Comparative epidemiology and quantitative neuroimaging of neuromyelitis optica spectrum disorder and multiple sclerosis

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ABSTRACT

Background: Central Nervous System (CNS) inflammatory syndromes represent a spectrum of diseases caused by infiltration of inflammatory cells into the tissue of the brain and spinal cord. They comprise a group of diseases that can be acute or chronic, including optic neuritis, transverse myelitis, acute disseminated encephalomyelitis (ADEM), neuromyelitis optica (NMO) and its spectrum disorders (NMOSD), clinically isolated syndrome (CIS) and multiple sclerosis (MS). MS is the most common one while NMO/NMOSD is relatively rare although the exact population-based epidemiological parameters are lacking. The distinction of MS and NMO/NMOSD in adult patients and of MS and ADEM in paediatric patients is important for considerations of prognosis and treatment. This thesis aims to differentiate NMO/NMOSD from MS using epidemiology analyses and quantitative magnetic resonance imaging at 7 Tesla. The hypothesis of the thesis is that NMO/NMOSD can be differentiated from MS by disease incidence, prevalence, severity, and the quantitative characteristics of the lesion and non-lesion white matter of the brain.

Methods: The thesis firstly employed a comparative epidemiological study to estimate incidence and prevalence of MS and NMO/NMOSD, and to estimate risks for associated comorbidities and mortality between NMO/NMOSD and MS. The thesis then applied two quantitative techniques to compare the white matter lesions and non-lesion white matter (so-called normal-appearing white matter [NAWM]) of both NMO/NMOSD and MS.

Results: The results are presented in seven chapters. Chapter 1 provides a general review of CNS inflammatory syndromes. Chapter 2 shows the estimated incidence and prevalence of CNS inflammatory diseases in 2012 using the Clinical Practice Research Datalink (CPRD). MS is estimated to be the most prevalent and the NMO/NMOSD the rarest. Chapter 3 shows the case-controlled analytic results on the impact of the burden of comorbidity in MS. The burden is cumulative after MS diagnosis and impacts on patient survival compared to non-MS patients. With an interest of new-onset epilepsy in the course of MS compared to non-MS controls, chapter 4

shows that MS patients have a higher risk of having the first medical recorded epilepsy at and after MS diagnosis. Chapter 5 shows the comorbidity burden in NMO/NMOSD, and estimates the risk for mortality between NMO/NMOSD and age- and gender-matched MS populations. Chapter 6 compares in vivo white matter lesions and NAWM of the brain between adult patients with NMO/NMOSD or MS and healthy subjects using quantitative T1 relaxation time and magnetisation transfer ratio (MTR) magnetic resonance imaging at 7 Tesla. The results show that myelin content of white matter lesions is relatively better-preserved in NMO/NMOSD than that in MS; and they also show some changes in the NAWM of NMO/NMOSD, despite the fact that the metrics of the histogram of both sequences are not different to those in MS.

Conclusions: In the UK, NMO/NMOSD is rare with a higher disease severity compared to MS, which is the most prevalent inflammatory disease. In vivo, using T1 relaxation time and MTR imaging at 7 Tesla, the white matter lesions of the brain have more preserved myelin in patients with NMO/NMOSD than in those with MS. There is at least a small proportion of intracranial NAWM having subtle changes in patients with NMO/NMOSD that is detectable, but the changes could not be differentiated from MS in the current study. This thesis helps to better understand the epidemiology of NMO/NMOSD and MS, and MRI detectable changes of lesions and NAWM in NMO/NMOSD.

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2. Subjective discomfort in children receiving 3 T MRI and experienced adults' perspective on children's tolerability of 7 T: a cross-sectional questionnaire survey. Chou IJ, Tench CR, Gowland P, Jaspan T, Dineen RA, Evangelou N, Abdel-Fahim R, Whitehouse WP, Constantinescu CS. BMJ Open. 2014 Oct 15;4(10):e006094. doi: 10.1136/bmjopen-2014-006094.
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GLOSSARY

Terms	Description
Acoustic noise	Sound waves caused by vibrations of the gradient coils of the magnetic resonance scanner.
Acute disseminated encephalomyelitis	An inflammatory disease that involving the brain and the spinal cord and must present with encephalopathy (i.e. altered consciousness that are caused by the lesion in the brain and cannot be explained by fever or other systemic conditions)
Antibody	Specific proteins in the blood that functions as an immune response to an antigen.
Antiphospholipid antibody syndrome	An autoimmune syndrome that has a tendency for recurrent thrombosis with recurrent abortion, thrombocytopenia, and neurologic disease
Aquaporin-4 (AQP-4)	Proteins that comprise water channels and are abundant at the end-feet of astrocytes in the brain and spinal cord
Artifacts	False features of images produced by the imaging process
Astrocytes	A kind of cells inside the brain that are multifunctional with biochemical and nutritional support of endothelial cells that constitute the blood–brain barrier
Autoimmune diseases	Disorders in which loss of function or destruction of normal tissue caused by immune responses to the body's own tissue
B cells	A subpopulation of lymphocytes that express B cell receptors and may differentiate into memory B cells or antibody secreting plasma cells after encountering with antigens. B cells also present foreign antigens or secret cytokines to active T cells.
B1	A conventional symbol to denote the radiofrequency magnetic induction field used in a magnetic resonance system
Bandwidth	A term referring to a range of frequencies
Basal ganglia	Large subcortical nuclear masses in the brain consisting of the several components including caudate, putamen, globus pallidus, subthalamus, and substantia nigra
Blood–brain barrier	A semipermeable membrane barrier that separates the circulating blood from the fluid in the brain and the spinal cord; it comprises of specialised cells including capillary endothelial cells that strictly limits transport of materials from the blood into the brain through both physical (tight junctions) and metabolic (enzymes) barriers
Brainstem	The medulla, pons, and midbrain
Case	A particular disease that meets selected criteria in a study
CD (Cluster of Differentiation)	Cell-membrane bound molecule used to differentiate leukocyte populations.
CD4	A receptor often found on helper T cells and monocytes which binds to HLA class II.

CD8	A receptor found on cytotoxic T lymphocytes and natural killer cells which binds to HLA class I.
Central nervous system (CNS)	The brain and the spinal cord
Cerebral aqueduct	A portion of the ventricular system in the dorsal midbrain connecting the third and fourth ventricles
Cerebral cortex	Convoluted grey matter layer that constitutes the outermost layer of the cerebrum
Cerebral hemisphere	Symmetrical half of the cerebrum separated by the sagittal sulcus and containing the cerebral cortex, basal ganglia, hippocampus, and amygdala
Cerebrospinal fluid (CSF)	Clear and colorless fluid bathing the entire surface of the brain and spinal cord
Clinically isolated syndrome	The first disease that may involve the optic nerves, the cerebrum, the brainstem, or spinal cord and 80% of which will convert to multiple sclerosis in 2 decades
Cognitive function	Intellectual functions such as thinking, remembering, and reasoning.
Coil	Loops of wire designed to produce a magnetic field from electrical current flowing through the wire, or to detect a change of magnetic field by the voltage induced in the wire.
Comorbidity	The co-existing of one or more additional diseases with a primary disease
Complement	A system of more than 30 proteins in the plasma and on cell surfaces that leads to the generation of potent proinflammatory mediators
Conditional logistic regression	Logistic regression is a statistical method to analyze a dataset in which there are at least two independent variables that determine an outcome. Conditional logistic regression is an extension of logistic regression by considering the matching to ensure that controls and cases are similar in variables.
Confidence interval	A range of values for a measure to show a specified probability (often 95%)
Confounding factor	A variable is related to an exposure and a health outcome and distort the examined association between the exposure and the health outcome
Contrast	The difference of the signal intensities in two adjacent regions divided by the average intensity.
Contrast-to-noise ratio	The ratio of the absolute intensity difference between two regions to the level of intensity fluctuations caused by noise.
Controls	A group of persons without the examined health problem and to be included for study
Covariate	A variable that can affect the outcome in the logistic regression model.
Crude	When referring to a rate or summary rate for a population, without adjustment
Cytokine	A group of secreted, low-molecular weight molecules that regulate immune responses
Cytotoxic T lymphocyte	An effector T cell (CD8+) that can mediate lysis of target cells

Dawson's fingers	Ovoid-shaped lesions of demyelination with their long axis perpendicular to the surface of ventricle which can be found in patients with multiple sclerosis
Denominator	The lower portion of a fraction; for a rate, the denominator is usually the mid-interval population
Demyelinating	Myelin sheath that covers the neuron axons was damaging and loss, resulting in impaired nerve impulse conduction
Disease-modifying Therapies (DMTs)	Agents that apparently alter the course and progression of inflammatory disease
Dose-response	An association between an exposure and health outcome increased or decreased consistently as the amount of exposure (dose) increases
Encephalopathy	Diffuse brain dysfunction that presents with an impaired level of consciousness
Eosinophil	A kind of granulocyte that plays a role in allergies and defense against extracellular parasites
Epilepsy	Chronic disorder of the brain with various etiologies characterized by recurrent unprovoked seizures and related symptoms
Epitope	The part of an antigen that is recognized by an antibody or T cell receptor
Fatigue	A mental status with a decreased capacity for work and reduced efficiency of accomplishment, often accompanied by a feeling of weariness, sleepiness, or irritability
Gadolinium-enhancing lesion	MRI abnormalities that become bright and visible after injection of the chemical compound gadolinium; the brightness indicates local breakdown in the blood-brain barrier
Gliosis	Scar in an area of injury in the brain and spinal cord
Granulocyte	Leukocytes containing cytoplasmic granules; including neutrophils, eosinophils and basophils
Grey matter	Part of the brain and spinal cord consisting of neuronal cells
Histocompatibility	The property of transplant acceptance among different individuals
Histogram	A visual representation of the frequency distribution of a continuous variable
Human Leukocyte Antigen (HLA)	Part of the Major Histocompatibility Complex (MHC), which are a set of genes that direct T cell mediated immunity, and is associated with different diseases
Image acquisition time	Time required to perform a magnetic resonance imaging procedure
Incidence	A measure of the frequency with which new cases of the examined health condition occurs among a population during a specified period
Incident cases	Those that are newly diagnosed (ideally those in whom the disease has just developed) with the disease of interest
Inflammatory	Active changes of tissue involved infiltration of immune cells or related soluble materials
Infratentorial	Below the tentorium cerebelli, including brainstem and cerebellum

Inhomogeneity	Degree of lack of homogeneity such as the fractional deviation of the local magnetic field from the average field value. Inhomogeneity tends to be most severe in brain regions close to bone-tissue interfaces or air cavities, such as the nasal cavity and in the proximity of the ears, where they can lead to signal loss.
Interferon	A group of cytokines that induces an antiviral state or regulates immune responses
Interleukin-6 (IL-6)	A kind of cytokines that is involved in the acute phase response in the inflammatory tissue
Inversion	A nonequilibrium state in which the magnetization vector is opposite to the magnetic field; usually produced by adiabatic fast passage or 180° RF pulses.
Inversion-recovery (IR)	Pulsed magnetic resonance imaging sequence in the nuclear magnetization is inverted at a time in the order of T1 before the regular imaging pulse-gradient sequences
Lesion	Pathological changes visible by naked eye or neuroimaging
Longitudinally extensive transverse myelitis	The visible tissue changes on the T2 weighted magnetic resonance imaging extend continuously over at least three vertebral segments
Macrophage	A type of white blood cells that plays a role in immunity by engulfing and digesting cellular debris, foreign substances, microbes
Magnetic field	The region surrounding a magnet resonance scanner
Magnetization transfer ratio	A measure based on the exchange of magnetization between free and bound protons and principally dependent on myelin content
Microglia	Tissue-resident macrophages in the brain and the spinal cord and are involved in immune reactions
Multiple sclerosis	An inflammatory disease that is characterized by demyelination and various extent of remyelination along the axons of neurons in the brain and spinal cord, and must fulfill the criteria of dissemination in time and dissemination in space
Myelin	Fatty substance produced by oligodendrocytes in the brain and spinal cord, to insulate around axons and increased nerve conduction velocity
Myelitis	Inflammatory disease of the spinal cord
N-acetyl-aspartate (NAA)	An amino acid metabolite which has been shown to be contained almost exclusively within neurons and is used as a marker of neuronal and axonal viability
Natural killer (NK) cells	Large, granular lymphocytes that do not express T or B cell receptors.
Neuromyelitis optica	An inflammatory disease that involves the optic nerves and the spinal cord at the same time or subsequently
Neuromyelitis optica spectrum disorders	A collective inflammatory disease that involve the optic nerves and/or the spinal cord at the same time or subsequently. It includes neuromyelitis optica according to the updated disease consensus published in 2016

Noise	The component of the reconstructed image (or spectrum) due to random and unpredictable processes as opposed to the signal within the image
Odds ratio	A measure of association between an exposure and a health outcome used in comparative studies, particularly case-control studies
Off resonance	A state occurring when the Larmor frequency of a spin isochromat is different from that of the exciting radiofrequency field
Oligoclonal bands	Immunoglobulins seen as bands on protein electrophoresis of cerebrospinal fluid and a total of 2 or more bands indicates positive
Oligodendrocytes	Glia cells in the brain and spinal cord that synthesis myelin
Optic neuritis	Inflammatory reaction involving the optic nerves
Partial volume effect	A phenomenon that more than one tissue type occupies the same voxel (or pixel), therefore the voxel (or pixel) is classified as one tissue but containing other tissue entity
Pia	The innermost layer of the meninges that is intimately contact the surface of the central nervous system
Pixel	A unit that represents the smallest part of any digital image display at 2-dimension
Prevalence	A term in epidemiology that is the proportion of a particular population found to have been affecting by a given medical condition within a specific period of time
Prevalence	The number or proportion of cases among a given population
Primary care	Care of a patient by a member of the healthcare system who has initial contact with the patient
Primary progressive multiple sclerosis	A subtype of multiple sclerosis that usually without clinical disabling relapse but clinical function (especially mobility) deteriorates gradually
Radiofrequency (RF)	Wave frequency intermediate between auditory and infrared
Receiver coil	Coil of the radiofrequency receiver; “picks up” the magnetic resonance signal
Relapsing remitting multiple sclerosis	The most common type of multiple sclerosis with repetitive episodes of acute disease activity which cause disability at a relapsing stage, and then disability recovers to a certain degree at a remitting stage
Relapsing-remitting	Episodic attacks of symptoms with a certain degree of recovery to normal function
Relative risk	The ratio of the probability of an event occurring (for example, developing a disease) in an exposed group to the probability of the event occurring in non-exposed group (controls)
Relaxation times	A measure of how quickly the spins, after radiofrequency excitation (in high energy state), will return to their equilibrium distribution (low energy state), in which no transverse and longitudinal magnetization is at the maximum value and oriented in the direction of the static magnetic field
Remyelination	Myelin regrowth at the region where myelin sheath was damaged
Retina	Light-sensitive neuronal layer lining the back of the eye

Secondary progressive multiple sclerosis	A subtype of multiple sclerosis at stages that patients with remitting relapsing multiple sclerosis no longer have disabling relapses but clinical function (especially mobility) deteriorates gradually
Segmentation	Segmentation can be based on sets of measured or calculated features, such as signal intensity. As such, a pixel or a voxel that containing a tissue can be classified into white matter, gray matter and cerebrospinal fluid.
Seizure	A discrete event due to sudden, transient, excessive neuronal discharges in the brain.
Signal-to-noise ratio	The relative contributions between a detected signal of the true signal and random unexpectedly superimposed signals (“noise”)
Spatial resolution	The distance between two points in the object that can be distinguished as separate details in the image
Spike-and-wave complex	Generalized synchronous pattern, consisting of a sharply contoured fast wave followed by a slow wave, that was detected on electroencephalogram
T cells	A subpopulation of lymphocytes that mature in the thymus and expresses a T cell receptor
T1 relaxation time	A measure of how quickly the excited nuclei return from the high-energy state to the low energy or ground state is associated with loss of energy to the surrounding nuclei
T1-weighted (T1W)	An image contrast between tissues is due to differences in tissue T1
T2-weighted (T2W)	An image contrast between tissues is due to differences in tissue T2
Tesla	A unit describing the static field strength generated by the magnetic resonance scanner
Thalamus	Mass of grey matter that is symmetrically localized in the dorsal aspect of the diencephalon
Thymus	Primary lymphoid organ located in the thorax where T cell maturation takes place
Transmit/receive coil	A radiofrequency coil that acts as two functions: a transmitter producing the B1 excitation field and a receiver of the MR signal
Transmitter coil	Coil of the radiofrequency transmitter, used in excitation of the spins
Transverse myelitis	Inflammatory disease involving both sides of the spinal cord
Treg cells	A subpopulation of lymphocytes that function as the maintenance of self-tolerance and tissue homeostasis
Voxel	A unit that represents the smallest part of any digital image display at 3-dimension
White matter	Part of the brain and spinal cord that contains axons

ABBREVIATIONS

Abbreviations	Definition
ADEM	Acute disseminated encephalomyelitis
APS	Area postrema syndrome
AQP4	Aquaporin protein 4
BBB	Blood-brain-barrier
CI	Confidence interval
CIS	Clinically isolated syndrome
CNS	Central nervous system
CPRD	Clinical Practice Research Data-link (previously named General Practice Research Database)
CSF	Cerebrospinal fluid
DIS	Dissemination in space
DIT	Dissemination in time
DMT	Disease modifying therapy
DTI	Diffusion tensor imaging
EDSS	Expanded Disability Status Scale
FLAIR	Fluid attenuated inversion recovery
FSS	Fatigue Severity Scale
GWAS	Genome-wide association study
GPRD	General Practice Research Database
HES	Hospital Episode Statistics
HLA	Human leukocyte antigen
^{1}H-MRS	Proton magnetic resonance spectroscopy
HR	Hazard Ratio
ICD-10	International Classification of Diseases, tenth revision
IQR	Interquartile range
LETM	Longitudinally extensive transverse myelitis
IgG	Immunoglobulin G
MHC	Major histocompatibility complex
MPRAGE	Magnetization-Prepared Rapid Acquisition and multiple Gradient Echoes
MRI	Magnetic resonance imaging
MS	Multiple sclerosis

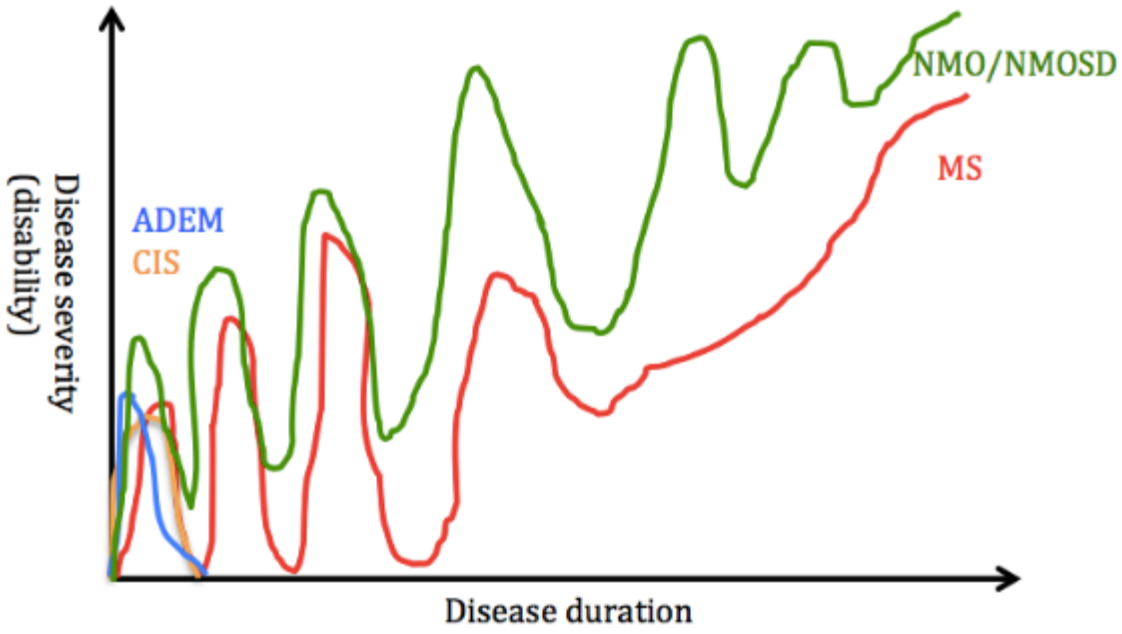
MSFC	Multiple Sclerosis Functional Composite
MTR	Magnetization transfer ratio
NAA	N-acetyl-aspartate
NAGM	Normal appearance gray matter
NAWM	Normal appearance white matter
NMO	Neuromyelitis optica
NMOSD	NMO spectrum disorders
OCBs	Oligoclonoal bands
ON	Optic Neuritis
OR	Odds ratio
PPMS	Primary progressive multiple sclerosis
RRMS	Relapsing remitting multiple sclerosis
SD	Standard deviation
SPMS	Secondary progressive multiple sclerosis
T	Tesla
TM	Transverse Myelitis
T1-RT	T1 relaxation time

Chapter 1. INTRODUCTION

Central Nervous System (CNS) inflammatory syndromes represent a spectrum of diseases caused by infiltration of inflammatory cells into the tissue of the brain and spinal cord (Adams and Kubik 1952) which impairs the conduction of signals in the affected nerves. Multiple sclerosis (MS), clinically isolated syndrome (CIS) (e.g. the first attack of MS), neuromyelitis optica (NMO) and its spectrum disorders (NMOSD), acute disseminated encephalomyelitis (ADEM), optic neuritis and transverse myelitis are the main entities of CNS inflammatory syndromes and are a major health problem in the United Kingdom (UK) and throughout the world because of disability caused by impairment of visual, locomotor, bowel, bladder, somatosensory as well as cognitive disturbances. Patients with these conditions need a high level of medical service utilisation and have low quality of life in comparison with other major chronic diseases (Benito-Leon, Morales et al. 2003, McCrone, Heslin et al. 2008, Kanamori, Nakashima et al. 2011). The diseases can affect individuals at any age, and mostly start in adulthood with exception of ADEM, which more commonly affects children. There is a female predominance but men comprise a larger proportion of ADEM patients whose onset age is usually younger than 10 years old (Pohl 2008).

The course of these diseases is usually an acute onset of CNS dysfunction followed by a variable recovery. CIS and ADEM are often monophasic while MS and NMO/NMOSD are chronic conditions with a relapsing-remitting course which may lead to cumulative disability (Figure 1-1). The term NMO represents as a classic presentation of simultaneous optic neuritis and transverse myelitis, whilst NMOSD represents a wider range of conditions including classic NMO. However, some patients with CIS and ADEM have repeated bouts of events and convert to MS or NMO/NMOSD. For example, about two-thirds of CIS patients eventually receive a diagnosis of MS after relapses and need a long-term disease-modifying treatment (Scalfari, Neuhaus et al. 2014).

Figure 1-1 Clinical courses of MS, NMO/NMOSD, CIS and ADEM.

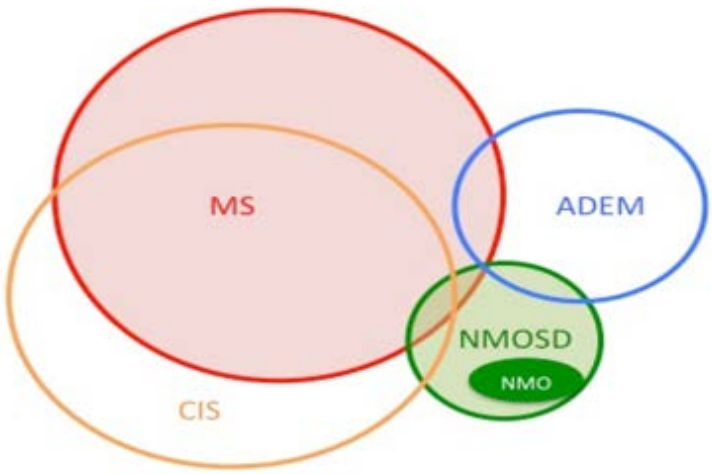


ADEM (blue) and CIS (orange) are monophasic diseases and may be the first manifestation of MS and therefore both overlap with the first bout of MS. Many of ADEM and CIS can remit to baseline daily function of living activities after the attack, albeit the severity of ADEM tends to be higher than that of CIS. MS (red) and NMO/NMOSD (green) are mostly relapsing-remitting courses with acute episodic relapsing and cumulative disability after clinical attacks. The severity of an attack can be worse and the recovery is less in NMO/NMOSD than in MS.

Figure 1-2 illustrates the overlaps of the clinical presentations of four CNS inflammatory syndromes. It is important to make a clear diagnosis for patients with CNS inflammatory syndromes for prognostic and treatment considerations. The long-term therapy for NMO/NMOSD and MS is different. Disease may worsen if a patient with NMO/NMOSD is treated with disease-modifying therapies for MS, such as fingolimod (Kira, Itoyama et al. 2014), natalizumab (Jurynczyk, Zaleski et al. 2013, Kitley, Evangelou et al. 2014), or interferon-β (Palace, Leite et al. 2010, Shimizu, Hatanaka et al. 2010).

In the paediatric population, the life-long immunomodulatory agents typically used in MS are not justified for patients with monophasic or recurrent ADEM (Poser and Brinar 2007). In contrast, childhood-onset MS cases that usually present with ADEM tend to be more severe; the median time from onset to fixed disability is longer than adult-onset MS patients (Harding, Liang et al. 2013), but the median age at evolution to secondary progression is 10 years younger than adult-onset MS (Renoux, Vukusic et al. 2007). Of those with a younger disease onset before 20 years, there is a 1.55-fold higher risk of becoming bedridden from onset of progression compared to those with patients with onset age older than 30 years (Scalfari, Neuhaus et al. 2014). Therefore, early and aggressive treatment for these patients is needed. Differences in treatment strategies underline the importance of early and accurate distinction between ADEM and MS.

Figure 1-2 The clinical presentations of MS, CIS, NMO/NMOSD and ADEM overlap**.**



About 80% of CIS convert to MS. Up to 30% of ADEM convert to MS. Early clinical and imaging characteristics in a small proportion of NMO/NMOSD patients are indistinguishable with MS or ADEM.

The aims of the present thesis are to describe and compare some of the epidemiological and imaging features of MS and NMO/NMOSD. I used a nationally representative database to

investigate the incidence, prevalence and comorbidities of MS and NMO/NMOSD. In addition, a prospective study using ultra-high-field magnetic resonance imaging (MRI) was conducted to compare lesion and non-lesion white matter of MS and NMO/NMOSD quantitatively.

This chapter summarises the current knowledge of epidemiology, aetiology, clinical features of MS, NMO/NMOSD, CIS and ADEM. In addition, a summary of quantitative MRI techniques and their application in this study is presented.

1.1 EPIDEMIOLOGY

1.1.1 Incidence and prevalence

The incidence and prevalence of CNS inflammatory syndromes vary from disease to disease and from region to region. The most common CNS inflammatory syndrome is MS. The UK is a high prevalence region for MS (a prevalence ≥ 30 cases/100,000 people (Kurtzke 2000). The prevalence of MS was 285.8 per 100,000 in women and 113.1 per 100,000 in men in 2010 and the incidence was 9.64 per 100,000/year (Mackenzie, Morant et al. 2014).

NMO/NMOSD is rarer with an estimated prevalence ranging from 0.52 to 4.40 per 100,000 population and incidence from 0.053 to 0.40 per 100,000 population worldwide (Marrie and Gryba 2013). In the UK, regional prevalence of NMO/NMOSD in South East Wales and North West England was 1.96 and 0.72 per 100,000 people, respectively (Cossburn, Tackley et al. 2012, Jacob, Panicker et al. 2013). The incidence in North West England was 0.08 per 100,000 person-years (Jacob, Panicker et al. 2013). Table 1-1 summarises global estimation of the incidence and prevalence NMO/NMOSD.

The epidemiology of CIS and ADEM is difficult to estimate because both conditions can be an isolated clinical entity as well as a part of clinical presentation of MS or NMO/NMOSD. The incidence of CIS has been estimated in children living in southern California at a rate of 4.72 per 100000 person-years (Langer-Gould, Zhang et al. 2011). ADEM is more common in children than adults. The estimated incidence in children is 0.2 to 0.7 per 100,000 person-years in the United States, Canada, Germany and Japan (Leake, Albani et al. 2004, Pohl, Hennemuth et al. 2007, Banwell, Kennedy et al. 2009, Torisu, Kira et al. 2010, Langer-Gould, Zhang et al. 2011).

The number of people living with CNS inflammatory diseases seems to have increased during past several decades (Kingwell, Marriott et al. 2013, Mackenzie, Morant et al. 2014).

Table 1-1 Epidemiology of NMO/NMOSD

Region	Study period	Incidence (95% CIs) per 100,000	Prevalence (95% CIs) per 100,000	Data sources	References
Europe, UK (Wales, South East)	01/05/2010		1.96 (1.22-2.97)	Regional database, Hospital records, Neurologists reports, Laboratory records	(Cossburn, Tackley et al. 2012)
Europe, UK (Merseyside, North West England)	31/12/2010	0.08 (0.03-0.16)	0.72 (0.31-1.42)	Regional database, Hospital records, Neurologists reports, Laboratory records	(Jacob, Panicker et al. 2013)
Europe, Demark	01/01/1998 – 31/12/2008	0.4 (0.30-0.54)	4.4 (3.1-5.7)	National administrative database, trial data, patient registry	(Asgari, Lillevang et al. 2011)
North America, Mexico	1993 – 2005		1.3 (0.9-1.8)	a tertiary care referral centre	(Rivera, Kurtzke et al. 2008)
North America, French West Indies (Martinique)	11/1997 – 10/1999		3.64	Population-based survey	(Cabre, Heinzlef et al. 2001)
North America, French West Indies (Martinique and Guadeloupe)	01/01/1998 – 31/12/2004		Martinique 2.3 (0.6-3.9); Guadeloupe 2.9 (1.1-4.7)	Population-based survey	(Cabre, Signate et al. 2005)
North America, French West Indies (Martinique and Guadeloupe)	07/2002-06/2007	0.20 (0.05-0.35)		Population-based survey	(Cabre 2009)
South America, Cuba	1/10/2003 – 30/11/2004	0.053 (0.040-0.068)	0.52 (0.39-0.67)	Hospital/clinical records, trial data, Media	(Cabrera-Gomez, Kurtzke et al. 2009)
Asia, Japan	10/1972 – 10/1973		0.32 (0.26-0.40)	Population-based survey	(Kuroiwa, Igata et al. 1975)
Asia, Japan (Tokachi province in northern Japan)	03/2011		0.9	Hospital data from10 MS-related institutions	(Houzen, Niino et al. 2012)
Asia, India (Urban Mangalore in south India)	01/2011– 06/2013		2.6	Questionnaire to medical professionals plus collect respondent patient data	(Pandit, Mustafa et al. 2013)
Oceania, New Zealand (Auckland)		0.24 (0.09 –0.68)	1.6 (1.06 – 2.43)		ECTRIMS conference proceedings, 2013

1.1.2 Comorbidity and mortality

The comorbidity impacts the quality of life on patients with MS or NMO/NMOSD and associates with an increased mortality compared to the general population (Wingerchuk and Weinshenker 2003, Marrie, Elliott et al. 2015). Among patients with MS, comorbidity delays diagnosis and increases disability at diagnosis (Marrie, Horwitz et al. 2009), and comorbid cardiovascular diseases may contribute to the increased mortality rate (Lalmohamed, Bazelier et al. 2012, Lavela, Prohaska et al. 2012). Other associated comorbidities include epilepsy, mental illness, hyperlipidaemia, hypertension, migraine, autoimmune thyroid disease, and irritable bowel syndrome (Allen, Lichtman et al. 2008, Kister, Caminero et al. 2010, Kister, Caminero et al. 2010, Marrie, Cutter et al. 2011, Marrie, Yu et al. 2012, Jalkanen, Saraste et al. 2013, Marrie, Fisk et al. 2013, Marrie, Yu et al. 2013). Patients with NMO/NMOSD are reported to present simultaneously with systemic lupus erythematous, Sjögren's syndrome, myasthenia gravis (Jarius, Paul et al. 2012), thyroid dysfunction, narcolepsy, and myopathy (Guo, Lennon et al. 2014).

1.2 PATHOLOGY

The pathological features of MS and NMO/NMOSD are different as shown in figure 1-3. The MS lesion is consistent with focal demyelinated plaques with various degrees of perivascular inflammation, remyelination, astrocyte activation, axonal injury or loss (Trapp, Peterson et al. 1998, Lucchinetti, Bruck et al. 2000). Lesion heterogeneity exists in individual MS patient (Lucchinetti, Bruck et al. 2000). The most common pathological pattern is CNS infiltration of myelin-laden macrophages/microglia and activated inflammatory cells, many CD8+ cytotoxic T lymphocytes, and fewer CD4+ helper T cells, B cells, and plasma cells. Other patterns include oligodendrocyte (i.e. myelin-sheath producing cell) dystrophy or extensive loss, and deposition of both immunoglobulins and complement C9neo antigens. The demyelination of the periventricular area is around a subependymal vein, and these coalesce with adjacent lesions forming confluent sheets (Adams, Abdulla et al. 1987). The lesions may extend into the central white matter, so-called "Dawson's fingers' (Adams, Abdulla et al. 1987). Severe tissue destruction is observed in chronic demyelinated lesions without active inflammation. The demyelination is also seen in the cerebral cortex, the deep grey matter, the grey matter of the cerebellum and the spinal cord (Gilmore, Donaldson et al. 2009). Demyelination mainly affects the subpial layers of the cerebral cortex, associated with the inflammatory cell infiltration in the adjacent meninges, in MS patients at an early or progressive stage (Kutzelnigg, Lucchinetti et al. 2005, Lucchinetti, Popescu et al. 2011). Other patterns include purely intracortical demyelinating lesions with few lymphocyte infiltration, and inflammatory demyelination lesions across grey matter and subcortical white matter regions (also named leukocortical lesions) (Geurts, Bo et al. 2005). Despite demyelination, remyelination is observed in white matter lesions including in new-forming lesions (Prineas, Barnard et al. 1993) and cortical lesions (Chang, Staugaitis et al. 2012). The remyelinated area is vulnerable to acute inflammatory process and becomes an expanding demyelination lesion or a focus of inflammation (Bramow, Frischer et al. 2010). Remyelination capacity is regional different (i.e. remyelination in

cerebral cortex lesion is more extensive than that in white matter lesion) (Chang, Staugaitis et al. 2012) and is negatively associated with disease chronicity (Goldschmidt, Antel et al. 2009).

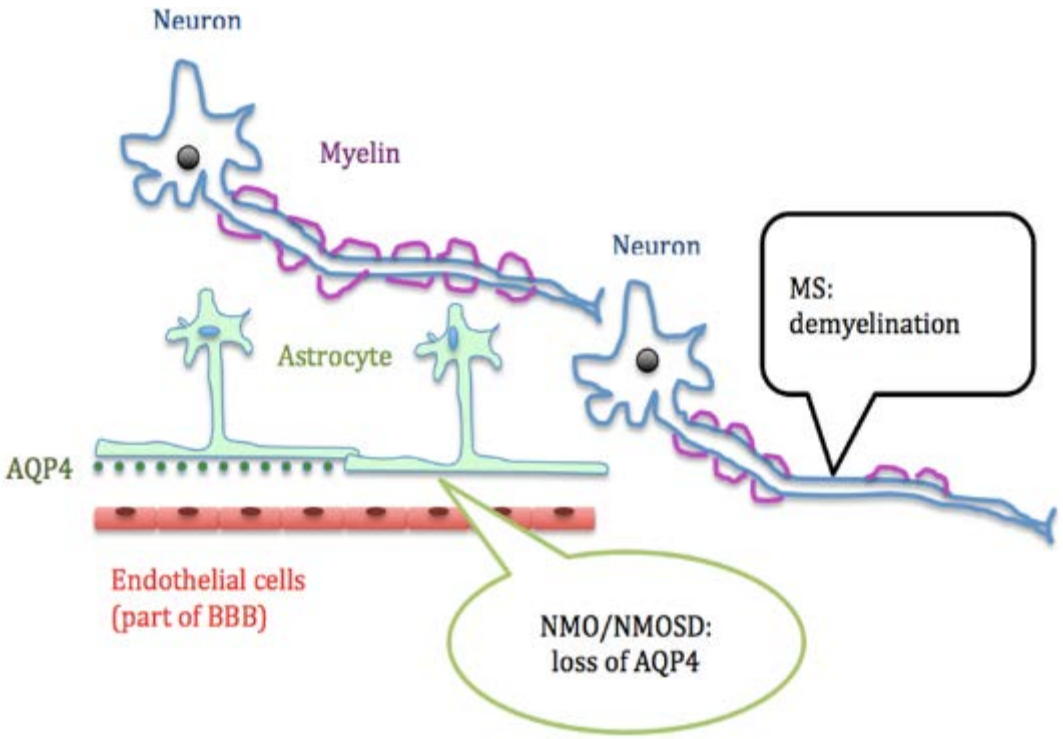
The pathological hallmark of NMO/NMOSD lesions is a loss of the immunostaining for aquaporin-4 (AQP4) protein, which is a plasma membrane water-transporting channel. AQP4 is concentrated at the end-feet of astrocytes forming an integral part of the barrier between the brain and the blood vessels (i.e. blood–brain barrier [BBB]) or between brain and the cerebrospinal fluid (CSF) (i.e. the blood–CSF barrier [BCSFB]) (Nielsen, Nagelhus et al. 1997, Amiry-Moghaddam and Ottersen 2003, Jarius and Wildemann 2010). A recent histopathological study showed that a decreased immunostaining for AQP4 proteins at pial and ependymal surfaces and in the fourth ventricle choroid plexus was observed in patients with NMO/NMOSD compared to MS and normal controls (Guo, Weigand et al. 2017). In addition, compared to patients with MS, the decreased AQP4 immunoreactivity collocated with more prominent reactive microglia and complement deposition (Guo, Weigand et al. 2017).

