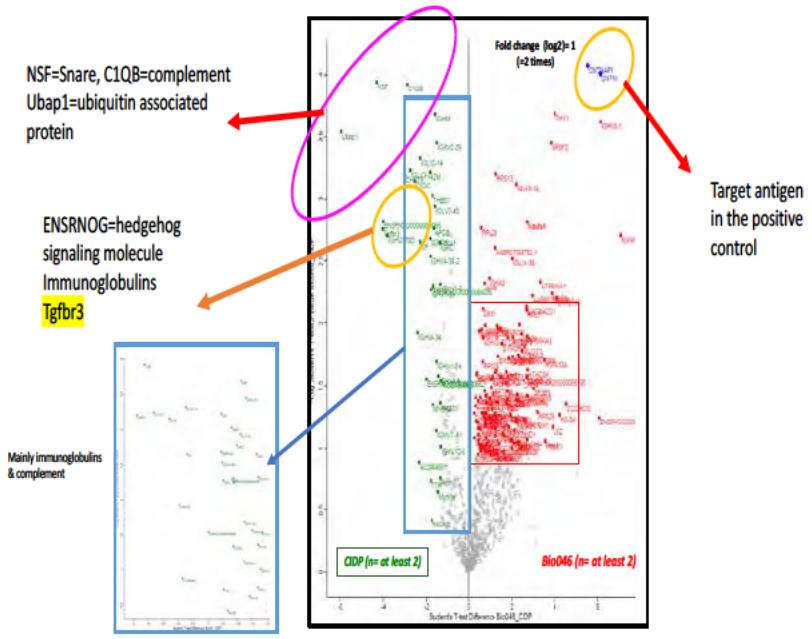


Figure 10.6: Volcano plot showing relative enrichment of various proteins in CIDP-1 compared to Bio046. Majority of the relevant proteins identified are complement, immunoglobulins, signaling molecules and intracellular molecules. The only protein that may have relevance to neural tissue is Tgfbr3.

Volcano plot comparing Blank (negative control) vs CIDP 1

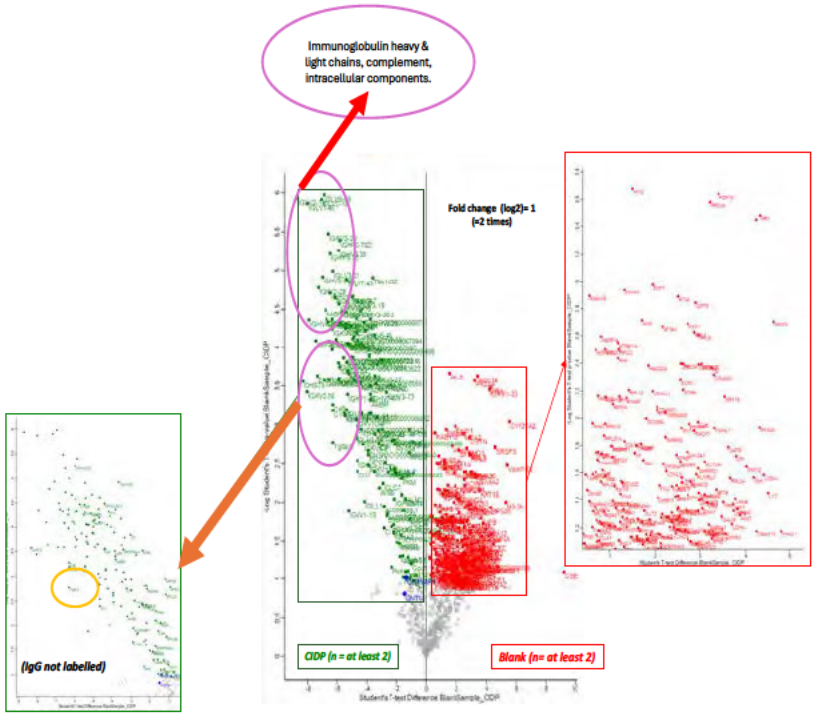


Figure 10.7: Similarly, among the relatively most enriched proteins, comparing CIDP-1 to blank, Tgfbr3 appears to be the only protein of relevance.

Relevance of Tgbr in peripheral nerve function[460]

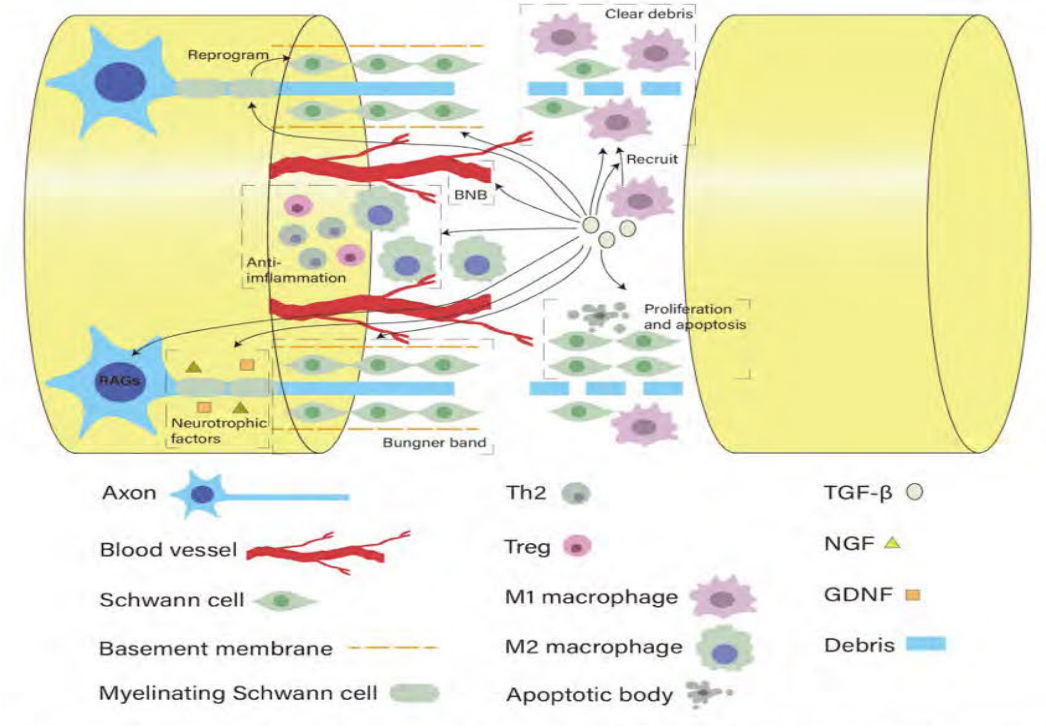


Figure 10.8: Demonstrates the diverse role of Tgfbr in neural regeneration which includes recruitment of macrophages, enhancing capacity of the SC (Schwann cell) to clear myelin debri, iniates programming of the Schwann cell, modulates BNB permeability and sustains Treg and Th2 cells to create a supportive environment for nerve growth and repair.

Tgfbr has multiple functions (demonstrated above) and is upregulated in various neuropathies including diabetic neuropathy[461]. This experiment shows that Tgfbr3 is more likely enriched in the immunoprecipate of CIDP1 sera. This may be as a consequence of nerve injury or inflammation and not necessarily pathogenetic. Despite the above there still remains a possibility that antibodies targeting Tgfr3 may impair Schwann cell function. However, more studies are required to explore its relevance and pathogenicity in inflammatory demyelinating polyneuropathies. Future tests will include: confirmation of the target using an antigen specific assay (transient transfection to produce over-expression of the protein of interest in HEK cells) or other techniques such as gene silencing techniques discussed chapter 3.

Limitations of this experiment using mass spectrometry:

1. Mass spectrometry is useful for protein antigens and, thus, lipid or carbohydrate antigens cannot be identified without modifications to the processing and analysis
2. Sensitivity for large molecules is poorer than for peptide analysis because the signal is distributed over many charge states. Furthermore, the molecular weight of a protein cannot be predicted precisely from its database entry, because of N- and C-terminal processing, posttranslational modifications, and chemical modifications introduced during sample purification.
3. More filtering out of immunoglobulins, complement, intracellular components is required to better interpret results in the above experiment
4. Tgfbr3 antigen is a potential candidate antigen targeting Schwann cells, however this needs to be confirmed and pathogenicity explored in future experiments, as its distribution and function is widespread
5. Target antigen may not be present in the data base.
   Although rodent and human protein databases cover a very significant proportion of the proteome, their completeness, accuracy and detail may be insufficient for novel antigens that may have not been studied in any other disease or model, and thus it is difficult to assign relevance to the identified antigens.
6. In HIV, multiple antibodies are produced and non-specific binding to multiple neural antigenic targets maybe possible without actually producing disease
7. In this experiment one may require additional controls who are HIV-infected without inflammatory neuropathy or HIV-infected controls with autoimmune disease (not neuropathy) to improve interpretation of results in future studies

Non- antibody mediated demyelination (Patient 3 and 21):

Despite the absence of visible binding of antibodies to myelin co-cultures at 1hr and 24hrs respectively, there was axonal degeneration/fragmentation and myelin blebbing in patient 3 and 21 at 1hr and 24hrs respectively and not in controls. There are clumps of degenerated axons and no intact axon between These findings prompted us to consider non-antibody mediated demyelination and axonal degeneration as a plausible explanation in these 2 patients and in the other patients in this cohort who despite having clinical demyelinating neuropathies with secondary axonal loss tested negative for various antibodies described in the respective manuscripts.