The NMO/NMOSD is proposed to be primary astrocytopathy, rather than primary demyelinating disease because active early lesions in autopsied white matter tissue show absence of astrocyte or clasmatodendrosis of astrocytes (i.e. loss of astrocyte distal processes) and the myelin is relatively preserved (Parratt and Prineas 2010). Many forms of lesions have been found, including astrocyte loss or dystrophic changes, blood vessel wall thickening and hyalinization, rim or rosette of immunoglobulin and complement, infiltration of eosinophils or granulocytes, no or some degree of demyelination, focal necrosis and cavity formation (Roemer, Parisi et al. 2007, Misu, Hoftberger et al. 2013, Guo, Lennon et al. 2014). In the cerebral cortex, histopathology shows cortical lesions show neuronal loss in cortical layers II, III and IV (Saji, Arakawa et al. 2013), and cortical demyelination is not found (Popescu, Parisi et al. 2010, Saji, Arakawa et al. 2013).

Pathology of ADEM lesions shows demyelination and infiltration of macrophages, lymphocytes, or granulocytes, both limited to perivenous ‘sleeves’. Cortical demyelination is accompanied with

lesser extent of inflammation than that in the white matter. Microglia aggregation is prevalent in the myelin preserved cortex (Young, Weinshenker et al. 2010).

Figure 1-3 Cardinal pathological changes of CNS in MS and NMO/NMOSD.



The main pathological feature of MS is demyelination of axons of neurons. NMO/NMOSD is characterised by a loss of immunostaining of aquaporin-4 (AQP4) protein at the end-feet of astrocytes, which are an integral part BBB.

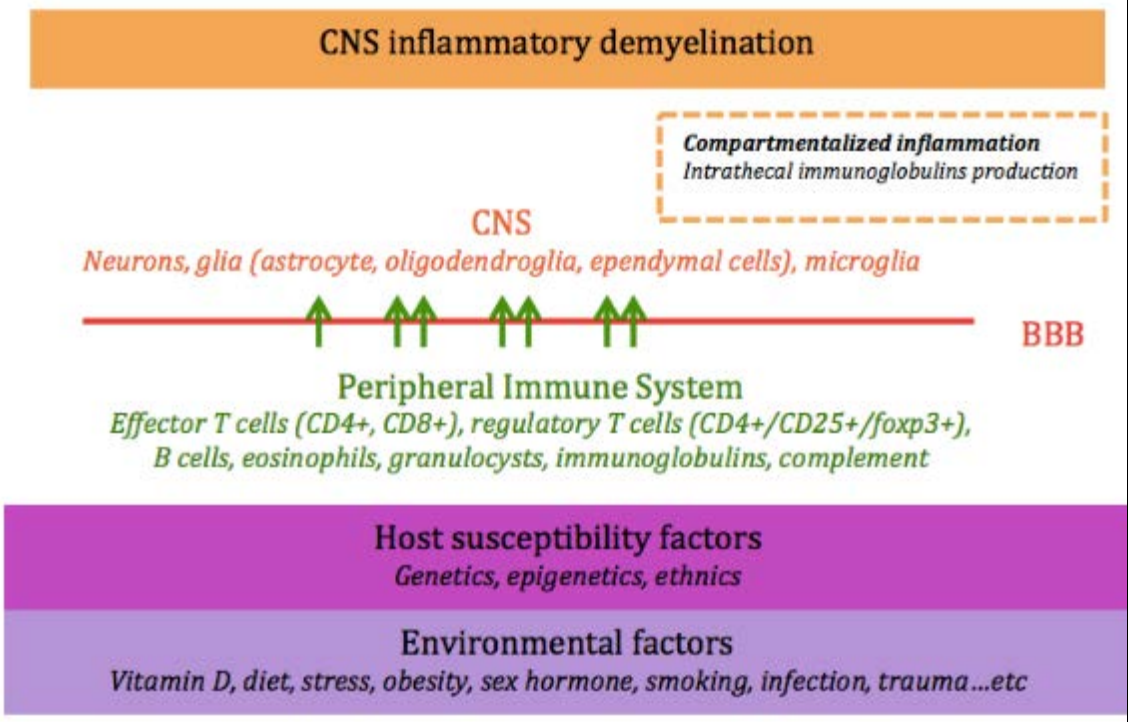
Neuropathologists attempted to explore the aetiology of demyelination in MS through analysis of macroscopically (visible to the naked eye) normal white matter because the most popular hypothesis of the disease is that myelin represents the primary target of the immune system. Allen et al. (Allen and McKeown 1979) demonstrated that 72% of normal-appearing white matter (NAWM) samples were abnormal when combined both histological and biochemical methods were used. The abnormalities included gliosis, the presence of small round blue cells and macrophages on Haematoxylin and Eosin stained sections and variation of myelin density (Allen

and McKeown 1979). These blue cells were identified to be activated microglia, as defined by the altered shape and immunophenotype with the expression of human leukocyte antigen (HLA)-DR (Allen, McQuaid et al. 2001). HLA-DR is a major histocompatibility complex (MHC) class II cell surface receptor that presents antigens to helper T lymphocytes and thus is responsible for immunoreactivity. In addition, axonal damage within NAWM in MS was also quantified (Trapp, Peterson et al. 1998, Evangelou, Esiri et al. 2000).

1.3 AETIOLOGY

The aetiology of CNS inflammatory syndromes is not entirely understood but several lines of evidence indicate an immune-mediated process (Lassmann 2011, Ontaneda, Hyland et al. 2012, Papadopoulos, Bennett et al. 2014). The range of all types of CNS resident cells and peripheral immune cells are seen in lesions as described in section 1.2. The disease occurrence and progression are influenced by both genetic and environmental factors. Figure 1-4 illustrates factors involved in the pathogenesis of MS.

Figure 1-4 Illustration of the aetiology of MS.



Host susceptibility and environmental factors are essential for eliciting immune system to attack CNS which results in the typical inflammatory demyelinating features of MS.

1.3.1 Genetic and environmental factors for MS

1.3.1.1 Genetics

Host susceptibility factors are genetically influenced. The concordance rate of MS in monozygotic twins (25.9%) was significantly higher than in dizygotic twins (2.3%) and in non-twin siblings (1.9%) among 5,463 patients across Canada (Ebers, Bulman et al. 1986). After a 7.5 years of following up, the concordance rate in monozygotic rate increased from 25.9 to 30.8% and the dizygotic concordance from 2.4 to 4.7% (Sadovnick, Armstrong et al. 1993). From another point of view, the high discordance of monozygotic twins implied that nonheritable factors such as environmental effects are also important for MS susceptibility (Sadovnick, Armstrong et al. 1993). In addition, studies of genome-wide association study (GWAS) (International Multiple Sclerosis Genetics, Wellcome Trust Case Control et al. 2011) and functional genomics (Disanto, Kjetil Sandve et al. 2014) found that genomic regions associated with MS are particularly active in immune cells such as Th1, Th17, and CD8 cytotoxic T cells, CD19+ B cells and natural killer (NK) cells.

1.3.1.2 Environmental factors

Environmental influence plays a role not only in host susceptibility, but also in symptom exacerbation and disease progression.

Vitamin D

The vitamin D deficiency and less sunshine exposure during early childhood were both suspected to be related to the MS occurrence (Munger, Zhang et al. 2004, Islam, Gauderman et al. 2007, Lucas, Ponsonby et al. 2011). A latitude gradient was associated with prevalence in MS (Simpson, Blizzard et al. 2011).

Infection

Infection is a common trigger of the disease exacerbation. For example, Epstein–Barr virus (EBV) infection seems always to precede disease occurrence of MS with serological evidence (Ascherio and Munger 2007, Levin, Munger et al. 2010). In addition, subjective infections (i.e. symptoms related to the respiratory tract, gastrointestinal system, or urinary tract) relate to disease relapse (Buljevac, Flach et al. 2002). Furthermore, Human Endogenous Retrovirus (HERVs), which originate from exogenous infectious retroviruses and then integrate into the human genome, have been thought as a risk factor for developing MS and for disease progression as HERVs genome expression were more commonly detected in MS patients than controls (Morandi, Tanasescu et al. 2017).

Other

The lifestyle, including tobacco smoking, is associated both with disease susceptibility and with disease progression (Manouchehrinia, Tench et al. 2013).

1.3.1.3 Interaction between genetics and environment

These complex interactions between genetics and environment factors are supported by a cohort study comprising 302 children with a first inflammatory demyelinating episode, which found that children with all three risk factors (low serum 25-hydroxyvitamin D concentrations, remote Epstein-Barr-virus infection, HLA-DRB1*15 alleles) were more likely to have a diagnosis of MS eventually than those with no or only one risk factor (Banwell, Bar-Or et al. 2011).

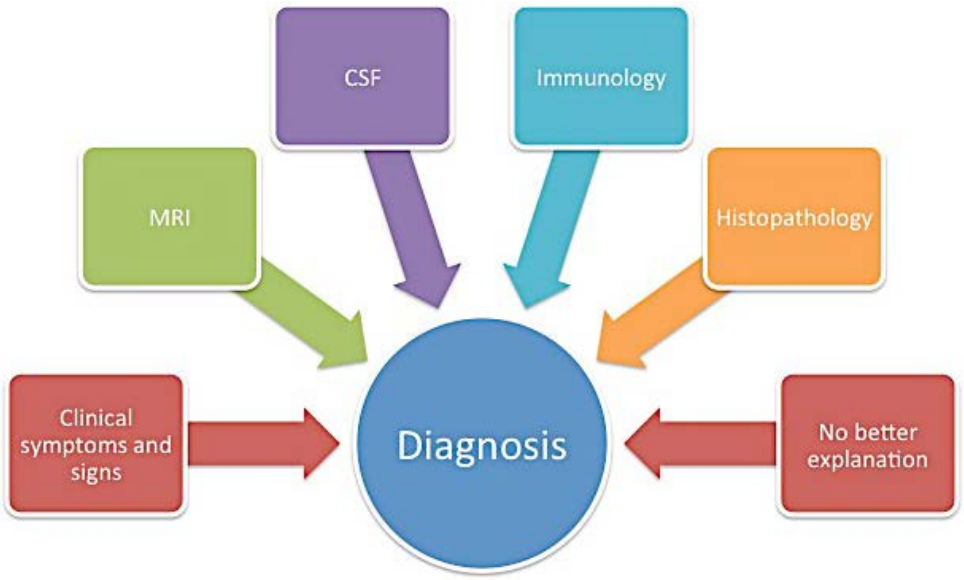
1.3.2 Genetic and environmental factors for NMO/NMOSD

The pathogenesis of NMO/NMOSD is still elusive. A small proportion (about 3%) of patients are familial cases with more tendency of maternal-sibling than paternal-sibling pairs (Matiello, Kim et al. 2010). The exposure to tobacco smoking, EBV infection, or presence of HLA-DR15 gene (specific to MS) seems not associated with disease susceptibility (Graves, Grandhe et al. 2014, Simon, Schmidt et al. 2015).

1.4 CHARACTERISTICS OF CNS INFLAMMATORY SYNDROME

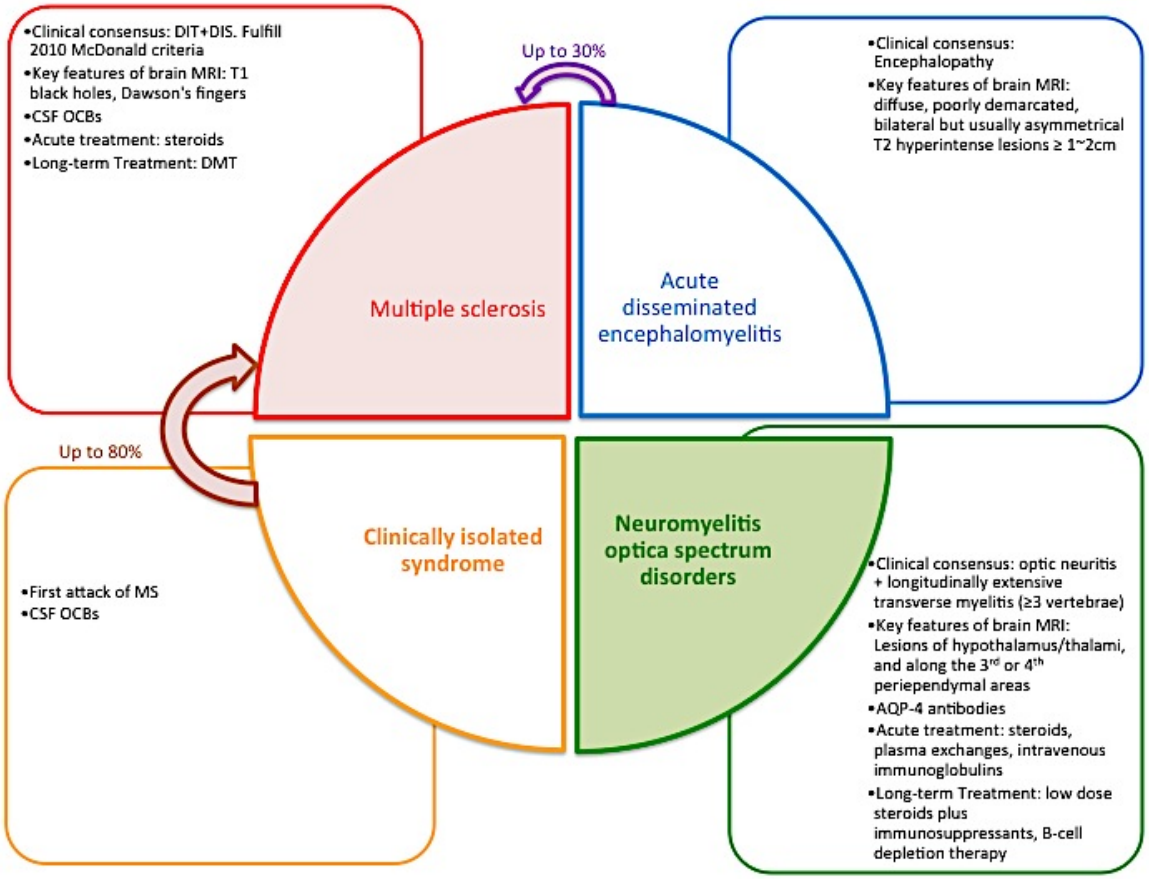
The diagnoses of MS, CIS, NMO/NMOSD, and ADEM are based on clinical symptoms and signs, neuroimaging features, laboratory characteristics, clinical course, and by excluding other possible disease entities such as tumour, infection, or vasculitis (Figure 1-5). There are several characteristics to distinguish entities of CNS inflammatory syndromes and are summarized in Figure 1-6.

Figure 1-5 Diagnosis of CNS Inflammatory syndromes.



Clinical, para-clinical and neuroimaging information that can help differentiate CNS inflammatory syndromes. Most importantly it should have no better explanation by excluding of other possibilities.

Figure 1-6 Clinical characteristics of multiple sclerosis (MS), neuromyelitis optica spectrum disorders (NMOSD), clinically isolated syndrome (CIS) and acute disseminated encephalomyelitis (ADEM).



The cardinal feature of MS is dissemination in time (DIT) and in space (DIS), usually the presence of cerebrospinal fluid (CSF) oligoclonal bands (OCBs), and needs long-term disease-modifying therapy (DMT). The clinically isolated syndrome (CIS) is presumed to be the first attack of MS, and about 80% will convert to MS in 2 decades. The feature of NMOSD is optic neuritis and longitudinally extensive transverse myelitis simultaneously or subsequently, the presence of autoantibodies to aquaporin 4 (AQP-4) proteins, and needs long-term immunosuppressants. Adapted from (Chou, Wang et al. 2016)

1.4.1 CIS/MS

CIS refers to the first clinical CNS demyelinating event. It may convert later to MS at the time of a next attack. CIS can have variable presentations, involving the optic nerves, brain stem, cerebellum, spinal cord, and cerebral hemisphere (Swanton, Rovira et al. 2007, Miller, Chard et al. 2012). In 2006, the International Advisory Committee on Clinical Trials in MS of the US National MS Society proposed 5 types of CIS (Miller, Weinshenker et al. 2008) based on clinical presentations and MRI findings (mostly brain images):

Type 1 CIS: clinically monofocal, at least one asymptomatic MRI lesion

Type 2 CIS: clinically multifocal, at least one asymptomatic MRI lesion

Type 3 CIS: clinically monofocal, MRI may appear normal; no asymptomatic MRI lesions

Type 4 CIS: clinically multifocal, MRI may appear normal; no asymptomatic MRI lesions

Type 5 CIS: no clinical presentation to suggest demyelinating disease, but MRI is suggestive

Type 5 CIS is also named radiologically isolated syndrome (RIS). RIS is a more complicated situation, and is not considered an MS subtype per se because brain MRI findings alone without clinical manifestation may be nonspecific (Lublin, Reingold et al. 2014).

In general, a second episode of idiopathic inflammatory demyelination in the CNS with a clear dissemination in time and space in a CIS patient indicates the diagnosis of MS. The evolution rate from CIS to MS can be as high as 80% up to 15 years (Fisniku, Brex et al. 2008, Miller, Chard et al. 2012).

MS affects more than one part of the CNS (dissemination in space) and occurring on more than one occasion (dissemination in time) (Poser, Paty et al. 1983, Polman, Reingold et al. 2011). The diagnosis of MS requires both clinical and paraclinical assessments to document dissemination of lesions in space as well as time and to exclude alternative diagnoses (Poser, Paty et al. 1983). The

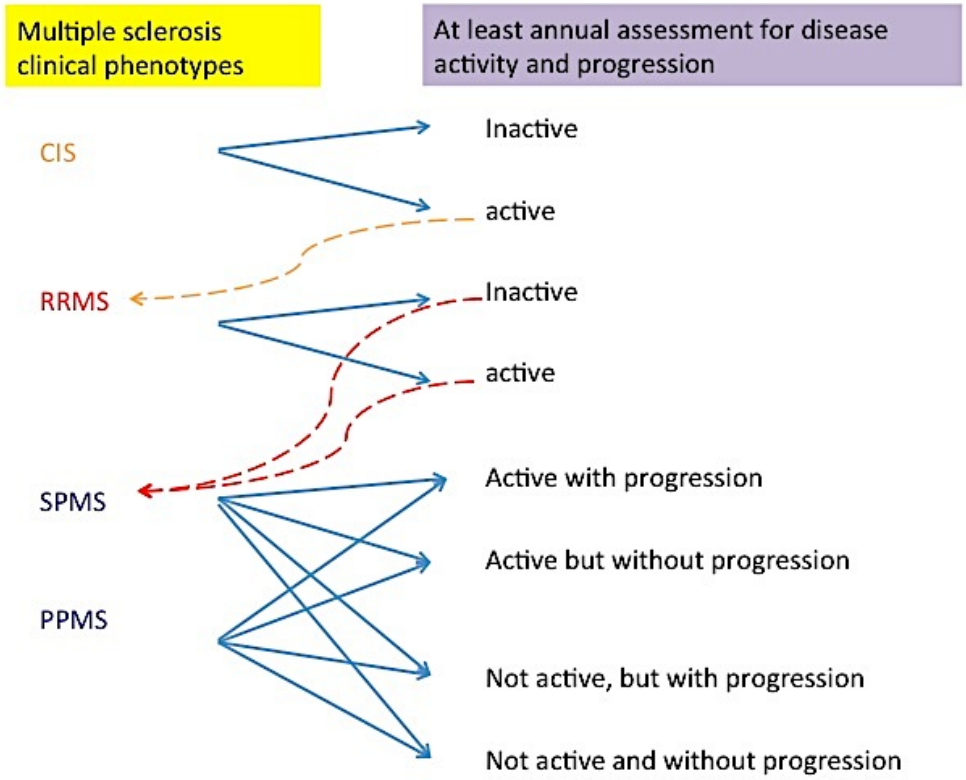
diagnosis can be made solely based on clinical presentations and neurological examinations. However, supplementary MRI evidence gives greater diagnostic certainty and earlier diagnosis of MS (McDonald, Compston et al. 2001). In 2005, the modified McDonald criteria for MS were proposed to demonstrate dissemination of lesions over time, to clarify the role of spinal cord lesions and therefore to simplify and speed the diagnosis of MS (Polman, Reingold et al. 2005).

A revision of the McDonald criteria in 2010 has been proposed by the International Panel on Diagnosis of MS to further simplify the use of imaging for demonstration of dissemination of CNS lesions in space and time (Polman, Reingold et al. 2011). The dissemination in space is defined as T2 lesions in at least 2 of 4 MS-typical regions (periventricular, juxtacortical, infratentorial areas, or spinal cord). The dissemination in time can be demonstrated by the simultaneous presence of asymptomatic lesions with or without gadolinium-enhances at any time or a new T2 and/or gadolinium-enhancing lesion on follow-up MRI. The diagnosis can be designated as "MS", "possible MS" or "not MS". The specifics of the criteria were shown in Table 1-2. In patients with CIS who are 12 years and older, if the 2010 McDonald MRI criteria (Polman, Reingold et al. 2011) are fulfilled with MRI evidence of dissemination in time and in space facilitate diagnoses for MS before the second clinical attack (Swanton, Rovira et al. 2007). For children under 12 years old, two clinical events are required for the definite diagnosis of MS. Table 1-3 summarises the diagnostic requirements for paediatric MS. Figure 1-7 shows the updated consensus on disease courses of MS phenotypes includes clinical isolated syndrome (CIS), relapsing-remitting (RRMS), primary progressive MS (PPMS), and secondary progressive (SPMS) (Lublin, Reingold et al. 2014).

After the first attack suggestive of MS or CIS, more than 85% of adult-onset patients experience a relapsing-remitting course (i.e. RRMS); 10% have a primary progressive onset with gradual worsening (i.e. PPMS) of function (Thompson, Polman et al. 1997). In contrast to heterogeneous clinical presentations of clinical subtypes in adult MS, more than 98% of paediatric-onset MS

patients have a RRMS course (Duquette, Murray et al. 1987, Ghezzi, Deplano et al. 1997, Renoux, Vukusic et al. 2007, Harding, Liang et al. 2013). In general, approximately two-thirds of RRMS patients eventually evolve within 2 decades to SPMS (Scalfari, Neuhaus et al. 2014), which is characterized by a progressive worsening of disability with fewer relapses.

Figure 1-7 Clinical phenotypes and courses of multiple sclerosis.



Definition of active is determined by clinical relapses in the absence of fever or infarction and/or MRI activity (gadolinium contrast-enhancing lesions; new or unequivocally enlarging T2 lesions). Progression is measured by clinical evaluation of functional disability. If assessments are not available, activity and progression are "indeterminate". Adapted from (Lublin, Reingold et al. 2014)

Table 1-2 The 2010 McDonald Diagnostic criteria for multiple sclerosis (MS) for adults and can be for paediatric patients aged 12 years and above.

Clinical presentation	Addition data needed for MS diagnosis
≥2 attacks;[a] objective clinical evidence of ≥2 lesions or objective clinical evidence of 1 lesion with reasonable historical evidence of a prior attack[b]	None
≥2 attacks[a]; objective clinical evidence of 1 lesion	Dissemination in space, demonstrated by: ≥1 T2 lesion in at least 2 of 4 MS-typical regions of the CNS (periventricular, juxtacortical, infratentorial, or spinal cord)[d]; Await a further clinical attack[a] implicating a different CNS site
1 attack[a]; objective clinical evidence of ≥2 lesions	Dissemination in time, demonstrated by: Simultaneous presence of asymptomatic gadolinium-enhancing and nonenhancing lesions at any time; A new T2 and/or gadolinium-enhancing lesion(s) on follow-up MRI, irrespective of its timing with reference to a baseline scan; Await a second clinical attack[a]
1 attack[a]; objective clinical evidence of 1 lesion (clinically isolated syndrome)	For dissemination in space, demonstrated by: ≥1 T2 lesion in at least 2 of 4 MS-typical regions of the CNS (periventricular, juxtacortical, infratentorial, or spinal cord)[d]; Await a second clinical attack[a] implicating a different CNS site; For dissemination in time, demonstrated by: Simultaneous presence of asymptomatic gadolinium-enhancing and nonenhancing lesions at any time; A new T2 and/or gadolinium-enhancing lesion(s) on follow-up MRI, irrespective of its timing with reference to a baseline scan; Await a second clinical attack[a]
primary progressive multiple sclerosis (PPMS): Insidious neurological progression suggestive of MS	1 year of disease progression (retrospectively or prospectively determined) plus 2 of 3 of the following criteria[d]: Evidence for dissemination in space in the brain based on≥1 T2 lesions in the MS-characteristic (periventricular, juxtacortical, or infratentorial) regions Evidence for dissemination in space in the spinal cord based on ≥2 T2 lesions in the cord Positive CSF (isoelectric focusing evidence of oligoclonal bands and/or elevated IgG index)

a An attack (relapse; exacerbation) is defined as patient-reported or objectively observed events typical of an acute inflammatory demyelinating event in the CNS, current or historical, with duration of at least 24 hours, in the absence of fever or infection. It should be documented by contemporaneous neurological examination, but some historical events with symptoms and evolution characteristic for MS, but for which no objective neurological findings are documented, can provide reasonable evidence of a prior demyelinating event. Reports of paroxysmal symptoms (historical or current) should, consist of multiple episodes occurring over not less than 24 hours. Before a definite diagnosis of MS can be made, at least 1 attack must be corroborated by findings on neurological examination, visual evoked potential response in patients reporting prior visual disturbance, or MRI consistent with demyelination in the area of the CNS implicated in the historical report of neurological symptoms. b Clinical diagnosis based on objective clinical findings for 2 attacks is most secure. Reasonable historical evidence for 1 past attack, in the absence of documented objective neurological findings, can include historical events with symptoms and evolution characteristics for a prior inflammatory demyelinating event; At least 1 attack must be supported by objective findings. c No additional tests are required. However, it is desirable that any diagnosis of MS be made with access to imaging based on these Criteria. If imaging or other tests (for instance, CSF) are undertaken and are negative, extreme caution needs to be taken before making a diagnosis of MS, and alternative diagnoses must be considered. There must be no better explanation for the clinical presentation, and objective evidence must be present to support a diagnosis of MS. d Gadolinium-enhancing lesions are not required; symptomatic

lesions are excluded from consideration in subjects with brainstem or spinal cord syndromes. MS = multiple sclerosis; CNS = central nervous system; MRI = magnetic resonance imaging; CSF =cerebrospinal fluid; IgG= immunoglobulin G. References: The 2010 McDonald Diagnostic criteria for multiple sclerosis (MS) for adults (Polman, Reingold et al. 2011) and paediatric patients aged 12 years and above. (Krupp, Tardieu et al. 2013)

Table 1-3 Diagnostic consensus for paediatric multiple sclerosis.

The 1st clinical CNS event	**Patient age**	**Number of clinical CNS events with presumed inflammatory demyelination**	**No. of MRI scans**	**Limited time gap to have the 2nd clinical CNS event**	**2nd clinical CNS event requirement**	**Other MRI requirements**		
						Dissemination in Space (DIS)*	Dissemination in Time (DIT)*	Note
CIS (Non-encephalopathic episode)	≥ 12 y	≥ 1	≥ 1	n.a.	n.a.	Yes	Yes	
	< 12 y	≥ 1	≥ 2	n.a.	n.a.	Yes	Yes	2nd MRI scan showed ≥ 1 new lesion
	< 18 y	≥ 2	≥ 1	> 30 days	Non-encephalopathic episode	Yes		Involving ≥2 areas of the CNS
ADEM (Encephalopathic episode)	< 18 y	≥ 2	≥ 2	≥ 3 months	Non-encephalopathic episode	Yes		MRI scan of event 2 showed new lesions fulfilled DIS

Each row constitutes a separate case definition for MS. n.a., not applicable
Adapted from (Krupp, Tardieu et al. 2013, Chou, Whitehouse et al. 2014)

1.4.1.1 Typical neuroimaging findings for MS

The early disease diagnosis is based on topographic distributions and morphological findings of T2 hyperintense lesions in MRI, which represent inflammatory changes. The diagnosis can be made if ≥ 1 T2 lesion in at least 2 of 4 MS-typical regions of the CNS (periventricular, juxtacortical, infratentorial, or spinal cord) (Figure 1-8). The MRI features of spinal cord lesions in MS patients are single or multiple focal, sharply delineated, T2 hyperintense lesions extending one vertebra (maximum two segments) in length (Bot, Barkhof et al. 2004, Patrucco, Rojas et al. 2012). Two morphological findings of brain MRI are characteristic in MS. One is the presence an ovoid lesion perpendicular to long axis of the corpus callosum, which corresponds to previous histopathological findings of "Dawson's finger" (Graves, Grandhe et al. 2014) (Figure 1-9). The other is the presence of "black holes", which were defined as non-enhancing hypointensity on T1 and hyperintensity on T2 imaging lasting for more than three months (Figure 1-10). Black holes suggest tissues of long-standing destruction.

During the course of disease, MRI is important to monitor disease activity or evident subclinical inflammation. The inflammatory activity can be assessed by the appearance of contrast-enhancing lesions, indicating the breakdown of the BBB. To evaluate disease progression, it can be estimated with the cumulative number or volume of T2 lesions or the extent of brain atrophy. In addition, changes in NAWM can be characterised by using MRI techniques to estimate the myelin density, tissue integrity, or axonal density.

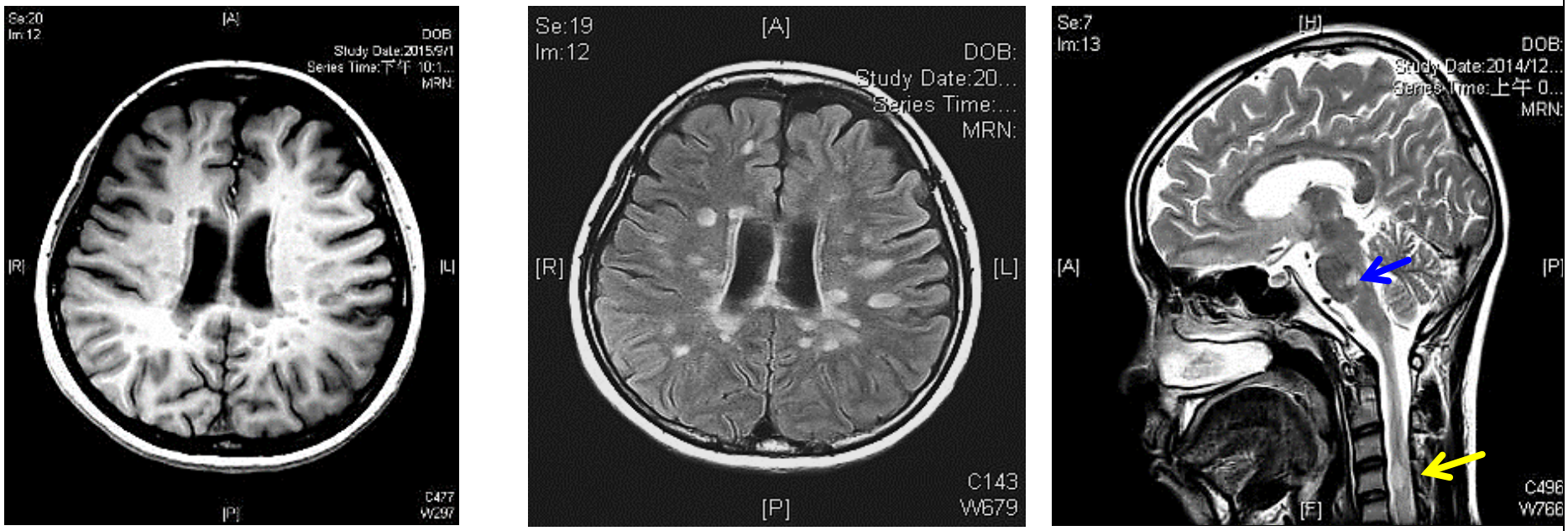
1.4.1.2 Laboratory tests for MS

Cerebrospinal fluid (CSF) is obtained for two main purposes in MS: (1) to confirm the diagnosis of MS. CSF findings supporting MS include an elevated immunoglobulin G (IgG) index; and/or at least two oligoclonal bands (OCBs; each band represents a subtype of immunoglobulins) in the CSF but not in the corresponding serum samples on isoelectric focusing (IEF) (Freedman, Thompson et al. 2005, Link and Huang 2006). Both biomarkers indicate inflammation inside the

CSF but not in the sera. Conversely, a negative OCB test often prompts the consideration of other diseases mimicking MS or CIS (2) to rule out other treatable diseases e.g. infection, systemic inflammatory disease, or tumours. However, OCBs do not always occur in MS (positive in 85-90%), and can present in ADEM (positive in up to 29%) (Dale, de Sousa et al. 2000, Hung, Wang et al. 2012), and in NMO/NMOSD (positive in up to 30%) (de Seze, Lebrun et al. 2003). In addition, if intrathecal IgG synthesis was detected, it tends to disappear in the ADEM and NMO patients while it is persistently detectable in MS patients.

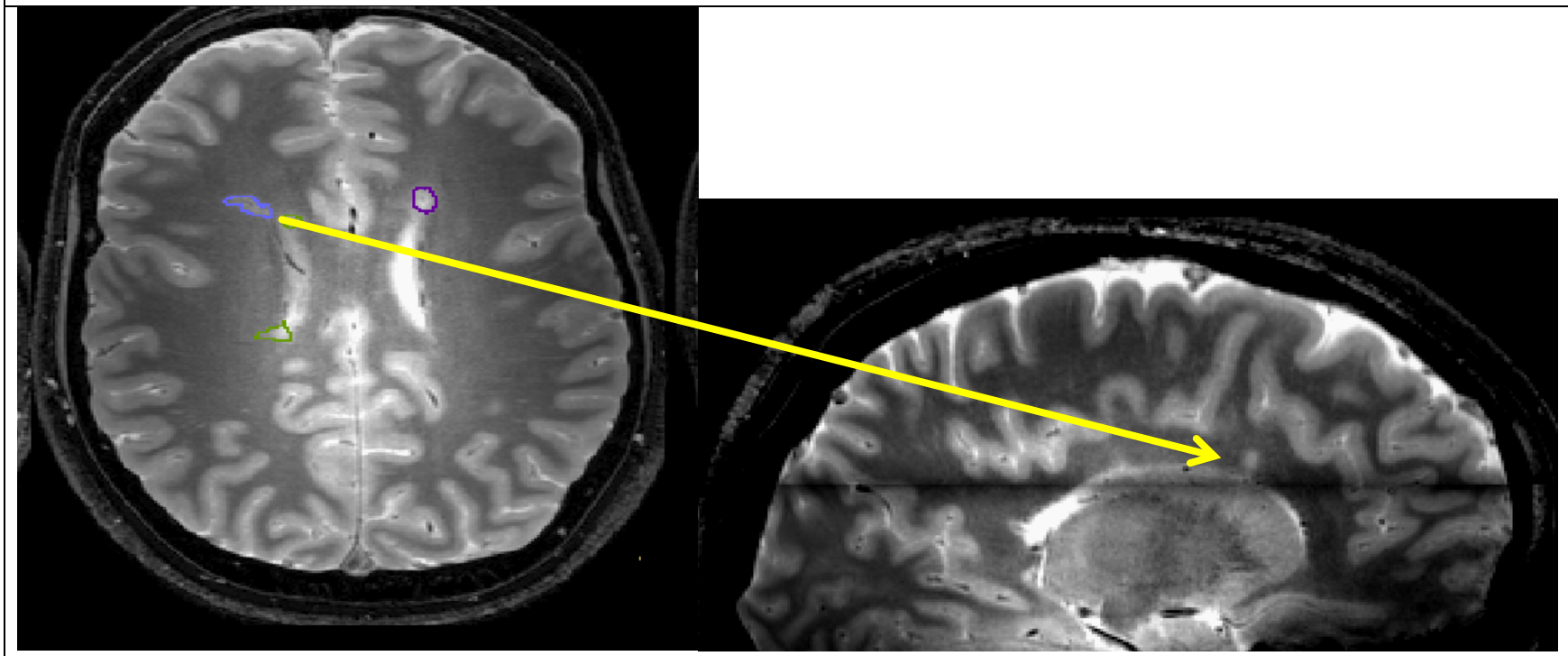
Figure 1-8 Topographic MRI findings of MS.