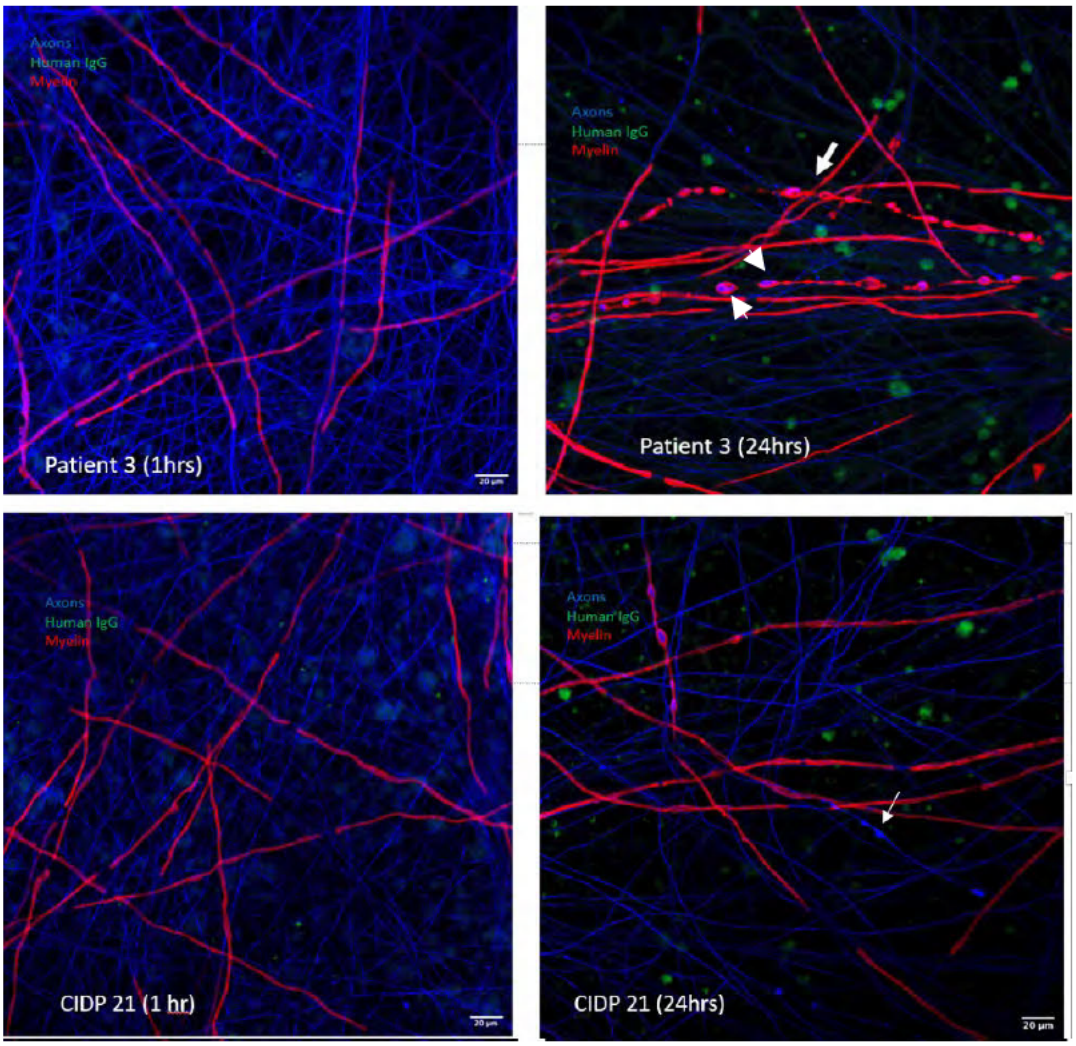


Figure 10.9: Patient 3 showing myelin blebbing (arrows) and clumps of NF200 within myelin fragments indicating axonal degeneration (arrowheads) and patient 21 showing less myelin blebbing and axonal fragmentation (arrow) at 24hrs. Note there is minimal neural injury at 1hour.

However various factors including laboratory factors may account for the axonal degeneration and demyelination without visible antibody binding. These are as follows:

1. Low antibody binding (low affinity IgG1 antibodies, or low antigen expression in the myelin co-culture system) resulting in no visible binding on fluorescent confocal light microscopy.
2. Myelin and axonal injury can occur in normal sera (<0.5%) and therefore requires objective quantification to decide if pathological or within normal limits. The use of automated imaging to capture multiple and consistent images/locations within each well, which is then analysed by computer software to objectively quantify fragmentation of axons and myelin as a marker of injury, avoids observer bias. Furthermore, quantifying what is regarded as normal/acceptable myelin damage in controls is important to establish normative data in future studies.
3. Repeating the experiment with duplicate/triplicate wells of control (NHS) vs patient sera will be useful in order to control for culture variability.
4. Other factors, other than antibodies in the sera may account for neural injury if objectively quantified and reproducible. These include non-antibody induced demyelination due to factors listed below discussed briefly in this chapter:
    a. TNF-alpha
    b. cytokines
    c. macrophages
    d. complement

Theories of non-humoral factors that may result in demyelination or neural injury:

TNF alpha[462, 463]

Several observations suggest that tumour necrosis factor (TNF) plays a role in demyelination, although direct evidence for the above is lacking. Studies have examined ultra-structurally rat sciatic nerve injected with TNF- alpha [433]. Initially, occasional myelinated axons associated with macrophages showed signs of mild myelin damage. By day 3 there were features of demyelination. By 6-7 days, the vascular changes had resolved, and the endoneurium contained significant numbers of demyelinating and degenerating axons. Control nerves, which received injections of placebo (saline), showed no vascular changes or demyelination.
Recombinant human tumor necrosis factor (rhTNF) has been tested for its effect on myelinated cultures of mouse spinal cord tissue. As controls, recombinant human interferon gamma (rhIFN) and interleukin-2 (rhIG2) were tested, as well as T-cell supernatants, anti-galactocerebroside serum, and normal culture medium. It was found that rhTNF induced delayed-onset (18-24 hr) oligodendrocyte

necrosis and demyelination. Some nerve fibres progressed to demyelination by 72 hours. In contrast, rhIFN, rhIL2, T-cell supernatants, and normal medium had little or no effect on myelin cultures. Whether TNF alone induces demyelination remains uncertain. Macrophages may remain an essential component of the immune attack and further studies are required to determine the role of TNF alpha WITHOUT macrophages in the demyelinating process .

The above is a plausible consideration in HIV, where there are high levels of TNF due to chronic immune activation, however demyelination has been described in the presence of macrophages (many of which contain HIV and may therefore not be functional)

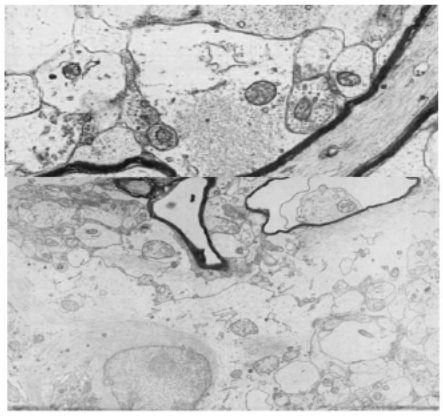


Fig 10.10: Selmaj KW, Raine CS. Tumor necrosis factor mediates myelin and oligodendrocyte damage in vitro. Ann Neurol 1988; 23:339-346

Complement mediated cytotoxicity.[464]

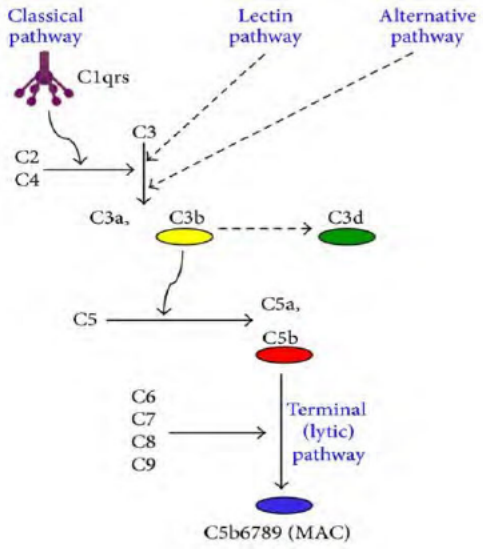


Figure: 10.11: Complement pathways: Classical. Lectin and alternative (Kuhn et al: Cells, 2003 *12*(6), 887)

Complement activation occurs via 3 pathways: namely the classical pathway, lectin pathway, alternative pathway. Complement involvement in peripheral nerve disease is evidenced by the demonstration of complement deposition in sural nerve biopsies from CIDP patients.

In a previous study, sural nerve biopsy specimens from seven CIDP patients showed deposition of IgM in intraneural blood vessels in all seven patients and deposition of C3 in 6 patients. Deposits of C3, were also noted on the Schwann cell plasmalemma of even non-demyelinated nerve fibres in CIDP patients. Complement may contribute to nerve injury by inducing vascular permeability changes, thereby increasing BNB permeability, and enhance the access of antibodies to nerve fibres. In another study, the presence of C3d as a part of the immune complex on myelin sheaths suggests a role of the complement system in the development of neuropathy.

A recent single case report of a patient with CIDP demonstrated the presence of a circulating IgG antibody to LM1, but not to NF155, CNTN1, GM1, and GD1b. Immunohistochemical analysis revealed deposition of C9 neoepitope, a component of MAC, on compact myelin. Macrophage infiltration was evident with the presence of several CD68- positive cells in each fascicle. Furthermore, deposition of complement was noted at the internodes, which comprise most of the length of myelinated fibres. This case highlights the possible role of complement-dependent cytotoxicity in the pathogenesis of CIDP with LM1 antibodies.

However, whether alternate complement pathway or lectin pathway activation occurs in CIDP (bacterial or viral induced) in the absence of the formation of Ag-Ab complexes is uncertain and requires further investigation. Current literature supports Ag-Ab complex formation to activate the classical pathway which did not occur in our patients. However, staining for complement deposition in culture models maybe useful in a future experiments, as complement deposition independent of Ag-Ab complex formation remains a possibility.

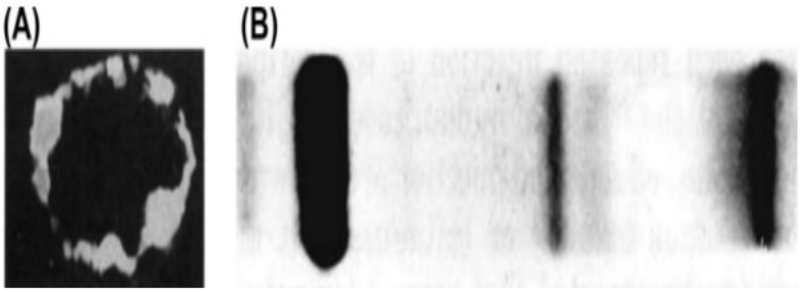


Fig 10.12: Complement deposition in sural nerve biopsy from CIDP patients (A) and (B) agarose gel electrophoresis patterns of complement in CSF of CIDP patients: Adapted from Dalakas MC and Engel WK

Viral Induced:[465, 466]

Several viruses can initiate CNS and PNS diseases that include demyelination as a major feature of neuropathology. In humans, the most prominent demyelinating diseases are progressive multifocal leukoencephalopathy, caused by **JC virus** which directly infects oligodendrocytes, and the measles

virus which causes subacute sclerosing panencephalitis. More recently the **Zika virus and the coronavirus** has also been implicated as causing GBS, CIDP and central demyelination [467-469].
Studies with neurotropic strains of mouse **hepatitis virus, Theiler's virus, and Semliki Forest** virus have been at the forefront of this research. These models demonstrate how viruses enter the brain, spread, persist, and interact with immune responses. Common features are an ability to infect and persist in glial cells and generation of predominantly CD8+ responses resulting in inflammatory demyelination. In most cases demyelination is to a limited extent the result of direct virus destruction of oligodendrocytes, but for the most part is the consequence of immune and inflammatory responses.
HIV gene sequences has been identified in HIV associated distal sensory neuropathy and has been implicated in axonal degeneration, but has not yet been described in demyelinating neuropathies.[10]

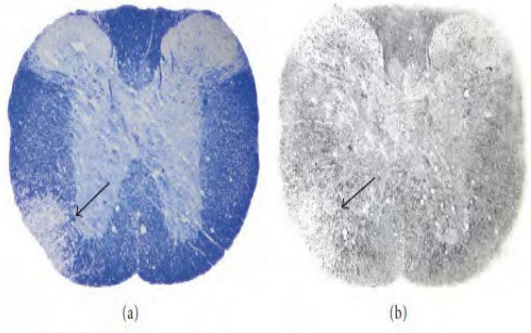


Fig 10.13: Demyelination and axonal loss in an infected mouse spinal cord, arrow demonstrates a large demyelinating plaque.

Cytokines:

Various combinations exist and differ in a disease model and/or tissue in which they function. Studies show that TNF-α, HGF, MIP-1β and IL-1β levels were significantly higher in total CIDP patients than in normal controls. Of these, HGF levels were elevated in typical CIDP patients, but not in MADSAM patients. Patients with high HGF levels showed good responses to steroid treatment. Different cytokine profiles among the CIDP subtypes presumably reflect differences in pathophysiology, stage of disease and differing microenvironments[470].
Other studies show that IL-12 may be involved as potential marker of immune activation in CIDP. The increase in its levels in CSF may be a marker of initiation of Th-1 cell-mediated immunity.[471]
However, whether cytokines alone or in specific combinations can cause nerve damage is unclear. Macrophages and/or antibodies may be required to induce or maintain demyelination.

Macrophage-induced demyelination

The above is the best described in CIDP, independent of the humoral arm.
Immunologic mechanisms underlying macrophage-induced demyelination remain to be elucidated, despite early reports having stressed the significance of this phenomenon. Macrophages play an important role in inflammatory demyelinating neuropathies, such as Guillain-Barre syndrome (GBS)/AIDP and CIDP. In HIV, M Trophic viruses infect macrophages early in the disease. These

macrophages may express viral antigenic components that cross react with the patients peripheral nerve antigens.

A concept of molecular mimicry of foreign epitopes in infectious agents to self-epitopes in the peripheral nervous system has been established in GBS. A similar mechanism may constitute the initial step in the immunologic cascade in a subpopulation of patients with CIDP, particularly those manifesting an acute progression mimicking GBS. Another possible first step may be initiated by resident macrophages in the peripheral nervous system that may act as antigen-presenting cells. Abnormal recognition of some myelin epitopes by these macrophages may act as the initial trigger for the pathogenesis in CIDP. Following the initial trigger of the inflammatory cascade, blood monocytes enter the endoneurium, guided by adhesion molecules, such as selectins or ICAM-1,35,36 and matrix metalloproteinases. They then differentiate into macrophages. Hence, both resident and blood-derived macrophages may contribute to inflammation in the peripheral nervous system. In addition to the production of proinflammatory cytokines that modulate the inflammatory process by macrophages in peripheral nerve tissue, direct damage to myelin leads to demyelination. Previous animal studies have suggested that proteases secreted by macrophages and TNF may also be involved in demyelination. Electron microscopic studies of nerve biopsy specimens obtained from patients with CIDP revealed that macrophages seem to actively destroy myelin that otherwise appears to be normal.
An antibody-mediated pathway is one of the putative mechanisms for macrophage-induced demyelination. This finding may suggest that the attachment of putative autoantibodies to myelin components provokes complement cascades and subsequent phagocytosis by macrophages.
Other cells: NK Cells, CD8>CD4 T cells

In Conclusion:

The inducing factor for demyelination is possibly viral, bacterial, trauma induced or exposure to neural antigens (pregnancy, surgery). This may sensitize the adaptive arm of immunity resulting in demyelination mediated by B cells, T cells and macrophages with the aid of cytokines. Cytokine and complement activation may vary depending on the environment in which they function and may require external co-factors example a virus or bacteria. Cytokines may differ not only in the tissue within which they are effective, but also the combination in which they exist and the stage of the immune response.

useful prospective experiment will be independent addition, or addition of different combinations of complement, cytokines, viruses, followed by immune cells and antibodies in a stepwise fashion or randomly to myelin co-cultures to determine which of the above independently or in combination induces demyelination. The HIV virus has shown to induce neural injury without the binding of antibodies in previous studies.

Chapter 11:

Discussion and synthesis

Despite, South Africa's effective ART rollout having decreased immunodeficiency syndromes, opportunistic infections and AIDS related neoplasms, autoimmunity still occurs. Chronic autoimmune disease occurs as a consequence of chronic immune activation, which includes the humoral arm and CTL responses. Additionally acute or subacute autoimmune diseases may occur during immune reconstitution and sero-conversion. Possible reasons for chronic immune activation, includes ongoing viral replication and incomplete immune restoration due to "immunological failure" or poor penetration of ART into the CNS compartment [472]. In addition, ongoing viral replication and emergence of circulating recombinant forms and "unique" recombinant forms, may occur due to poor compliance to ART [473]. In 2019, an estimated 15%–20% of patients on first-line ART and 30% on second-line ART in SA experienced virological failure [474, 475] and 19% on non-nucleotide reverse transcriptase inhibitor (NNRTI)-based ART, had failed virologically and immunologically. [269]

The main aim of the thesis was to describe the clinical, electrophysiological, laboratory and radiological findings of immune mediated peripheral nervous system disease in the setting of HIV, to compare these findings to an HIV-uninfected cohort, and to explore the immunopathogenesis of these unique disorders. This was achieved by assessing clinical response to immunotherapy, testing sera and CSF for potential pathogenetic peripheral nerve antibodies and screening for novel antibodies using myelin co-culture screens. Immunoprecipitation and mass spectrometry, although described in a single patient in chapter 10, will be explored further in prospective studies. The novel findings of each published manuscript are summarized below.

A comparison of clinical, electro-diagnostic, laboratory and treatment outcome differences in a cohort of HIV-infected and HIV-uninfected patients with Myasthenia Gravis

In the above study, significant differences are that a) HIV-infected MG patients were more likely to be young, black females. b) HIV-infected MG presented with more severe generalised disease as evidenced by higher MGFA grades and functional scores (MMT, MGQOL, and MGADL) and were more likely to present with bulbar-respiratory failure requiring ventilation. c) The majority of the patients were AcHR antibody negative compared to the HIV-uninfected category. HIV-infected patients often required rescue therapy with IVIG/PLEX or IVI Cyclophosphamide. Prospective studies of MuSK, the IgG subclass, novel antibodies, immune function studies, and possibly thymic histology may be valuable to explain the pathogenesis of MG in HIV and perhaps segregate alternate therapeutic avenues. MuSK a likely antibody, which accurately predicts for their phenotype has previously been described in the African ancestary[166], and may possibly be related to genetic factors

or HIV itself. Screening for the above will allow for the establishment of treatment guidelines of MG in HIV.

A comparative study of HIV-infected and HIV un-infected patients with motor neuron syndrome

HIV-infected patients with MNS are more functionally disabled at presentation and die within the first year if untreated. ART therapy results in improved functional recovery with possible reversal of the disease process, which supports a viral or immune pathogenesis. Future prospective studies are required to evaluate the pathogenesis of HIV-associated MNS, especially with respect to endogenous retroviruses[159]. This may extend clues to the "multi-step" pathogenesis of MND. Lastly, and most importantly, active support for patients with MNS in the long term is warranted as survival and improvement with ART is highly likely and the clinician should be aware of the reversible nature of this disease as it is not neurodegenerative.

A comparison of clinical, electro-diagnostic, laboratory and treatment outcome differences in a cohort of HIV-infected and HIV-uninfected patients with Chronic Inflammatory Demyelinating Polyneuropathy

This study suggests that treated HIV-infected CIDP patients have a short duration of disease, a benign course and are highly steroid responsive compared to their HIV-uninfected counterparts. Although the cohort satisfied the ENFS criteria for CIDP, the quick response to corticosteroids and the relatively benign monophasic course makes AIDP a possibility and argues for a possible steroid responsive nodo-paranodopathy. Perhaps the course and progression of CIDP in HIV-infected individuals is different and revision of criteria for the diagnosis of CIDP in HIV-infected individuals is required. This study also shows that steroids are a cost-effective and safe option in HIV-infected CIDP patients especially in a resource limited setting. Further prospective studies confirming a rapid corticosteroid response in HIV-infected CIDP patients as well as unravelling the immune mechanisms responsible for CIDP in these patients is required to define future therapy.

Pure motor lumbo-sacral radiculopathy in HIV:

The HIV pure motor lumbosacral radiculopathy cohort, is the largest cohort of the above condition and the second reported case series in the world. The above is an unusual presentation of PM LSP affecting the lumber-sacral roots only. Presentation is subacute and patients often respond to corticosteroid therapy suggesting that the above condition is possibly a variant of CIDP and not AIDP. Another postulate is that it is antibody mediated, a possible nodo-paranodopathy with ventral root predilection. Future studies are required to better understand the pathogenesis of the above disease.