(a) (b) (c)



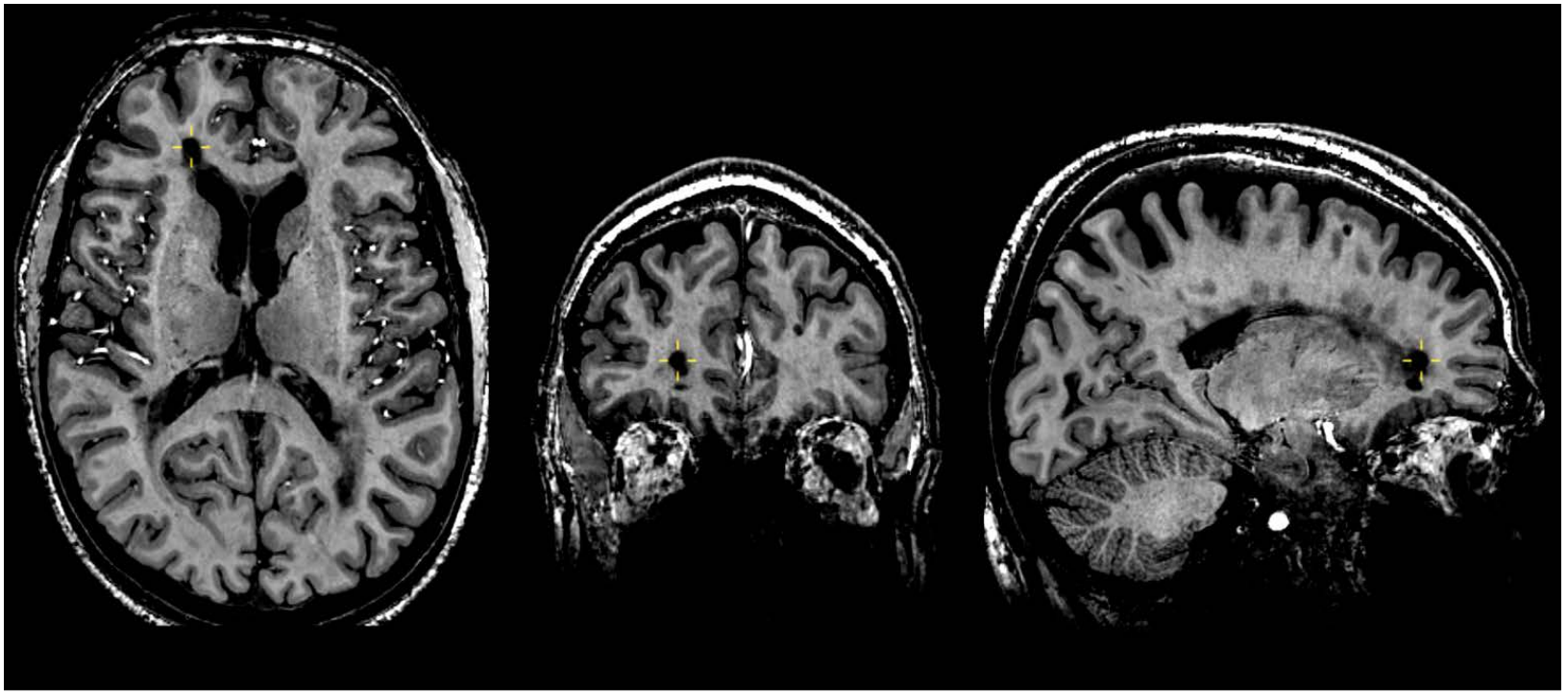
Multiple white matter lesions with T1 hypointense (a) and T2 FLAIR (b, c) hyperintense over periventricular, deep white matter, juxtacortical, infratentorium (figure c, blue arrow showed a plague in the pons), and spinal cord regions in a 16-year-old girl with multiple sclerosis presenting with ataxia. The length of spinal cord lesion (c, yellow arrow) is not longer than 2 vertebral segments. (1.5 Tesla, clinical MRI) (Personal collection from a patient in Chang Gung Memorial Hospital, Taiwan)

Figure 1-9 Typical morphological lesions in the corpus callosum in a MS patient.



The 'Dawson's finger', an ovoid lesion perpendicular to long axis of the corpus callosum. It localizes at the periventricular area, abutting any portion of the lateral ventricles. (Magnetisation transfer imaging, 7 Tesla, research MRI, voxel size 0.6 mmx0.6 mmx 0.6 mm) (Imaging from a patient volunteer of this thesis with permission)

Figure 1-10 Typical neuroimages of MS – black holes.



The image shows the so-called 'black-holes' (persistent hypointense lesion for at least for 3 months on T1 weighted images) in a middle-aged man with secondary progressive multiple sclerosis. (T1 MPRAGE imaging, 7 Tesla, research MRI, voxel size 0.6 mmx0.6 mmx 0.6 mm) (Imaging from a patient volunteer of this thesis with permission)

1.4.2 NMO/NMOSD

Classic NMO was defined by inflammatory demyelination of optic neuritis and spinal cord (i.e. transverse myelitis) simultaneously. In 1999, Wingerchuk et al. proposed the clinical criteria for NMO as monophasic and relapsing NMO (accounting for about 90%), which can be simultaneous or subsequent occurrence of optic neuritis and transverse myelitis (Wingerchuk, Hogancamp et al. 1999). About 1%– 2% of patients have a primary progressive course without any acute episode (Wingerchuk, Pittock et al. 2007). Later, neuromyelitis optica immunoglobulin G (NMO-IgG) was discovered in the serum of 78 patients with clinically defined NMO (Lennon, Wingerchuk et al. 2004) and its target antigen was identified as the astrocyte water channel protein, aquaporin protein 4 (AQP4) (Lennon, Kryzer et al. 2005).

Recently, the International Panel for NMO Diagnosis (IPND) has reached a consensus on the new nomenclature of NMO related diseases, with a unified term "NMO spectrum disorders" (NMOSD) to represent NMO with or without AQP4-IgG (Wingerchuk, Banwell et al. 2015). More than half patients with NMOSD is AQP4-IgG positive if the test is available. To diagnose NMOSD, patients with AQP4-IgG positive have to present with one of the core clinical and/or MRI findings involving optic nerves, spinal cord, area postrema syndrome (an episode of unexplained hiccups or nausea and vomiting lasting for at least 48 hours), brainstem, diencephalic and typical symptomatic cerebral lesions (Wingerchuk, Banwell et al. 2015). Table 1-4 summarises the diagnostic criteria for NMOSD. For paediatric population, the consensus is applied (Tenembaum, Chitnis et al. 2016).

Table 1-4 Diagnostic consensus for neuromyelitis optica spectrum disorders (NMOSD).

Classification	
NMOSD with AQP4	**NMOSD without AQP4 or Unknown AQP4-IgG Status**
(Wingerchuk, Banwell et al. 2015) 1. At least 1 core clinical characteristic 2. Positive test for AQP4-IgG using best available detection method (cell-based assay strongly recommended) 3. Exclusion of alternative diagnoses	1. At least 2 core clinical characteristics resulting from 1 or more clinical attacks and satisfying all the following requirements: a) At least one of: ON, acute myelitis with LETM, or APS b) Dissemination in space (>2 different core characteristics) c) MRI requirements, if applicable (see below) 2. Negative test(s) for AQP4-IgG or testing unavailable 3. Exclusion of alternative diagnoses
Core clinical characteristics	**Supporting MRI for NMOSD without AQP4**
Most common: 1. Optic neuritis (ON) 2. Acute myelitis (TM) 3. Area postrema syndrome (APS): episode of otherwise unexplained hiccups or nausea and vomiting Less common: 4. Acute brain stem syndrome 5. Symptomatic narcolepsy or acute diencephalic clinical syndrome with NMOSD-typical diencephalic MRI lesions 6. Symptomatic cerebral syndrome with NMOSD- typical brain lesions	**1. Acute optic neuritis:** brain MRI normal or demonstrating only nonspecific white matter lesions; OR optic nerve MRI with T2-hyperintense lesion or T1- weighted gadolinium-enhancing lesion extending over >1/2 optic nerve length or involving optic chiasm **2. Acute myelitis:** spinal cord MRI showing attack-associated lesion extending >3 contiguous segments (LETM); OR >3 contiguous segments of focal cord atrophy in patients with prior history of acute myelitis **3. Area postrema syndrome:** dorsal medulla/area postrema MRI lesion **4. Acute brain stem syndrome:** peri-ependymal brain stem lesions

Abbreviation: AQP-4, aquaporin-4; APS, area postrema syndrome: IgG, immunoglobulin G; LETM, longitudinally extensive transverse myelitis lesions; MRI, magnetic resonance imaging; NMOSD, neuromyelitis optica spectrum disorders.

References: Diagnostic consensus for neuromyelitis optica spectrum disorders (NMOSD) (Wingerchuk, Banwell et al. 2015).

1.4.2.1 Typical imaging findings in NMO/NMOSD

The cardinal features of NMO/NMOSD are optic neuritis and long-segment transverse myelitis over at least 3 vertebral segments simultaneously or subsequently (Figure 1-11). Most patients have normal brain MRI at disease onset, and later develop various brain MRI lesions in up to 60% of cases. The specific locations of some MRI lesions, accompanied by certain clinical symptoms, are important clues suggesting NMO/NMOSD rather than MS. For example, the signal abnormality in NMO/NMOSD is typically contiguous throughout the periventricular and along the third or fourth ventricular periependymal tissues, and involves the hypothalamus (Figure 1-12, a, b) (Pittock, Weinshenker et al. 2006). Besides, a linear medullary or medullospinal lesion in an individual manifesting intractable hiccups and nausea lasting more than 48 hours are typical for NMO and extremely rare in MS (Figure 1-12, c) (Misu, Fujihara et al. 2005). These locations are corresponding to location of AQP4 proteins along the BBB and Blood-CSF barrier. In some cases, diffuse, poorly demarcated lesion > 1-2 cm involving mainly the cerebral white matter are revealed on MRI (as shown in Figure 1-11 which was obtained from a 3-year-old boy).

1.4.2.2 Laboratory tests in NMO/NMOSD

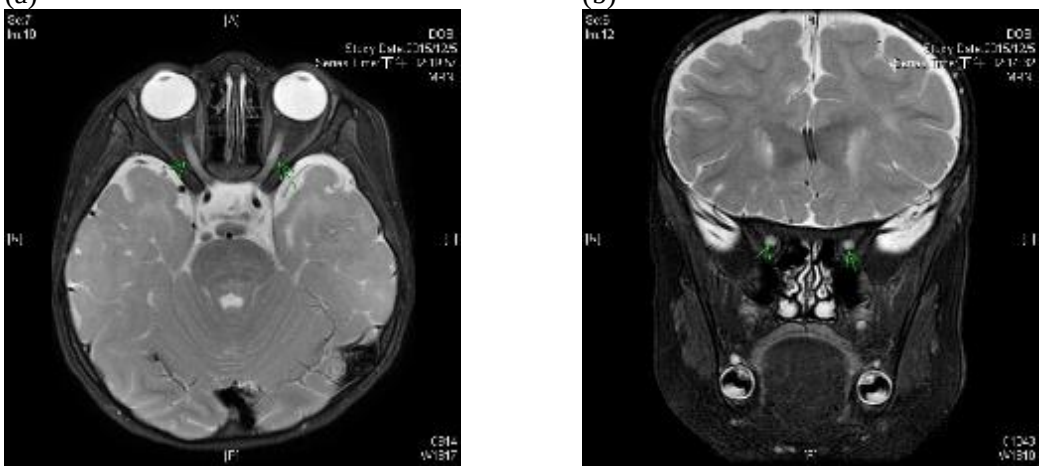
Currently, clinically relevant biomarkers include autoantibodies against AQP4 and possibly myelin oligodendrocyte glycoprotein (MOG). They may increase the confidence of disease diagnosis and facilitate decision making for long-term treatment.

The presence of Anti-AQP4 IgG antibody in the serum or CSF is one diagnostic biomarker for NMO/NMOSD. It is detectable in two-thirds of patients with clinically identified NMO/NMOSD. The seroprevalence of this antibody in other inflammatory demyelinating diseases, including MS, is very low. Approximately a quarter of AQP4 seronegative NMO/NMOSD patients have antibodies against MOG (Kitley, Waters et al. 2014, Sato, Callegaro et al. 2014), an autoantigen expressed exclusively in outer sheath of CNS myelin and oligodendrocytes (Linington, Bradl et al. 1988). MOG-Ab-positive patients are more like to present with simultaneous or sequential optic neuritis and myelitis with better recovery than AQP4-Ab-positive patients (Kitley, Waters et al.

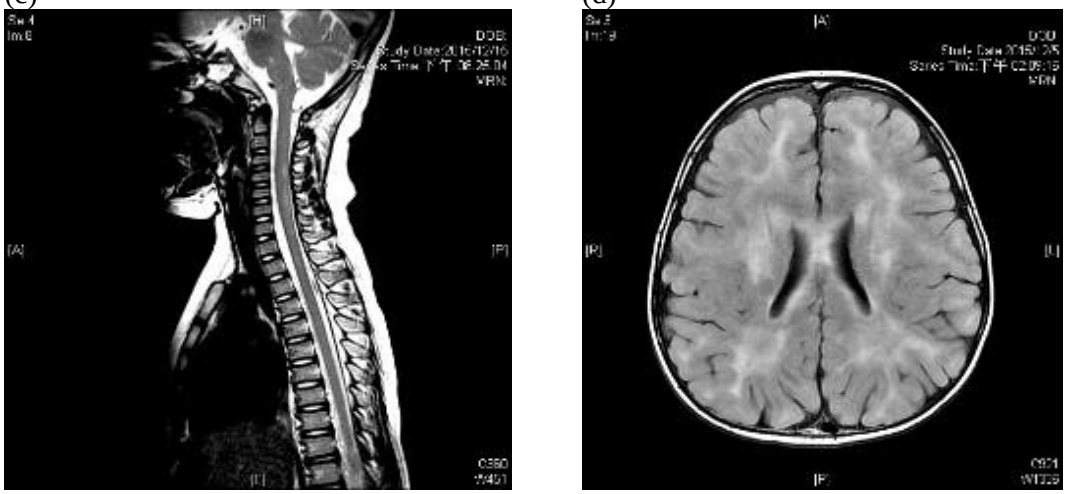
2014). Although its pathogenic role is still elusive, some evidence shows that the immunoglobulins to native MOG at high titres are capable of inducing complement activation (Mader, Gredler et al. 2011) and antibody-dependent cytotoxicity when natural killer cells are present in vitro (Brilot, Dale et al. 2009); both mechanisms contribute to CNS demyelination.

Figure 1-11 Typical neuroimages of NMO/NMOSD – patient 1.

(a) (b)



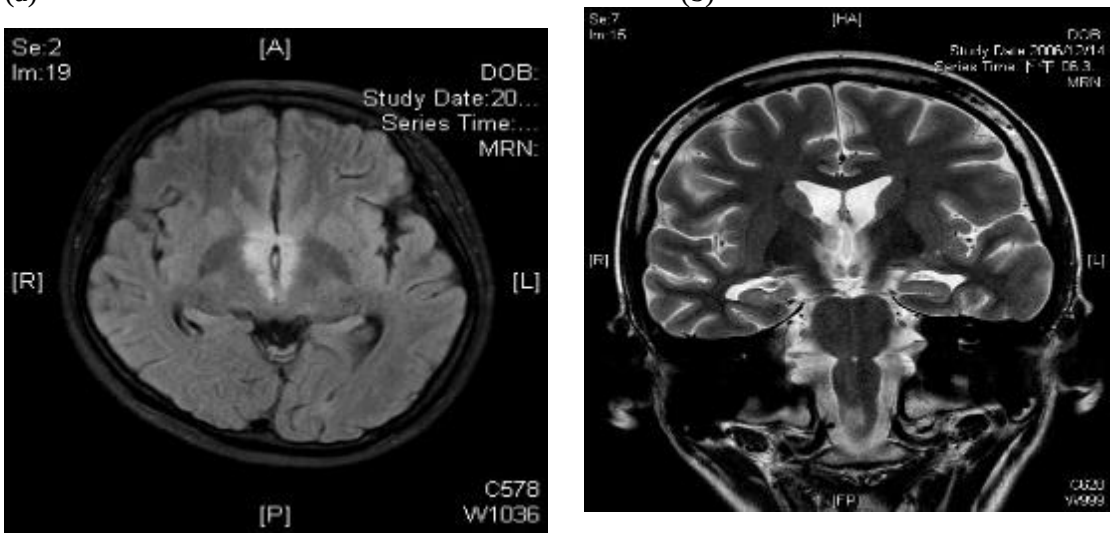
(c) (d)



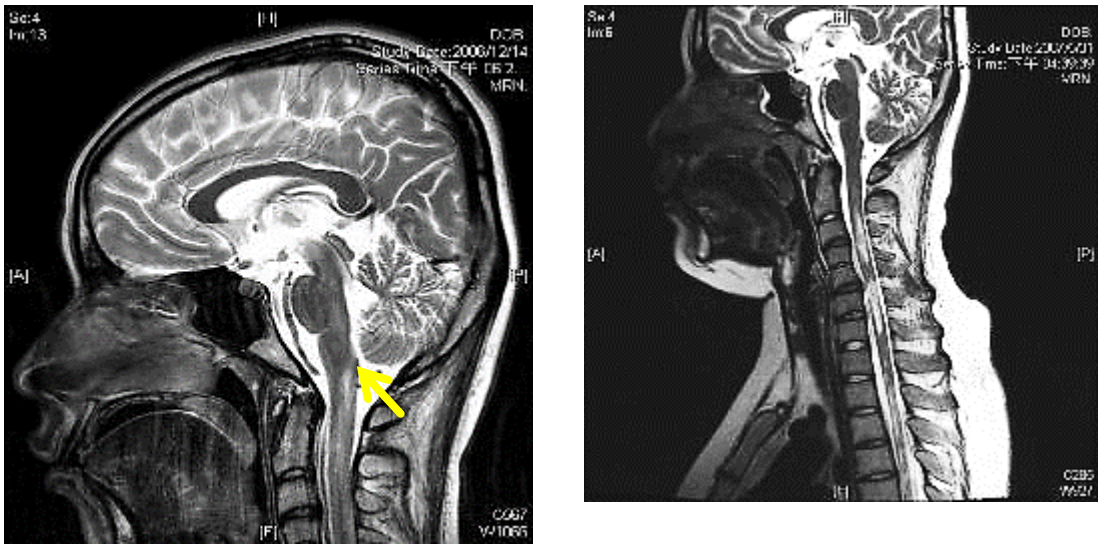
A 3-year-old boy with NMO had simultaneous bilateral optic neuritis with hyperintense in T2 imaging (a, b, green arrow) and long segmented transverse myelitis from C4-7 (c). The white matter was diffusely hyperintense in T2 FLAIR imaging (d). The serum antibodies to aquaporin 4 proteins was positive and fundus showed papilledema, the ambulation and bladder function were normal, but he complained of feet pain and tingling during sleep. (1.5 Tesla, clinical MRI) (Personal collection from a patient in Chang Gung Memorial Hospital, Taiwan)

Figure 1-12 Typical neuroimages of NMO/NMOSD – patient 2.

(a) (b)



(c) (d)



A teen girl with NMOSD, unknown AQP4 status, had T2 signal abnormality typically contiguous hyperintense signals throughout the periventricular and along the third or fourth ventricular periependymal tissues, and involves the hypothalamus (a, b, c). Prominent hyperintense signals at the upper cervical cord and pontomedullary junction, including Area postrema (c, yellow arrow). The spinal cord lesion is longer than 3 vertebral segments (d). (1.5 Tesla, clinical MRI) (Personal collection from a patient in Chang Gung Memorial Hospital, Taiwan)

1.4.3 ADEM

ADEM is usually monophasic and characterised by the presence of multifocal involvement and encephalopathy (Dale, de Sousa et al. 2000, Krupp, Banwell et al. 2007). Encephalopathy is defined as an alteration in consciousness or behavioural change unexplained by fever, systemic illness, or postictal epileptic symptoms (Krupp, Tardieu et al. 2013). Early fatal cases exhibited diffuse cerebral oedema, vascular congestion, with or without lymphocytic infiltration and petechial haemorrhages but without demyelintion (Van Bogaert 1950, Adams and Kubik 1952, Gibbons, Miller et al. 1956). The perivenous lymphocytic infiltration with or without myelin loss was a feature that can be differentiated from MS, which showed the confluent demyelination instead (Van Bogaert 1950, Young, Weinshenker et al. 2010). This group of disease was proposed to be a kind of allergic reaction with a spectrum from acute fatal to completely recovery. The differences between ADEM in children and in adults have not been fully elucidated. Table 1-5 summarises the updated consensus. Previous studies have shown an evolution rate from ADEM to MS ranging from 9% to 35%, based on different case definition, ethnicities, and durations of follow-up (Schwarz, Mohr et al. 2001, Tenembaum, Chamoles et al. 2002, Mikaeloff, Suissa et al. 2004, Liao, Huang et al. 2011). Many of these studies have tried to identify risk factors associated with the conversion to MS, but the findings are inconsistent. Currently, predicting which types of patients with the first episode of ADEM will subsequently convert to MS remains difficult.

Table 1-5 Diagnostic consensus for acute disseminated encephalomyelitis (ADEM).

Classification	Monophasic ADEM	Multiphasic ADEM
Requirement	**All of them are required** A first polyfocal clinical CNS event with presumed inflammatory demyelinating cause Encephalopathy that cannot be explained by fever No new clinical and MRI findings emerge ≥ 3 months after the onset Brain MRI is abnormal during the acute phase (< 3 month) Typical brain MRI findings: Diffuse, poorly demarcated, > 1-2 cm lesions involving mainly the cerebral white matter "Rare" T1 hypointense lesions in the white matter Deep grey matter lesions can be present	Two events consistent with ADEM attacks separated by ≥ 3 months

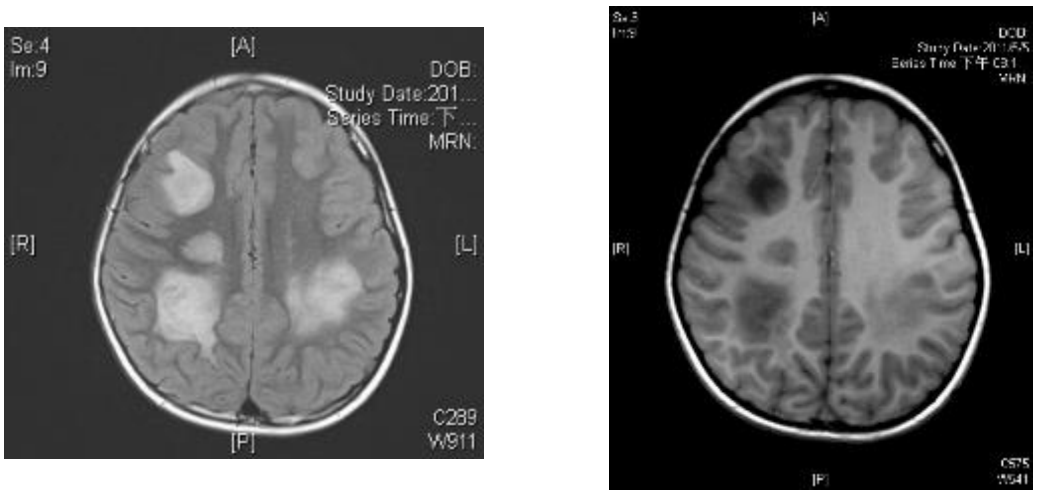
Reference: Diagnostic consensus for acute disseminated encephalomyelitis (ADEM). (Krupp, Tardieu et al. 2013)

1.4.3.1 Typical imaging/laboratory findings for ADEM

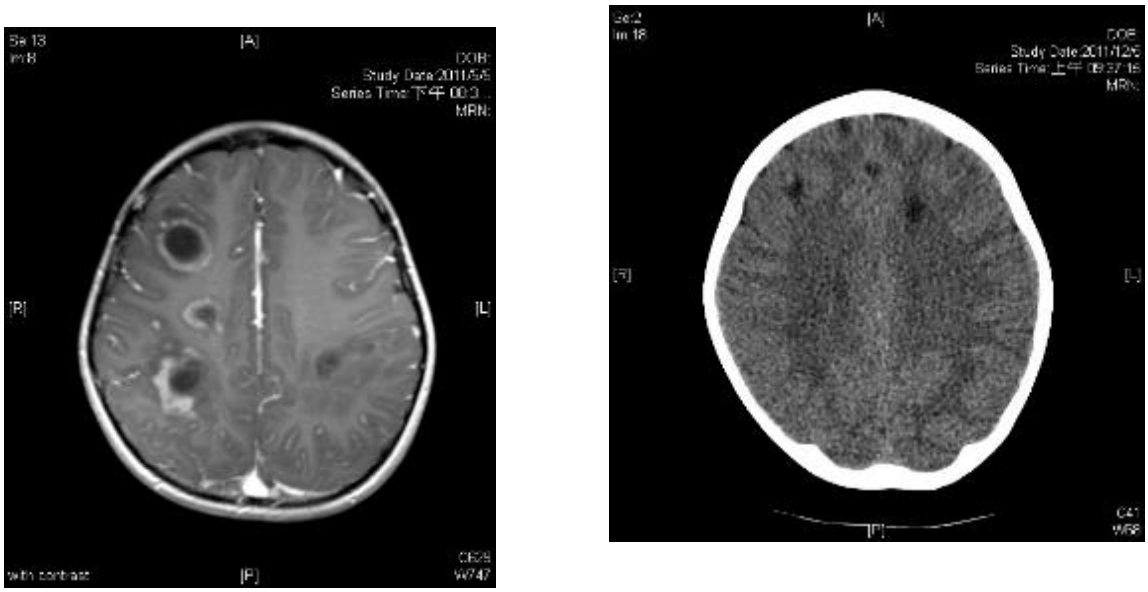
Typically, the MRI shows diffuse, poorly demarcated, bilateral, but usually asymmetrical T2 hyperintense lesions larger than one to two centimetres (Figure 1-13). Persistent T1-weighted hypointense lesions in the white matter are rare. The spinal cord lesion, if present, can be long extending over more than three contiguous segments. There has yet no specific laboratory test for ADEM.

Figure 1-13 Typical neuroimages of ADEM.

(a) (b)



(c) (d)



Diffuse, poorly demarcated, > 2 cm lesions of the cerebral white matter with hyperintense on T2 FLAIR imaging. (a), hypointense on T1-weighted imaging (b), with contrast-enhancing on post-contrast T1-weighted imaging(c) in a 5-year-old boy with ADEM. Most lesions resolved with residual encephalomalacia (hypodense lesion) over bilateral frontal white matter on the CT scan after 6 months. (1.5 Tesla, clinical MRI) (Personal collection from a patient in Chang Gung Memorial Hospital, Taiwan)

1.4.4 Treatment of MS, NMO/NMOSD and ADEM

The treatment scheme of MS generally includes the acute management and long-term prevention for relapses. During an acute disabling relapse, patients with MS are commonly treated with corticosteroids. The most commonly used regimen is intravenous methylprednisolone at a dose of 1 g/day for 3 days for adult patients and 30 mg/kg/day for 3–5 days for paediatric patients, with or without tapering according to clinical disability persistence. Oral form methylprednisolone (5 g/day for 5 days or 1 g/day for 3 days) is alternatively used in adults. For persistent life-threatening conditions such as respiratory compromise, 5–7 cycles of plasma exchange in 2 weeks has been shown to prevent mortality and decreased the disability severity (Tumani 2008, Bigi, Banwell et al. 2015). Alternative management includes intravenous immunoglobulin G (IVIG) (Elovaara, Kuusisto et al. 2011) or cyclophosphamide (La Mantia, Milanese et al. 2007, Yang, Huang et al. 2014) have been reported.

The life-long immunomodulatory agents are used in patients with MS to prevent relapses and treatment appears to be more effective when disease-modifying agents are used soon after onset of symptoms (Bates 2011). Clinical trials in CIS showed that patients who received treatment earlier in the course had more clinical benefit in terms of decreased relapse rates and new MRI lesions (Comi, Filippi et al. 2001, Bates 2011). Immunomodulators are classified into first- and second-line and there are different regulatory approvals between countries, although there is no consensus on the optimal order (Stuve and Cutter 2015). The first-line disease modifying therapies (DMTs) for MS include interferon beta-1a, interferon beta-1b, glatiramer acetate, Teriflunomide, Dimethyl fumarate (Gajofatto and Benedetti 2015). The second-line DMTs include natalizumab, fingolimod, mitoxantrone, alemtuzumab, and daclizumab (Gajofatto and Benedetti 2015) and cladribine (Giovannoni, Comi et al. 2010, Baker, Herrod et al. 2017).

The treatment scheme of NMO/NMOSD includes the acute management and life-long immunosuppressant. For debilitating or life-threatening relapses in patients with NMO/NMOSD, the intravenous methylprednisolone at a dose of 30 mg/kg/day for 3–5 days needs to be promptly

used. For steroid pulse-therapy unresponsive patients, plasma exchange (5 to 7 cycles in 2 weeks), IVIG, or rituximab are recommended to be administrated. The life-long immunosuppressive agents are typically used in patients with NMO/NMOSD to prevent relapses. After the first attack, patients with AQP4 IgG antibodies are recommended to be kept on low dose prednisolone. Other agents include oral azathioprine (Bichuetti, Lobato de Oliveira et al. 2010, Costanzi, Matiello et al. 2011, Mealy, Wingerchuk et al. 2014, Trebst, Jarius et al. 2014), oral mycophenolate (Tillema and McKeon 2012, Mealy, Wingerchuk et al. 2014), methotrexate (Kitley, Elsone et al. 2013), or B cell depletion therapy with rituximab (Jacob, Weinshenker et al. 2008, Kim, Kim et al. 2011, Tillema and McKeon 2012, Kim, Huh et al. 2013, Mealy, Wingerchuk et al. 2014). If a breakthrough relapse occurs after adequate dosage and time, steroid pulse therapy and other acute therapies will need to be used and regular rituximab is considered. Other alternative methods include periodic plasma exchanges, regular IVIG, cyclophosphamide, tacrolimus, mitoxantrone (Cabre, Olindo et al. 2013), and methotrexate (Kitley, Elsone et al. 2013).

Patients with ADEM usually need acute intravenous corticosteroid treatment, but do not need long-term medication because it is typically a single acute event (Krupp, Tardieu et al. 2013).

1.4.5 Perspectives from paediatric MS with general implications for the pathogenesis of MS

1.4.5.1 Clinical manifestations

Paediatric onset acute CNS inflammatory syndromes comprise a small proportion of all disease population. For example, 2-5% of MS patients and 4% of NNO/NMOSD patients have a paediatric onset course. Although paediatric MS and NMO/NMOSD can be diagnosed using the same diagnostic consensus criteria as adult patients, the clinical manifestations in paediatric patients are age and gender conditioned.

Firstly, MRI presentations for MS and NMO/NMOSD patients whose onset age less than 11 years old are undifferentiated from those in ADEM. For example, the spinal cord lesions longer than 3

vertebral segments can be the first presentations in ADEM, NMO/NMOSD, and rarely in paediatric MS (Pidcock, Krishnan et al. 2007, Absoud, Lim et al. 2015). Secondly, the female to male ratio increases after age 11 years since sex hormones start to take effect during puberty (Pohl 2008, Huppke, Ellenberger et al. 2014, Chitnis, Ness et al. 2016). Female sex is a well-recognised risk factor for MS. The earlier onset of menarche was associated with a higher risk of MS in women in a Canadian population-based study (Ramagopalan, Valdar et al. 2009). For patients with disease onset at or before menarche, relapses were significantly more common during the peri-menarche period than the post-menarche period (Lulu, Graves et al. 2016). Furthermore, obesity was also associated with an increased risk for MS in teenage girls but not in boys (Langer-Gould, Brara et al. 2013).

Thirdly, the positive rate of immunological markers (e.g. AQP4 antibodies in NMO/NMOSD, and OCBs in MS) is lower in patients aged younger than 11 years than those aged 12 years and above. However, there is a trend to become detectable in serial tests in the same individual. Previous studies indicated that there was a higher frequency of OCBs in CSF among paediatric MS patients with disease onset at 11 years and older (68%) than those with a younger onset age (43%; odds ratio 2.6 with a 95% confidence interval 0.8 and 8.8) (Chabas, Ness et al. 2010). Although the presence of OCBs was less common in MS patients diagnosed before puberty, many of these patients eventually had positive OCBs in CSF after serial lumbar punctures (Yeh, Chitnis et al. 2009, Chabas, Ness et al. 2010, Pfeifenbring, Bunyan et al. 2015). The exact mechanisms of the serial changes have not yet been studied.

1.4.5.2 Immunology

Epitope spreading

Epitope spreading was noted in patients with MS. Epitope spreading is a phenomenon of the development of new T-lymphocyte related or antibody-mediated autoimmune responses to new endogenous epitopes, which are possibly secondary to the release of self-antigens during

inflammatory responses (Vanderlugt and Miller 1996). In adults with RRMS, intrathecal produced autoantibodies (immunoglobulin class G) recognise autoantigens related to myelin protein peptides and the pattern of antigen recognition was heterogeneous in individual patient (Quintana, Farez et al. 2012). In children and adolescents with MS, an expansion of the initial antibody response to CNS antigens was noted at 3 months after clinical onset, but not in patients with monophasic CNS inflammatory demyelination (Quintana, Patel et al. 2014). Possibly, the intrathecal autoantibody response in MS results from the release of self-antigens during the T cell–mediated attack on the CNS.

T cells

MS is related to immune dysregulation, and T cells and B cells play important roles in disease development. Regulatory T (Treg) cells are a heterogeneous population of lymphocytes and central to the maintenance of self-tolerance and tissue homeostasis. Treg cells are dysfunctional in patients with autoimmune diseases including MS. Treg function is age conditioned. In healthy children and adolescents aged 1 to 18 years, Treg cell levels decrease with age (Arismendi, Kallas et al. 2012). Treg suppressive capacity declines with age and is highest in children aged below 12 years (Balint, Haas et al. 2013). Treg suppressive capacity is the ability to suppress inappropriate T cell responses. Treg suppressive function crucially depends on a subgroup of Treg cells, recent thymic emigrants (RTE)–Treg. The recent thymic emigrants (RTE) are the youngest peripheral T cells underwent post-thymic maturation in the peripheral lymphoid tissue (Fink and Hendricks 2011). High Treg suppressive function is associated with a high proportion of RTE-Treg (Balint, Haas et al. 2013). In children with MS, the ratios of naïve Treg and RTE-Treg to CD4+ T cells are both lower than that in healthy controls, and the ratios between both T-cells subsets are similar to those in healthy young adults (Balint, Haas et al. 2013). Therefore, premature immune senescence is present in children with MS compared to healthy children. Such premature immune senescence may reflect that a relative failure in these young patients with MS to regulate aberrant CNS-directed

autoantibody responses facing the first CNS inflammation and subsequently developing chronic autoimmunity (Viglietta, Baecher-Allan et al. 2004, Quintana, Patel et al. 2014).

B cells

The role of B cells in MS pathogenesis is intriguing. Peripheral blood B cells have two major subpopulations: naïve B cells and memory B cells. Naïve B cells produce IgM and IgD. After activation and through interaction with specific signaling molecules, they switch to memory B cells and begin producing IgG, IgA or IgE. Naïve B cells comprise more than 90% of the total peripheral leukocytes in healthy neonates and infants aged less than 6 months (Duchamp, Sterlin et al. 2014). After 6 months of age, the percentage of naïve B cells out of the total peripheral leukocytes declines with the increase in age up to 55 years (Morbach, Eichhorn et al. 2010, Duchamp, Sterlin et al. 2014, Schwarz, Balint et al. 2017). Conversely, memory B cells increase with age (Morbach, Eichhorn et al. 2010, Schwarz, Balint et al. 2017). In a recent study, Schwarz et al. found that age-dependent changes of ratio of naïve to memory B cells in the peripheral blood are lost in patients with MS aged between 12 and 55 years (Schwarz, Balint et al. 2017).

A proportion of naïve B cells, after activation, will switch to antibody producing plasmablasts and then plasma cells. Plasmablasts are short-lived immature predecessors of long-lived tissue plasma cells (Nutt, Hodgkin et al. 2015). Schwarz et al. found that there is an expansion of plasmablasts in intrathecal B cell subpopulations during acute relapses in paediatric MS (plasmablasts: plasma cells ratio = 3:2), in comparison to intrathecal B cell subpopulations of adult MS (plasmablasts : plasma cells = 2:3) (Schwarz, Balint et al. 2017).

B cells in paediatric MS are likely to serve similar function to B cells in adult MS. First, the presence of OCBs in CSF is a biomarker for MS diagnosis. Second, the epitope spreading of antibody responses (Quintana, Patel et al. 2014) in childhood MS reflects diversification of B cell repertoire in early MS (Schwarz, Balint et al. 2017). Third, B cells are the main reservoir of EBV and most children with MS have already been exposed to EBV (Banwell, Bar-Or et al.

2011). Morandi et al. reported one possible mechanism of EBV-infected B cells to the MS susceptibility is to induce autophagy and then to be linked with autoimmunity (Morandi, Jagessar et al. 2017). Although the pathogenic role of B cells has remained relatively under-explored in paediatric MS, lines of evidence suggest that B cells are likely to represent a significant role.

1.5 MRI

Neuroimaging findings of CNS inflammatory syndromes are the key to diagnosis. MRI usually demonstrates spots of hyperintensity on T2-weighted images. Topographic distributions and morphological features of the lesions are important for disease diagnosis. In addition, asymptomatic T2 hyperintensity does occur in patients with CNS inflammatory syndromes, and it is a component of MS diagnostic criteria at the first clinical attack. Furthermore, it is clinically useful for differentiating difficult cases. They are relatively non-invasive, more accessible, and may reflect biochemical or pathological mechanisms.

Currently 1.5 Tesla (T) MRI is the routine imaging modality for clinical use and clinical 3T MRI is becoming more common. With clear depiction of submillimetre anatomical details, better imaging contrast and potentially shorter scanning time than 1.5 or 3 Tesla MRI, 7T MRI may become a useful diagnostic imaging tool for brain and musculoskeletal system in the near future.

1.5.1 Safety of MRI

MRI does not use ionising radiation (e.g. X-ray) and is considered safe for the human body. In 2003, the United States Food and Drug Administration guideline stated that magnetic field up to and including 8 Tesla poses no significant risk to adults, children, and infants aged > 1 month. Over the past decade, the safety of human exposure up to 8 Tesla has been investigated looking at changes of cognitive function, body temperature, blood pressure, heart rate, and cellular information (Kangarlu, Burgess et al. 1999, Chakeres, Bornstein et al. 2003, Chakeres, Kangarlu et al. 2003, Kangarlu, Baudendistel et al. 2004, Chakeres and de Vocht 2005, Miyakoshi 2005). Miyakoshi extensively reviewed the literature regarding cellular changes exposed to various magnetic fields and concluded that there are no or extremely small effects on those tested cells (Miyakoshi 2005). For example, Hirose et al revealed no significant changes of protein expression produced by proto-oncogenes of the human promyelocytic leukaemia cell line HL-60 after exposure to 6 or 10 Tesla static magnetic field for 1 to 48 hours (Hirose, Nakahara et al. 2003). It

is of note that an increased trend of expression of a proto-oncogene, namely C-Jun, in HL-60 was noted after exposing to spatially inhomogeneous magnetic field gradient for 24, 36, 48, and 72 hours (Hirose, Nakahara et al. 2003).

Acoustic noise produced by MRI has been linked to a temporary shift in hearing thresholds, brief hearing loss (Brummett, Talbot et al. 1988) and a decrease in otoacoustic emissions even with the use of earplugs (Radomskij, Schmidt et al. 2002). To reduce potential noise-related hearing impairment, hearing protection can be done by choosing high quality earplugs and carefully visualise-assistant fitting of earplugs to achieve good attenuation (Toivonen, Paakkonen et al. 2002).

Other theoretical hazards of MRI include burns and electric shocks. This usually happens when the participants have metal objects in the body or on the skin (including a large tattoo or a dermal patch) and the limbs crossed during the exam. This only very rarely occurred and should never happen if standard safety procedures are followed. The safety procedures in our research centre include the follows: (1) A primary screen of participants for MRI is firstly performed by investigators. Participants with cardiac pacemaker, surgical clip for an aneurysm, metal objects in the body, claustrophobia and pregnancy will be excluded. (2) A second screen of participants for MRI is performed by using the MRI safety questionnaire at least 2 days before the MRI scan. (3) A third screen is performed before the participants enter the hall of 7T MRI device. A trained member of staff in the research MRI centre reminds and observes the participants to remove any movable metal objects. (4) During the scan, a rest device for legs will be placed under the participants' knees to prevent the crossing of the legs. (5) During the scan, a handheld track call button can be used whenever the participants have uncomfortable feelings or sensation and need to talk to the researcher.

1.5.2 MS lesions compared to histopathological findings

T2 weighted techniques are sensitive for detecting inflammatory demyelinating lesions. Therefore, T2 weighting has been used to monitor the burden of the disease in MS patients with and without treatment (Daumer, Neuhaus et al. 2009). T2 lesion load can also be a parameter to predict the long-term outcome and disability in MS (Fisniku, Brex et al. 2008). However, the correlations of T2 lesion load with short-term clinical disability in patients with MS are poor (Li, Held et al. 2006). This is because T2-detectable hyperintense lesions are not specific for the magnitude of oedema and inflammation, the degree of myelin loss or regeneration; and do not distinguish between acute or chronic patterns.

In a MRI-guided histopathological analysis in post-mortem MS brains, the microscopic findings of T2-detectable lesions ranged from oedema without demyelination, mild demyelination through to destroyed lesions with different extent of inflammatory cell infiltration (De Groot, Bergers et al. 2001). Other studies showed that T2-weighted imaging cannot quantify the level of axonal loss within T2 lesions (Miller, Grossman et al. 1998), and cannot differentiate demyelination from remyelination changes (Barkhof, Bruck et al. 2003). More specific modalities to capture the specific substance that was damaged in MS lesions are desirable. Magnetization transfer imaging (MTR) is one of the advanced techniques to quantify MS lesions and will be introduced in the following section.

About 10-30% of T2 hyperintense lesions are hypointense on T1-weighted images, whether acute transient lesions or persistent chronic foci (Filippi, Rocca et al. 2012). The former represents probably a consequence of oedema and demyelination with or without matrix destruction and can completely disappear as inflammation resolves (van Waesberghe, van Walderveen et al. 1998). The chronic hypointense lesions correlate with severe matrix destruction and axonal loss (De Groot, Bergers et al. 2001) and are dark on T1-weighted images and thus commonly called “black holes”. The cumulative volume of T1 hypointense lesions correlates with the severity of clinical disability,

brain atrophy, and disease progression (Truyen, van Waesberghe et al. 1996, van Walderveen, Lycklama et al. 2001).

However, the degree of T1-hypointensity is affected by different histopathological abnormalities. The strongly hypointense lesions were shown to be associated with a larger amount of extracellular fluid (Vavasour, Li et al. 2007), or greater myelin loss (Schmierer, Scaravilli et al. 2004); while partially or fully remyelinated lesions showed lesser degrees of hypointensity (Barkhof, Bruck et al. 2003). Therefore, quantitative methods may be better to describe the extent of those changes.

The appearance of Gadolinium diethylenetriamine-pentaacetic acid (Gd-DTPA) enhancement is the most important marker for active inflammation of the demyelinating lesion, and commonly appears in new lesions. Enhancing lesions generally reflect the breakdown of the blood-brain-barrier (BBB) and correlate with the extent of perivascular inflammation (Bruck, Bitsch et al. 1997). About 20% of enhancing lesions originate from a pre-existing area of chronic T2 lesions (Campbell, Sahm et al. 2012), suggesting that acute lesions may recur in previously affected areas. This finding was proven in one histopathological study, which showed that some acute lesions were centred on the same vessel (Prineas, Barnard et al. 1993). Most enhancing lesions disappear within 4 weeks, and about half of them evolve into chronic T1 hypointense lesions (van Waesberghe, van Walderveen et al. 1998).

1.5.3 Importance of normal appearing white matter

MRI defined normal appearing white matter (NAWM) in MS refers to microanatomical abnormalities in the white matter that cannot be detected by vision on conventional images but is indeed abnormal histopathologically, with ongoing inflammation, demyelination and axonal damage. The NAWM in MS is important for understanding why the MRI detectable lesions in total (the so-called lesion load) do not correlate well with the clinical dysfunction and disabilities (the so-called imaging –clinical paradox) (Barkhof and van Walderveen 1999, Li, Held et al. 2006). Despite the fact that no apparent abnormality can be detected using conventional MRI imaging,

NAWM is in fact abnormal and contributes significantly to clinical manifestations. The NAWM may be active, inflammatory, symptom-determining lesions, may be precursor areas to detectable T2 lesions (Goodkin, Rooney et al. 1998, Werring, Brassat et al. 2000), and may play a role in driving the atrophy of brain white matter in MS (Steenwijk, Daams et al. 2014). Besides, BBB disturbance was also evident in some areas of NAWM as shown by a histopathological study with abnormalities of endothelium tight junctions (Plumb, McQuaid et al. 2002) and by a MRI study with water diffusion abnormalities (Werring, Brassat et al. 2000), which can evolve to T2 detectable or contrast-enhancing lesions.

In a pathology-imaging correlative study on post-mortem tissue from MS patients, NAWM could be demonstrated having swollen axons and increased number of activated microglia or macrophages (Moll, Rietsch et al. 2011). High field MRI has been proposed to assist the assessment of NAWM. This thesis uses 7T MRI and quantitative imaging techniques to identify abnormal signals that cannot be captured by conventional MRI, to determine the extent of NAWM involvement in MS and other demyelinating inflammatory disorders.

1.5.4 Current advanced MRI techniques to quantify injury in the brain white matter lesions and normal-appearing white matter in MS

1.5.4.1 T1 relaxation time mapping

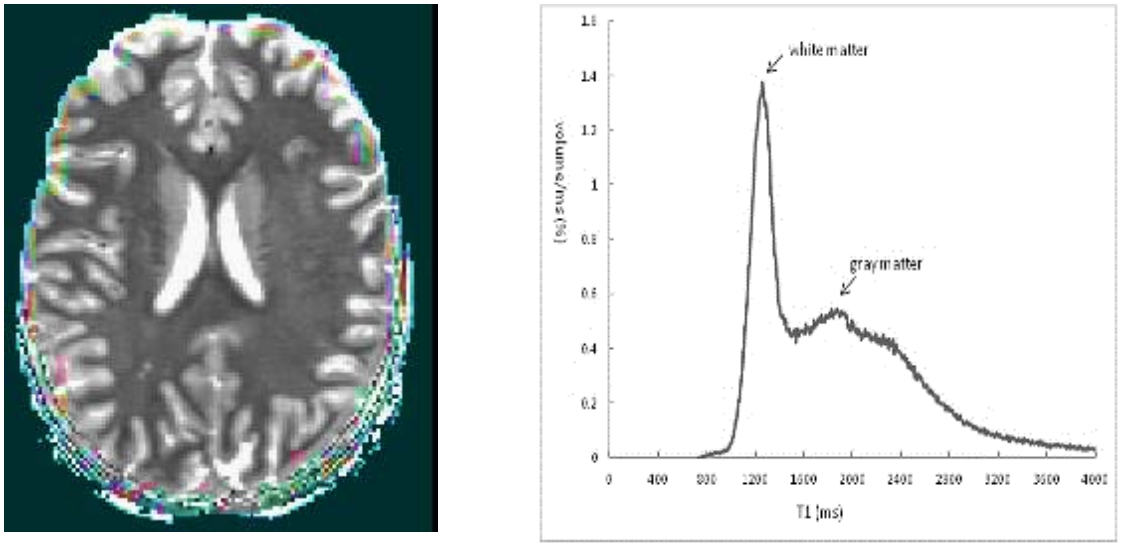
T1 value is an intrinsic biophysical property of the tissue detected by MRI. Therefore, the measurement of T1 value is important for tissue characterization. A T1 map can be used to measure T1 value from two or several T1-weighted images of the brain (Figure 1-16a). This T1 map is calculated from the ratio of each corresponding part of all T1 images. The value of an individual unit of map (pixel or voxel) represents a numerical T1 value (millisecond) rather than visual detectable signal intensity.

The histogram of T1 map, which was a result from collecting data from all voxels of the brain, is generated to give a peak for white matter and a second peak for grey matter (Figure 1-16b). In

general, T1 relaxation time is much longer (hypointensity on images) in MRI-detectable lesions of MS patients than the corresponding normal tissue from healthy controls (Ormerod, Bronstein et al. 1986). T1 relaxation time mapping can provide the quantitative information in a numeric way to differentiate very subtle lesions from normal tissues and to quantify the change of disease-related tissue.

Figure 1-14 T1 map and the histogram of whole brain derived from T1 map.

(a) (b)



(a) Whole brain T1 map was derived from seven T1-weighted images in a 40-year-old female with clinically isolated syndrome at a 7 Tesla scanner. (b) A histogram of whole brain showed information of T1 relaxation time (T1-RT) of white matter and grey matter of a 46-year-old female with relapsing-remitting multiple sclerosis. The T1-RT histogram showed peak centred on approximate 1260 milliseconds, this number is correspondent to the peak location of the white matter. The images were produced using in-house software NeuRoi. (Imaging from patient volunteers of this thesis with permission)

1.5.4.2 T1 value of NAWM in MS is different from that in healthy controls

Most studies on MS have found an increase in T1 relaxation times within NAWM compared with the normal white matter of healthy controls. These abnormalities have been proved to be diffuse in secondary progressive MS (Vrenken, Geurts et al. 2006). As the extent of T1 hypointensity was

related to both axonal loss and demyelinating activity (Bitsch, Kuhlmann et al. 2001), a measure of T1 value within NAWM has the potential to predict clinical relapse or disability in MS.

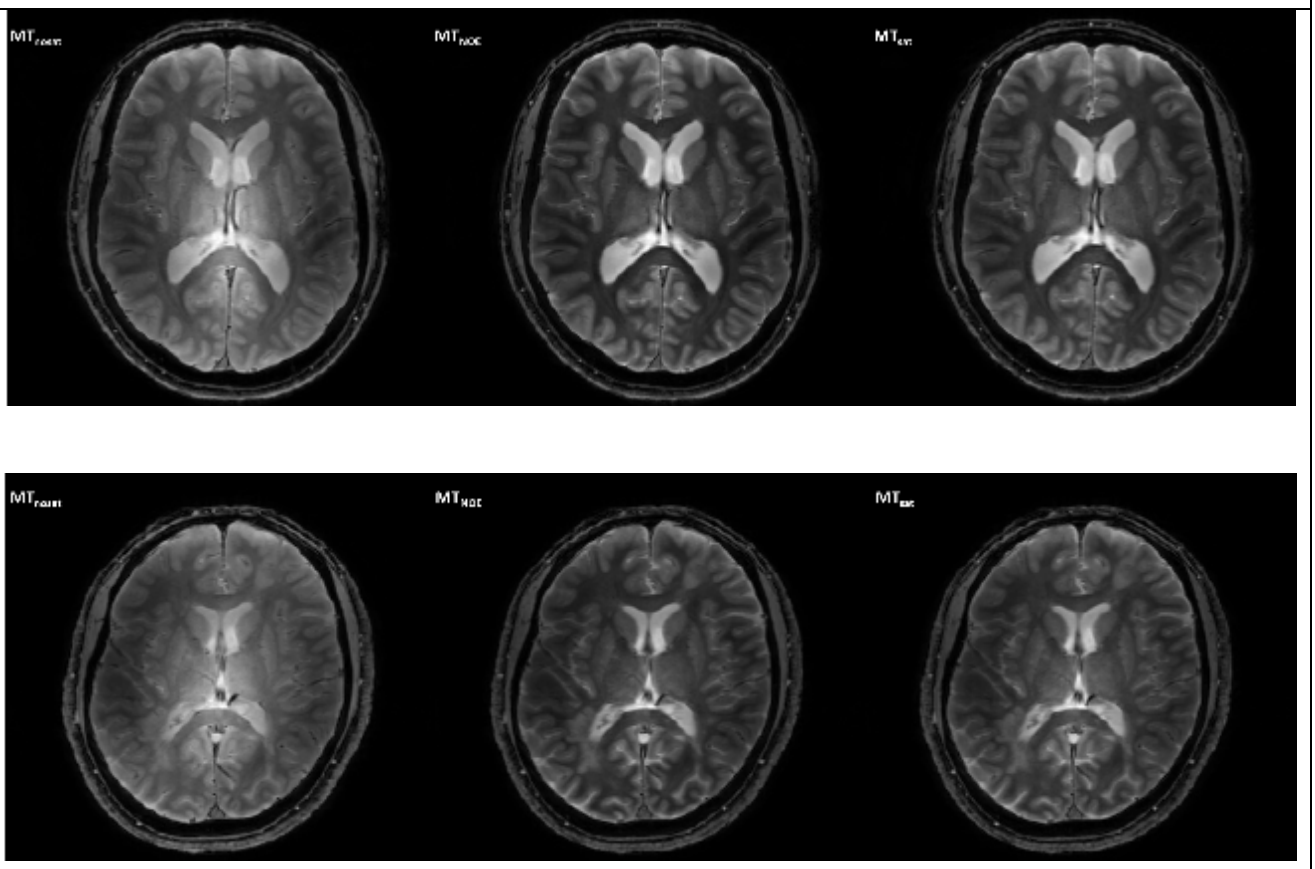
1.5.4.3 Magnetization transfer imaging (MTR)

MTR is an approach to explore ^{1}H proton of non-water components in tissue. The major non-water tissue constituents in the brain containing protons are large molecules such as myelin. Signals transmitted as a result of energy transfer between myelin-bound and water protons can be detected by MTR (Wolff and Balaban 1989). MTR maps derived pixel by pixel are obtained from Magnetisation Transfer prepared-Turbo Field Echo (MT-TFE) sequences (Figure 1-17) and further produce histograms for quantitative analysis of a region of interest. This technique provides relatively specific and quantitative information on the integrity of myelin. There is also evidence from studies of MRI–pathology correlation in MS lesions supporting the strong correlation between MTR and the degree of myelination (Schmierer, Scaravilli et al. 2004). The demyelinated tissues have severely reduced MTR. This may be a useful tool to monitor MS patients for the evolution of both demyelination and remyelination in white matter (Schmierer, Tozer et al. 2007).

Magnetization transfer ratios (MTR) have been found to be abnormal in brain NAWM in MS comparing to brain white matter in healthy controls (Table 1-6 and Table 1-7). The abnormalities of indices used in MTR were shown to be associated with myelin content and axonal density (Mottershead, Schmierer et al. 2003, Schmierer, Scaravilli et al. 2004). Furthermore, there has been a correlation between the abnormal indices of these measures and clinical disability (Vaithianathar, Tench et al. 2002, Bellmann-Strobl, Stiepani et al. 2009).

The extent of MTR decrease in NAWM of MS is more obvious at the area with the proximity to T2 white matter lesions. The MRI-pathology correlation showed the MTR decrease was associated with myelin density, axon swelling and density (Moll, Rietsch et al. 2011). It is also important to notice that brain NAWM has been shown to be abnormal in paediatric patients with MS (Absinta, Rocca et al. 2010, Oguz, Kurne et al. 2010, Tillema, Leach et al. 2012).

Figure 1-15 Magnetization transfer images at 7 Tesla MRI in a healthy subject and an MS patient.



Axial 7T 3-dimensional MR imaging examples of MTnosat, MT_{NOE} and MTsat in a healthy subject and (upper row), and in a 27-year-old male patient with MS (lower row). Magnetization transfer images were acquired using a 3-dimensional (3D) Magnetisation Transfer prepared-Turbo Field Echo (MT-TFE) sequence in 3 volumes: (Mougin, Coxon et al. 2010, Mougin, Clemence et al. 2013) (1) with no saturation pulse (MTnosat = proton imaging), (2) with -1.05 kHz off resonance to water [MT_{NOE}; signal sensitive to magnetization transfer effect and Nuclear Overhauser Effects], and (3) with off resonance saturation pulses (MTsat; signal weighted by the magnetization transfer effect). (Imaging from volunteers of this thesis with permission)

1.5.4.4 Other techniques

Diffusion tensor imaging (DTI) has been found to be abnormal in brain NAWM in adult and paediatric patients with MS (Table 1-6 and Table 1-7) (Absinta, Rocca et al. 2010, Oguz, Kurne et al. 2010, Tillema, Leach et al. 2012). Proton magnetic resonance spectroscopy (^{1}H-MRS) studies have demonstrated reduced N-acetyl aspartate (NAA, a derivative of aspartic acid) to creatine (Cr) level ratios in NAWM, suggesting neuro-axonal dysfunction and increased total creatine and myo-inositol, indicating an increased density of glia cells (Table 1-8).

The cumulative evidence has shown that NAWM in MS has significant abnormalities. It can contribute to clinical disability and future white matter atrophy. Therefore, advanced MRI techniques, with more sensitivity and pathological specificity in detecting NAWM change in MS, would usefully supplement clinical information.

Table 1-6 Comparative results of diffusion tensor imaging (DTI) of NAWM in MS, CIS, ADEM and NMO/NMOSD

Studies	Scanner	MS	CIS	ADEM	NMO/NMOSD
(Werring, Clark et al. 1999)	1.5T	abnormal			
(Filippi, Iannucci et al. 2000)	1.5T	abnormal			
(Inglese, Salvi et al. 2002)	1.5T	abnormal		(-)	
(Rocca, Agosta et al. 2004)	1.5T				(-)
(Gallo, Rovaris et al. 2005)	1.5T	abnormal			
(Vrenken, Pouwels et al. 2006)	1.5T	abnormal			
(Tortorella, Rocca et al. 2006)*	1.5T	abnormal			
(Yu, Lin et al. 2006, Yu, Lin et al. 2008, Liu, Duan et al. 2012)	1.5T				Abnormal; restricted to optic radiation and cortico-spinal tract
(Yu, Lin et al. 2008)	1.5T	abnormal	abnormal		
(Rashid, Hadjiprocopis et al. 2008)*	1.5T	abnormal			
(Dineen, Vilisaar et al. 2009)	3T	abnormal			
(Vishwas, Chitnis et al. 2010)*	1.5T	abnormal			
(Absinta, Rocca et al. 2010)*	1.5T	abnormal	(-)		
(Pichiecchio, Tavazzi et al. 2012)	1.5T				Abnormal; restricted to optic pathway including lateral geniculate nuclei
(Tillema, Leach et al. 2012)*	1.5T	abnormal	abnormal	Abnormal	
(Testaverde, Caporali et al. 2012)	3T	abnormal			
(Zhao, Zhou et al. 2012)	3T				Abnormal; medulla oblongata, genu of corpus callosum and optic radiation

*Studies include children aged under 18 years; T, Tesla; (-), normal

Table 1-7 Comparative results of magnetization transfer ratio (MTR) of NAWM in MS, CIS, ADEM and NMO/NMOSD.

Studies	Scanner	MS	CIS	ADEM	NMO/NMOSD
(Filippi, Campi et al. 1995)	1.5T	Abnormal; frontal lobe NAWM			
(Filippi, Rocca et al. 1999)	1.5T				(-)
(Brex, Leary et al. 2001)	1.5T		(-)		
(Cercignani, Bozzali et al. 2001)	1.5T	Abnormal			
(Inglese, Salvi et al. 2002)	1.5T	Abnormal		(-)	
(Davies, Ramio-Torrenta et al. 2004)	1.5T	Abnormal			
(Rocca, Agosta et al. 2004)	1.5T				(-)
(Mezzapesa, Rocca et al. 2004)*	1.5T	Abnormal			
(Tortorella, Rocca et al. 2006)*	1.5T	Abnormal			
(Pichiecchio, Tavazzi et al. 2012)	1.5T				(-)

*Studies include children aged under 18 years; T, Tesla; (-), normal

Table 1-8 Comparative results of proton magnetic resonance spectroscopy (^{1}H-MRS) of NAWM in MS, CIS and NMO/NMOSD.

Studies	Scanner	MS	CIS	NMO/NMOSD
(Tourbah, Stievenart et al. 1999)	1.5T	Abnormal; decreased NAA/Cr, NAA/Cho,	Abnormal in 27% of 14 subjects with ON; decreased NAA, increased choline, presence of free lipid peak	
(Brex, Gomez-Anson et al. 1999)	1.5T		(-)	
(Fernando, McLean et al. 2004)	1.5T		Abnormal; increased myo-inositol, increased tCr	
(Vrenken, Barkhof et al. 2005)	1.5T	Abnormal; increased tCr, normal tNAA, causing decreased NAA/Cr, increased myo-inositol		
(Bichuetti, Rivero et al. 2008)	1.5T			(-); NAA/Cr
(Oguz, Kurne et al. 2009, Oguz, Kurne et al. 2010)*	1.5T	Abnormal; increased citrulline		
(Aboul-Enein, Krssak et al. 2010)	3T			(-); NAA/Cr, Cho/Cr
(Aboul-Enein, Krssak et al. 2010)	3T	Abnormal; reduced NAA		
(de Seze, Blanc et al. 2010)	1.5T			(-); NAA/Cr, Cho/Cr, Myo-inositol
(Pichiecchio, Tavazzi et al. 2012)	1.5T			(-); NAA/Cr, Cho/Cr

*Studies include children aged under 18 years; Cho = choline; CIS = clinically isolated syndrome; Cr = creatine; MRS = ^{1}H - Magnetic Resonance Spectroscopy; MS = multiple sclerosis; NAA = N-acetyl aspartate; NMO = neuromyelitis optica; ON = optic neuritis; T = Tesla; tCr = total creatine; tNAA = total N-acetyl aspartate;(-), negative

1.5.5 NAWM in NMO/NMOSD

In NMO/NMOSD, occult changes in brain NAWM were not observed using MTR or MRS methods at 1.5T field strength (Table 1-7 and Table 1-8). Using DTI and other newer techniques to analyse selected white matter tracts, some abnormalities can be demonstrated using 1.5T and 3T MRI scanners (Table 1-6). These abnormalities were localised in optic tracts (internal capsule, lateral geniculate nuclei), corpus callosum, medulla oblongata, and corticospinal tracts (Yu, Lin et al. 2006, Yu, Lin et al. 2008, Pichiecchio, Tavazzi et al. 2012, Zhao, Zhou et al. 2012). It is of note that using 3T MRI scanners can provide more precise results than 1.5T MRI scanners can as it is better for anatomical resolution and tract identification (Zhao, Zhou et al. 2012).

There is very little information on the involvement of NAWM in NMO/NMOSD at ultra-high field MRI. This is subject to some of the studies in this thesis work.

1.5.6 NAWM in ADEM

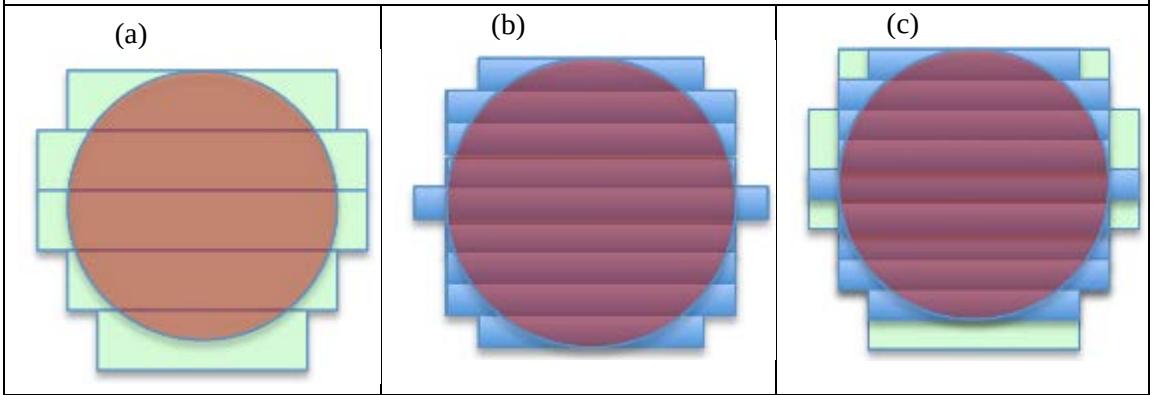
In ADEM, there were only two studies focusing on NAWM compared with healthy controls as well as MS in the literature (Table 1-6 and Table 1-7). One study demonstrated that parameters of MTR and DTI in normal-appearing brain tissue and cervical cord in patients with ADEM are not different from those in healthy controls (Inglese, Salvi et al. 2002). Another study showed that NAWM in children with combined ADEM and CIS was abnormal but the extent was less severe than MS group (Tillema, Leach et al. 2012). However, whether ultra-high field MRI can detect subtle NAWM changes that cannot be captured at lower field strengths is unknown, and is addressed in this thesis work.

1.5.7 More precise estimation of tissue volume in a shorter scanning time at 7 Tesla MRI than that at a lower magnet field strength

MRI depends on a static magnetic field to generate signals that are detectable and decipherable after proper processing. Higher magnetic field strength potentially improves signal-to-noise and

contrast-to-noise ratio, leading to images with a better spectral and spatial resolution. This is possible because the acquired images using 7T MRI can have thinner slices and a smaller pixel or voxel size in a shorter time than using conventional MRI systems (Figure 1-18). Therefore, in addition to a better imaging resolution, better volume estimation is possible.

Figure 1-16 The difference of quantitative measure of a lesion between conventional MRI and 7 Tesla MRI.



A simulation of lesion estimation of a round lesion (red) between conventional MRI (a, green) and 7 T MRI (b, blue), and the difference between the prior two estimations (c). The lesion volume estimation is conventional MRI employs a slice thickness of 1-2mm (a) while 7T MRI (b) can support a smaller slice thickness (0.5-0.6mm). Therefore, there will be discrepancy in lesion volume estimation with 7T MRI being more accurate.

1.6 GAP OF KNOWLEDGE

Overlapping features exist between MS and ADEM, between MS and NMO/NMOSD, between ADEM and NMO/NMOSD, especially in paediatric population. The uncertainty of diagnosis may prevent clinicians to provide appropriate medications to ensure a better outcome. Therefore, it is desirable to have a better understanding of each disease entity and to identify disease-specific biomarkers in adults and paediatric patients.

NAWM can be a potential target to help differentiate NMO/NMOSD or ADEM from MS for several reasons. Firstly, NAWM is known to be abnormal in progressive type MS, but to a lesser extent in relapsing remitting MS and even in CIS using conventional field strength MRI scanners. A recent quantitative MRI study of NAWM at 7T showed that abnormality can be measured in both CIS and early MS when compared to age- and sex-matched healthy controls (Al-Radaideh, Mougin et al. 2015). The precision of 7T MRI potentiates early delineation between ADEM, NMO/NMOSD, and MS at their first acute exacerbation. Using high-field MRI, it is possible to depict the lesions specifically and to quantify the abnormality in the NAWM of the brain more precisely to distinguish entities of CNS inflammatory syndromes. The NAWM in NMO/NMOSD has not been evaluated extensively at 7 T MRI. Furthermore, there is no comparative study to evaluate the histopathology to understand the MRI detected lesion and non-lesion white matter changes in NMO/NMOSD.

1.7 USE OF 7T MRI IN THIS THESIS

High field MRI can take pictures in a shorter time to allow smaller voxels of the brain to be acquired and is ideally employing precision estimation of the content of NAWM to collect more voxels and to avoid partial volume effect of tissue contamination in the normal appearing white matter. This strength enables fewer patient numbers to achieve an appropriate assessment. Practically it facilitates the study of a rare disease such as ADEM or NMO/NMOSD. One of the aims of this thesis was to identify abnormalities of NAWM in patients with MS and NMO/NMOSD using 7T MRI and quantitative imaging techniques. Despite MS being a relatively common demyelinating disease, NMO/NMOSD is a rare disease and the enrolment was expected to be difficult. Therefore, an effort was made to perform epidemiological studies, and a pre-protocol power calculation and estimation of potential case numbers using a representative primary care database.

1.7.1 Ethical approval and funding

This study was approved by the Institutional Review Board of Chang Gung Memorial Hospital in Taiwan (approval number: 97-1564C), and by Nottingham Research Ethics Committee in the UK (REC reference no. 07/H0408/115 and 13/EM/0080). This study was funded by the Chang Gung Memorial Hospital in Taiwan (Research Grant CMRPG4A0121, CMRPG4A0122, CMRPG4A0123, and CMRPG4A0124), and by the University of Nottingham in the UK (Sponsor reference: 13009).

1.7.2 Power calculation of estimation number for 7T MRI study

Because there was never a patient with NMO/NMOSD who underwent 7T quantitative MRI study by the time our study started, we tried to make a power calculation from previous MS studies using 7T MRI techniques in the Sir Peter Mansfield Magnetic Resonance Centre, University of Nottingham. According to a prior 7T MRI study into MS and healthy controls, whole brain T1 histogram between 9 relapsing-remitting MS subjects and 11 healthy controls showed the

difference of the peak location was 50 milliseconds with a standard deviation of 25.7 milliseconds. On the basis of literature which showed specific tract abnormality in normal appearing white matter at 1.5 T or 3 T, (Yu, Lin et al. 2006, Yu, Lin et al. 2008, Pichiecchio, Tavazzi et al. 2012, Zhao, Zhou et al. 2012) the expected difference between MS and NMO/NMOSD subjects was calculated to be 60% of the difference between MS subjects and healthy controls. Using 2-tailed t-test to identify the expected difference between NAWM of NMO/NMOSD and MS, 10 subjects per subgroup are required to give 85% power at the significant level of 0.05. Expecting difficulty of 7T MRI studies on NMO/NMOSD is the number of patients we can successfully recruit to having an acceptable quality of images for analysis. Some patients are known to be too disabled (e.g. bed-ridden, limb spasticity) or living from a long distance to visit the 7T suit in the University of Nottingham.

1.7.3 Feasibility of 7T MRI study

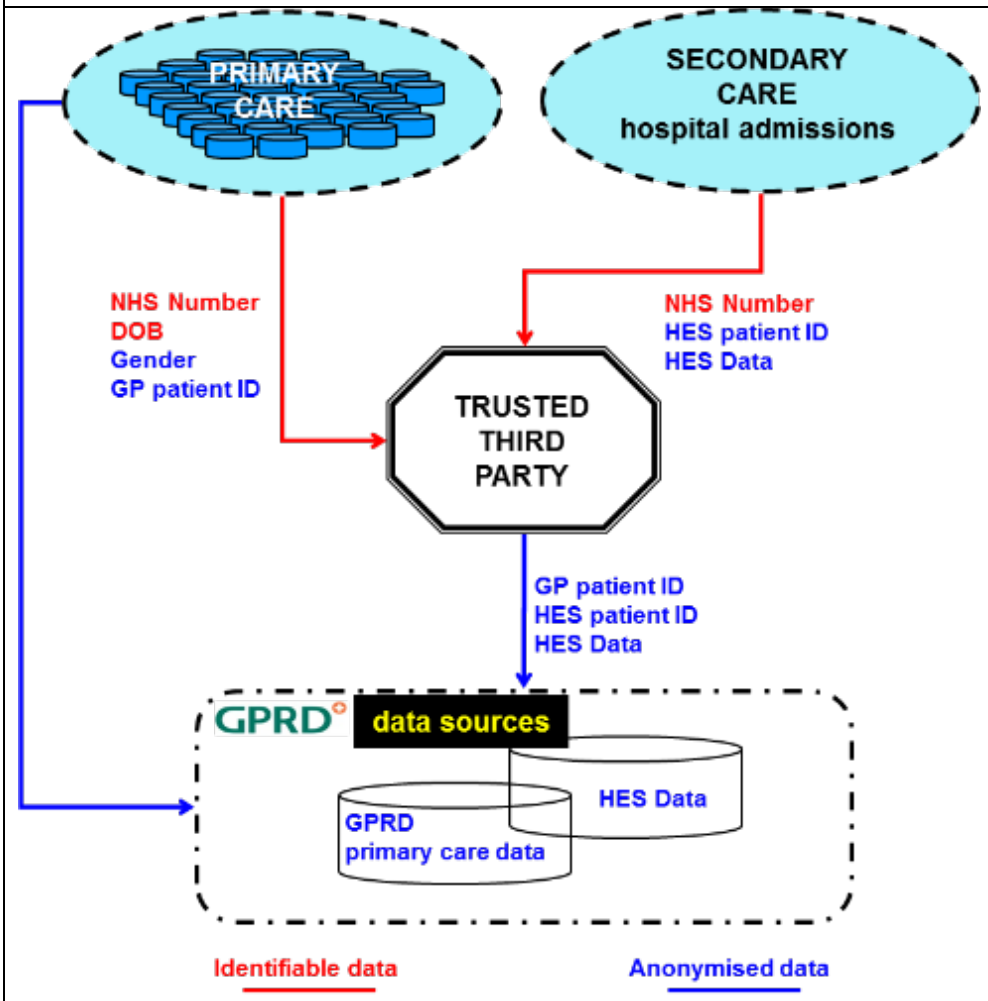
The power calculation requires 10 MS patients, 10 NMO/NMOSD and 10 healthy volunteers. For rarity of NMO/NMOSD, I managed to estimate the true number of MS and NMO/NMOSD patients around Nottingham. The Clinical Practice Research Data-link (CPRD, previously called General Practice Research Database) is a database focused on primary care in the UK, which contains medical records of research standard prospectively collected from around 14 million individuals registered in 684 general practices until August 2014. The data were prospectively collected from as early as 1987. As the UK National Health System provides universal coverage, no segment of the population is excluded from the CPRD. The patients enrolled in the database are largely representative of the UK population regarding age, gender and geographical distribution. The database contains comprehensive information on patient demographics, date of birth, date of death, body mass index (BMI), smoking status, alcohol consumption, medical diagnoses, results of laboratory tests and examinations, immunisation, and prescribed medications. In addition to comprehensive data from participating general practices, CPRD provides data on secondary care admissions and ethnicity from Hospital Episode Statistics data, index of multiple deprivation (IMD)

scores, and mortality data from the Office for National Statistics from patients from the CPRD practices which have data linked to these sources (Figure 1-19). The database is fully anonymised to protect participant confidentiality. Therefore, the CPRD provides access to multi-linked longitudinal data that may enhance healthcare research (website: www.cprd.com).

Therefore, CPRD is an ideal database to estimate the number of NMO/NMOSD patients in England and to help evaluate the feasibility of the 7T study. Patients with NMO/NMOSD are very rare and some patients are disabled and there was only one 7T MRI in the UK during this thesis period. Epidemiology studies using CPRD can estimate the incidence and prevalence of NMO/NMOSD. Using the database, we are able to estimate the exact number of NMO/NMOSD within reasonable distance around Nottingham. In addition, we are able to look into clinical details of these patients, facilitating our understanding of NMO/NMOSD patients in the real world. Despite the fact that the database is totally anonymized, the information taken from CPRD can greatly facilitate the development of protocol, power calculation, estimation of cases within reach and assistance for ethical review.

Although global epidemiology reveals a low prevalence of NMO/NMOSD, the estimate in England has not been conducted. We hypothesized that the evolutionary changes in the diagnostic criteria proposed on 1999, 2006, and 2007 and 2016 had increased recognition of NMO/NMOSD in a population and increased the incidence. The descriptive study of incidence, prevalence, comorbidity, and mortality in NMO/NMOSD and MS in the same England population have not been carried out. This is an important issue to researchers who intend to carry out MRI studies. The hypothesis of this study was that the prevalence of NMO/NMOSD is much lower than MS in England and that the comorbidity may be different between these two populations. The epidemiology study was approved by the Independent Scientific Advisory Committee (ISAC) for the Medicines and Healthcare Products Regulatory Agency Database Research (protocol 14-070).

Figure 1-17 The Clinical Practice Research Datalink (CPRD).



General Practice Research Database (GPRD) was established in 1987 and named as Clinical Practice Research Datalink since April 2012, when there were 684 primary care practices, collected anonymised clinical records from around 12 million individuals, representing (8% of the UK population). The Hospital Episode Statistics (HES) contains details of all admissions to the National Health Service (NHS) hospitals in England only; while the GPRD main dataset covers practices in England, Scotland, Wales, and Northern Ireland. Data were available for every year from 1989 onwards, and linked to CPRD main dataset from 1st April 1997. (Adapted from the information on https://www.cprd.com/intro.asp)

1.8 HYPOTHESIS OF THESIS

After extensive reviews of literature, I identified several questions that remain to be elucidated and require further exploration. Firstly, what are the quantitative characteristics of white matter in normal subjects observed using high-field MRI. Secondly, what are the characteristics of cerebral white matter lesions in NMO/NMOSD compared to that in MS. Thirdly, is normal appearing white matter abnormal in NMO/NMOSD? If yes, what is the degree of difference from that in MS?