Nodal-paranodal antibodies in HIV-immune mediated radiculo-neuropathies: Clinical phenotypes and relevance:

The study, although small, is the first such study in an HIV infected cohort. The study suggests that nodal-paranodal antibodies occur at a similar frequency in HIV-infected and HIV-uninfected IMRN. However, interpretation of results in the context of HIV infection, especially with IgG1 subtypes and low antibody titres is challenging as many antibodies occur as an epiphenomenon in HIV and may therefore be non-specific and non-pathogenetic. Larger prospective studies are required to better define the relevance of these antibodies in the context of HIV and to determine if they define a specific clinical entity. Potential novel antibodies may exist in this cohort as evidenced by the positive myelin co-cultures and results of the mass spectrometry described in chapter 10. These will by defined by larger prospective studies and confirmatory tests. The study provides direction for future research, in terms of the importance of developing panels of antibodies or other biomarkers that select for specific phenotypes, namely pure motor, pure sensory, CCPD and CIDP with optic neuritis. This will ultimately provide direction for the establishment of targeted immunotherapy.

Case Report: Immune mediated or genetic? Combined central and peripheral demyelination in 2 Siblings of African Origin

The novel cases of CCPD among two sisters, provide a platform to explore the confluence of genetic, immune, and environmental factors in the context of autoimmune disease, where pregnancy and exposure to other infections such as HIV or toxins are potential triggers.
Future studies using a human derived myelin culture model of oligodendrocytes and Schwann cells in their native environment to screen for common novel antigens is potentially useful. Identification of the causative genes in autoimmunity and tolerance may require whole-genome sequencing or long-read sequencing technologies or genome mapping to cover for non-coding and structural genetic variations. The cases highlight the need for multicentre genome-wide association studies. This will allow better understanding of single nucleotide polymorphisms in different populations, and in complex diseases such as immune mediated demyelination, which is likely due to an interplay between multiple genes and various environmental factors such as pregnancy. However, in the context of this uncommon disorder, antibodies against NF155, lactosylceremide, AQP-4, GQ1B, MOG, CV2/CRMP5 should be excluded, despite most reported cases being antibody negative. Future discovery of novel pathogenetic antibodies will direct therapy, for example B cell-depleting therapy, if anti-NF155 positive.

Relevant questions that arise from this project, in addition to the disease specific discussions in each chapter, includes the following:

What is the relationship between CD4 counts and emergence of CIDP, MNS ,PM LSP, Nodopathies and MG?

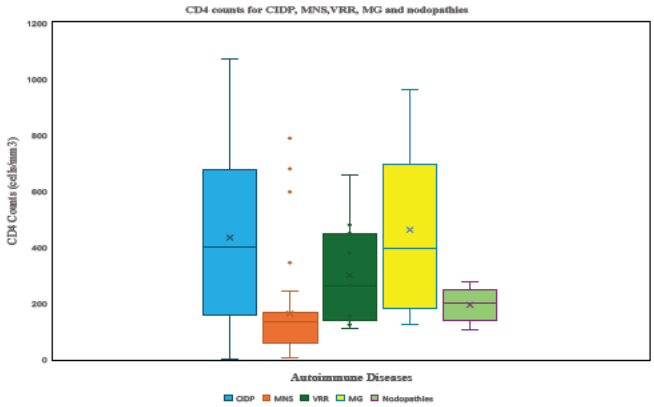


Figure 11.1: CD4 counts for CIDP, MNS,PM LSP, MG and nodopathies

The above graph indicates that HIV-infected CIDP (median CD4 count: 384, IQR 126-423) and MG (median CD4 count: 390, IQR 186-461) are more likely to occur at higher CD4 counts compared to MNS (median CD4 count: 161, IQR 84-210), nodopathies (median: 210, IQR 195-229) and PM LSP (327, IQR 146-457), PV <0.003. MNS and nodopathies are likely to occur during immunosuppression, CD4 counts < 250cells/mm^3 and PM LSP at CD4 counts less than 400 cells/mm^3, although not absolute.

As documented in previous articles, CIDP and MG may occur during immune reconstitution or seroconversion when the immune system is preserved [180, 261], whereas MNS occurs during CD4 lymphopenia and therefore possibly viral, humoral or CD8 mediated[156, 186]. Literature regarding nodopathies and PM LSP in HIV is limited and numbers are small to draw reliable conclusions. However, one needs to exercise caution when interpreting CD4 counts as there are many confounding factors that need consideration which include circadian cycle, concomitant nicotine and recreational drug use, infections such as viruses and tuberculosis and ART[476].

Is CIDP and MG an immune reconstitution inflammatory syndrome (IRIS) ?

IRIS is a potential complication in 30% of HIV-infected patients initiated on ART. It is a state of hyper-inflammatory response against latent infections or antigens after a rise in CD4 cell count. Infections such as cytomegalovirus, mycobacterium, cryptococcus, Epstein-Barr virus, pneumocystis, JC virus, hepatitis B, and C infection and malignancies such as Kaposi sarcoma and non-Hodgkin's lymphoma has been described as an IRIS phenomenon [459].

In the case of HIV patients receiving HAART the following risk factors have been described [459]

1. Young Age, male gender
2. CD4+T cell count less than 100 cells/mm^{3}at the time of initiating ART.

3. An accelerated rise in CD4 count following treatment with ART.
4. Rapid HIV RNA viral suppression within ninety days of ART
5. Genetic factors example (HLA-A, -B44, -DR4 associated with herpes virus IRIS).

Applying criteria 2, 3 and 4 to our cohorts of patients who received ART, 41% (10/24) patients with MG and 41% (16/39) patients with CIDP met the criteria for IRIS, showing either emergence of disease or clinical deterioration with rising CD4 counts. This has been reported in previous studies[180, 233, 261]. Similarly other autoimmune diseases may also occur as an IRIS phenomenon in HIV such as Graves' disease and sarcoidosis. This is therefore a plausible explanation for the prompt response of HIV-infected CIDP patients to CST with no relapses, in contrast to HIV-uninfected CIDP. Future studies with larger numbers are required to explore this concept further.

Is immunomodulatory therapy safe in HIV?

This studies demonstrates safe and effective use of CST, IVIG,PLEX and even cyclophosphamide in HIV-infected patients. Various studies have also reported safe use of the above and additional agents such as B cell depleting therapy in HIV[270]. High frequency PLEX without the use of immunosuppressive therapy has been successfully used in HIV glomerular nephritis[477]. This theoretically may be a "safer" treatment option in advanced immunosuppression. Prospective studies are required to design clinical protocols to monitor the safe and effective administration of immunotherapy in HIV.

Interpreting results for live CBA and pathogenetic antibody assessment in myelinating co-cultures

Live CBA:

Unpublished data of an inter-laboratory validation of nodal/paranodal antibody testing study, across four different laboratories in Europe, which includes the Nuffield Department of Clinical Neurosciences laboratory, demonstrated an interlaboratory agreement in the overall results with high concordance for 142/159 (89.3%) of samples[235, 478]. Overall sensitivity ranged from 80%-87.3% and specificity from 98.3% to 100% with accuracy between 96.9-99% among different tests. Live cell-based assays was most accurate (98.9- 99.2%) followed by other CBA (96.6%-98.8%) and ELISA (91-99%).This data demonstrates that live CBA are accurate and reliable methods to determine the presence or absence of nodal/paranodal antibodies as depicted in figure 11.2. CBAs were also shown to be more sensitive than ELISA, particularly for the detection of pan-neurofascin antibodies, where ELISA misses 25-30% of CBA seropositive cases[235, 478].

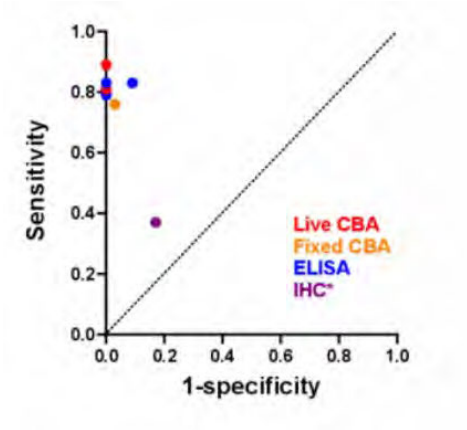


Figure 11.2: Sensitivity and specificity by assay type. Cell based assay and ELISA have high sensitivity and specificity, Immunohistochemistry on teased nerve fibres was only performed by 1 laboratory on a limited number of equivocal samples, but within this population had a more limited ability to distinguish positives from controls.

(Rinaldi, S; Poster Presentation, Annual PNS Meeting, Miami,2022)[235]

However factors such as end-point titre (EPT) for NF155 antibodies and non-IgG4 subclass testing are variable among laboratories which may be due to differing laboratory methods and variations in the subclass specific secondary antibodies used by the different centres respectively, figure 11.3

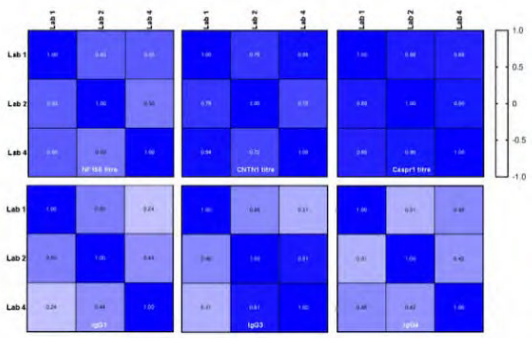


Fig 11.3: Spearman's correlation matrices for antibody titre and subclass determination between laboratories:
Although absolute titres varied, relative end-point titres were highly correlated for CNTN1 and Caspr1, but less well correlated for NF155. There was also generally lower correlation in the detection of specific IgG subclasses.

(Rinaldi, S ; Poster Presentation, Annual PNS Meeting Miami,2022)[235]

Therefore the detection of IgG1 monospecific antibodies, especially NF 186, although variable among laboratories in Europe, are likely related to our cohort of black- African -HIV-infected patients or due to differing laboratory methods and variations in subclass specific secondary antibodies. NF186 monospecific antibodies are also described as uncommon among the centres testing for paranodal antibodies in Europe and are more likely to be detected by centres using live CBA than fixed CBA or ELISA. Larger prospective studies may be useful to confirm or exclude laboratory factors that may influence results, confirm population specificity and to harmonise EPT and IgG subclass detection among laboratories and different population groups.

Myelinating co-cultures:

The myelinating co-cultures used in the experiments are contemporary and novel techniques to detect antibodies. Hence testing across different population groups is limited, there are no clear guidelines regarding parameters such as sensitivity (proportion of positive results in the diseased population, correctly identified by the test), specificity (proportion of patients in the control population, excluded by the test), non-specific binding, binding in controls and comparative studies with other methods example teased nerve fibres.