To address the above questions, the availability of high-field MRI, techniques of quantitative MRI and adequate patient enrolment is pivotal. Therefore, I proposed this clinical study to enroll patients with MS and NMO/NMOSD. NMO/NMOSD is expected to be rare. Therefore, an epidemiology study is required to test the feasibility of the study.

The hypotheses underlying the work done for this thesis include:

1. The normal-appearing white matter at 7T MRI is not normal in NMO/NMOSD, and the qualitative features can differentiate NMO/NMOSD from MS.
2. The prevalence of NMO/NMOSD is very rare in England at about 1:100 MS in the region near Nottinghamshire and the case number is expected to be 10 which is the required number for this study.
3. Comorbidity is relevant to premature mortality in patients with MS or NMO/NMOSD in a dose effect.
4. Cortical demyelinating lesions accumulate along the clinical course of MS, which may expose patients to a higher risk of epilepsy.

1.9 OBJECTIVES AND PURPOSE

1.9.1 Primary objectives

To quantify the normal appearing white matter in NMO/NMOSD and compared to that in healthy volunteers and MS

1.9.2 Secondary objectives

To estimate prevalence and incidence of NMO/NMOSD and MS in England

To evaluate the mortality of NMO/NMOSD and MS in the relevant to comorbidity

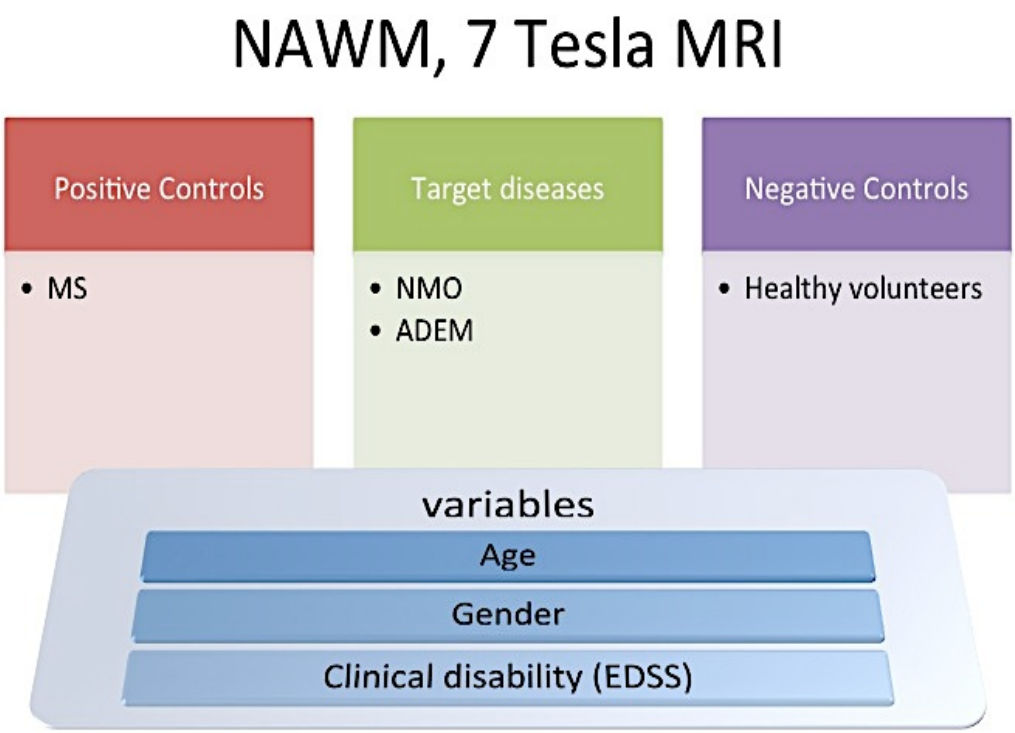
To evaluate the incidence of epilepsy in MS along the time-course of disease chronicity

1.9.3 Study design

This is a prospective cohort study enrolling patients with MS, NMO/NMOSD, and healthy volunteers for a clinical study involving clinical assessment, neurological examination, functional and quality of life questionnaires and 7T MRI and subsequent quantitative imaging analysis focusing on NAWM (Figure 1-20). As NMO/NMOSD are rare diseases, I used the CPRD to estimate the prevalence and incidence of CNS inflammatory syndromes, the comorbidities and mortality of MS and NMO/NMOSD and also test the hypothesis that cortical involvement of MS would increase the risk of epilepsy.

Since the initial plan of the study involved the paediatric population, I performed a pre-protocol exercise to assess the feasibility of 7T MRI in children (appendix 1, (Chou, Tench et al. 2014)) and these data were incorporated into the application for ethical review, which was later approved. Therefore, a parallel study is to enrol paediatric patients who are aged 12 years old or above and have ADEM or MS, and matched healthy volunteers. However because the rarity of the CNS inflammatory syndromes in the paediatric population hampered MRI and epidemiology comparison between ADEM and MS, the thesis shifted the focus on NMO/NMOSD and MS.

Figure 1-18 MRI study plan.



The protocol is designed to recruit NMO/NMOSD patients, and age-, gender-, and clinical disability- matched MS patients (as positive controls), and healthy controls (as negative controls) for normal-appearing white matter (NAWM) investigation at 7 Tesla MRI.
In addition, a parallel protocol is to recruit paediatric patients aged 12 years and older and with ADEM or MS, and matched healthy controls.

1.9.4 Sample size and justification

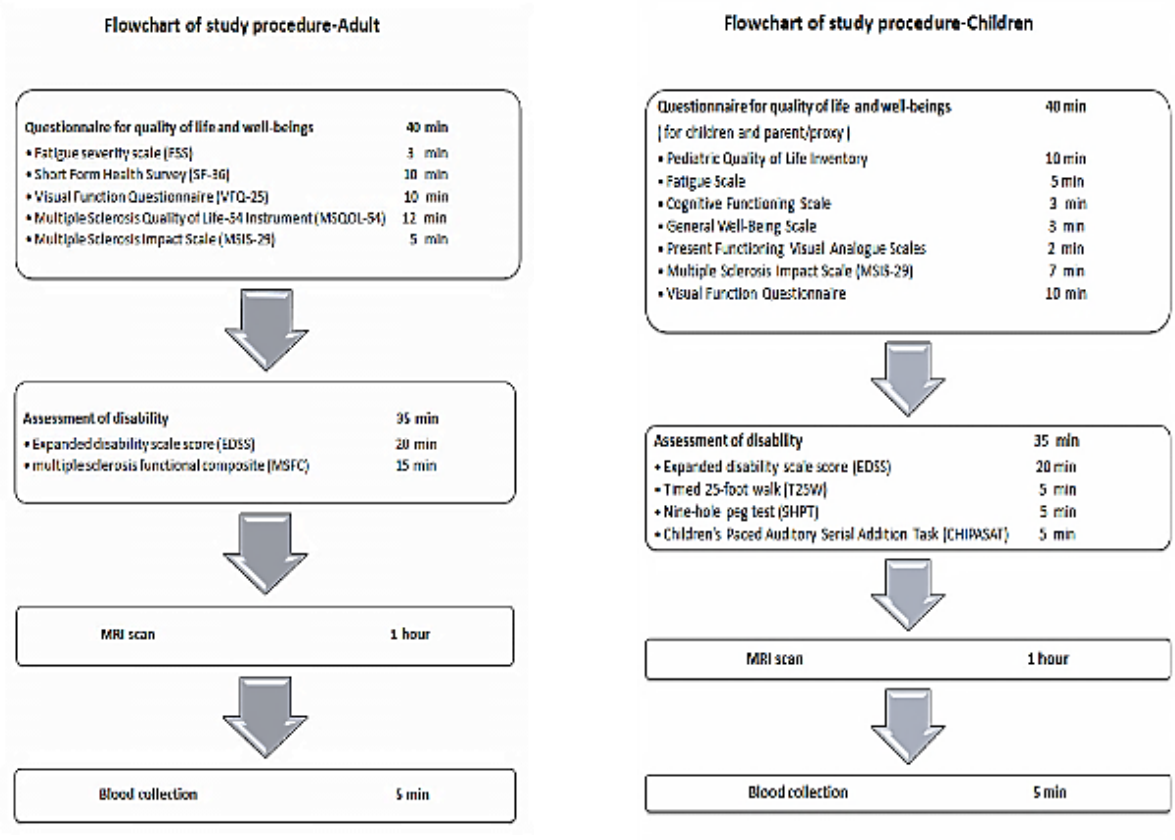
Based on the previous power calculation, we expected to enroll at least 10 subjects with NMO/NMOSD first. Then, enrolment of age-, sex-, and clinical disability-matched at least 10 MS patients were done. The clinical disability is measured as Expanded Disability Status Scale (EDSS; range, 0 to 10; higher scores indicate more severe disability and 10 indicates death) (Kurtzke 1983)

by trained neurologists. At the same time, at least 10 healthy controls were recruited via displaying posters in the public areas.

1.9.5 Study procedure per clinical visit

Enrolees, including patients in the retrospective and the prospective study and healthy controls, will undertake the following study steps during their visit as shown in flowcharts (Figure 1-21):

Figure 1-19 Study flowchart per visit for adult and paediatric patients.



The study applied age-appropriate, validated, self-filled questionnaires for the quality of life in MS patients. Expanded Disability Status Scale (EDSS) was used to evaluate the overall clinical disability by trained neurologists. Hand function, ambulatory ability, and cognition were also examined before the scan. The scanning time of MRI at the 7 Tesla suite is about 1 hour, followed by blood sample collection for further immunological study.

1.10 STRUCTURE OF THIS THESIS

The thesis consists of two parts: the epidemiology study and the advanced MRI study. The epidemiology analyses involved estimation of prevalence and incidence of MS, NMO/NMOSD and other CNS inflammatory syndrome, case-control study to assess the risk of comorbidities and mortality before and after the formal diagnosis of MS and NMO/NMOSD, and a cohort study to estimate the relative risk of epilepsy in MS patients. In the MRI study, I focus on NAWM in both MS and NMO/NMOSD compared with white matter in normal subjects, and discuss the pathological entities of white matter lesions between both diseases. In this part, I employed new quantitative imaging analysis techniques to transform qualitative imaging into comparable sets of data. Several different algorithms are used in this part.

Chapter 2 shows the estimation of incidence and prevalence of NMO/NMOSD and MS, along with other CNS inflammatory syndromes in 2012. Two methods of patient identification, by using both CPRD database alone and CPRD plus linkage dataset, were conducted and compared to optimise the estimate.

Chapter 3 estimates the burden of comorbidities in incident MS patients at diagnosis and estimates the relative risk of comorbidities in MS patients compared to controls. Then the mortality risk was analysed with adjustment for an extensive list of comorbidities.

Chapter 4 shows a case-control study of the risk of having new onset epilepsy in patients with MS. In incident MS population, the first diagnosis of epilepsy was analysed in the time course of MS disease, comparing to age- and sex-matched general population with comprehensive comorbidity adjustment.

Chapter 5 is to estimate the comorbidity burden and mortality of NMO/NMOSD compared to the matched non-NMO/NMOSD controls and matched MS using the case-control design. The disease severity was measured with comorbidity and mortality since the first recorded diagnosis of NMO/NMOSD. This chapter firstly estimated the burden of comorbidities in incident

NMO/NMOSD patients at diagnosis and after diagnosis compared to the matched general population. Then the risk of mortality was estimated between NMO/NMOSD to matched general population and between NMO/NMOSD to matched MS.

Chapter 6 presents a cross-sectional, case-control study using T1 relaxation time and MTR histogram analysis of white matter lesions and normal appearing white matter between NMO/NMOSD, MS, and healthy controls.

Finally, chapter 7 is the general discussion summarising this thesis with implications of findings and future research questions.

Chapter 2. INCIDENCE AND PREVALENCE OF NEUROMYELITIS OPTICA, MULTIPLE SCLEROSIS, OPTIC NEURITIS, AND TRANSVERSE MYELITIS IN THE UNITED KINGDOM

2.1 INTRODUCTION

In the previous chapters of this thesis we introduce the entities of CNS inflammatory diseases. The incidence and prevalence of NMO/NMOSD, MS, optic neuritis and transverse myelitis have not been estimated in the same population before. Therefore, in this chapter we use the CPRD and its linked HES dataset to perform the estimation and compare the difference between the results using CPRD alone and CPRD plus linked HES dataset.

NMO/NMOSD are devastating inflammatory diseases of the CNS, characterized by optic neuritis and transverse myelitis simultaneously or subsequently. NMO/NMOSD is the first CNS inflammatory disease whose diagnosis can be supported by a positive serological test of autoantibodies against aquaporin-4 (AQP-4) (Chou, Whitehouse et al. 2014). However, it is sometimes difficult to differentiate NMO/NMOSD from other CNS inflammatory diseases such as MS because some clinical features overlap (Jurynczyk, Craner et al. 2015). NMO/NMOSD is generally considered to be more severe than MS in terms of poorer recovery and quality of life, and higher neurological pain and disease-related mortality (Wingerchuk and Weinshenker 2003, Cabre, Gonzalez-Quevedo et al. 2009, Qian, Lancia et al. 2012). The incidence and prevalence of NMO/NMOSD have been reviewed (Marrie and Gryba 2013), including population-based estimates, and the evidence suggests it is a rare disease. The incidence and prevalence of other CNS inflammatory diseases vary from disease to disease and from region to region. The most common one is MS and the UK is a high prevalence region for MS.

Most NMO/NMOSD and MS patients have a relapsing remitting course and the time from the first symptom (such as optic neuritis or transverse myelitis) to a definite diagnosis can range from several months to decades. For example, a recent study found that approximately half of patients with optic neuritis converted to MS by 15 years follow up (Optic Neuritis Study 2008) and another study found that one-third patients with transverse myelitis converted to MS by 30 years follow up (Young, Quinn et al. 2009). Although the risk of converting to NMO/NMOSD after single attack

of optic neuritis or transverse myelitis is rarely reported, a Danish retrospective study estimated that 15% of incident optic neuritis or transverse myelitis would fulfil criteria of NMO/NMOSD with or without serological evidence (Asgari, Lillevang et al. 2011). Taken together, optic neuritis and transverse myelitis can be an isolated presentation; they may progress to MS or NMO/NMOSD as well. The epidemiology of optic neuritis or transverse myelitis is therefore difficult to estimate. However, apart from MS (Alonso and Hernan 2008), reliable population-based estimates for prevalence and incidence of NMO/NMOSD, optic neuritis, transverse myelitis and other CNS inflammatory diseases are rarely reported (Young, Quinn et al. 2009, Klein, Ray et al. 2010, Langer-Gould, Brara et al. 2014, Martinez-Lapiscina, Fraga-Pumar et al. 2014). In addition, the epidemiology of these diseases has not been compared in a population representative of the general population in England.

The purpose of this study was to estimate the prevalence and incidence of NMO/NMOSD, MS, isolated optic neuritis, isolated transverse myelitis without NMO/NMOSD or MS, as well as other CNS inflammatory diseases in 2012 using the Clinical Practice Research Datalink (CPRD). We hypothesised that NMO/NMOSD is rare in England, and the prevalence of NMO/NMOSD to MS is about 1:100.

2.2 METHODS

The study was approved by the Independent Scientific Advisory Committee for the Medicines and Healthcare Products Regulatory Agency Database Research (protocol 14-070).

2.2.1 Source of data

The UK CPRD is one of the largest electronic databases containing medical records prospectively collected from primary care in the world. Established in 1987 and named the General Practice Research Database (GPRD) until 2012, the CPRD has collected anonymised clinical records from around 14 million individuals, broadly representing approximately 8% of the UK population, with similar age and sex distributions to the UK general population (Mackenzie, Morant et al. 2014). The participating practitioners have been trained to record the clinical and demographic information up to research standard (Walley and Mantgani 1997). The results from a recent systematic review supported a high validity of recorded diagnoses in CPRD for 183 different conditions; among them the median accuracy rate was 89% (Herrett, Thomas et al. 2010).

The CPRD is also linked to external datasets such as Hospital Episode Statistics (HES; recording data in the secondary care), the Office for National Statistics cause of death data and information from specific disease registries. The HES data was linked to CPRD main dataset from 1st April 1997. HES data contain details of all admissions to NHS hospitals in England only (while the GPRD main dataset covers practices in England, Scotland, Wales and Northern Ireland). Patient-level linkage was done internally by GPRD using National Health Service (NHS) number, date of birth and the linked data were anonymised before releasing to researchers. This study was based on August 2014 version of CPRD and set 10 HES dataset. Information available for each hospitalization episode includes date of hospital admission and discharge and, for each episode, a starting date (episode date). For more details please refer to 1.7.3.

2.2.2 Study population

We constructed two study populations. The first included all people contributing to both the linked datasets of primary (CPRD main database) and secondary care (HES) in 2012. In 2012, primary care data of 80.4% of English general practices were linked to HES. The second population contained all participants who contributed to the main CPRD database in 2012. The HES only covered 398 English practices. Therefore, the second population was used to estimate the prevalence and incidence of CNS inflammatory diseases in the primary care of the UK.

2.2.3 Case definitions of CNS inflammatory diseases

CNS inflammatory diseases were classified as MS, NMO/NMOSD, isolated optic neuritis, isolated transverse myelitis, and other. Patients with isolated optic neuritis or transverse myelitis are those with the specified conditions in the absence of an MS or NMO/NMOSD diagnosis. For isolated optic neuritis or transverse myelitis, any concurrent or prior diagnosis of MS or NMO/NMOSD excluded them from the categories. The case definition of any CNS inflammatory disease for the first population was a diagnosis in the main database or the HES dataset. For the second population, case definition was any diagnosis in the main database only.

The CPRD main database records diagnoses using Read codes and the HES uses ICD-10 (International Classification of Diseases, tenth revision) codes for the primary diagnosis. Read codes for demyelinating diseases were those beginning at F20, F21, and some codes beginning at F4H and F03. ICD-10 codes for demyelinating diseases were those beginning at G35, G36, G37 and H46. Details of specific codes, based on the opinions of two senior neurologists and a general practitioner, are listed in table 2-1 and 2-2.

The validity of CNS inflammatory diseases has not been tested for each entity. The validity of MS diagnosis in the CPRD has been investigated and found to be high (Hernan, Jick et al. 2004). In that study, 632 MS patients with medical records for review were identified from CPRD. After a

full review by two senior neurologists, 580 cases (91.8%) of them were possible MS and 521 cases (82.4%) were confirmed MS. Among confirmed MS, the MS diagnoses were further supported by positive results on MRI in 85% of them (Hernan, Jick et al. 2004).

2.2.4 Estimation of prevalence and incidence

The denominator for prevalence estimation (eligible population) included all individuals registered on 1st July 2012 (mid-year population) with the up-to-standard general practices Prevalence was calculated using the number of people who were diagnosed with CNS inflammatory diseases at any time before the mid-point of 2012 as the numerator, and the number of all people contributing CPRD data at the same time point as the denominator.

For incidence of CNS inflammatory diseases in 2012, we constructed at-risk cohorts, which comprised all individuals registered with up-to-standard practices during the year who had no history of demyelinating disease diagnosis before 1st January of 2012 or current registration date plus 365 days to ensure a minimum of one-year observation. Person-years of follow-up were then calculated from 1st January 2012 or the date of registration plus 365 days to the earliest date of transfer-out, incident diagnosis with any CNS inflammatory diseases, death or 31st December 2012. Incidence was calculated by dividing the number of incident cases with CNS inflammatory diseases during 2012 (numerator) by the total person-years occurring in the database during the same year (denominator).

2.2.5 Statistical analysis

The 95% confidence intervals (CIs) for prevalence and incidence were calculated based on the assumption of a Poisson distribution of the observed prevalent cases. The significance level was set at 0.05. All statistical analyses were performed by using SAS statistical software, version 9.4.

Table 2-1 Read codes for inflammatory diseases of the Central Nervous System.

Read codes	Description
Multiple sclerosis	
F20..00	Multiple sclerosis
F20..11	Disseminated sclerosis
F200.00	Multiple sclerosis of the brain stem
F201.00	Multiple sclerosis of the spinal cord
F202.00	Generalised multiple sclerosis
F203.00	Exacerbation of multiple sclerosis
F204.00	Benign multiple sclerosis
F206.00	Primary progressive multiple sclerosis
F207.00	Relapsing and remitting multiple sclerosis
F208.00	Secondary progressive multiple sclerosis
F20z.00	Multiple sclerosis NOS
Neuromyelitis optica	
F210.00	Neuromyelitis optica
F210.11	Devic's disease
Optic neuritis*	
F4H3.00	Optic neuritis
F4H3000	Unspecified optic neuritis
F4H3100	Optic papillitis
F4H3200	Acute retrobulbar neuritis
Transverse myelitis*	
F03..13	Transverse myelitis
F037.00	Transverse myelitis
Other	
F21..00	Other central nervous system demyelinating diseases
F211.00	Schilder's disease
F211.11	Balo's concentric sclerosis
F212.00	Acute and subacute haemorrhagic leukoencephalitis [Hurst]
F213.00	Clinically isolated syndrome
F21X.00	Acute disseminated demyelination, unspecified
F21y.00	Other specified central nervous system demyelinating disease
F21y400	Subacute necrotizing myelitis
F21y500	Concentric sclerosis
F21yz00	Other specified central nervous system demyelination NOS
F21z.00	Central nervous system demyelination NOS

*** isolated conditions were those without MS and NMO/NMOSD**

Table 2-2 ICD-10 codes for inflammatory demyelinating diseases of the Central Nervous System.

ICD-10 codes	**Description**
G35	Multiple sclerosis
G36.0	Neuromyelitis optica [Devic]
H46*	Optic neuritis
G37.3*	Acute transverse myelitis in demyelinating disease of central nervous system
Other	
G36.1	Acute and subacute haemorrhagic leukoencephalitis [Hurst]
G36.8	Other specified acute disseminated demyelination
G36.9	Acute disseminated demyelination, unspecified
G37.0	Diffuse sclerosis
G37.4	Subacute necrotizing myelitis
G37.5	Concentric sclerosis [Baló]
G37.8	Other specified demyelinating diseases of central nervous system
G37.9	Demyelinating disease of central nervous system, unspecified

* isolated condition**s** were those without MS and NMO/NMOSD

2.3 RESULTS

In 2012, there were 2,098,285 eligible individuals contributing data to HES linked dataset within CPRD, with the median prior observation duration of 9 years (interquartile range [IQR] 3–20 years). Of them, 8,122 prevalent cases of CNS inflammatory diseases were identified, giving a prevalence of 387.08 (378.72–395.57) per 100,000 people. Women had a significantly higher prevalence than men with a female to male prevalence ratio of 1.86. The gender specific prevalence of MS, NMO/NMOSD, isolated optic neuritis, isolated transverse myelitis, and other CNS inflammatory diseases were shown in table 2-3. MS was the most common CNS inflammatory disease, with a prevalence of 260.16 (253.32–267.15) per 100,000 people while NMO/NMOSD was rare with a prevalence of 3.72 (2.94–4.64) per 100,000 people. Figure 2-1 shows age-specific prevalence of CNS inflammatory diseases. In general, CNS inflammatory diseases were rare before the age of 20 years and more frequently observed in the people in young adulthood and middle age.

There was a total of 1,903,493 person-years of follow-up in 2012 and 372 incident cases of CNS inflammatory diseases were identified, equivalent to an incidence of 19.54 (95% CI, 17.61–21.63) per 100,000 person-years. The mean age of initial diagnosis of CNS inflammatory diseases was 44.9 ± 15.5 years. Women had a higher incidence 25.40 [95% CI, 22.44–28.64] per 100,000 person-years) than men (12.38 [95% CI, 10.14–14.97]) per 100,000 person-years) with a female to male incidence ratio of 2.05. The incidence of MS, NMO/NMOSD, isolated optic neuritis, isolated transverse myelitis, and other CNS inflammatory diseases were shown in table 2-4 with the estimated incidence of 10.34 (8.94–11.88) per 100,000 person-years for MS and 0.42 (0.18–0.83) per 100,000 person-years for NMO/NMOSD. As shown in figure 2-2, the number of patients with incidence of MS or isolated optic neuritis was greatest at the age of 30-39 years. Isolated transverse myelitis tended to occur in elder people. Incident cases of NMO/NMOSD were identified at any age without an appreciable peak.

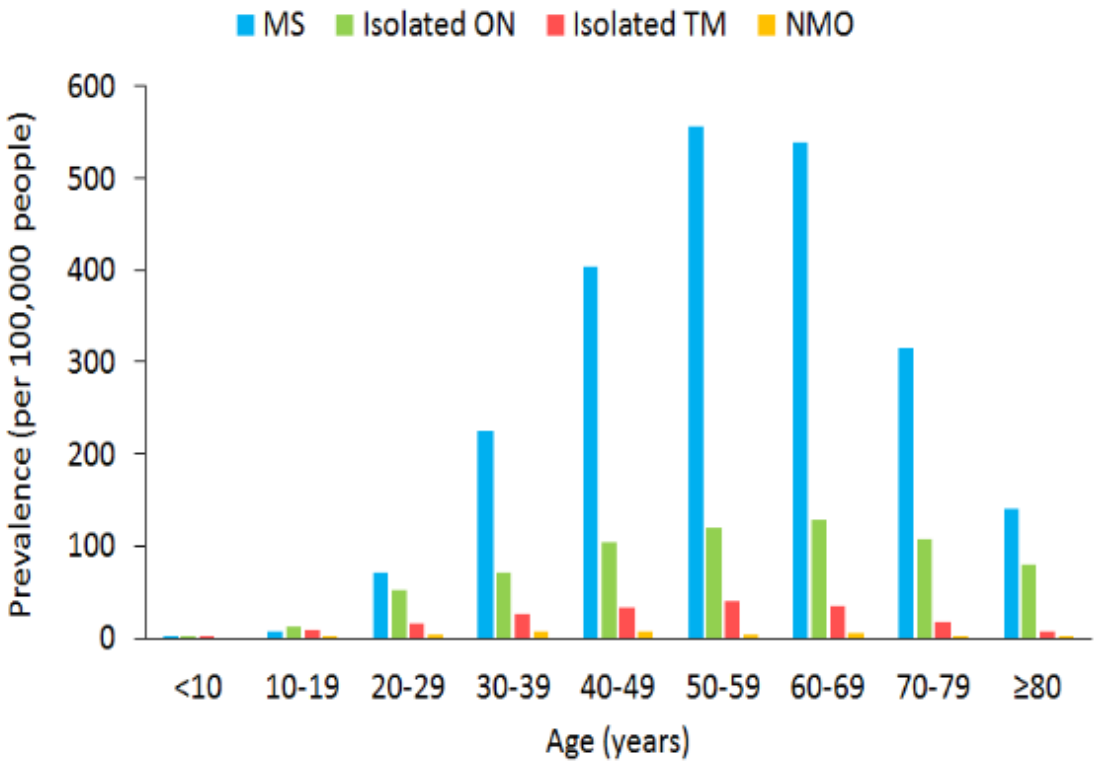
We also estimated the prevalence and incidence of CNS inflammatory diseases based on recordings in the main database (table 2-5). In 2012, there were 5,660,943 patients contributing data to CPRD and their mean age was similar to those contributing to HES linked dataset within CPRD; among them, 14,183 patients with CNS inflammatory diseases were identified. The prevalence of CNS inflammatory diseases was 237.52 (233.63–241.46) cases per 100,000 people in 2012 based on CPRD main database, which is approximately 61% of estimate based on HES linked dataset. There were 5,504,792 person-years in 2012 and 604 incident cases were identified. The incidence of CNS inflammatory diseases was 10.97 (10.11–11.88) per 100,000 person-years based on CPRD main database, which was 56% of estimate based on HES linked dataset.

Table 2-3 Prevalence of CNS inflammatory diseases in 2012 in patients contributing data to HES linked dataset within CPRD.

	Overall n = 2,098,285		**Women n = 1,157,998**		**Men n = 940,287**		**F/M ratio**
	n	Prevalence (95% CI)	N	Prevalence (95% CI)	n	Prevalence (95% CI)	
MS	5,459	260.16 (253.32–267.15)	3,930	339.38 (328.87–350.14)	1,529	162.61 (154.57–170.96)	2.09
NMO/NMOSD	78	3.72 (2.94–4.64)	60	5.18 (3.95–6.67)	18	1.91 (1.13–3.03)	2.71
Isolated TM[a]	441	21.02 (19.10–23.07)	267	23.06 (20.37–25.99)	174	18.50 (15.86–21.47)	1.25
Isolated ON[a]	1,563	74.49 (70.84–78.27)	1,050	90.67 (85.27–96.33)	513	54.56 (49.94–59.49)	1.66
Other	628	29.93 (27.63–32.36)	385	34.25 (30.01–36.74)	243	25.84 (22.70–29.30)	1.33
All[b]	8,122	387.08 (378.72–395.57)	5,658	488.60 (475.98–501.47)	2,464	262.05 (251.81–272.59)	1.86

Values represent numbers of the affected patients and prevalence of the disease (per 100,000 people).
Abbreviations: CI: confidence interval; CNS: central nervous system; CPRD: Clinical Practice Research Datalink; F/M: female to male; HES: Hospital Episode Statistics; IQR, interquartile range; MS: multiple sclerosis; NMO/NMOSD: neuromyelitis optica; ON: optic neuritis; TM: transverse myelitis
a: isolated conditions were those without MS and NMO/NMOSD.
b: patients with any diagnosis of MS, NMO/NMOSD, isolated TM, isolated ON, or other CNS inflammatory diseases.

Figure 2-1 Age-specific prevalence of CNS inflammatory diseases in 2012.



The prevalence rate is the highest for MS (blue) and the lowest for NMO/NMOSD (orange). In MS, the prevalence rate peaked in the 50-59 age group. In NMO/NMOSD, the prevalence rate peaked in the 30-39 age group.

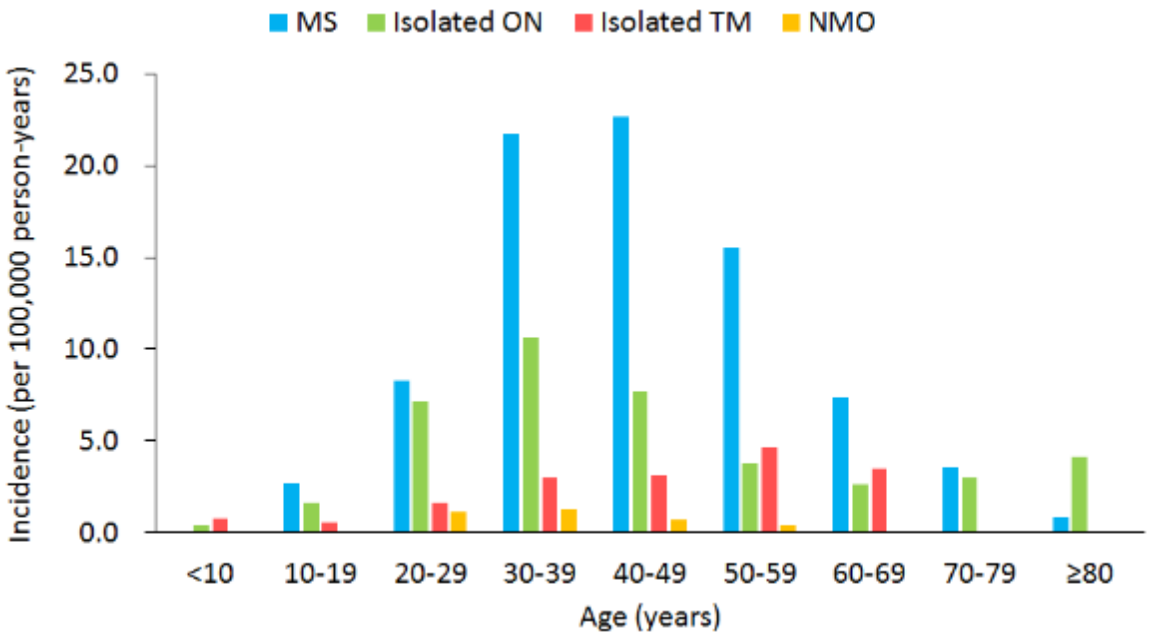
Table 2-4 Incidence of CNS inflammatory diseases in 2012 contributing data to HES linked dataset within CPRD.

	Overall			Women		Men		
	Mean age (SD, years)	n (total person-years)	Incidence (95% CI, per 100,000 person-years)	n (total person-years)	Incidence (95% CI, per 100,000 person-years)	n (total person-years)	Incidence (95% CI, per 100,000 person-years)	**F/M ratio**
MS	44.3 (12.9)	197 (1,905,938)	10.34 (8.94–11.88)	147 (1,048,958)	14.01 (11.84–16.47)	50 (856,979)	5.83 (4.33–7.69)	2.40
NMO/NMOSD	35.6 (11.6)	8 (1,911,185)	0.42 (0.18–0.83)	7 (1,052,733)	0.66 (0.27–1.37)	1 (858,451)	0.12 (0.30–0.65)	5.50
Isolated TM[a]	44.5 (16.2)	41 (1,905,354)	2.15 (1.54–2.92)	29 (1,048,583)	2.77 (1.85–3.97)	12 (856,771)	1.40 (0.72–2.45)	1.98
Isolated ON[a]	43.4 (17.8)	89 (1,904,328)	4.67 (3.75–5.75)	61 (1,047,875)	5.82 (4.45–7.47)	28 (856,454)	3.27 (2.17–4.72)	1.78
Other	46.7 (16.9)	80 (1,903,326)	4.20 (3.33–5.23)	52 (1,047,264)	4.97 (3.71–7.48)	28 (856,061)	3.27 (2.17–4.73)	1.52
All[b]	44.9 (15.5)	372 (1,903,493)	19.54 (17.61–21.63)	266 (1,047,381)	25.40 (22.44–28.64)	106 (856,113)	12.38 (10.14–14.97)	2.05

Abbreviations: CI: confidence interval; CNS: central nervous system; CPRD: Clinical Practice Research Datalink; F/M: female to male; HES: Hospital Episode Statistics; MS: multiple sclerosis; NMO/NMOSD: neuromyelitis optica; ON: optic neuritis; TM: transverse myelitis

a: isolated conditions were those without MS and NMO/NMOSD. b: patients with any diagnosis of MS, NMO/NMOSD, isolated TM, isolated ON, or other CNS inflammatory diseases.

Figure 2-2 Age-specific incidence of CNS inflammatory diseases in 2012.



The incidence rate is the highest for MS (blue) and the lowest for NMO/NMOSD (orange). In MS, the incidence rate peaked in the 40-39 and age group. In NMO/NMOSD, the incidence rate peaked in the 30-39 age group.

Table 2-5 Comparison of the demographics and estimated prevalence and incidence of CNS inflammatory diseases based on CPRD or HES-linked dataset within CPRD in 2012.

	CPRD		HES linked dataset within CPRD		Ratio of patients in CPRD to HES linked dataset
Demographics of the population					
Population	5,660,943		2,098,285		
Mean age (SD), years	41.7	(23.7)	40.9	(24.7)	
Women (%)	3,080,914	(51.60)	1,157,998	(55.19)	
Median prior observation (IQR), years	10	(4–19)	9	(3–20)	
Prevalence (95% CI), per 100,000 people					
MS	166.93	(163.68–170.24)	260.16	(253.32–267.15)	0.64
NMO/NMOSD	0.99	(0.75–1.27)	3.72	(2.94–4.64)	0.27
Isolated TM[a]	11.42	(10.58–12.31)	21.02	(19.10–23.07)	0.54
Isolated ON[a]	50.94	(49.15–52.79)	74.49	(70.84–78.27)	0.68
Other	12.29	(11.42–13.21)	29.93	(27.63–32.36)	0.41
All[b]	237.52	(233.63–241.46)	387.08	(378.72–395.57)	0.61
Incidence (95% CI), per 100,000 person-years					
MS	6.28	(5.64–6.98)	10.34	(8.94–11.88)	0.61
NMO/NMOSD	0.09	(0.03–0.21)	0.42	(0.18–0.83)	0.21
Isolated TM[a]	1.02	(0.77–1.32)	2.15	(1.54–2.92)	0.47
Isolated ON[a]	3.05	(2.61–3.55)	4.67	(3.75–5.75)	0.65
Other	1.20	(0.93–1.53)	4.20	(3.33–5.23)	0.43
All[b]	10.97	(10.11–11.88)	19.54	(17.61–21.63)	0.56

Abbreviations: CI: confidence interval; CNS: central nervous system; CPRD: Clinical Practice Research Datalink; HES: Hospital Episode Statistics; IQR, interquartile range; MS: multiple sclerosis; NMO/NMOSD: neuromyelitis optica; ON: optic neuritis; SD: standard deviation; TM: transverse myelitis. a: isolated conditions were those without MS and NMO/NMOSD diagnosis; b: patients with any diagnosis of MS, NMO/NMOSD, isolated TM, isolated ON, or other CNS inflammatory diseases.

2.4 DISCUSSION

This nationwide population study estimated that approximately one in 250 people was affected by CNS inflammatory diseases in the UK in 2012. NMO/NMOSD was the rarest CNS inflammatory disease, while MS was the most prevalent one with isolated optic neuritis the second. All CNS inflammatory diseases are female predominant and occurred most commonly in the fourth decade of life, except for transverse myelitis which mainly occurred later. We found that prevalence and incidence based solely on primary care data were grossly underestimated by 30-40% compared to that using data derived from linked datasets containing both primary and secondary care data. We further found that the extent of the underestimation was most prominent for NMO/NMOSD and transverse myelitis compared to other CNS inflammatory diseases. This discrepancy may reflect that NMO/NMOSD are conditions with high risk for disability or death, and needed for diagnoses and management in hospitals. In contrast, MS or optic neuritis is a more insidious condition and likely diagnosed in outpatient settings of hospitals, and therefore patients' records transferred from their previous practices back to their original general practitioners are sooner and better accomplished than in NMO/NMOSD (Leite, Andrews et al. 2017).

The prevalence of NMO/NMOSD ranges from 0.52 to 4.40 per 100,000 population and the incidence from 0.053 to 0.40 per 100,000 population worldwide as reported by a systematic review (Marrie and Gryba 2013). Our estimates for NMO/NMOSD prevalence (3.72 per 100,000 people) is higher than previous regional surveys carried out by specialists in South East Wales and in North West England (1.96 and 0.72 per 100,000 people, respectively) (Cossburn, Tackley et al. 2012, Jacob, Panicker et al. 2013), and slightly lower than a population-based study in Demark (4.4 per 100,000 people) (Asgari, Lillevang et al. 2011). The incidence of NMO/NMOSD estimated in our study was also higher than the previously regional estimate in North West England (0.08 per 100,000 person-years) (Jacob, Panicker et al. 2013), in which the authors recognized their underestimation by not including paediatric patients and patients with optic neuritis not having

been seen by specialists. The annual incidence of NMO/NMOSD estimated in our study (0.42 per 100,000 person-years) was very similar to the population-based estimation in Denmark (0.4 per 100,000 person-years) (Asgari, Lillevang et al. 2011).

The prevalence and incidence of NMO/NMOSD based on primary care data alone may be underestimated because patients with NMO/NMOSD were predominately cared by specialists in secondary case. However, HES only contains hospitalisation data. Therefore, we may not capture patients who were cared by specialists in outpatient clinics but not hospitalised. This may lead to an underestimation of both prevalence and incidence of NMO/NMOSD. We are not sure of the extent of this underestimation. The underestimation may be less prominent as the North West England study (Jacob, Panicker et al. 2013) since their estimates were based on multiple overlapping sources, including a medical centre hospital electronic records in Liverpool, regional district general hospital data, central laboratory data for AQP-4 autoantibodies in Oxford and the British Neurological Surveillance Unit for adults during a shorter observation period than the current study. The true prevalence and incidence of NMO/NMOSD may be higher than our estimates. However, it is difficult to conduct a nationwide study to ascertain the prevalence and incidence of NMO/NMOSD because it is too rare. Any reasonable population sample may result in unreliable data since the robustness of the sampling strategy may not be good enough to capture cases with such rarity. Currently our data seem to be acceptable estimates.

The incidence and prevalence of MS have been estimated using CPRD during the past 20 years. The recent point prevalence estimation using similar methods (CPRD plus HES during 1997 to 2010) for beginning of 2010 was 283.5 per 100,000 for women and 110.2 per 100,000 for men. (Mackenzie, Morant et al. 2014) The estimation in mid-2012 was 339.4 and 162.6 per 100,000 for women and men respectively and supported their observation of rising prevalence of MS annually. The incidence in 2010 was 14.52 and 6.28 per 100,000 person-years for women and men respectively (Mackenzie, Morant et al. 2014). Our study showed the incidence of 14.01 and 5.83 per 100,000 person-years for women and men respectively, and supported the findings of a

downward trend in the incidence of MS in the UK that was found by Mackenzie et al. (Mackenzie, Morant et al. 2014). Alonso and colleagues reported a lower incidence rate of 5.3 per 1,000 women and 2.3 per 1,000 men during 1993 to 2000 (Alonso, Jick et al. 2007). The lower incidence rate in this study may be attributed to the use of main CPRD dataset only, a maximum observation time that was shorter than the current study (7 years vs. 20 years), and the performance of medical record reviews included only confirmed MS (47.1% of cases) and excluded possible MS or prevalent MS. The overall estimated incidence of MS in the UK is relatively high among European countries (Kingwell, Marriott et al. 2013).

Isolated optic neuritis was the second most common inflammatory disease. There are few reports of the incidence estimation of optic neuritis since 2005. Our estimate of the optic neuritis incidence (4.67 cases per 100,000 person-years) was lower than that reported in a recent hospital database in Barcelona, Spain (5.36 per 100,000 person-years) (Martinez-Lapiscina, Fraga-Pumar et al. 2014), but higher than that in a recent study at Kaiser Permanente Southern California (2.64 per 100,000 person-years) (Langer-Gould, Brara et al. 2014) and some other countries (0.56–5.1 cases per 100,000 person-years) (Martinez-Lapiscina, Fraga-Pumar et al. 2014). The incidence estimate in our study was higher than an earlier study in 1996 using HES linked datasets containing 13 primary practices in central London (1 per 100,000 person-years) (MacDonald, Cockerell et al. 2000).

The incidence of isolated transverse myelitis in our study (2.15 per 100,000 person-years) was close to the recent research in the USA (2.26–3.1 per 100,000 person-years) (Klein, Ray et al. 2010, Langer-Gould, Brara et al. 2014) and New Zealand (2.46 per 100,000 person-years) (Young, Quinn et al. 2009). The mean onset age of transverse myelitis was slightly older in the UK than in the USA and New Zealand (44.5 versus 43.5 and 36.8 years, respectively) with a similar female predominance.

There are several limitations to our study. Firstly, we based our case definition on physician-recorded diagnoses, rather than according to classification criteria for specific inflammatory

diseases. However, the validity of MS diagnosis in the CPRD has been investigated and found to be high (Hernan, Jick et al. 2004). Secondly, we used patients who have primary care data and linked hospitalisation data in secondary care. Patients who were cared for by specialists in the hospitals but had never been hospitalised may not have been identified using our current linked data strategy. Therefore, our estimates based on CPRD and HES data may still underestimate the incidence and prevalence of demyelinating diseases. Thirdly, we cannot analyse the ethnicity of the patients because that data was not recorded reliably in HES (Mathur, Bhaskaran et al. 2014). However, this study is strengthened by estimating incidence and prevalence of MS, NMO/NMOSD, optic neuritis, and transverse myelitis in England altogether for the first time, and showing that NMO/NMOSD is a rare disease with prevalence ratio of NMO/NMOSD to MS of approximately 1:70.

2.5 CONCLUSION

In conclusion, this population-based study provides estimates for prevalence and incidence of CNS inflammatory diseases in England in 2012. MS was the most common and NMO/NMOSD was the rarest. The peak age of initial diagnosis of NMO/NMOSD is in 30–39 age group, which is a similar age range to that of isolated optic neuritis and close to multiple sclerosis, but a younger age range than that of isolated transverse myelitis.

Chapter 3. HIGHER RISK OF COMORBIDITY AND MORTALITY IN PATIENTS WITH MULTIPLE SCLEROSIS: A NATIONWIDE POPULATION CASE-CONTROL STUDY

3.1 INTRODUCTION

In the previous chapters of this thesis we estimated the incidence and prevalence of MS, NMO/NMOSD, optic neuritis and transverse myelitis. We found that MS is the most common CNS inflammatory disease. In this chapter we focus on the disease severity in MS, using comorbidity and mortality as surrogate parameters, compared to general population.

MS is a chronic progressive and disabling disorder that causes substantial negative social and economic consequences. A recent population-based study in the UK showed that the prevalence of MS has increased by about 2.4% per year over 20 years and estimated that 126,669 people had MS in 2010 (203.4 per 100,000 population) (Mackenzie, Morant et al. 2014). Analyses of two multiple sclerosis specialist clinic databases at Nottingham (Manouchehrinia, Tench et al. 2013) and Oxford (Piccolo, Kumar et al. 2015), show that patients have disabilities that are severe enough to impair activities of living as measured by Kurtzke Expanded Disability Status Scale (EDSS) (Kurtzke 1983). MS also has long-standing impact on the quality of life of those affected (Murphy, Confavreux et al. 1998), and is associated with increased mortality compared to the general population (Manouchehrinia, Tanasescu et al. 2015).

MS is also associated with several comorbidities, which can delay diagnosis, reduce functional status at diagnosis, and impact patient survival. For example, of 8,983 MS patients registered in the North American Research Committee on Multiple Sclerosis (NARCOMS), 24% already had physical comorbidities and 8.4% had mental comorbidities at the onset of MS (Marrie, Horwitz et al. 2011). According to their research, existing comorbidity not only delayed disease diagnosis but also increased severity of disability at diagnosis (Marrie, Horwitz et al. 2009). In addition, vascular comorbidity was associated with more rapid progression of disability (Marrie, Rudick et al. 2010). In a study of UK population database, cardiovascular comorbidty was shown to contribute to the increased mortality

rate than matched general population (Lalmohamed, Bazelier et al. 2012). Although the awareness of diverse comorbidities in patients with multiple sclerosis is growing (Marrie and Horwitz 2010, Jick, Li et al. 2014, Marrie, Reider et al. 2015, Marrie, Reider et al. 2015, Marrie, Reingold et al. 2015), temporal changes of comorbidity burden and the dose effect of comorbidity on survival relative to a comparator population have seldom been addressed.

This study aimed to examine the burden of comorbidities in new cases of MS during 1997-2006 in the Clinical Practice Research Data-link (CPRD), which is known to contain data representative of the general population of the UK. We further followed these incident patients and matched non-MS controls to compare their subsequent accrual of comorbidities and all-cause mortality. Firstly, we hypothesised that MS patients have a higher risk of comorbidities at diagnosis and also have a higher risk of developing comorbidities following diagnosis. Secondly, we hypothesised that the extent of comorbidity at MS diagnosis has a dose effect on survival.

3.2 METHODS

This case-control study utilised both data prior to diagnosis and subsequent follow-up data (after diagnosis) within the CPRD. The study was approved by the Independent Scientific Advisory Committee for the Medicines and Healthcare Products Regulatory Agency Database Research (protocol 14-070).

3.2.1 Data source

The CPRD is a database focused on primary care in the UK, which contains prospectively collected up-to-research-standard medical records from around 16.5 million individuals registered in 701 general practices (August 2016 version). The participating doctors have been trained to record clinical information according to a set of standards ensuring the quality meets the research requirement. The database has been described previously (Walley and Mantgani 1997) and has been validated for many diagnoses under scrutiny in this study, including MS (Hernan, Jick et al. 2004) and various comorbidities (Herrett, Thomas et al. 2010). The validity of the diagnoses recorded in the database is high, as shown in a recent systematic review, reporting confirmation of diagnosis in a median of 89% for 183 different conditions (Herrett, Thomas et al. 2010). The database contains comprehensive information on patients from participating general practices, and provides data linked to other sources including mortality data from the Office of National Statistics. The database is fully anonymised to protect participant confidentiality. For more details please refer to 1.7.3.

3.2.2 Definition of incident MS

Our source population comprises all participants who contributed data to the CPRD between 1st January 1997 and 31th December 2006. This data range was chosen for consideration of sample size

and length of follow-up. We used Read codes (codes beginning with F20, Table 3-1) to identify MS patients. Eligibility criteria for an incident MS patient included (1) participants had to have no evidence of MS prior to the time of diagnosis (index date) and (2) at least had a three-year continuous registration prior to the index date (Hernan, Jick et al. 2004, Cea Soriano, Rothenbacher et al. 2011). The case definition was based on physician-diagnosis using Read codes indicative of incident MS. The code list has been used (Mackenzie, Morant et al. 2014) and validated previously (Hernan, Jick et al. 2004). Hernán et al. (Hernan, Jick et al. 2004) identified 710 patients with a first diagnosis of MS recorded in the CPRD between 1993 and 2000. Among them, medical records of 632 patients were available. After reviewing medical records, 580 of them (91.8%) were confirmed or possible cases of MS.

Table 3-1 Read code of multiple sclerosis

Read code	Description
F20..00	Multiple sclerosis
F20..11	Disseminated sclerosis
F200.00	Multiple sclerosis of the brain stem
F201.00	Multiple sclerosis of the spinal cord
F202.00	Generalised multiple sclerosis
F203.00	Exacerbation of multiple sclerosis
F204.00	Benign multiple sclerosis
F206.00	Primary progressive multiple sclerosis
F207.00	Relapsing and remitting multiple sclerosis
F208.00	Secondary progressive multiple sclerosis
F20z.00	Multiple sclerosis NOS

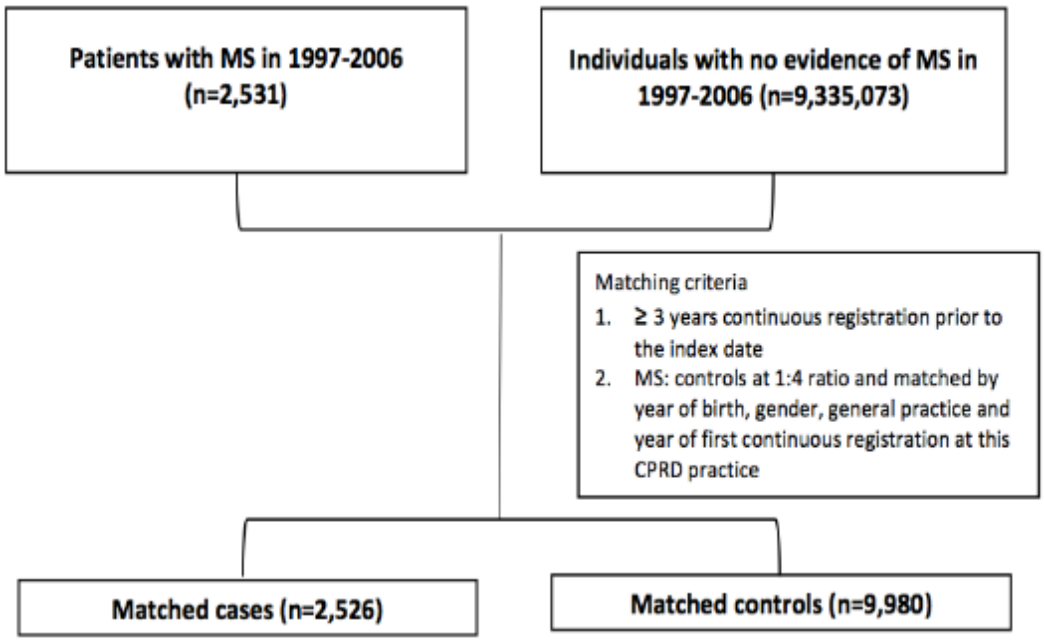
3.2.3 Selection of controls

From active CPRD participants who had an registration from 1997 to 2006, we included at random up to 4 controls per case who had no record of MS diagnosis matched by year of birth (±2 years), gender, general practice and year of first continuous registration (±2 years) at this CPRD practice (Figure 3-1). The design of matching general practice and year of first continuous registration is to control for possible confounding by the general practice with which participants were registered. The same index date was assigned to each of the matched controls. As with incident patients with MS, the matched controls were required to have at least three years of registration prior to the index date.

3.2.4 Study period

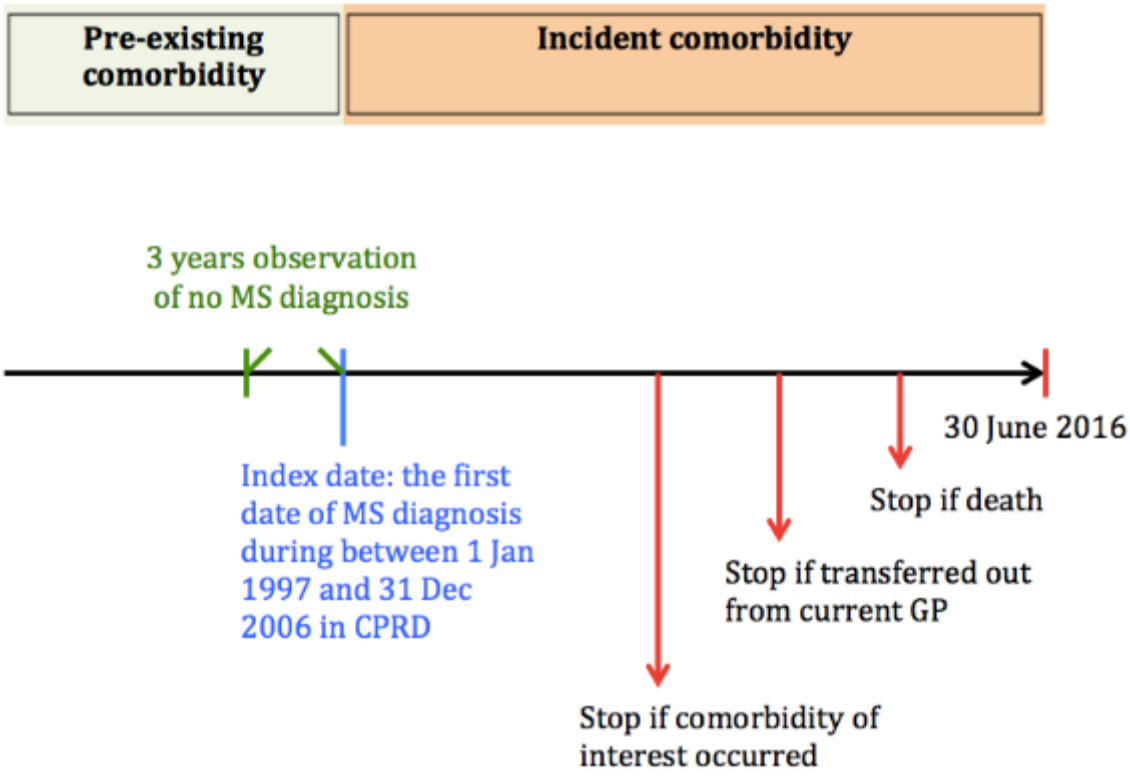
We defined two periods to assess comorbidity: (a) the 10 years prior to diagnosis and (b) the period from diagnosis to the earliest date of occurrence of comorbidities, death, transfer out, last data collection time for practice or end of study (30 June 2016), whichever came first (Figure 3-2).

Figure 3-1 Flowchart of eligible MS patients with multiple sclerosis and matched controls



The study comprised incident MS patients and 4 matched non-MS controls who were continuously registered for at least three years before the date MS was firstly recorded during 1997 to 2006.

Figure 3-2 The observation of comorbidity in an incident MS patient.



A patient is defined as an incident case if the diagnosis of MS was recorded between 1997 and 2006 and no priori MS diagnosis for ≥ 3 years. The pre-existing comorbidity was observed before the first recorded MS diagnosis (index date) and the incident comorbidity are those occurred afterwards until 30 June 2016.

Abbreviations: MS, multiple sclerosis; GP, general practitioner; CPRD, Clinical Practice Research Datalink

3.2.5 Comorbidities of interest

We defined MS as the index disease in this study and comorbidities were defined by Feinstein's definition for comorbidity, specifically: "*any distinct additional entity that has existed or may occur during the clinical course of a patient who has the index disease under study*" (Feinstein 1970). To estimate and compare the burden of comorbidity in MS to controls, we used the Charlson comorbidity index (Table 3-2). Charlson comorbidity index was developed in medical centres in 1987 (Charlson, Pompei et al. 1987), and comprises 17 categories of comorbid disease which are weighted derived from the initial tested hospitalized patients who died within one year's follow-up (Charlson, Pompei et al. 1987). The Charlson's comorbidity score is calculated as the resultant weighted sum to represent individual health status (Charlson, Szatrowski et al. 1994). It has been extensively studied as valid and reliable method (Lindhardsen, Ahlehoff et al. 2011) to measure comorbidity for controlling the confounding factors in contemporary clinical research (Peterson, Paget et al. 2012) to predict mortality (D'Hoore, Bouckaert et al. 1996, Crooks, West et al. 2015) and high medical costs (Charlson, Wells et al. 2014). Although it is not perfect, it has been studied as a suitable tool regarding medical costs for paediatric patients (Charlson, Wells et al. 2014) and mortality except for those aged less than 1 year (D'Hoore, Bouckaert et al. 1996). The index had been adapted for use with Read/OXMIS coded data of the GPRD (Khan, Perera et al. 2010). Deyo et al produced a validated version for use with the ICD-9 based database (Deyo, Cherkin et al. 1992), which was the basis of the validated Read code version (Khan, Perera et al. 2010). We calculated Charlson comorbidity index score for each study person at index date by adding scores assigned to each specific diagnosis. We categorised Charlson comorbidity index as (1) 0, 1-2, 3-4 or ≥ 5 scores and (2) dichotomised it as 0 and ≥ 1 score.

To estimate the risk of an individual developing a comorbidity, potentially associated with MS, we grouped the pre-specified comorbidities into 9 categories: neoplasms, cardiovascular diseases, renal

diseases, metabolic and endocrine diseases, gastrointestinal and hepatic diseases, chronic pulmonary diseases, musculoskeletal and connective tissue diseases, neurological and mental disorders, and other.

Table 3-2 The 17 comorbid disease categories and assigned weight in Charlson's comorbid index. An individual's combined comorbidity score is calculated by summing the weights.

Charlson Diagnostic Category	Weighted score	Charlson Diagnostic Category	Weighted score
AIDS	6	Congestive heart disease	1
Metastatic tumour	6	Myocardial infarction	1
Moderate or severe liver disease	3	Mild liver disease	1
Diabetes with complications	2	Diabetes	1
Hemiplegia and paraplegia	2	Chronic pulmonary disease	1
Renal disease	2	Cerebrovascular disease	1
Cancer	2	Dementia	1
Peptic ulcer disease	1	Peripheral vascular disease	1
Rheumatological disease	1		

3.2.6 All-cause mortality

To analyse whether the extent of existing comorbidity in patients with MS at diagnosis would affect their mortality, we used all-cause mortality for patients and controls. Mortality and date of death were based on the recording in the main CPRD database. The CPRD has developed an algorithm to identify death and death dates of participants. In order to validate the recorded death and date of death in CPRD, we requested data from the death registration for all patients registered between 2004 and 2006 who had consented to participate in the linkage scheme, which covered the period between 1st January 1998 and 10th January 2012. The results have been reported before (Kuo, Grainge et al. 2014). In brief, the sensitivity, specificity, positive predictive value and negative predictive value of a CPRD recorded death were 0.99, 0.99, 0.93 and 1.00, respectively. The dates of death recorded in CPRD were identical to those recorded in death registration in 89.0% of deceased patients identified by both CPRD and death registration. The difference between recorded death dates was less than 3 months in 98.5% of deceased patients. Therefore the recording of death and date of death in the CPRD is generally consistent with death registration.

3.2.7 Covariates

Information on individual's demographic factors (age and gender) (Jick, Li et al. 2014) and lifestyle factors (body mass index [BMI], smoking status and alcohol consumption) at diagnosis was extracted. BMI, smoking and alcohol consumption have been recognised as potentially modifiable factors affecting MS risk (Sundstrom, Nystrom et al. 2008, Hedstrom, Olsson et al. 2012, Hedstrom, Hillert et al. 2014) and survival (Manouchehrinia, Weston et al. 2014).

3.2.8 Statistical analysis

The prevalence of each comorbidity was calculated using the number of people ever diagnosed with a given comorbidity during the past 10-year period before the index date as the numerator and the number of incident MS patients or matched non-MS controls as denominator. Odds ratios (OR) and 95% confidence intervals (CI) were used to estimate the association between MS and each co-existing medical condition. Conditional logistic regression was used to calculate ORs and 95% CIs adjust for age, gender, index year, body mass index (BMI) category, smoking status and alcohol consumption. Missing data for BMI, smoking and alcohol status were coded as 'unknown'. Kaplan–Meier plots were used to estimate the cumulative probability of the occurrence of new Charlson comorbidity and the all-cause mortality in people with incident MS and controls. In individuals without any Charlson comorbidity at index date, cumulative probabilities of new Charlson comorbidity was estimated at 1, 2, 5, and 10 years following the time of diagnosis (index date).

Only people not having that comorbidity at the index date were considered to estimate hazard ratios (HR) of a specific comorbidity. HRs with 95% CIs were calculated using a Cox proportional hazards model for a Charlson comorbidity index ≥ 1, for each comorbidity separately, and for all-cause mortality. The HRs were adjusted by BMI categories, smoking status and alcohol consumption in

addition to the matching variables at index date. We further evaluate the relationships between Charlson scores at diagnosis and all-cause mortality in MS patients using Cox proportional hazards models adjusted by BMI, smoking and alcohol in addition to the matching variables. Finally, to compare all-cause mortality between MS patients and controls, we adjusted for aforementioned covariates and also Charlson comorbidity index at index date. All statistical analyses were performed using SAS statistical software, version 9.3.

3.3 RESULTS

We found 2,526 incident MS patients during 1997 to 2006 with a mean age of 45.0 ± 12.4 years and the majority were female. The median length of observation periods (interquartile range) before and after index date was 12 (7–21) and 10 (5–13) years, respectively. There were 9,980 non-MS controls with similar age and gender distributions and observation periods before and after the index date (Table 3-3).

Table 3-3 Baseline characteristics of patients with incident MS and controls

	Incident MS (n=2,526)		**Controls (n=9,980)**		**Adjusted ORs† (95% CI)**	
Age (years)	45.03±12.37		45.17±12.43			
Gender						
Men	736	(29.14)	2,910	(29.16)		
Women	1,790	(70.86)	7070	(70.84)		
Median observation years (IQR)						
Prior to index date	12	(7-21)	12	(7-20)		
After index date	10	(5-13)	10	(6-13)		
BMI (kg/m^2)						
<18.5	983	(38.92)	3,452	(34.59)	1.44	(1.07-1.93)*
18.5-24.9	69	(2.73)	169	(1.69)	Reference	
25.0-29.9	685	(27.12)	2,670	(26.75)	0.90	(0.80-1.00)
≥30	469	(18.57)	1,871	(18.75)	0.88	(0.77-0.99)
Unknown	320	(12.67)	1,818	(18.22)	0.58	(0.50-0.68)
Smoking						
Non-smoker	720	(28.50)	2,953	(29.59)	Reference	
Current smoker	554	(21.93)	1,542	(15.45)	1.53	(1.34-1.75)*
Ex-smoker	878	(34.76)	3,261	(32.68)	1.09	(0.97-1.24)
Unknown	374	(14.81)	2,224	(22.28)	0.62	(0.54-0.72)
Alcohol consumption (units/week)						
Never/Ex-drinker	333	(13.18)	1,085	(10.87)	Reference	
Current 1-9	1,321	(52.30)	4,814	(48.24)	0.90	(0.99-1.08)
Current ≥ 10	268	(10.61)	1,030	(10.32)	0.83	(0.68-1.00)*
Unknown	604	(23.91)	3,051	(30.57)	0.59	(0.50-0.70)*

Abbreviation: BMI, body mass index; IQR, interquartile range
†adjusted for age, gender and index year; *P<0.05

3.3.1 Retrospective observation

At time of the index date, a higher proportion of MS patients (22.72%) had at least one Charlson comorbidity than controls (16.80%; $p<0.001$). As Table 3-4 shows, incident MS patients tended to have higher Charlson comorbidity index than non-MS controls did before index date. Cerebrovascular diseases were 11-fold more common in MS patients one year before MS diagnosis than that in matched non-MS controls. Other conditions that were more common in incident MS patients than matched controls one year before the MS diagnosis included neurological and mental disorders (OR, 3.60), peripheral vascular diseases (OR, 3.30), myocardial infarction (OR, 3.07), hyperlipidaemia (OR, 1.93), and anaemia (OR, 1.81).

3.3.2 Follow-up data after index date

Table 3-5 shows cumulative probability of any or specific comorbidity after diagnosis of multiple sclerosis. Among people with a Charlson score of zero at index date, the cumulative probabilities of having new Charlson comorbidity at 1, 2, 5 and 10 years from index date were 2.31%, 4.16%, 8.57% and 17.23% in incident MS patients and 1.64%, 3.10%, 7.83% and 15.78% in non-MS controls (Figure 3-3, $p = 0.2333$).

Table 3-4 Comorbidities present 10 years and 1 year before index date

	10-year period before index date								1-year period before index date							
	Cases		Controls		Unadjusted OR		Adjusted OR†		Cases		Controls		Unadjusted OR		Adjusted OR†	
Charlson index																
0	1,952	(77.28)	8,303	(83.2)	Reference		Reference		2,250	(89.07)	9,294	(93.13)	Reference		Reference	
1-2	501	(19.83)	1,518	(15.21)	1.43	(1.27-1.60)*	1.31	(1.17-1.47)*	258	(10.21)	658	(6.59)	1.63	(1.40-1.90)*	1.46	(1.25-1.71)*
3-4	59	(2.34)	141	(1.41)	1.82	(1.33-2.49)*	1.65	(1.20-2.26)*	16	(0.63)	26	(0.26)	2.45	(1.30-4.61)*	2.40	(1.27-4.53)*
≥5	14	(0.55)	18	(0.18)	3.48	(1.70-7.12)*	3.26	(1.58-6.70)*	2	(0.08)	2	(0.02)	4.48	(0.63-32.0)	4.80	(0.66-35.0)
Neoplasms	64	(2.53)	226	(2.26)	1.11	(0.83-1.47)	1.08	(0.81-1.45)	17	(0.67)	65	(0.65)	1.02	(0.60-1.76)	0.97	(0.56-1.66)
Solid malignancy, leukaemia and lymphoma	61	(2.41)	210	(2.1)	1.15	(0.86-1.54)	1.12	(0.84-1.51)	16	(0.63)	64	(0.64)	0.98	(0.56-1.70)	0.92	(0.52-1.60)
Metastatic solid tumours	4	(0.16)	7	(0.07)	2.00	(0.57-7.01)	1.96	(0.56-6.91)	1	(0.04)	2	(0.02)	2.00	(0.18-22.1)	2.30	(0.20-26.1)
Cardiovascular diseases	325	(12.87)	1,065	(10.67)	1.27	(1.10-1.46)*	1.18	(1.02-1.37)*	134	(5.3)	383	(3.84)	1.46	(1.18-1.80)*	1.35	(1.09-1.67)*
Hypertension	222	(8.79)	867	(8.69)	1.00	(0.85-1.18)	0.93	(0.79-1.10)	86	(3.4)	329	(3.3)	1.05	(0.81-1.35)	0.96	(0.75-1.24)
Cardiac arrhythmias	34	(1.35)	84	(0.84)	1.65	(1.10-2.47)*	1.60	(1.06-2.41)*	7	(0.28)	25	(0.25)	1.12	(0.48-2.61)	1.13	(0.48-2.64)
Cerebrovascular disease	71	(2.81)	94	(0.94)	3.18	(2.31-4.38)*	2.98	(2.15-4.12)*	37	(1.46)	14	(0.14)	11.96	(6.23-23.0)	11.09	(5.74-21.4)*
Congestive heart failure	13	(0.51)	24	(0.24)	2.01	(1.01-4.01)*	1.96	(0.97-3.95)	13	(0.51)	24	(0.24)	2.88	(0.86-9.64)	2.87	(0.85-9.68)
Myocardial infarction	23	(0.91)	60	(0.6)	1.55	(0.95-2.54)	1.37	(0.84-2.26)	6	(0.24)	8	(0.08)	3.19	(1.06-9.56)*	3.07	(1.00-9.43)*
Peripheral vascular disease	20	(0.79)	47	(0.47)	1.68	(0.99-2.85)	1.48	(0.87-2.53)	6	(0.24)	6	(0.06)	3.78	(1.21-11.8)*	3.30	(1.05-10.4)*
Valvular heart disease	9	(0.36)	32	(0.32)	1.13	(0.54-2.37)	1.10	(0.52-2.31)	3	(0.12)	12	(0.12)	1.00	(0.28-3.54)	0.97	(0.27-3.44)
Genitourinary diseases	18	(0.71)	79	(0.79)	0.89	(0.53-1.49)	0.84	(0.50-1.41)	7	(0.28)	15	(0.15)	1.87	(0.76-4.58)	1.69	(0.69-4.18)
Urolithiasis	10	(0.4)	48	(0.48)	0.83	(0.42-1.65)	0.77	(0.39-1.53)	5	(0.2)	6	(0.06)	3.33	(1.02-10.9)*	3.25	(0.98-10.7)
Renal diseases	8	(0.32)	32	(0.32)	0.95	(0.44-2.08)	0.93	(0.42-2.03)	2	(0.08)	10	(0.1)	0.80	(0.18-3.65)	0.71	(0.15-3.25)
Metabolic and endocrine diseases	208	(8.23)	696	(6.97)	1.20	(1.01-1.42)*	1.10	(0.92-1.30)	86	(3.4)	263	(2.64)	1.29	(1.00-1.66)*	1.18	(0.91-1.52)
Uncomplicated diabetes mellitus	67	(2.65)	250	(2.51)	1.04	(0.79-1.37)	0.94	(0.71-1.24)	41	(1.62)	151	(1.51)	1.04	(0.73-1.49)	0.93	(0.65-1.33)
Diabetes mellitus with complications	20	(0.79)	44	(0.44)	1.77	(1.04-3.01)*	1.56	(0.91-2.67)	7	(0.28)	14	(0.14)	1.97	(0.80-4.89)	1.90	(0.76-4.75)
Hyperlipidaemia	72	(2.85)	255	(2.56)	1.12	(0.85-1.48)	1.01	(0.76-1.33)	25	(0.99)	49	(0.49)	2.14	(1.29-3.53)*	1.93	(1.16-3.19)*
Hypothyroidism	84	(3.33)	260	(2.61)	1.28	(0.99-1.65)	1.20	(0.93-1.56)	22	(0.87)	63	(0.63)	1.36	(0.83-2.23)	1.31	(0.79-2.16)
Gastrointestinal and hepatic diseases	37	(1.46)	90	(0.9)	1.68	(1.14-2.49)*	1.58	(1.06-2.36)*	6	(0.24)	17	(0.17)	1.41	(0.56-3.58)	1.49	(0.58-3.80)
Peptic ulcer disease	29	(1.15)	69	(0.69)	1.72	(1.10-2.69)*	1.60	(1.02-2.50)*	4	(0.16)	9	(0.09)	1.78	(0.55-5.77)	1.69	(0.52-5.52)
Mild liver disease	7	(0.28)	18	(0.18)	1.57	(0.65-3.81)	1.49	(0.61-3.66)	1	(0.04)	5	(0.05)	0.80	(0.09-6.85)	0.96	(0.11-8.29)
Moderate to severe liver disease	3	(0.12)	7	(0.07)	1.71	(0.44-6.63)	2.08	(0.53-8.19)	1	(0.04)	3	(0.03)	1.33	(0.14-12.8)	1.64	(0.16-16.4)
Chronic pulmonary diseases	290	(11.48)	970	(9.72)	1.21	(1.05-1.39)*	1.09	(0.95-1.26)	117	(4.63)	370	(3.71)	1.25	(1.01-1.55)*	1.10	(0.89-1.37)
Musculoskeletal and connective tissue diseases	148	(5.86)	509	(5.1)	1.15	(0.94-1.39)	1.06	(0.87-1.29)	51	(2.02)	167	(1.67)	1.19	(0.86-1.65)	1.10	(0.80-1.53)
Osteoarthritis	63	(2.49)	260	(2.61)	0.94	(0.70-1.25)	0.91	(0.68-1.21)	15	(0.59)	49	(0.49)	1.15	(0.63-2.09)	1.16	(0.64-2.12)
Rheumatological disease	70	(2.77)	282	(2.83)	0.95	(0.72-1.24)	0.86	(0.65-1.13)	28	(1.11)	121	(1.21)	0.88	(0.58-1.33)	0.80	(0.52-1.22)
Gout	16	(0.63)	81	(0.81)	0.79	(0.46-1.35)	0.76	(0.44-1.31)	5	(0.2)	24	(0.24)	0.83	(0.32-2.18)	0.82	(0.31-2.17)
Neurological and mental disorders	882	(34.92)	1,783	(17.87)	2.65	(2.39-2.93)*	2.49	(2.24-2.76)*	411	(16.27)	503	(5.04)	3.83	(3.32-4.42)*	3.60	(3.11-4.17)*

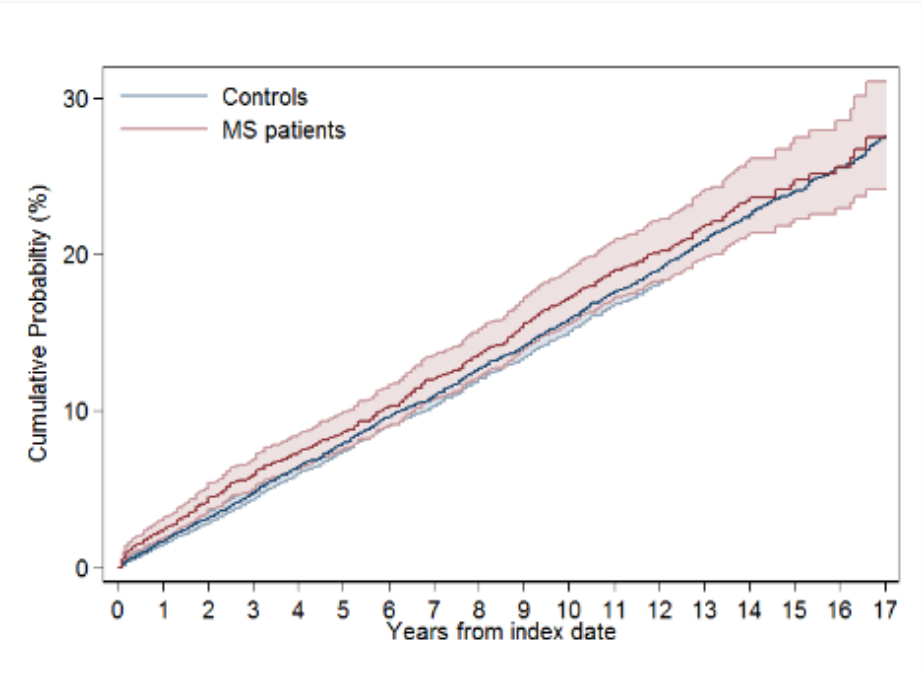
Dementia	0	(0)	5	(0.05)	n.a.		n.a.		0	(0)	1	(0.01)	n.a.		n.a.	
Other neurological diseases	297	(11.76)	119	(1.19)	11.3	(9.00-14.2)*	10.72	(8.52-13.5)*	175	(6.93)	37	(0.37)	20.16	(14.0-29.1)*	18.89	(13.1-27.3)*
Psychosis	14	(0.55)	31	(0.31)	1.81	(0.96-3.40)	1.70	(0.90-3.22)	2	(0.08)	7	(0.07)	1.14	(0.24-5.50)	1.06	(0.22-5.14)
Depression	616	(24.39)	1,683	(16.86)	1.67	(1.49-1.86)*	1.54	(1.38-1.72)*	215	(8.51)	463	(4.64)	1.96	(1.65-2.33)*	1.84	(1.54-2.19)*
Other comorbidities	252	(9.98)	871	(8.73)	1.16	(1.00-1.35)*	1.10	(0.94-1.28)	56	(2.22)	141	(1.41)	1.58	(1.15-2.17)*	1.50	(1.09-2.07)*
Anaemia	210	(8.31)	671	(6.72)	1.27	(1.07-1.50)*	1.19	(1.01-1.41)*	44	(1.74)	91	(0.91)	1.93	(1.33-2.79)*	1.81	(1.25-2.63)*
Psoriasis	48	(1.9)	211	(2.11)	0.90	(0.66-1.24)	0.88	(0.64-1.21)	12	(0.48)	50	(0.5)	0.96	(0.51-1.80)	0.94	(0.50-1.77)
HIV infection	0	(0)	2	(0.02)	n.a.		n.a.		0	(0.00)	0	(0.00)	n.a.		n.a.	