However, data from small studies suggest that for known antibodies the sensitivity varies between 50-90% and is different for different antibodies for example 50% for NF155 and 80-90% (CNTN1/Caspr). Figure 11.4 shows results of 56 patients with positive NF155 and CNTN1 antibodies detected on live CBA and how they compare to a myelin co-culture system[479]

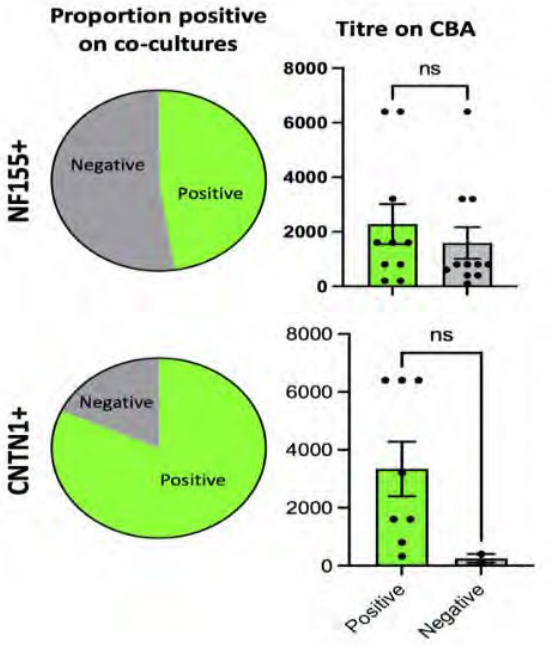


Figure 11.4: Proportion of patients (Total number 56) with NF155 and CNTN1 antibodies by CBA also detected using co-cultures. Graphs show CBA titres of samples positive (green) or negative (grey) on co-cultures.

This study shows that with blinded repeat evaluation of 56 samples, overall agreement of sample reactivity using Fleiss' Kappa statistic was 0.737 ($z = 31.6$) across 8 different rater combinations. This small study demonstrated that false positive antibody binding is rare in controls (0.5%), blinded, inter-rater agreement is high, and similar results are obtained across multiple experimental runs using different hiPSC clones [479].

For unknown or novel antibodies, it is not possible to determine a sensitivity or specificity as we do not know if the antibody is present in the sample tested and if the antigen of interest is expressed in the culture system myelinated by rat Schwann cells and not human Schwann cells.

False negative (a negative result in the diseased population who have neural antibodies, type 2 error) may occur with low binding, that is below the positivity threshold, or the antigen of interest is not expressed in the myelin co-culture system that leads to undetectable binding. Technical errors and

poor-quality myelin co-cultures are possible but avoided by using control samples and quantifying the ratio of myelin to axonal coverage as discussed in other studies[237].

False positives (positive result in the control population, type 1 error) is rare (<0.5%) and the pattern of binding often lacks specificity. Myelin and axonal injury can uncommonly occur in normal sera and therefore requires objective quantification to decide if pathological or within normal limits as in (patient 3 and 21 in chapter 10). As already mentioned, quantifying what is regarded as normal/acceptable myelin damage in controls is important to establish normative data in future studies. The degree of myelin destruction by pathogenetic antibodies can be quantified as discussed in the methods section by Davies et al[480]. Factors other than known antibodies in the sera may also account for neural injury if objectively quantified and reproducible. These confounding factors, may include TNF-alpha, cytokines, macrophages , complement discussed briefly in chapter 10.

The above highlights the challenges of calculating sensitivity, specificity, false negatives and false positives for a novel and contemporary assay like myelin co-culture assay. Presumably, the assay has a lower than expected true positive rate (proportion of patients who have neural antibodies that are detected by the assay). However, this is not possible to calculate, as there isn't a more definitive or accurate "gold standard test" to confirm this.

The above studies, although small, show that live CBA and myelin co-culture systems are useful tests to detect antibodies in inflammatory neuropathies with a high degree of accuracy for live CBA and a variable sensitivity and specificity for different known or unknown antibodies using the myelin co-culture system. The myelin culture system which expresses antigen in its "live native form, not altered by fixative or freeze artefact" is theoretically an attractive system for the detection of novel or unknown, peripheral nerve reactive antibodies, in addition to known nodal, paranodal and ganglioside antibodies not detected by other tests. The cultures systems described have also been used to demonstrate conduction block, axonal degeneration and demyelination induced by both IgM human monoclonal disialosyl/ganglioside antibodies and neuropathy-associated serum IgG[237].

These assays are therefore valuable with respect to further prospective research to detect pathogenic peripheral nerve reactive antibodies in inflammatory conditions such as CIDP, AIDP, PM LSP, DRG. However, cost, skill, category 4 laboratory facilities, and time required for testing are limiting factors especially in a resource deficient country like SA. The use of fixed CBA will be explored in a future study and is possibly useful in resource deficient countries such as SA.

CHAPTER 12 :

Recommendations for future research

Despite the changing landscape with ART, the above diseases, although uncommon in the global arena, remain relevant to our future clinical practise in Africa, where the HIV prevalence remains relatively high. This is due to the complexities that surround the clinical diagnosis, challenges with interpretation of laboratory tests and use of immunotherapy in the setting of HIV induced immune dysregulation, and concomitant infections.

It therefore remains necessary to pedantically tease out and document these cases as treatment avenues may differ due to differing pathogenesis. These diseases, therefore, deserve our current focus of attention and it is not merely "stamp collecting" for journals that they are published in.
There is little doubt that unless a vaccine or cure for HIV is discovered, these immune mediated diseases will continue to exist. Hopefully, an explanatory mechanism will emerge for cases such as pure motor lumbosacral polyradiculopathy, combined peripheral and central inflammatory demyelination, HIV associated MNS, MG, and CIDP, just as explanations ultimately emerged for other unexplained disorders in the past. An obvious guess for the current cases would be an unexpected or novel antibody, unmasked by chronic inflammation and viraemia such as HIV or HERV-K.
Appropriately designed clinical and laboratory research studies will provide a future foundation for investigating the cellular and molecular pathogenesis of CIDP, MG, PM LSP, MNS and nodopathies in patients with HIV infection. Simultaneously, these findings may be applied to those patients who do not have HIV infection. In both situations, further research will provide opportunities to improve clinical diagnostic algorithms, laboratory testing, therapeutic options and patient outcomes. Our recommendations for future research focuses on rapidly evolving and growing fields of neurogenetics and neuroimmunology documented below:

A: CIDP,PM LSP, Autoimmune nodopathies:
Identification of a "NOVEL" antibody which resulted in a demyelinating neuropathy in patient 1, 30, 49.
In our study 3/76 (4%) of patients with inflammatory neuropathies were positive against neural antigens on myelin co-culture screens. Future experiments to discover this novel antibody, targeting specific neural antigen, example non-myelinating Schwann cells as in CIDP 1 , will be undertaken. These experiments will include IP using magnetic beading and mass spectroscopy, gene silencing

techniques and recreating disease in animal models which is discussed in Chapter 10. Tgfr3 may be a potential Schwann cell target that requires further investigations and exploration as in CIDP-1.

Categorizing inflammatory neuropathies into 2 broad serological groups, namely IgG1 and IgG4 related disease

The above concept has emerged from the IgG1 monospecific antibodies in HIV autoimmune nodopathies from our SA cohort versus IgG4 nodopathies seen in Europe. Theoretically, patients with IgG1 disease had a more benign disease course relative to those with IgG4 disease from Europe. The above categorization is likely generalizable to HIV-uninfected cohorts with inflammatory neuropathy and other autoimmune diseases. This classification will help to define abnormalities of B cell tolerance, antibody production by plasma cells, and somatic hypermutations of immune cells (chapter 1) . Identifying these abnormalities, systematically and longitudinally, may help explain the differences in clinical presentation, temporal progression of disease (acute vs chronic, progressive vs relapsing remitting), risk of relapse and treatment response in IgG1 vs IgG4 disease. Studying B cell populations and exposing B cells to the different conditions required to produce varying IgG antibodies, example HIV may lend relevance to IgG class switching. Studies documenting changes in IgG subtypes during treatment or spontaneous remissions and relapses may also provide clues. Above concept may apply to all immune mediated disorders including MG (MUSK vs AChR MG)

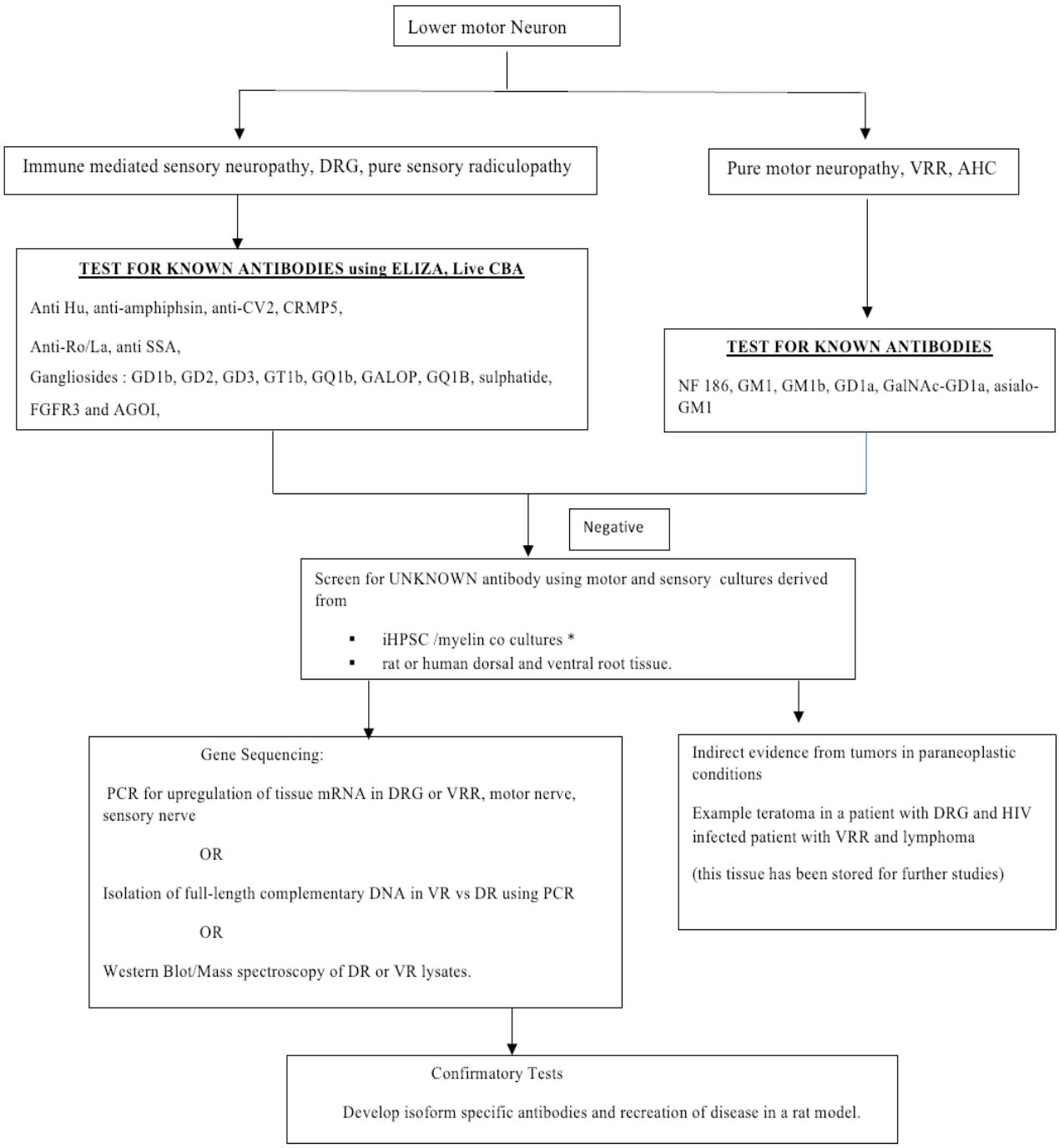
Defining the antigenicity of the DRG/pure sensory nerve vs PM LSP/pure motor nerve/motor neuron. Flow chart 10.1.1

The following categories of patients from previous studies in chapter 8 and 9 (PM LSP, MNS, CIDP, DRG) will be included as well as prospective patients from other centres will be recruited to increase the number of patients in each subcategory as these phenotypes are rare :

a. Motor phenotypes: pure motor neuropathy, PM LSP, MNS
b. Sensory phenotype: pure sensory neuropathy, DRG, pure sensory polyradiculopathy

The following flow chart reflects the various steps that will be undertaken in a prospective study to define pure sensory vs pure motor antigenic targets. Patients who test positive for known antibodies will be excluded from the study. Those who are negative will undergo the following screening tests using:

a. Ventral root and sensory neuron cultures derived from hiPSC or rodent tissue
b. Genetic Techniques
c. Western Blot and
d. Indirect evidence from tumour tissue as in paraneoplastic syndromes



*To initiate the differentiation of HiPSC into sensory and motor neurons, the cells will be cultured according to a previously described protocol until the cell cultures reach a 50% confluency[62]. At this point differentiation will be commenced using the AD2 line according to a previously described protocol [63]. Motor neurons will be differentiated using the AD3 HiPSC lines [64]. Co-cultures will be generated by seeding the rat Schwann cells onto neuronal cultures at 4 weeks. Stains to confirm motor neuron=motor neuron specific choline-acetyl transferase and neuron specific β 111 tubulin and sensory neurons = BRN3A will be used.

Figure 12.1: Algorithm for determining antigenicity of the DRG vs VR or AHC

Subtypes of CIDP: Optic Neuritis:

Similarly, defining antigenic targets in the subcategory of patients with CIDP and ON published in chapter 8 is an exciting new project. This will be undertaken following exclusion of alternate immune mediated diagnoses of ON such as MS, MOGAD, NMO , GFAP, Miller Fischer syndrome and autoimmune nodopathies. Defining antigenic targets of the optic nerve using hiPSC optic nerve or oligodendrocyte culture screens or human/ rodent optic nerve tissue in the following categories will assist with future diagnosis of disease and therapy. Potential categories of patients include :

a. CIDP with optic Neuritis
b. Idiopathic ON

Thus far studies include cultures of astrocytes from the retina and optic nerve as well as optic nerve demyelination induced by intraneural injection of antibodies example galactocerebroside antibodies [481, 482]. A useful future clinical study, in addition to novel antibody detection, will include comparing clinical presentation, MRI findings, response to therapy, and serology in idiopathic ON versus MS, NMO, MOG associated ON to identify bedside and radiological features that will predict for each phenotype.

Defining antigens common to PNS and CNS in CCPD:

Antibodies common to both the central and peripheral nervous system currently include antibodies against the following :

a. NF155
b. MAG,
c. MOG,
d. AQ4
e. GQ1b,
f. lactosylceremide

The above antibodies were negative in our patients with CCPD. Future studies to identify new myelin antigenic targets common to both the CNS and PNS may be possible using myelin cultures from rat or human oligodendrocytes, in addition to Schwann cells. Other techniques may include immunoblot of lysates of rat brain or genetic techniques such as PCR comparing antigens common to both central and peripheral myelin antigens An in-vitro myelination model for the central nervous system remains to be established[483].

Creating a myelin culture model that is species, environment and disease specific for HIV inflammatory neuropathy:

The ideal model to overcome species differences will be myelin derived from human Schwann cells (not rat Schwann cells), exposed to HIV and/or co-factors that result in chronic inflammation. This may alter the antigenic expression and will be most reflective of myelin antigens in their "live, native conformation". An inflammatory environment could possibly be achieved by attempting to grow myelin cultures in patients serum, after filtering out antibodies. Assessment of cell mediated demyelination induced by HIV-itself, can be achieved by adding HIV-infected macrophage's to the culture system. Alternatively, a novel dorsal root ganglion culture system comprising of sensory neurons, macrophages and Schwann cells from transgenic rats expressing human CD4 and CCR5 can be used as described in other studies [10].

Use of HIV-infected Simian nerve tissue for immunohistochemistry

The above has closest similarity to HIV-infected human nerve tissue and will therefore be useful to assess for binding of antibodies. However storage, ethical issues and distortion of antigenic expression due to fixation may be prohibitive. Comparing and contrasting immune responses and modification of the CD4 receptors over time in natural and unnatural Simian hosts may also provide clues to autoimmunity in HIV.

Immune cell profiling in the following categories:

I. Acute onset CIDP vs AIDP

Immune cell profiling of CSF and serum for CD4 + cells, CD 8+ cells , NKT cells, NK cells, intermediate, classical monocytes using flow cytometry in patients with HIV-associated AIDP vs CIDP. Previous studies have observed an expansion of specific cell types such as the NKT and monocytes cell clones in CIDP and not in AIDP [484].

11. Subtypes of CIDP

Immune cell profiling will help determine if the CIDP subtypes differ in their T helper response against specific antigens, example Th1 response against NF155 may be used as a biomarker for MADSAM or Th2 response against GM1 is a marker for MMN [485]

Biomarkers in HIV-infected vs HIV-uninfected inflammatory neuropathies[87]

Periaxin, has recently been described as a potential novel biomarker for peripheral nerve demyelination[87]. Measuring periaxin levels in the serum and CSF using novel techniques such as ultrasensitive immunoassays example single molecule arrays (Simoa) or ECL pre-treatment and post treatment as described in other publications, in HIV-infected and uninfected patients with inflammatory neuropathies may be useful. Biomarkers may be used to herald a relapse or monitor response to therapy . This may also provide an indirect assessment of disease severity in the 2 cohorts of patients.

Genome Wide association studies for CIDP:

We have joined a multicentre study being conducted by Professor Henry Houlden at University College London focusing on genetic mutations in CIDP, including mutations that predispose to autoimmunity. We have thus far recruited 76 patients from South Africa and will continue to increase our patient numbers. This cohort includes HIV-infected patients. Future studies can explore the influence of HIV on genetic mutations.

B: MNS:

The following prospective studies will be considered:

HERV-K:

Identification and measurement of CSF HERV-K titres in HIV-infected MNS compared to HIV uninfected MND patients using PCR or other techniques such as a CSF or blood bioinformatic pipeline that identifies microbial sequences in mammalian RNA-sequence data, including sequences with no significant nucleotide similarity hits in GenBank[486]. This may also be useful in other inflammatory diseases such as PM LSP and CIDP to identify viral or microbial co-factors that precipitate autoimmunity. This study will also allow us to assess the effect of ART on CSF HERV-K levels and on the disease evolution.

CSF Proteomics in HIV-infected MNS:

Proteomic analysis, using techniques such as liquid chromatography mass spectroscopy, has contributed significantly to the study of the neurodegenerative disease [147].

It has helped to define the pathological change common to nearly all cases of MND/ALS, namely intracellular aggregates of phosphorylated TDP-43 and chitinase, shifting the focus of pathogenesis in ALS toward RNA biology. Measuring the above in the CSF of MND and MNS will help define and possibly contrast pathogenesis in the 2 cohorts of patients.

Similarly proteomics, using liquid chromatography mass spectroscopy may be useful in inflammatory diseases such as CIDP patients to identify novel biomarkers in serum or CSF.

C) MG:

The following prospective studies will enable us to better understand the immunopathological differences between HIV-infected MG and HIV-uninfected MG.

a. Identification of antibodies example MUSK, agrin, LRP4 or an unknown antibody in HIV-infected AChR-ve MG and IgG subtypes of these antibodies
b. Evaluating differences in thymic histology in HIV-infected and HIV-uninfected patients with MG and understanding the role of the thymus and value of thymectomy in the 2 subcategories
c. Possible identification of an immune marker that heralds a clinical relapse, such as CD27 B cells may herald a relapse in MUSK-MG patients treated with rituximab [487]

D) Myositis in HIV

A retrospective chart review of myositis in HIV infection focusing on the following:

a. Clinical differences in myositis in the HIV-infected category compared to HIV-uninfected patients with focus on polymyositis, necrotising myopathy and IBM.
b. Differences in myositis specific antibodies: example Ro, La, Ku, RNP, AMA, M2, NTC51a, SRP,HMGCR in the 2 subcategories
c. Response and adverse events of HIV-infected patients with myositis to corticosteroids, IVIG, Rituximab, ART compared to the HIV-uninfected counterparts.
d. Histological differences between HIV-infected and uninfected categories
e. CD4 counts and VL over time and effects on clinical progression of disease .