Charlson index includes myocardial infarction, congestive heart failure, peripheral vascular disease, cerebrovascular disease, dementia, chronic pulmonary disease, rheumatologic disease, peptic ulcer disease, mild liver disease, moderate or severe liver disease, diabetes mellitus [DM], DM with chronic complications, renal diseases, any malignancy [including leukaemia and lymphoma], metastatic solid tumour and human immunodeficiency virus [HIV] infection.

†adjusted for BMI class, smoking status and alcohol consumption

* $p<0.05$; n.a., not applicable

Figure 3-3 The cumulative probability of having new Charlson comorbidity in MS patients (red) and matched non–MS controls (blue) with a Charlson score of zero at index date.



Among people with a Charlson score of zero at index date, the cumulative probabilities of having new Charlson comorbidity from index date in incident MS patients were not different from those in non-MS controls (p=0.2333).

Table 3-5 Cumulative probability of comorbidity after diagnosis of multiple sclerosis

	Patients with incident MS					Controls					
	At diagnosis	**1 year**	**2 years**	**5 years**	**10 years**	**At diagnosis**	**1 year**	**2 years**	**5 years**	**10 years**	**p Value**
Charlson index ≥1	22.72	25.45	27.28	31.63	40.03	16.80	18.73	20.18	24.87	32.64	<0.0001*
Any increase in Charlson index	0	2.31	4.16	8.57	17.23	0	1.64	3.1	7.83	15.78	0.2333
Neoplasms	2.53	33.33	39.09	54.32	82.3	2.26	30.64	35.26	49.25	78.15	0.0754
Solid malignancy, leukaemia and lymphoma	2.41	3.21	3.8	5.48	9.72	2.1	2.55	2.96	4.38	8.08	0.0236*
Metastatic solid tumours	0.16	0.28	0.37	0.47	0.84	0.07	0.12	0.21	0.36	0.81	0.9448
Cardiovascular diseases	12.87	14.91	16.41	20.61	27.61	10.67	12.28	13.99	18.86	26.77	0.2208
Hypertension	8.79	10.31	11.68	15.24	20.41	8.69	10.02	11.43	15.7	22.2	0.0513
Cardiac arrhythmias	1.35	1.54	1.63	2.16	3.37	0.84	1.01	1.22	1.78	3.11	0.4422
Cerebrovascular disease	2.81	3.17	3.51	4.09	5.53	0.94	1.15	1.48	1.98	3.52	<0.0001*
Congestive heart failure	0.51	0.53	0.57	0.76	1.33	0.24	0.38	0.5	0.74	1.23	0.5057
Myocardial infarction	0.91	1.1	1.31	1.55	2.44	0.6	0.71	0.86	1.33	2.02	0.2653
Peripheral vascular disease	0.79	0.98	1.06	1.45	1.83	0.47	0.56	0.69	0.99	1.45	0.1062
Valvular heart disease	0.36	0.49	0.53	0.77	1.28	0.32	0.36	0.4	0.57	1.11	0.2754
Genitourinary diseases	0.71	0.85	0.9	1.52	2.53	0.79	0.96	1.12	1.53	2.32	0.8418
Urolithiasis	0.40	0.45	0.45	0.64	1.08	0.48	0.54	0.55	0.71	0.88	0.4859
Renal diseases	0.32	0.41	0.45	0.88	1.45	0.32	0.43	0.58	0.85	1.49	0.6481
Metabolic and endocrine diseases	8.23	9.22	10.55	14.44	20.89	6.97	8.09	9.12	12.64	18.3	0.0424*
Uncomplicated diabetes mellitus	2.65	3.01	3.48	4.83	7.78	2.51	2.89	3.29	4.54	6.77	0.3760
Diabetes mellitus with complications	0.79	0.98	1.06	1.4	5.06	0.44	0.54	0.71	1.31	4.42	0.3959
Hyperlipidaemia	2.85	3.45	3.97	6.19	8.97	2.56	3.08	3.64	5.37	8.37	0.7182
Hypothyroidism	3.33	3.7	4.34	5.6	7.56	2.61	3.04	3.36	4.74	6.45	0.0358*
Gastrointestinal and hepatic diseases	1.46	1.79	2.05	2.38	3.27	0.9	1.06	1.12	1.47	2.11	0.0027*
Peptic ulcer disease	1.15	1.34	1.56	1.65	2.03	0.69	0.79	0.82	0.95	1.2	0.0129*
Mild liver disease	0.28	0.41	0.41	0.65	1.09	0.18	0.24	0.28	0.49	0.86	0.1296
Moderate to severe liver disease	0.12	0.12	0.16	0.16	0.23	0.07	0.08	0.1	0.16	0.21	0.9593
Chronic pulmonary diseases	11.48	12.36	12.87	14.75	17.43	9.72	10.64	11.18	13.21	16.02	0.2456
Musculoskeletal and connective tissue diseases	5.86	6.62	7.48	10.08	15.87	5.1	5.85	6.52	8.84	13.42	0.0003*
Osteoarthritis	2.49	2.76	2.85	4.3	7.56	2.61	3.09	3.52	5.27	8.1	0.2089
Rheumatological disease	2.77	3.01	3.44	4.45	6.47	2.83	3.18	3.57	4.62	6.68	0.4502
Gout	0.63	0.93	1.08	1.55	2.61	0.81	0.69	0.86	1.06	1.56	0.0182*
Neurological and mental disorders	34.92	41.18	44.24	50.24	57.42	17.87	19.89	21.36	25.08	31.33	<0.0001*
Dementia	0.00	0	0.04	0.14	0.46	0.05	0.06	0.1	0.16	0.43	0.9758
Other neurological diseases	11.76	13.9	14.24	15.43	17.37	1.19	1.36	1.57	1.96	2.66	<0.0001*
Psychosis	0.55	0.85	1.11	1.11	1.62	0.31	0.36	0.4	0.54	0.75	<0.0001*
Depression	24.39	30.09	33.64	40.06	46.94	16.86	18.75	20.11	23.52	29.26	<0.0001*
Other comorbidities	9.98	11.19	12.14	14.69	18.87	8.73	9.63	10.36	13.02	17.1	0.0361*
Anaemia	8.31	9.25	10.02	12.14	15.88	6.72	7.33	7.95	10.23	13.7	0.0066*
Psoriasis	1.90	2.07	2.29	2.92	3.99	2.11	2.33	2.50	2.98	4.00	0.8035
HIV infection	0.00	0	0	0	0	0.02	0.02	0.02	0.02	0.02	0.4768

Figures are probability of incident comorbidity (percentage); *p<0.05

Table 3-6 Risk of incident comorbidity after diagnosis of multiple sclerosis.

	At-risk cases		At-risk controls		Unadjusted HRs		Adjusted HRs	
Charlson index ≥1	1952	(20.34)	8303	(18.85)	1.19	(1.05-1.34)*	1.13	(1.00-1.29)*
Any increase in Charlson index	2526	(15.72)	9980	(15.68)	1.08	(0.96-1.22)	1.05	(0.92-1.19)
Neoplasms	2462	(7.27)	9754	(6.55)	1.24	(1.03-1.50)*	1.22	(1.01-1.48)*
Solid malignancy, leukaemia and lymphoma	2464	(7.22)	9763	(6.59)	1.23	(1.02-1.48)*	1.21	(1.00-1.46)*
Metastatic solid tumours	2522	(0.59)	9973	(0.74)	0.79	(0.44-1.41)	0.73	(0.40-1.32)
Cardiovascular diseases	2201	(15.77)	8915	(17.52)	1.00	(0.87-1.13)	0.97	(0.84-1.11)
Hypertension	2304	(11.46)	9113	(14.38)	0.91	(0.78-1.05)	0.89	(0.76-1.03)
Cardiac arrhythmias	2492	(1.97)	9896	(2.33)	0.98	(0.70-1.36)	0.97	(0.69-1.36)
Cerebrovascular disease	2455	(3.01)	9886	(2.83)	1.12	(0.85-1.47)	1.08	(0.81-1.44)
Congestive heart failure	2513	(0.76)	9956	(0.92)	1.01	(0.61-1.67)	1.00	(0.61-1.65)
Myocardial infarction	2503	(1.28)	9920	(1.32)	1.09	(0.72-1.65)	1.04	(0.67-1.61)
Peripheral vascular disease	2506	(1.00)	9933	(0.94)	1.32	(0.82-2.13)	1.42	(0.84-2.39)
Valvular heart disease	2517	(0.87)	9948	(0.71)	1.41	(0.87-2.28)	1.32	(0.79-2.21)
Genitourinary diseases	2508	(1.59)	9901	(1.55)	1.08	(0.77-1.52)	1.03	(0.72-1.47)
Urolithiasis	2516	(0.64)	9932	(0.43)	1.47	(0.83-2.63)	1.52	(0.81-2.85)
Renal diseases	2518	(0.95)	9948	(1.14)	0.90	(0.58-1.38)	0.83	(0.53-1.31)
Metabolic and endocrine diseases	2318	(11.69)	9284	(11.75)	1.08	(0.93-1.26)	1.08	(0.93-1.27)
Uncomplicated diabetes mellitus	2459	(4.51)	9730	(4.44)	1.10	(0.88-1.38)	1.08	(0.84-1.40)
Diabetes mellitus with complications	2506	(4.27)	9936	(4.58)	1.07	(0.84-1.37)	1.03	(0.78-1.35)
Hyperlipidaemia	2454	(5.26)	9725	(5.68)	1.03	(0.82-1.29)	1.00	(0.80-1.26)
Hypothyroidism	2442	(3.97)	9720	(3.72)	1.07	(0.85-1.35)	1.03	(0.81-1.30)
Gastrointestinal and hepatic diseases	2489	(1.69)	9890	(1.32)	1.43	(0.99-2.07)	1.39	(0.94-2.05)
Peptic ulcer disease	2497	(0.76)	9911	(0.61)	1.47	(0.85-2.54)	1.45	(0.82-2.55)
Mild liver disease	2519	(0.87)	9962	(0.7)	1.28	(0.76-2.17)	1.11	(0.61-2.00)
Moderate to severe liver disease	2523	(0.08)	9973	(0.14)	0.62	(0.14-2.85)	1.08	(0.27-4.29)
Chronic pulmonary diseases	2236	(5.37)	9010	(6.57)	0.86	(0.70-1.06)	0.83	(0.67-1.03)
Musculoskeletal and connective tissue diseases	2378	(10.26)	9471	(8.72)	1.24	(1.07-1.45)*	1.20	(1.02-1.40)*
Osteoarthritis	2463	(4.51)	9720	(5.47)	0.90	(0.74-1.10)	0.92	(0.75-1.14)
Rheumatological disease	2456	(3.46)	9698	(4.10)	0.89	(0.70-1.15)	0.86	(0.66-1.11)
Gout	2510	(1.00)	9899	(1.72)	0.64	(0.42-0.97)	0.59	(0.37-0.94)

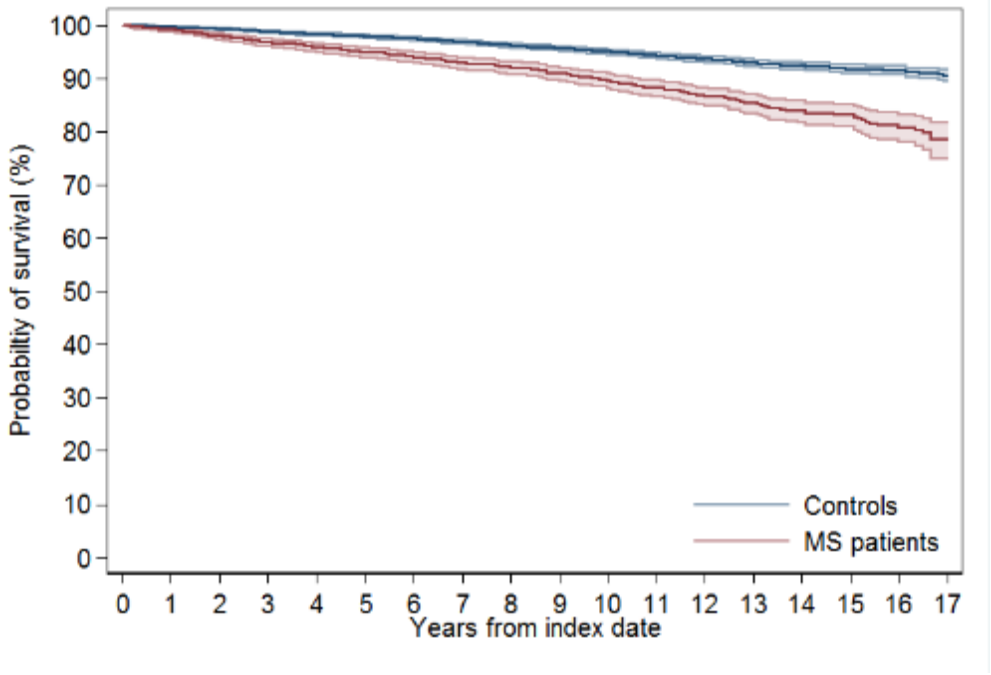
Neurological and mental disorders	1644	(30.54)	8197	(15.08)	2.61	(2.28-2.99)*	2.50	(2.17-2.88)*
Dementia	2526	(0.51)	9975	(0.51)	1.12	(0.56-2.25)	1.28	(0.66-2.49)
Other neurological diseases	2229	(5.29)	9861	(1.53)	4.33	(3.21-5.83)*	4.49	(3.28-6.13)*
Psychosis	2512	(1.00)	9949	(0.42)	2.24	(1.32-3.80)*	2.36	(1.34-4.16)*
Depression	1910	(26.54)	8297	(13.49)	2.43	(2.14-2.76)*	2.32	(2.04-2.64)*
Other comorbidities	2274	(8.62)	9109	(8.54)	1.02	(0.86-1.20)	0.99	(0.83-1.18)
Anaemia	2322	(7.49)	9331	(7.35)	1.01	(0.85-1.21)	0.99	(0.81-1.19)
Psoriasis	2478	(1.74)	9769	(1.73)	1.13	(0.79-1.62)	1.18	(0.80-1.73)
HIV infection	2526	(0.00)	9978	(0.00)	n.a.		n.a.	

Abbreviation: BMI, body mass index
Figures are number of at-risk population (percentage of at-risk population who developed incident comorbidity) and HRs (95% CIs)
†Adjusted for BMI class, smoking status and alcohol consumption
*$p<0.05$; n.a., not applicable

MS was associated with an adjusted HR (95% CI) of 1.13 (1.00–1.29) for having a Charlson comorbidity index ≥ 1. Table 3-6 shows MS patients had a higher risk of developing incident comorbidity including psychosis (adjusted HR 2.36), depression (HR 2.32), and malignancy (HR 1.21). MS patients had a lower risk for gout (HR 0.59).

Figure 3-4 shows the survival curves for MS patients and non-MS controls. After index date, mortality at 5 years and 10 years from any cause was 5.11% and 10.27% in incident MS patients, compared with 2.09% and 5.08% in matched controls (log-rank test, $p<0.0001$) (Table 3-7). MS was associated a crude HR for death of 2.38 (95% CI, 2.02-2.81). The hazard ratio was further adjusted for Charlson comorbidity index at diagnosis as well as for BMI class, smoking status, and alcohol consumption in addition to the matching variables and MS was associated with an HR for all-cause mortality of 2.29 (95% CI, 1.81-2.73; $p<0.05$) (Table 3-8).

Figure 3-4 Survival curve of incident multiple sclerosis (MS) patients (red) and matched non–MS controls (blue).



After index date, cumulative mortality from any cause was higher in incident MS patients than matched controls (log-rank test, p<0.0001).

Table 3-7 Cumulative probability of all-cause mortality after diagnosis of multiple sclerosis

	Patients with incident MS					Controls					
	At diagnosis	1 year	2 years	5 years	10 years	At diagnosis	1 year	2 years	5 years	10 years	p Value
Overall	0	0.89	2.03	5.11	10.27	0	0.38	0.72	2.09	5.08	<.0001
Gender											
Men	0	1.81	2.83	6.36	13.08	0	0.60	0.96	2.71	6.40	<.0001
Women	0	0.51	1.71	4.59	9.12	0	0.29	0.62	1.83	4.54	<.0001

Figures are probability of mortality (percentage)

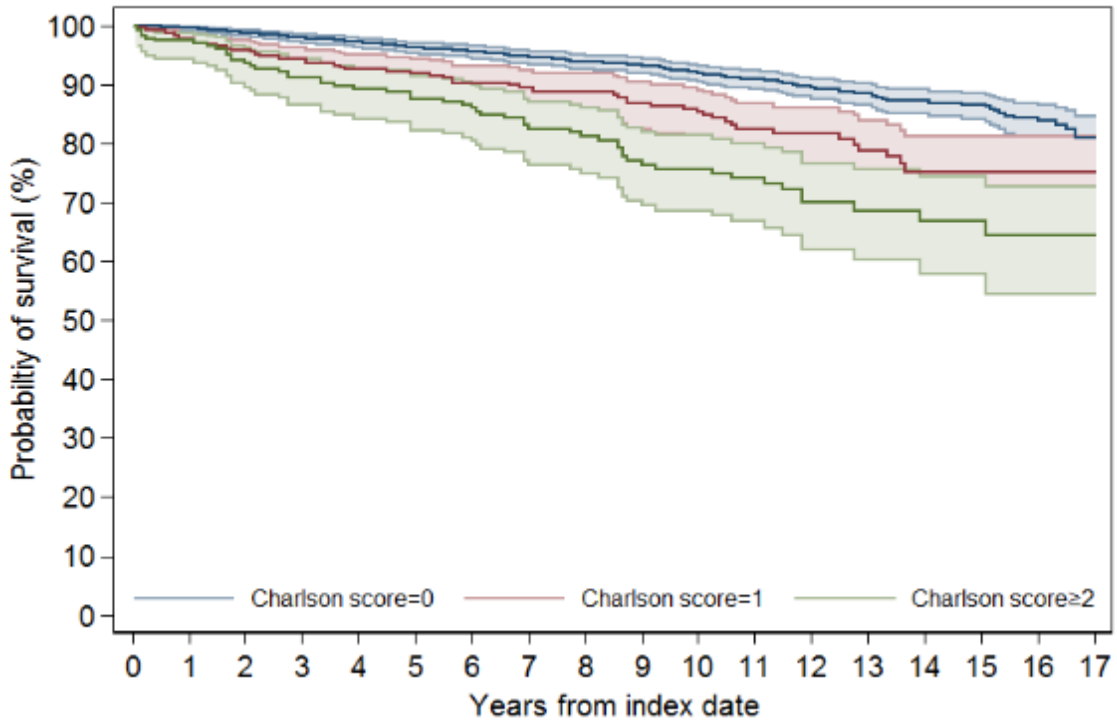
Table 3-8 Predictors for all-cause mortality

	Hazard ratios (95% CI confidence intervals)		
Characteristics	**All patients**	**Men**	**Women**
Model 1: unadjusted	2.38 (2.02-2.81)*	2.56 (1.96-3.33)*	2.28 (1.85-2.82)*
Model 2: adjusted for BMI class, smoking status and alcohol consumption	2.33 (1.98-2.73)*	2.58 (1.99-3.34)*	2.19 (1.78-2.69)*
Model 3: adjusted for BMI class, smoking status and alcohol consumption and all comorbidities at index date	2.29 (1.81-2.73)*	2.65 (1.96-3.59)*	2.27 (1.79-2.86)*

*$p<0.05$

To evaluate the impact of existing comorbidities on survival of MS patients at diagnosis, we further limited analyses within MS patients and evaluated the association between baseline Charlson score and all-cause mortality. As shown in Figure 3-5, all-cause mortality in MS patients at 5-years (95% CIs) was 3.67%, 8.22% and 12.45% for those with a Charlson score of 0, 1 and ≥ 2 at index date, respectively (log-rank test, $p<0.001$). Crude HRs (95% CI) for all-cause death associated with a Charlson score of 1 and ≥ 2 were 1.90 (1.37-2.64) and 2.97 (2.21-3.97), respectively. After the adjustment for BMI class, smoking status and alcohol consumption in addition to the matching variables at diagnosis, Charlson score of 1 and ≥ 2 were associated with adjusted HRs (95% CI) for all-cause death of 1.94 (1.36-2.75) and 3.27 (2.42-4.42), respectively.

Figure 3-5 Survival curve of incident MS patients with a Charlson score of 0 (blue), 1 (red), and ≥ 2 (green) at diagnosis.



After index date, cumulative mortality from any cause was higher in incident MS patients with pre-existing Charlson index 1 (red), or ≥ 2 (green) than those with 0 (blue) (log-rank test, $p<0.0001$).

3.4 DISCUSSION

This study found that approximately a quarter of MS patients already had a Charlson comorbidity index score ≥ 1 at diagnosis comparing to only one-sixth of matched non-MS controls with any Charlson comorbidity. When patients were diagnosed with MS, the prevalence of cerebrovascular diseases, neurological and mental disorders, peripheral vascular diseases, myocardial infarction, hyperlipidaemia, and anaemia were all significantly higher than that in matched non-MS controls. After diagnosis, MS patients were more likely than controls to develop psychosis, depression, and any malignancy. Despite the burden of comorbidity is already higher at diagnosis of MS, the risk of having new comorbid conditions in incident MS patients is not different from controls. These comorbidities at diagnosis affected survival of patients with MS with a dose effect. However, MS itself is associated with increased mortality. Comorbidity is not uncommon in patients with MS and the accumulation of comorbidities in an individual with MS over time complicates the management of the disease. Therefore, a good understanding of comorbid illness in MS is needed to improve health outcomes.

Recently, several systematic reviews summarised previous literature regarding prevalence and incidence of various comorbidities and their associations with MS (Marrie, Reider et al. 2015, Marrie, Reingold et al. 2015). In general comorbidity seems to be common in MS patients but the associations between MS and comorbidity were reported with conflicting results. Various study designs and a limited number of population-based studies are the main causes leading to conflicting results. Furthermore, many previous studies did not explicitly define MS patients in terms of their clinical course relative to initial diagnosis. However, cardiovascular diseases including cardiac arrhythmia, ischaemic heart disease and congestive heart failure (Marrie, Reider et al. 2015), psychiatric disorders (particularly depression) (Marrie, Reingold et al. 2015) and probably certain types of cancer (Marrie, Reider et al. 2015) and autoimmune diseases (Edwards and

Constantinescu 2004, Marrie, Reider et al. 2015) seem to be more common in MS population than a comparator population.

The relationships between coexistence of index disease and comorbidity in the same individual are very complex (Valderas, Starfield et al. 2009). Previous work broadly classified this relationship into three models: direct causation, associated risk factors and heterogeneity (Rhee, Hewitt et al. 2004). In the case of MS, psychiatry problems in patients are not uncommon (Marrie, Reingold et al. 2015). Although the physiopathology is not fully understood, both MS lesions and microstructural white matter changes may lead to psychiatric manifestations (Feinstein, Roy et al. 2004, Feinstein, O'Connor et al. 2010, Lagopoulos, Hermens et al. 2013). For example, previous studies have shown that MS patients with depression have an increased lesion load and more detectable subtle changes in normal appearing brain tissue using diffusion tensor imaging than non-depressed MS patients (Feinstein, Roy et al. 2004, Feinstein, O'Connor et al. 2010). Other factors, such as genetic, biochemical, immunological, and psychosocial factors, have also been implicated for the link between depression and MS (Feinstein, Magalhaes et al. 2014). Our result supports the complex association between MS and psychiatric illnesses.

The relationship between MS and cerebrovascular disease is more complex. MS is an immune-mediated disease targeting the myelin of axons but some evidence also suggests that inflammation to endothelium could contribute to the disruption of the blood-brain-barrier (Wakefield, More et al. 1994, Minagar and Alexander 2003). The association between MS and peripheral vascular disease that is shown in our study also supports that vascular inflammation may be part of MS pathogenesis, while vascular inflammation is a well-known factor contributing to the development of cerebrovascular diseases. In addition, smoking has been found to be a risk factor for both MS (Hedstrom, Baarnhielm et al. 2009, Manouchehrinia, Tench et al. 2013) and cerebrovascular diseases (Shinton and Beevers 1989). Therefore, the association between MS and cerebrovascular disease and peripheral vascular disease may be related by associated risk factors.

Clinical management implications of comorbidity are simpler to address than the complexity of aetiological associations (Feinstein 1970). Firstly, the existence of comorbidity may complicate the management of MS. For example, fingolimod is contraindicated for patients who have recently experienced ischemic heart disease, symptomatic cerebrovascular disease, heart failure, or other cardiovascular problems (Wingerchuk and Carter 2014). Depression in MS patients may result in poorer adherence to treatment (Mohr, Goodkin et al. 1997). Secondly, given that one-third of all MS patients are expected to have one or more comorbidities at 5 years from diagnosis, this study supports the case for an assessment of comorbidities at diagnosis and during their medical follow-up. Thirdly, using comorbidity as an indicator of prognosis is widely used clinically. For instance, the Charlson comorbidity index can be used to estimate comorbidity burden with inference to mortality in MS as shown in this study.

Two main limitations need to be discussed. Firstly, there may be misclassification bias since the identification of MS patients was based purely on physician diagnosis, rather than according to accepted criteria (Poser, Paty et al. 1983, McDonald, Compston et al. 2001, Polman, Reingold et al. 2005). However, the validity of MS diagnosis in the CPRD has been investigated and validated previously (Hernan, Jick et al. 2004). Similarly there may have been misclassification of comorbidities, although again, most of the comorbidities in this study have been validated previously (Herrett, Thomas et al. 2010). Since we used a unified case definition for comorbidities in both MS patients and non-MS controls, the potential differential misclassification between MS patients and non-MS controls is expected to be kept to a minimum. Also, ascertainment bias between incident MS patients and controls cannot be excluded entirely. However, the observation periods before and after the index date were similar for cases and controls. Second, some of potential confounders, such as race, education, annual household income, marital status, and treatment, were not included. However, this study was strengthened by considering the timing of disease onset and the burden of various comorbidities in the disease course of MS and by

comparing age, gender, and registered general practice matched non-MS controls from the general population for the robust risk estimation.

3.5 CONCLUSIONS

A higher proportion of MS patients in the UK already have comorbidity at MS diagnosis, especially neurological and mental disorders and cardiovascular diseases, compared to a matched general population. After MS diagnoses were made, the patients have higher risks of comorbid neoplasms in addition to neurological and mental disorders. All-cause mortality is higher in MS patients and the increased risk is attributed to both MS and comorbidities.

Chapter 4. RISK OF EPILEPSY IN INCIDENT PATIENTS WITH MULTIPLE SCLEROSIS PRIOR TO AND FOLLOWING DIAGNOSIS

4.1 INTRODUCTION

In the previous chapter, we studied comorbidities of MS and found that they are associated with neurological comorbidities. Cortical lesions are not uncommon in MS., The demyelinated cortical lesions are more common in secondary progressive and primary progressive MS than relapsing remitting MS (Kutzelnigg, Lucchinetti et al. 2005). Epilepsy, generalizing from disturbed neuronal activities, has been reported to be higher in MS than in general population. In a hospital-based study in Norway, 'active' epilepsy was estimated to affect 7.8% of MS patients in 2003 (Lund, Nakken et al. 2014) while the prevalence of active epilepsy in the general population was estimated to be 0.82% (Svendsen, Lossius et al. 2007). Several hospital-based studies also reported that MS patients were about 3 times more likely to have epilepsy than the general population (Olafsson, Benedikz et al. 1999, Nicoletti, Sofia et al. 2003, Cheng, Wai et al. 2012). In contrast, a population-based study in Rochester Minnesota did not find any difference in the incidence of epilepsy between MS patients and the general population (Nyquist, Cascino et al. 2002). Because previous epidemiologic studies reported conflicting results on the risk of epilepsy in MS patients, we aimed to focus on epilepsy to analysis whether epilepsy is a comorbid disease in MS population or just a coincident disease.

Using data representative of the UK general population from the CPRD, this chapter aimed to estimate the prevalence of epilepsy at the time of the MS diagnosis compared to matched controls without MS who were identified from a population in a primary care setting. We further followed incident MS patients and their matched controls after initial MS diagnosis to compare their subsequent risks of developing epilepsy. We hypothesised that MS patients have a higher risk of epilepsy when they were diagnosed and that the risk of epilepsy would continue to rise with time after the diagnosis of MS.

4.2 METHODS

This was a case-control study using both retrospective (prior to diagnosis) and subsequent follow-up data (after diagnosis) within the CPRD. The study was approved by the Independent Scientific Advisory Committee for the Medicines and Healthcare Products Regulatory Agency Database Research (protocol 14-070).

4.2.1 Data source

The CPRD is a primary care database in the UK, which contains prospectively collected medical records of research standard from around 16.5 million individuals registered in over 701 general practices (2016 version). For detail please refer to 3.2.1. and 1.7.3.

4.2.2 Study population

Our source population comprised all participants who contributed data to the CPRD between 1st January 1997 and 31th December 2006. For more detail please refer to 3.2.2

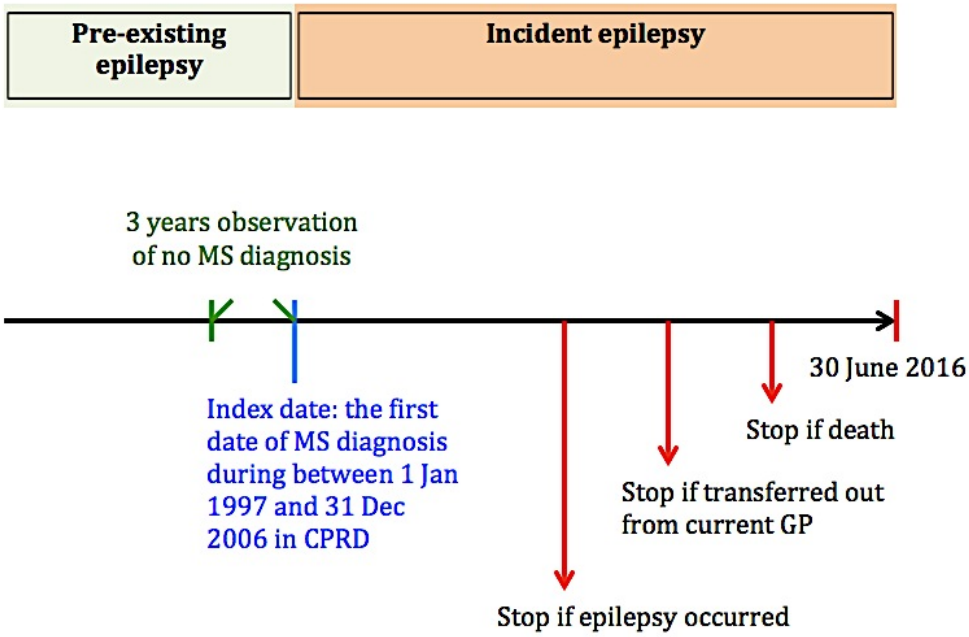
4.2.3 Selection of controls

From CPRD participants who had an active registration from 1997 to 2006, we identified up to 4 random controls per case who had no record of MS in the CPRD. For more detail please refer to 3.2.3 and Figure 3-1.

4.2.4 Study period

We defined two periods to assess epilepsy: (a) the 10 years prior to diagnosis and (b) from diagnosis to the earliest date of occurrence of the diagnosis of epilepsy, death, transfer out, last data collection time for practice or end of study (30 June 2016), whichever came first (figure 4-1).

Figure 4-1 The observation of epilepsy in an incident MS patient.



A patient is defined as an incident case if the diagnosis of MS was recorded between 1997 and 2006 and no priori MS diagnosis for ≥ 3 years. The pre-existing epilepsy were observed since 10 years before the first MS diagnosis (index date) and the incident epilepsy were those occurred afterwards until 30 June 2016. Abbreviations: MS, multiple sclerosis; GP, general practitioner; CPRD, Clinical Practice Research Datalink

4.2.5 Definition of epilepsy

A physician diagnosis of epilepsy was the primary outcome of interest. We developed the code lists of epilepsy (Table 4-1), based on opinions of two senior neurologists and a general practitioner. We were unable to validate epilepsy diagnosis in this study. Therefore, we performed a sensitivity analysis by using an alternative case definition based on a physician diagnosis (Read code) plus a prescription of antiepileptic drug (AED) use.

We performed two sensitivity analyses. One included AEDs that were used by more than 1% of patients with epilepsy in the UK according to a previous CPRD study. The AEDs included were valproate, carbamazepine, lamotrigine, phenytoin, levetiracetam, phenobarbital, clobazam,

topiramate, gabapentin, clonazepam, primidone, and pregabalin (Irizarry 2011). The other included the aforementioned AEDs except for gabapentin and pregabalin as both can be prescribed mainly for alleviating neurological pain rather than active epilepsy.

4.2.6 Covariates

Covariates included general characteristics (age, gender, body mass index [BMI]), lifestyle factors (smoking status and alcohol consumption) and Charlson comorbidity index, which were derived from 17 diagnostic categories (for detail please refer to Table 3-2) (Deyo, Cherkin et al. 1992). Charlson comorbidity index has been strongly associated with mortality in several medical conditions (Charlson, Szatrowski et al. 1994) and multiple sclerosis (for more detail please refer to chapter 3). Only GP records within the 10-year period before initial diagnosis of MS were used to evaluate Charlson comorbidity index scores. BMI, smoking and alcohol consumption have been recognised as potentially modifiable factors affecting MS risk (Sundstrom, Nystrom et al. 2008, Hedstrom, Olsson et al. 2012, Hedstrom, Hillert et al. 2014) and possibly epilepsy (Daniels, Nick et al. 2009, Dworetzky, Bromfield et al. 2010, Leach, Mohanraj et al. 2012). The definition of these categories was based on physician diagnoses recorded as a list of Read codes, as previously described (Deyo, Cherkin et al. 1992, Kuo, Grainge et al. 2014).

Table 4-1 Read Code for epilepsy

Read Code	Description
F132100	Progressive myoclonic epilepsy
F25..00	Epilepsy
F250.00	Generalised nonconvulsive epilepsy
F250000	Petit mal (minor) epilepsy
F250100	Pykno-epilepsy
F250400	Juvenile absence epilepsy
F250y00	Other specified generalised nonconvulsive epilepsy
F250z00	Generalised nonconvulsive epilepsy NOS
F251.00	Generalised convulsive epilepsy
F251000	Grand mal (major) epilepsy
F251011	Tonic-clonic epilepsy
F251500	Tonic-clonic epilepsy

F251y00	Other specified generalised convulsive epilepsy
F251z00	Generalised convulsive epilepsy NOS
F254.00	Partial epilepsy with impairment of consciousness
F254000	Temporal lobe epilepsy
F254100	Psychomotor epilepsy
F254200	Psychosensory epilepsy
F254300	Limbic system epilepsy
F254z00	Partial epilepsy with impairment of consciousness NOS
F255.00	Partial epilepsy without impairment of consciousness
F255000	Jacksonian, focal or motor epilepsy
F255011	Focal epilepsy
F255012	Motor epilepsy
F255100	Sensory induced epilepsy
F255200	Somatosensory epilepsy
F255300	Visceral reflex epilepsy
F255311	Partial epilepsy with autonomic symptoms
F255400	Visual reflex epilepsy
F255500	Unilateral epilepsy
F255y00	Partial epilepsy without impairment of consciousness OS
F255z00	Partial epilepsy without impairment of consciousness NOS
F257.00	Kojevnikov's epilepsy
F25A.00	Juvenile myoclonic epilepsy
F25B.00	Alcohol-induced epilepsy
F25C.00	Drug-induced epilepsy
F25D.00	Menstrual epilepsy
F25E.00	Stress-induced epilepsy
F25F.00	Photosensitive epilepsy
F25y.00	Other forms of epilepsy
F25y000	Cursive (running) epilepsy
F25y100	Gelastic epilepsy
F25y400	Benign Rolandic epilepsy
F25yz00	Other forms of epilepsy NOS
F25z.00	Epilepsy NOS
Fyu5000	[X]Other generalized epilepsy and epileptic syndromes
Fyu5100	[X]Other epilepsy

4.2.7 Statistical analysis

The prevalence of epilepsy at index date was calculated by dividing the number of people diagnosed with epilepsy before the index date (numerator) and the number of incident MS patients or non-MS controls (denominators). Odds ratios (OR) and 95% confidence intervals (CI) were used to estimate the association between MS and epilepsy at index date using conditional logistic

regression adjusting for age, gender, index year, body mass index (BMI) category, smoking status, alcohol consumption and Charlson comorbidity index. Missing data for BMI, smoking and alcohol status were coded as ‘unknown’. Kaplan–Meier plots were used to estimate the probability of epilepsy in people with incident MS and those without MS at 1, 5, and 10 years following diagnosis. Log-rank test was used to compare the probability of epilepsy between MS patients and non-MS controls. Only people at risk for epilepsy (not having epilepsy at index date) were considered to estimate the hazard ratios (HR) for developing an epilepsy. HRs and 95% CIs were calculated for incident epilepsy using a Cox proportional hazards model. The hazard ratios were adjusted by age, gender, index year, BMI categories, smoking status, alcohol consumption and Charlson comorbidity index. All statistical analyses were performed using SAS statistical software, version 9.3.