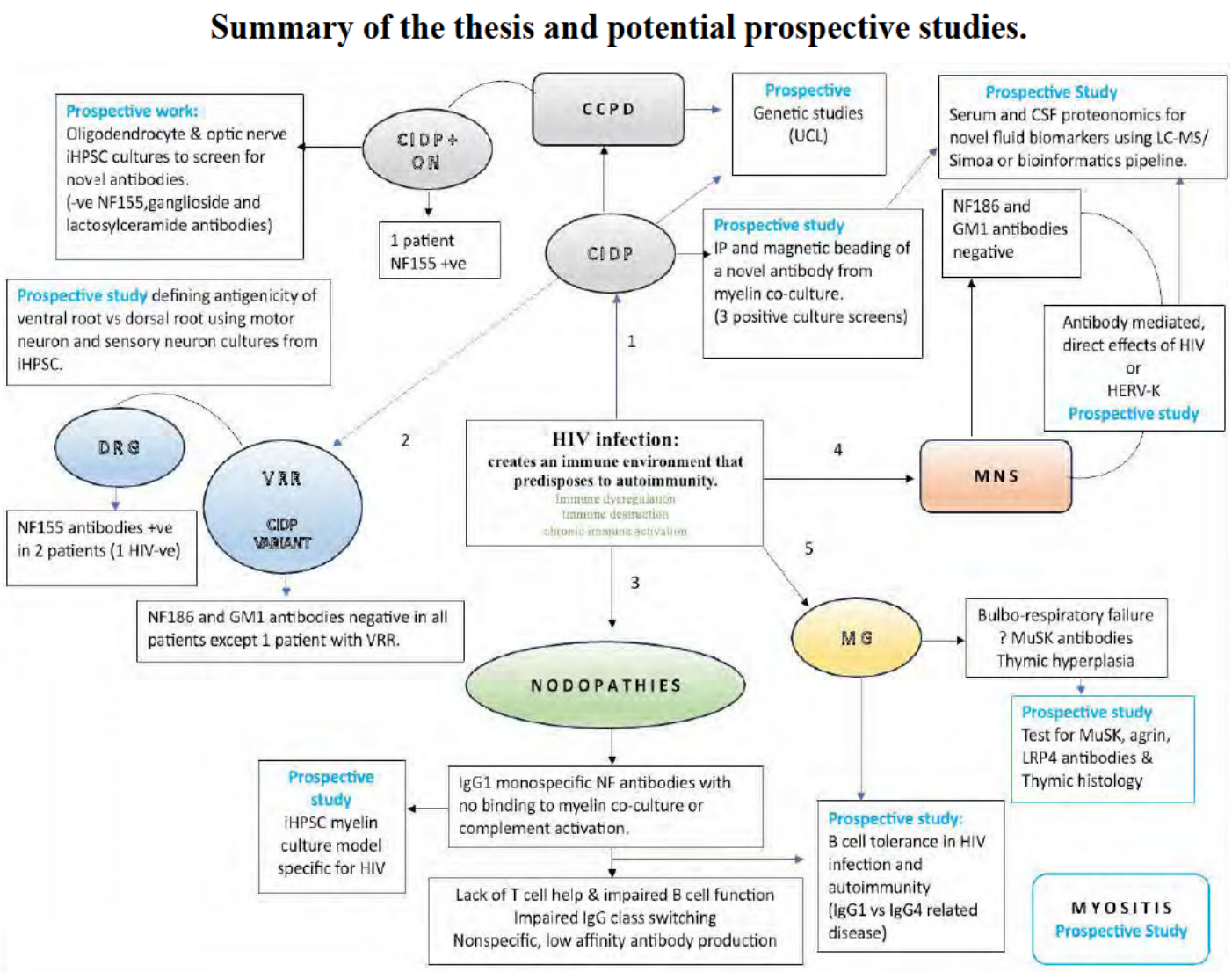
E) Clinical trials to establish protocols for the safe use of immunomodulatory therapy in HIV-infected patients:

In addition to investigating the cellular and molecular basis of neuromuscular condition in patients with HIV infection, clinical research studies need to be done to establish safety protocols for the use of immunomodulatory therapy in this cohort . Careful selection of patients and monitoring the clinical, laboratory and radiological parameters listed below are important to prevent complications of therapy example opportunistic infections in patients with grade 3 or 4 lymphopenia or neutropenia. Screening and monitoring for opportunistic infection such as TB, cryptococcal meningitis, herpes zoster and PML prior to initiating therapy and during the course of therapy is essential as these infections may resurge with the use of immunomodulatory therapy. Administration of prophylactic therapy and vaccines are useful to prevent infections. Careful monitoring of the following clinical and laboratory parameters are therefore important to design future protocols for the following :

a. Monitoring of laboratory parameters which include: CD4 counts and VL, white cell counts, platelets, haemoglobin levels, immunoglobulin levels, CD19 levels, T and B cell subsets using flow cytometry.
b. Lumbar punctures for JC virus index in CSF if using drugs that can potentially cause PML

c. Routine CXR to monitor for PTB
d. Use of prophylactic drugs for opportunistic infections in patients with moderate - severe lymphopenia which includes
    a. Antivirals: Acyclovir, valacyclovir
    b. Bactrim prophylaxis
e. Pre-treatment vaccinations : haemophilus influenza, Zoster, Covid, pneumococcal
f. Clinical trials assessing the safety and efficacy of novel therapies such as B cell depleting therapies (Rituximab), complement inhibitors, BAFF inhibitors and AHSCT, CAR T cell therapy in HIV
g. Identification and management of IRIS.
h. Selecting ARVs with optimal CNS, peripheral nerve and muscle penetration.
i. Monitoring drug-drug interactions with ARVs

Figure 12.2: Summary of the thesis and potential prospective studies



Conclusion

In conclusion, our work, highlights the need for more focused collaborative basic science work in immunology, genetics and virology. It provides a platform for a variety of neuroscience studies. This can be achieved using contemporary techniques in each of the conditions discussed to unravel the pathogenesis of these rare and intriguing disorders and to ultimately develop targeted and safe immune therapy for patients with HIV-immune mediated neuromuscular syndromes.
In years to come fewer difficult or rare neurological cases will remain cryptic. Whole genome sequencing, long read sequencing, genome mapping, metagenomic sequencing of CSF, sophisticated imaging, exposure to enigmatic infections such as HERV-K, which may potentially unmask autoimmunity, development of disease specific culture models exposed to HIV, CSF and serum proteomics, and liquid biopsies will plug remaining diagnostic gaps. This will provide clues to autoimmune disease in general and not confined to HIV-infected patients. Newer future immune therapies such as more humanised and targeted monoclonal antibodies or targeted complement inhibitors will allow for safe use even in the immunocompromised HIV-infected patient. Therefore, until then, as mentioned by Prof Neil Scolding (in his recent editorial on our published manuscript on "CCPD in 2 siblings, immune mediated or genetic?) there will always be a place for the careful clinical description, painstaking analysis, succinct and accurate case reporting and publication - Anorakish or not.

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APPENDIX

Table A1: Detailed NCS in patients with HIV-infected IMRN

Patient Number	Time from symptom onset	SNAP	Motor studies												EMG	Neurophysiologist Report
			DML		Slow CV			F waves			CB	TD	CMAP	In excitable LL nerves		
			> 120%	> 150%	< 80%	< 70 %	< 50%	> 120%	> 150%	Absent						
P1	8 weeks	Absent LL, reduced UL	4			2				3	2	2	N		No active denervation	Demyelinating
P2	16 weeks	Absent in all 4 limbs	2	3	2	2	1	2	2	2	4	2	<80% LLN	2	Positive SW in TA and ADM	Demyelinating with secondary axonal loss
P3	10 weeks	Absent in LL Normal in UL		4		4			4				<50% LLN	4	Absent denervation	Demyelinating
P4	12 weeks	Reduced SNAP in all 4 limbs	2	2	1	2	1	2	2		1	1	<70% LLN	4	Scant denervation	Demyelinating
P5	3 weeks	Absent SNAPs in UL	4	4	8			8			2		N		No active denervation	Demyelinating
P6	2 weeks	Normal						4	4				N		No active denervation	Demyelinating
P7	17 weeks	Reduced in all UL and LL	4	4	2	2		1	2			1	<70% LLN		Scant denervation	Demyelinating
P8	15 weeks	Absent in LL and reduced in UL	2			4			2		1	1	N	1	No active denervation	Demyelinating
P9	4 weeks	Globally absent	3				4						<50% LLN	4	Florid denervation	Primary demyelinating with secondary axonal loss
P10	6 weeks	Reduced in all 4 limbs	2			4		1	1	2	2	2		4	Scant denervation	Demyelinating
P12	22 weeks	Reduced in LL	4			4		4					<80%		Scant denervation	Demyelinating
P13	5 weeks	Reduced in UL	3	2		2		3			2	1		2	No denervation	Demyelinating

Patient Number	Time from symptom onset	SNAP	Motor studies												EMG	Neurophysiologist Report
			DML		Slow CV			F waves			CB	TD	CMAP	In excitable LL nerves		
			> 120%	> 150%	< 80%	< 70%	< 50%	> 120%	> 150%	Absent						
P14	7 weeks	Absent	4										<50% LLN		No denervation	Axonal ? Demyelinating
P15	6 weeks	Absent in LL Reduced in UL				3				2				2	No denervation	Demyelinating
P16	6 weeks	absent													Normal	Dorsal root ganglionopathy/ Pure sensory neuropathy
P17	4 weeks	absent													Normal	Dorsal Root Ganglionopathy
P18	10 weeks	absent													Normal	Dorsal Root ganglionopathy, Abnormal blink Response
P19	9 weeks	Absent													Normal	Dorsal Root Ganglionopathy/ Pure sensory Neuropathy
P20	7 weeks	Absent													Normal	Dorsal Root Ganglionopathy/ Pure sensory Neuropathy
P21	4 weeks	Normal								4			<70% LLN		Paraspinal denervation	VRR
P22	6 weeks	Normal								2			<50% LLN		Florid denervation including Lumbar paraspinal	VRR

DML=Distal motor latency, CV=Conduction velocity, SNAP=Sensory nerve action potential, CMAP=Compound muscle action potential, VRR=ventral root radiculopathy, Numbers refer to how many individual nerves met the criteria stated

Patient Number	Time from symptom onset	SNAP	Motor studies												EMG	Neurophysiologist Report
			DML		Slow CV			F waves			CB	TD	CMAP	In excitable LL nerves		
			> 120%	> 150%	< 80%	< 70 %	< 50%	> 120%	> 150%	Absent						
P23	5 weeks	Normal						2	2				<50% LLN		Denervation including lumbar paraspinal	VRR
P24	8 weeks	Normal								4			<70% LLN		Lumbar Paraspinal denervation	VRR

DML=Distal motor latency. CV=Conduction velocity. SNAP=Sensory nerve action potential. CMAP=Compound muscle action potential. VRR=ventral root radiculopathy. Numbers refer to how many individual nerves met the criteria stated

Table A2: Antibodies in HIV-infected Patients

Patient	Connective	SPEP	Paraneoplastic	Ganglioside	Aquaporin/	NF -155	NF186	NF-140	Contactin1	CasPr
P1		P		-	-	-	-	-	-	-
P2	1:160	P		-	-	-	-	-	-	-
P3		P		-	-	1:100(IgG1)	-	-	-	-
P4		P		-	-	-	-	-	-	-
P5				-	-	-	-	-	-	-
P6	1:160	P		-	-	-	-	-	-	-
P7		P		-	-	-	-	-	-	-
P8	Ro	P		-	-	-	-	-	-	-
P9				-	-	-	-	-	-	-
P10	1:160	P		-	-	-	-	-	-	-
P11				-	-	-	-	-	-	-
P12		P		-	-	-	-	-	-	-
P13				-	-	-	-	-	-	-
P14		P	+ Ma2	-	-	-	-	-	-	-
P15				-	-	-	-	-	-	-
P16				-	-	1:100 (IgG1	-	-	-	-
P17		P		-	-	-	-	-	-	-
P18				-	-	-	-	-	-	-
P19		P		-	-	-	-	-	-	-
P20				-	-	-	-	-	-	-
P21		P		-	-	-	1:400 (IgG1)	-	-	-
P22		P		-	-	-	-	-	-	-
P23		P		-	-	-	-	-	-	-
P24		P		-	-	-	-	-	-	-

P= Polyclonal, -ve +Negative

Table A3: ART, VL and CSF findings of HIV-infected IMRN

	ART	VL	CD4 count	CSF Protein	CSF OBs	CSF IgG index	CSF Neutrophil count	CSF Lymphocyte count	Auramine stain, TB culture, gene expert	VDRL FTA-abs	Cryptococcal Ag,India ink, cryptococcal culture	PCR HSV,CMV,HZ,EV	CSF Cytology
P1	Nil	ND	122	1.24g/L	T4	0,42	0	10	-	-	-	-	-
P2	Nil	94712	333	1.28g/L	T2	0,92	0	8	-	-	-	-	-
P3	Yes	2648	189	1.22g/L			2	16	-	-	-	-	-
P4	Yes	<20	288	1.06g/L	ND		0	22	-	-	-	-	-
P5	Yes	<20	356	1.41g/L	T4	0,57	0	18	-	-	-	-	-
P6	Nil	ND	123	1.12g/L	ND		0	28	-	-	-	-	-
P7	Yes	<20	366	1.76g/L	ND		3	22	-	-	-	-	-
P8	Nil	88452	168	0.99g/L	ND		0	7	-	-	-	-	-
P9	Nil	35412	432	0.88g/L	T4	0,77	0	16	-	-	-	-	-
P10	Nil	145963	132	1.72g/L	ND		0	6	-	-	-	-	-
P11	Yes	<20	312	1.15g/L	ND		0	18	-	-	-	-	-
P12	Nil	88745	196	0.77g/L	T4	0,56	0	5	-	-	-	-	-
P13	Nil	ND	122	1.08g/L	ND		1	7	-	-	-	-	-
P14	Nil	76542	166	1.33g/L	ND		0	4	-	-	-	-	-
P15	Nil	92587	196	0.77g/L	T4	0,63	0	10	-	-	-	-	-
P16	Yes	1247	277	0.95g/L	ND		0	3	-	-	-	-	-
P17	Nil	ND	180	0.88g/L	ND		0	5	-	-	-	-	-
P18	Yes	<20	223	0.61g/L	T4	0,58	0	8	-	-	-	-	-
P19	Yes	<20	214	2.13g/L	ND		2	15	-	-	-	-	-
P20	Nil	ND	122	1.21g/L	ND		0	8	-	-	-	-	-
P21	Nil	45214	210	2,34g/L	T2	0,91	0	14	-	-	-	-	-
P22	Nil	117458	112	2.23g/L	T3	0,96	4	8	-	-	-	-	-
P23	Nil	102004	208	1.66g/L	T2	1,06	0	5	-	-	-	-	-
P24	Nil	92743	212	2.56g/L	T2	1,18	0	6	-	-	-	-	-

ND=not done,CSF=cerebrospinal fluid, ART=antiretroviral therapy, OBs=oligoclonal bands, TB=tuberculosis,VDRL=venereal disease research laboratory, FTA=Fluorescent Treponemal antibody absorption test, HSV=Herpes Simplex virus,HZ= Herpes zoster, CMV=cytomegalovirus,EV=enterovirus, PCR=polymerase chain reaction

Table A4: Visual Acuity, VEPs and OCT in patients with Optic Neuritis

	Snellen's chart		Visual Evoked		OCT						
Patient Number	Visual Acuity		VEP-P100 (msec)		RNFL thickness (μm)				GCL thickness	MRI Orbits	Fundal Photos
	RE	LE	RE	LE	RE	LE	RE	LE		Optic disc swelling	Optic Atrophy
3	CF	20/100	160	144	154	148	92	98	X	X	
5	20/70	20/50	142	138	128	125	64	60	ND		X
6	20/50	20/70	144	152	133	141	84	86	X	X	
11	CF	20/100	Abs	Abs	146	138	72	78	X	X	

RE=Right eye, LE=Left eye, RNFL=retinal nerve fibre layer, OCT=optical coherence tomography, GCL=ganglionic cell layer, VEP=visual evoked potentials

Table A5: Blood and CSF Investigations

Table A5.1 Serological Blood Investigations

	Connective Tissue Screen (ANA, Ro/SSA/La/SSB/ANCA)	SPEP	Paraneoplastic antibodies	Aquaporin 4 & MOG antibodies	Paranodal antibodies (NF155, NF186, NF140, Caspr, CNTN1)	Very Long chain fatty acids	Leucocyte aryl sulphatase A Enzyme activity
Patient 1	Negative	No paraprotein	Negative	Negative	Negative	No abnormality detected	121.4 units
Patient 2	ANA 1:160	No paraprotein	Negative	Negative	Negative	No abnormality detected	105.2 units

Full Blood count, Liver function tests ,urea and electrolytes ,calcium, magnesium and phosphate and serum angiotensin converting enzyme levels were normal in both patients

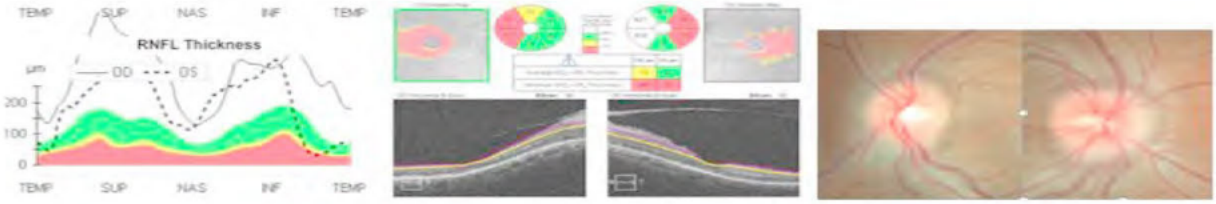
Table A5.2 CSF Investigations

	HIV Status	CD4 cct cells/uL	VL (copies/ml)	CSF Protein (g/L)	CSF OBs	CSF IgG index	CSF Neutrophils (cells/ul)	CSF Lymphocyte (cells/ul)	CSF Glucose (mmol/L)	Auramine stain, Gene expert Culture,VDRL,FTA abs, cryptococcal antigen, culture, HSV PCR,CMV PCR,HZ PCR, JCV PCR,	CSF Cytology
Patient 1	Negative	Not done	Not done	0.97	+ (T2 pattern)	0.81	0	0	3.5	Negative	No malignant cells
Patient 2	Positive	333	94712	1.28	+ (T2 pattern)	0.92	0	8	3.8	Negative	No malignant cells

VDRL=Venereal Disease Research Laboratory, TB=Tuberculosis, FTA abs= Fluorescent Treponemal antibody absorption Test, HSV=Herpes Simplex Virus, CMV=cytomegalovirus, HZ=Herpes Zoster, JCV=John Cunningham

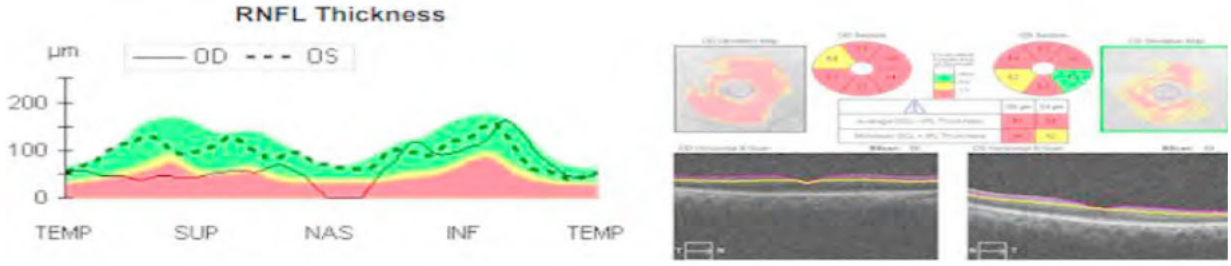
Figure A1: OCT and Fundal Images

Patient 1: OCT RNFL thickness, GCL at presentation and fundal photos 2 months after treatment



Above findings are consistent with bilateral swollen discs: RNFL OD: 293um, OS 191um.

Patient 1: OCT RNFL and GCL after a relapse



Above findings are consistent with optic atrophy on the R: RNFL OD: 65um, OS 93um