4.3 RESULTS

We identified 2,526 incident MS patients during 1997 to 2006 with a mean age of 45.0±12.4 years, 70.9% being women. The median observation period (interquartile range) was 12 (7–21) and 10 (5–13) years before and after the index date, respectively. They were matched to 9,980 controls with the similar age and gender structure and observation periods before and after index date (Table 4-2).

Table 4-2 Characteristics of incident multiple sclerosis (MS) patients and matched non-MS controls.

Characteristics	**Incident MS (n = 2,526)**	**Non-MS controls (n = 9,980)**	**Adjusted[a] odds ratios (95% CI)**
Age (years, SD)	45.04±12.37	45.17±12.43	
Women (n, %)	1,790 (70.86)	7,070 (70.84)	
Median observation (years, interquartile range)			
Prior to index date	12 (7–21)	12 (7–20)	
After index date	10 (5–13)	10 (6–13)	
BMI (kg/m2)			
<25.0	1052 (41.65)	3621 (36.28)	Reference
25.0 – 29.9	685 (27.12)	2670 (26.75)	0.88 (0.79–0.98)*
≥30	469 (18.57)	1871 (18.75)	0.86 (0.76–0.97)*
Unknown	320 (12.67)	1818 (18.22)	0.57 (0.49–0.66)*
Smoking			
Non-smoker	720 (28.5)	2953 (29.59)	Reference
Current smoker	554 (21.93)	1542 (15.45)	1.53 (1.34–1.75)*
Ex-smoker	878 (34.76)	3261 (32.68)	1.09 (0.97–1.24)
Unknown	374 (14.81)	2224 (22.28)	0.62 (0.54–0.72)*
Alcohol consumption (units/week)			
Never/ Ex-drinker	333 (13.18)	1085 (10.87)	Reference
Current 1-9	1245 (49.29)	4523 (45.32)	0.90 (0.78–1.03)
Current ≥10	344 (13.62)	1321 (13.24)	0.83 (0.68–1.01)
Unknown	604 (23.91)	3051 (30.57)	0.59 (0.50–0.70)*
Charlson comorbidity index			
0	1952 (77.28)	8303 (83.2)	Reference
1-2	501 (19.83)	1518 (15.21)	1.42 (1.27–1.60)*
≥ 3	73 (2.89)	159 (1.59)	1.99 (1.49–2.66)*

Values are numbers (percentages) unless stated otherwise.
a. adjusted by age, gender and index year; *P<0.05

4.3.1 Retrospective observation

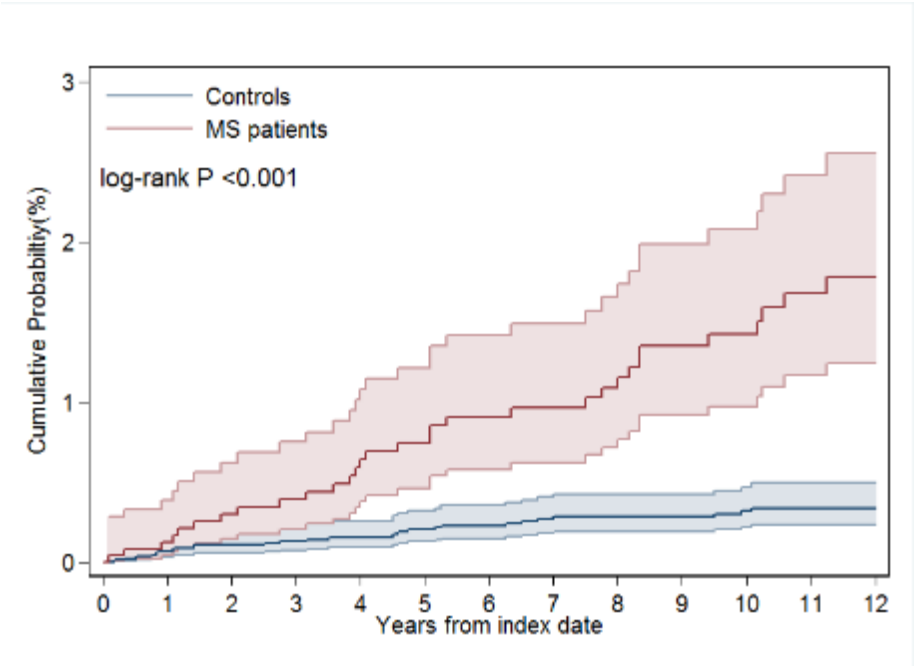
On the index date, 33 MS patients (21 female) already had an epilepsy diagnosis, equivalent to a prevalence of epilepsy of 1.30% (95% CI, 0.90%–1.83%). We also identified 57 controls with

epilepsy (38 female), with a significantly lower prevalence of 0.57% (95% CI, 0.43%–0.74%; <0.0001). In an adjusted logistic regression model, MS was associated with a crude OR (95% CI) of 2.27 (1.47–3.50) for epilepsy, with no significant gender difference (men: OR, 2.42 [95% CI, 1.17-5.00]; women, OR, 2.19 [95% CI, 1.28–3.77]). After adjusting for age, index year, BMI, smoking, alcohol consumption and Charlson comorbidity index, OR was 2.11 (1.36–3.27).

4.3.2 Follow-up data after index date

Figure 4-2 shows the cumulative probability of first recorded epilepsy in MS patients and non-MS controls who were epilepsy-free at index date (log-rank test, $p<0.001$). The cumulative probabilities of first recorded epilepsy at 1, 5 and 10 years from index date were 1.46%, 2.07% and 2.77% in incident MS patients and 0.65%, 0.78% and 0.90% in controls.

Figure 4-2 The cumulative probability of first recorded epilepsy in MS patients (red) and non-MS controls (blue).



Cumulative probability of first recorded epilepsy is higher in incident MS patients than that in matched controls (log-rank test, p<0.001).

Next we investigated relative risk of incident epilepsy diagnosis in MS patients compared to non-MS controls. Using Cox proportional hazard model, MS was associated with an unadjusted HR (95% CI) of 4.53 (2.66–7.72) for epilepsy. Men had a higher HR (6.25; 95% CI, 2.51–15.53) for epilepsy than women (3.98; 95% CI, 2.10–7.54), but these were not statistically different. After adjusting for age, index year, BMI, smoking, alcohol consumption and Charlson comorbidity index, MS was associated with an HR (95% CI) of 6.01 (2.94-12.29) (Table 4-3).

Table 4-3 Cox proportional hazard models examining relative risks of the first recorded epilepsy in patients with incident MS compared to matched controls.

Variables	Crude hazard ratios (95% CI)	Adjusted hazard ratios (95% CI)
Multiple sclerosis	4.53 (2.66-7.72)*	6.01 (2.94-12.29)*
BMI (kg/m2)		
<25.0	Reference	Reference
25.0 – 29.9	2.31 (1.20-4.46)*	4.83 (1.90-12.27)*
≥30	1.24 (0.53-2.91)	1.73 (0.56-5.30)
Unknown	0.65 (0.19-2.21)	2.49 (0.50-12.53)
Smoking		
Non-smoker	Reference	Reference
Current smoker	0.53 (0.24-1.17)	0.58 (0.20-1.64)
Ex-smoker	0.76 (0.31-1.83)	0.76 (0.21-2.73)
Unknown	0.25 (0.09-0.70)*	0.10 (0.02-0.51)*
Alcohol consumption (units/week)		
Never/ Ex-drinker	Reference	Reference
Current 1-9	0.92 (0.38-2.26)	0.58 (0.16-2.08)
Current ≥10	1.17 (0.52-2.65)	0.79 (0.24-2.59)
Unknown	0.52 (0.19-1.38)	1.39 (0.54-3.56)
Charlson comorbidity index		
0	Reference	Reference
1-2	2.20 (1.04-4.65)*	3.69 (1.31-10.44)*
≥ 3	3.97 (1.13-13.88)*	3.66 (1.00-13.36)*

Footnote: a. adjusted by age, gender, registered general practitioner and index year; *p<0.05.

Values are numbers (percentages) unless stated otherwise.

4.3.3 Sensitivity analysis

Using the alternative case definition of epilepsy (a diagnosis plus a prescription of AEDs), we identified 31 MS patients who already had epilepsy, equivalent to a prevalence of epilepsy of 1.23% (95% CI, 0.84%–1.74%), and 44 controls who already had epilepsy, with a significantly lower prevalence of 0.44% (95% CI, 0.32%–0.59%; p<0.0001). Using a logistic regression model

adjusted for BMI, smoking, alcohol consumption and Charlson comorbidity index, MS was associated with an OR (95% CI) of 2.40 (1.37-4.22). Among those without epilepsy at baseline, 31 MS patients and 20 controls developed epilepsy after the index date. Using Cox proportional hazard model, MS was associated with an adjusted HR of 6.79 (95% CI, 3.78–12.18).

In the second set of sensitivity tests using case definition of epilepsy with a diagnosis plus a prescription of AEDs excluding gabapentin and pregabalin, we identified 28 MS patients who already had epilepsy, equivalent to a prevalence of epilepsy of 1.11% (95% CI, 0.74%–1.60%), and 43 controls who already had epilepsy, with a significantly lower prevalence of 0.43% (95% CI, 0.31%–0.58%; $p<0.0001$). Using a logistic regression model adjusted for BMI, smoking, alcohol consumption and Charlson comorbidity index, MS was associated with an OR (95% CI) of 2.37 (1.33-4.24). Among those without epilepsy at baseline, 31 MS patients and 16 controls developed epilepsy after the index date. Using Cox proportional hazard model, MS was associated with an adjusted HR of 8.64 (95% CI, 4.63-16.09).

4.4 DISCUSSION

Based on a large UK primary care database, this study compared the risk of epilepsy at diagnosis in MS patients with non-MS controls and estimated the risks of incident epilepsy following diagnosis. Approximately one in 80 incident MS patients already had epilepsy at diagnosis, which is twice more likely than for controls. After 10 years of follow-up, 2.77% of epilepsy-free MS patients at diagnosis were diagnosed with epilepsy. After adjusting for age, gender, registered general practice, index year, BMI, smoking, alcohol consumption and Charlson comorbidity index, MS was associated a 6-fold risk of epilepsy. To our knowledge, this is the first population-based longitudinal epidemiological study to examine the risk of epilepsy in MS patients compared to matched non-MS controls. Overall, our results confirm the higher risk of epilepsy in MS patients at diagnosis and show a higher risk of developing epilepsy after diagnosis than in non-MS controls from general population.

Although the absolute risk of a patient with MS developing an epilepsy is not high, it is significantly higher relative to matched controls without MS. A recent systematic review summarised data from 24 studies and estimated that the prevalence of seizure disorders in MS patients ranged from 0.89% to 8.06% with high heterogeneity among studies (Marrie, Reider et al. 2015). A recent study in Norway by Lund et al. estimated a prevalence of epilepsy in 7.4% of MS population in 2003 (Lund, Nakken et al. 2014). Several studies reported a comparison of epilepsy risk in MS patients and controls. In general, the risk ratio between MS and controls ranged from one to 16 folds (Kinnunen and Wikstrom 1986, Engelsen and Gronning 1997, Olafsson, Benedikz et al. 1999, Eriksson, Ben-Menachem et al. 2002, Nyquist, Cascino et al. 2002, Nicoletti, Sofia et al. 2003, Adelow, Andersson et al. 2012, Allen, Seminog et al. 2013, Marrie, Reider et al. 2015). For example, Allen et al. found that MS patients has 3 to 4-fold higher risk of hospital admissions for epilepsy than other patients without MS, using both Oxford Record Linkage Study and English National Hospital Episode Statistics datasets (Allen, Seminog et al. 2013). A study from Taiwan

comparing epilepsy prevalence in 898 MS patients and 4,490 matched controls identified in a single year reported that MS was associated with an OR of 16 for epilepsy (Kang, Chen et al. 2010). Several studies also estimated incidence of epilepsy in MS patients. For example, Nicoletti et al identified four incident cases of epilepsy in 170 MS patients after onset of MS (Nicoletti, Sofia et al. 2003). These studies suggest that epilepsy is not uncommon in MS patients. However, the association between MS and epilepsy cannot be conclusively made because their results are hampered by selection bias due to dependence on hospital records to identify patients, a lack of proper population controls, and not considering the timing of epilepsy onset relative to the MS diagnosis.

The current study utilised data from the CPRD, which is representative of primary care population in the UK, to identify incident MS patients and matched non-MS controls selected from a primary care population therefore ensuring a minumum selection bias and a robust control. In addition, our design considered the timing of both MS diagnosis and epilepsy onset. Therefore we were able to estimate the risk of epilepsy in MS patients compared to the general population from diagnosis of MS to 10 years later. We found that MS patients already had a higher risk of epilepsy at the time of diagnosis and the risk was also higher after the diagnosis. Our findings support that concurrence of MS and epilepsy may not simply be a chance association. One hypothesis is that epilepsy is probably an integral part of MS, and close clinical follow-up and observation seems warranted.

The pathogenesis of epilepsy in MS remains elusive, although it seems relate to cortical and subcortical lesions. Thompson et al. reported seven MS patients with acute seizures and, at the same time, the magnetic resonance imaging (MRI) revealed correlated new evolving or enhancing lesions involving the cortex or subcortical area (Thompson, Kermode et al. 1993). One of seven patients underwent lesion biopsy, which revealed extreme oedema and subacute demyelination. Four of these patients had recurrent seizures and were treated for epilepsy, and two of them still had recurrent seizure attacks. The authors concluded that acute seizures might disappear as the

resolution of acute oedema of lesions, whilst recurrence of seizures may occur due to a damaged cerebral structure that becomes epileptogenic (Thompson, Kermode et al. 1993).

The hypothesis of epilepsy associated with focal cortical and subcortical lesions in MS is further supported by recent studies applying MRI (Calabrese, De Stefano et al. 2008, Calabrese, Grossi et al. 2012, Martinez-Lapiscina, Ayuso et al. 2013). Using T2 weighted and fluid attenuated inversion recovery (FLAIR) sequences at 1.5 Tesla (T) field strength MRI retrospectively, Martínez-Lapiscina et al. (Martinez-Lapiscina, Ayuso et al. 2013) found that 13 MS patients with epilepsy had a greater number of cortical or subcortical lesions than 26 matched MS patients without epilepsy. Calabrese et al. conducted both cross-sectional (Calabrese, De Stefano et al. 2008) and longitudinal (Calabrese, Grossi et al. 2012) studies using 1.5T double inversion recovery (DIR) sequences on relapsing-remitting (RR) MS patients, and showed that intracortical lesion loads were greater in MS patients with epilepsy than those without. In addition, patients with epilepsy showed a higher accumulation rate of new cortical lesions and faster reduction of grey matter fraction (Calabrese, Grossi et al. 2012).

However, the authors did not find anatomical association between intracortical lesions and EEG patterns or foci (Calabrese, De Stefano et al. 2008). They reported that in eight of twenty patients' inter-ictal EEG were essentially normal, and five of them showed diffuse spike-and-wave bursts with clinical generalized seizures. This observation implies that cortical or subcortical demyelination is not the only focus for epilepsy. Thalamus was shown to be involved in the discharges of diffuse spike-and-wave bursts, like the bilaterally symmetric activation of thalami among people with idiopathic generalized epilepsy (Aghakhani, Bagshaw et al. 2004), or symptomatic epilepsy associated with focal thalamic lesion (Kelemen, Barsi et al. 2006). Thalamic axons connect axons of cortical and subcortical area. Thalamic involvement might partially explain that cortical lesions are frequently found histopathologically in MS (Kutzelnigg, Lucchinetti et al.

2005, Lucchinetti, Popescu et al. 2011) but the absolute risk of developing epilepsy in MS is relatively small.

The mechanism whereby some of the MS lesions becoming epileptogenic is unknown. A recent histopathology study showed decreased immunostaining of Kir4.1 proteins in acute demyelinating and chronic active demyelinated lesions of the subcortical regions in MS (Schirmer, Srivastava et al. 2014). The Kir4.1 proteins are part of potassium channels localising at end-feet of astrocytes and oligodendrocytes, and loss of function of this channel increases seizure susceptibility in patients with mesial temporal epilepsy (Heuser, Eid et al. 2012). In MS, the pathogenesis of decrease of Kir4.1 proteins in demyelinated lesions is still elusive (Brickshawana, Hinson et al. 2014, Schirmer, Srivastava et al. 2014), but may be related to the presence of anti-Kir4.1 antibodies in a small proportion of people with MS (Srivastava, Aslam et al. 2012, Kraus, Srivastava et al. 2014), and these antibodies deplete the Kir4.1 protein in a similar manner to the depletion of AQP4 by ant-AQP4 antibodies in NMO/NMOSD (Lennon, Kryzer et al. 2005, Guo, Weigand et al. 2017).

The long-term prognosis of MS patients with epilepsy compared to those without epilepsy is not known. It has been suggested that the majority of MS patients with epilepsy fall into two major groups: those in whom epilepsy is a clinical manifestation associated with MS onset or relapse; and patients in whom epilepsy is associated with progressive cognitive involvement and disability (Spatt, Chaix et al. 2001). The occurrence of epilepsy has been reported not only in patients with RRMS but also in patients with secondary progressive (SPMS) and primary progressive (PPMS) (Catenoix, Marignier et al. 2011). Of note, the RRMS patients with epilepsy in the Calabrese et al. study had a more pronounced cognitive decline and a higher disability over 3 years (Calabrese, Grossi et al. 2012). The increased probability of having epilepsy at 5 and 10 years after MS diagnosis suggests that new-onset epilepsy during the MS disease course can be a sign of disease progression. Further studies are needed to monitor cognition and disease progression in this group

of MS patients, as cognitive decline may be more pronounced due to grey matter involvement, seizure recurrence and use of AEDs treatments.

There are several limitations to this study. Firstly, misclassification bias may occur since the identification of MS patients was based on physician diagnosis, rather than according to accepted criteria (Poser, Paty et al. 1983, McDonald, Compston et al. 2001, Polman, Reingold et al. 2005). However, the diagnosis of MS in the CPRD has been investigated and validated previously (Hernan, Jick et al. 2004, Alonso, Jick et al. 2005). Similarly there may have been some misclassification of epilepsy. However, we did a sensitivity analysis and found similar results. Furthermore, the models did not adjust for all potential risk factors for epilepsy, such as family history, previous head injuries, vascular diseases or history of epilepsy in childhood. However, part of these risk factors was accounted for by the Charlson comorbidity index, as it incorporated 17 different categories of illnesses. In addition, with such a large effect size of 4, it is unlikely that any or all of these potential sources of bias explain our findings completely. Furthermore, limited evidence suggests that some disease-modifying treatment (DMT) and symptomatic treatment may increase the risk of epilepsy (Kelley and Rodriguez 2009). We did not examine the use of DMT in this cohort because DMT are given in secondary-care, and data on their use is not completely linked in the current dataset.

4.5 CONCLUSION

A fraction of MS patients (approximately 1%) in the UK already have epilepsy at diagnosis. In epilepsy-free patients, the risk of incident epilepsy was also higher after diagnosis, affecting nearly 3% by 10 years. This six-fold increased risk of epilepsy is not widely recognised, but is important for patient counselling and for their medical follow-up.

Chapter 5. INCREASED MORTALITY IN NEUROMYELITIS OPTICA COMPARED TO THE GENERAL POPULATION AND MULTIPLE SCLEROSIS: A NATIONWIDE POPULATION CASE-CONTROL STUDY

5.1 INTRODUCTION

In the previous chapters of this thesis we found that the recording ratio of NMO/NMOSD in primary care is lower than MS when comparing to datasets using CPRD plus HES (chapter 2). We proposed that one possibility of the low recording ratio is because the disease severity of patients with NMO/NMOSD is higher, needing more hospitalisation, than patients with MS. To test this hypothesis, in this chapter we use associated comorbidity and mortality as proxies to compare disease severity between NMO/NMOSD and MS.

Patients with NMO/NMOSD, compared to those with MS, are thought to be more disabled and have a poor functional recovery in terms of visual acuity (Pirko, Blauwet et al. 2004) and mobility (Cabre, Heinzlef et al. 2001). Among patients with NMO/NMOSD, the 5-year mortality rate was estimated to be 15% and 32% of the prevalent patients with NNO/NMOSD in the geographic Caribbean database (including Cuba and French West Indies) (Cabre, Gonzalez-Quevedo et al. 2009) and at the Mayo clinic of the United States (Wingerchuk, Hogancamp et al. 1999), respectively. Patients with NMO/NMOSD are prone to death due to respiratory failure at disease onset or during subsequent relapses (Wingerchuk, Hogancamp et al. 1999). Wingerchuk et al showed that 18 of 80 patients died due to cervical myelitis associated respiratory failure (Wingerchuk and Weinshenker 2003). Although, the overall mortality of NMO/NMOSD is observed to be high, a prospective study to examine the mortality and compare it between NMO/NMOSD and general population or MS is lacking.

In patients with MS, comorbidity is associated with increased mortality compared to MS without comorbidity (Bronnum-Hansen, Koch-Henriksen et al. 2004, Lalmohamed, Bazelier et al. 2012, Krokki, Bloigu et al. 2014, Marrie, Elliott et al. 2015). NMO/NMOSD is associated with several comorbidities, of which other autoimmune diseases are the most commonly reported. Iyer et al

reviewed the literature and reported that about 20-30% of patients had coexisting autoimmune diseases before or after the diagnosis of NMO/NMOSD (Iyer, Elsone et al. 2014). There are at least 22 autoimmune conditions coexisting with NMO/NMOSD, including myasthenia gravis, systemic lupus erythematosus, antiphospholipid antibody syndrome (Vernant, Cabre et al. 1997, Wingerchuk, Hogancamp et al. 1999, Lucchinetti, Mandler et al. 2002, Pittock, Lennon et al. 2008, Iyer, Elsone et al. 2014). Some symptoms of the coexisting autoimmune conditions might be misclassified as treatment side effects or part of NMO/NMOSD (Iyer, Elsone et al. 2014). Other neurological conditions, such as posterior reversible encephalopathy syndrome (Magana, Matiello et al. 2009) have been reported in patients with NMO/NMOSD. The aforementioned comorbidity complicates the disease diagnosis and management (Pittock, Lennon et al. 2008), and influences quality of life of the patients affected (Zhao, Mutch et al. 2014), and increases the risk of death (Wingerchuk and Weinshenker 2003). The overall effects of comorbidity on survival in patients with NMO/NMOSD have rarely been evaluated.

The current study aims to evaluate the comorbidity of NMO/NMOSD, and to compare the disease severity between NMO/NMOSD and MS using mortality as a proxy measure. Firstly, we hypothesised that NMO/NMOSD patients have a higher risk of comorbidities at diagnosis and also have a higher risk of developing comorbidities following diagnosis. Secondly, we hypothesised that the amount of comorbidity at NMO/NMOSD diagnosis has a dose effect on survival. Thirdly, we hypothesised that NMO/NMOSD has a higher mortality than the general population. Finally, we hypothesised that NMO/NMOSD has a higher mortality risk than MS. This study prospectively observes new cases of NMO/NMOSD during 1997-2016 in the Clinical Practice Research Data-link (CPRD), and further followed these incident patients and matched general population or matched MS patients to compare their all-cause mortality.

5.2 METHODS

This case-control study used retrospective data prior to diagnosis and also subsequent follow-up data (after diagnosis) within the CPRD. The study was approved by the Independent Scientific Advisory Committee for the Medicines and Healthcare Products Regulatory Agency Database Research (protocol 14-070).

5.2.1 Data source

The CPRD is a database focusing on primary care in the UK, which contains prospectively collected medical records of research standard from around 16.5 million individuals registered in 701 general practices (2016 version). For details please refer to 3.2.1.

5.2.2 Definition of incident NMO/NMOSD

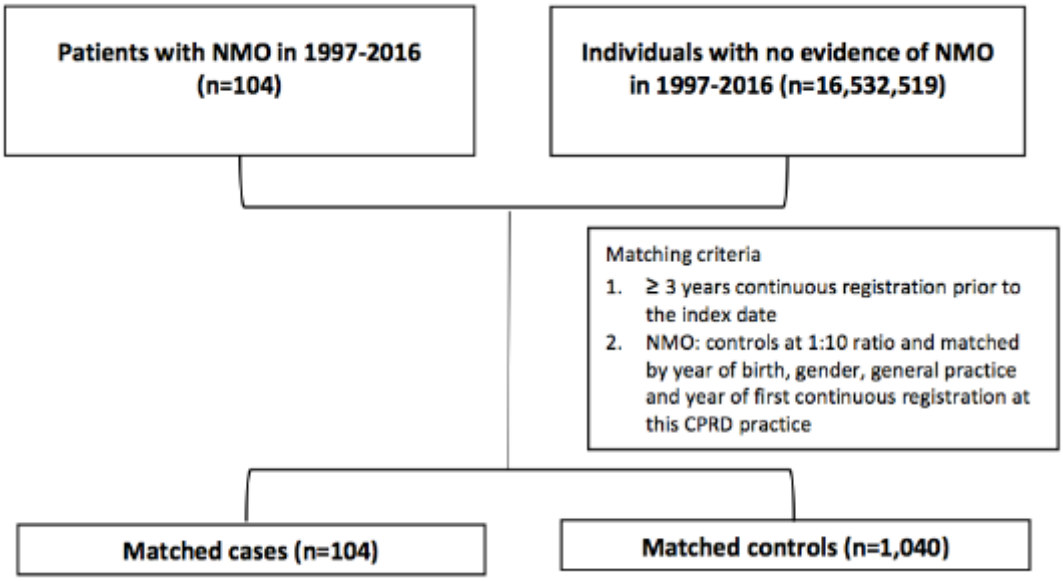
Our source population comprises all active participants who contributed data to the CPRD between 1 January 1997 and 30 June 2016. This data range was chosen for consideration of sample size and length of follow-up. Physician-diagnosis based on Read codes indicative of NMO/NMOSD were used to identify cases. Read code F201.00 represents neuromyelitis optica and F210.11 Devic's disease. To be eligible as an incident NMO/NMOSD patient, participants had to have no history of NMO/NMOSD and to have at least a three-year continuous registration prior to the index date (Hernan, Jick et al. 2004, Cea Soriano, Rothenbacher et al. 2011).

5.2.3 Selection of controls from general population

From CPRD participants who had an active registration from 1 January 1997 to 30 June 2016, we identified at random up to 10 controls per case who had no record of NMO/NMOSD diagnosis matched by year of birth (±2 years), gender, general practice, and year of first continuous registration (±2 years) at this CPRD practice (Figure 5-1). The design of matching general practice

and year of first continuous registration is to control for possible confounding by the general practice with which participants were registered. The same index date was assigned to each of the matched controls. As with incident NMO/NMOSD patients the matched controls had at least three years of registration prior to the index date.

Figure 5-1 Flowchart of eligible NMO/NMOSD patients and matched controls.

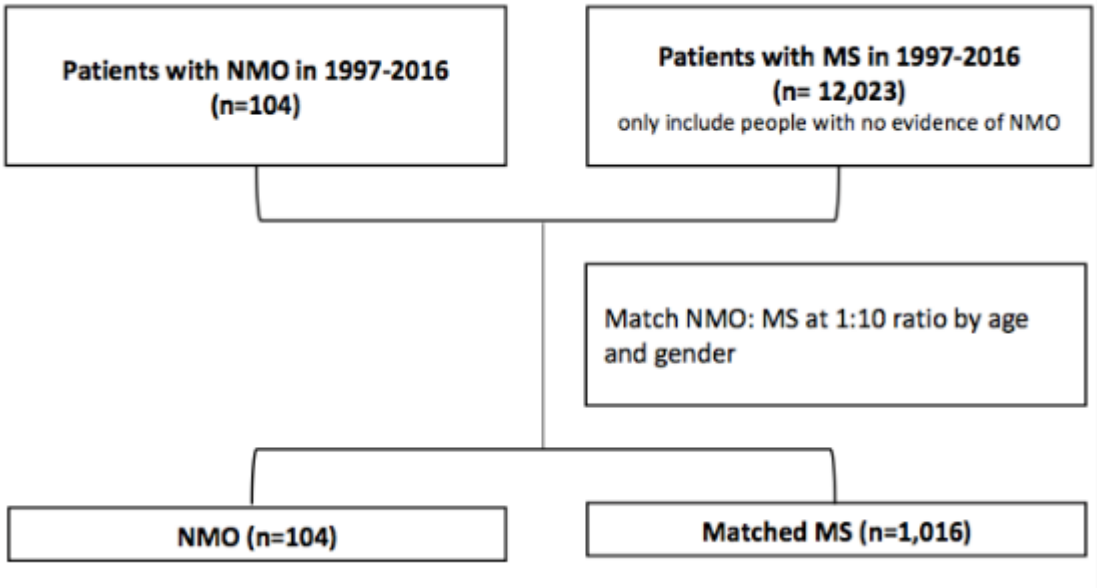


The study comprised incident NMO/NMOSD patients and 10 matched controls who were continuously registered for at least three years before the index date, which date NMO/NMOSD was firstly recorded during 1997 to 2016.

5.2.4 Selection of controls from patients with multiple sclerosis

From CPRD participants who had an at least 3-year active registration from 1 January 1997 to 30 June 2016, we identified at random up to 10 MS patients per case who had no record of NMO/NMOSD diagnosis, matched by year of birth (±2 years) and gender (Figure 5-2).

Figure 5-2 Flowchart of eligible NMO/NMOSD patients with matched MS.

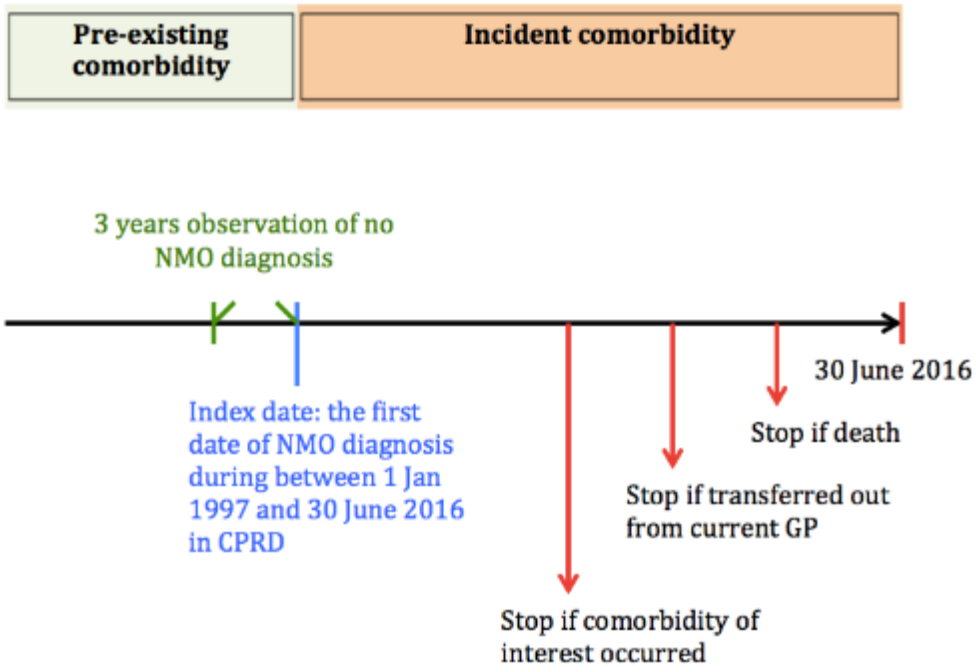


The study comprised incident NMO/NMOSD patients and 10 matched MS patients who were continuously registered for at least three years before the NMO/NMOSD or MS were firstly recorded during 1997 to 2016.

5.2.5 Study period

We defined two periods to assess comorbidity: (a) the 10 years prior to diagnosis and (b) the period from diagnosis to the earliest date of occurrence of comorbidities, death, transfer out, last data collection time for practice or end of study (30 June 2016), whichever came first (Figure 5-3).

Figure 5-3 The observation of comorbidity in an incident NMO/NMOSD patient.



A patient is defined as an incident case if the diagnosis of NMO/NMOSD was recorded between 1997 and 2016 and no priori NMO/NMOSD diagnosis for ≥ 3 years. The pre-existing comorbidity was observed before the first recorded NMO/NMOSD diagnosis (index date) and the incident comorbidity are those occurred afterwards until 30 June 2016. Abbreviations: MS, multiple sclerosis; GP, general practitioner; CPRD, Clinical Practice Research Datalink

5.2.6 Comorbidities of interest

We defined NMO/NMOSD as the index disease in this study and as previous chapters, we used Feinstein's definition for comorbidity (Feinstein 1970). To ensure the data are comparable to previous estimates for comorbidity burden in MS patients, we used identical definitions for comorbidity categorisation. In brief, to estimate and compare the burden of comorbidity in NMO/NMOSD to controls, we used the Charlson comorbidity index (For details please refer to section 3.2.5 and table 3-2). We categorised Charlson comorbidity index as (1) 0, 1-2, 3-4 or ≥ 5 scores and (2) dichotomised it as 0 and ≥ 1 score. To estimate the risk of an individual developing various types of comorbidity, we grouped the pre-specified comorbidities into 9 categories: neoplasms, cardiovascular diseases, renal diseases, metabolic and endocrine diseases, gastrointestinal and hepatic diseases, chronic pulmonary diseases, musculoskeletal and connective tissue diseases, neurological and mental disorders, and other.

5.2.7 Covariates

Information on individual's demographic factors (age and gender) (Jick, Li et al. 2014) and lifestyle factors (body mass index [BMI], smoking status and alcohol consumption) at diagnosis was extracted. Tobacco smoking seems not be associated with susceptibility to NMO/NMOSD but may associate with disease progression (Simon, Schmidt et al. 2015). BMI, smoking and alcohol consumption have been recognised as potentially modifiable factors affecting MS risk (Sundstrom, Nystrom et al. 2008, Hedstrom, Olsson et al. 2012, Hedstrom, Hillert et al. 2014) and survival (Manouchehrinia, Weston et al. 2014).

5.2.8 All-cause mortality

To analyse whether the extent of existing comorbidity in patients with NMO/NMOSD at diagnosis would affect their mortality, we used all-cause mortality for patients and controls. Mortality and date of death were based on the recording in the main CPRD database. The CPRD has developed an algorithm to identify death and death dates of participants. For details please refer to 3.2.6.

5.2.9 Statistical analysis

The prevalence of rach comorbidity was calculated using the number of all people with a given comorbidity during the past 10-year period before the index date (numerator) and the number of incident NMO/NMOSD patients or matched controls (denominator). Odds ratios (OR) and 95% confidence intervals (CI) were used to estimate the association between NMO/NMOSD and each co-existing medical condition and the estimates were calculated by using conditional logistic regression was used to adjust for age, gender, index year, body mass index (BMI) category, smoking status and alcohol consumption. Missing data were coded as 'unknown'. In individuals free of Charlson comorbidity at index date, cumulative probabilities of new Charlson comorbidity was estimated at 1, 2, 5, and 10 years following the time of diagnosis (index date). Kaplan–Meier plots were used to estimate the cumulative probability of the occurrence of new Charlson comorbidity and the all-cause mortality in people with incident NMO/NMOSD and controls.

Only at-risk people for a given comorbidity were considered to estimate hazard ratios (HR) of a specific comorbidity. HRs with 95% CIs were calculated for a Charlson comorbidity index ≥ 1, for each comorbidity separately, and for all-cause mortality using a Cox proportional hazards model. The HRs were adjusted by BMI categories, smoking status and alcohol consumption in addition to the matching variables at index date. We further evaluate the relationships between Charlson scores at diagnosis and all-cause mortality in NMO/NMOSD patients using Cox proportional hazards models adjusted by BMI, smoking and alcohol in addition to the matching variables. Finally, to compare all-cause mortality between NMO/NMOSD patients and non-NMO/NMOSD controls, we adjusted for aforementioned covariates and also Charlson comorbidity index at index date.

Furthermore, we compared mortality between NMO/NMOSD patients and age-and gender-matched MS controls, we adjusted for aforementioned covariates (except for practice) and also Charlson comorbidity index at disease diagnosis of each disease. All statistical analyses were performed using SAS statistical software, version 9.3.

5.3 RESULTS

We identified 104 incident NMO/NMOSD patients during 1997 to 2016 with a mean age of 40.4 ± 16.7 years and more than two-thirds were female. The median observation periods (interquartile range) before and after index date were 2 (0–10) and 4 (2–7.5) years, respectively. There were 1,040 matched controls with similar age and gender distributions and observation periods before and after the index date (Table 5-1).

Table 5-1 Baseline characteristics of patients with incident NMO/NMOSD and matched controls

	Incident NMO/NMOSD (n=104)		**Controls (n=1,040)**		**Adjusted ORs† (95% CI)**	
Age (years)	40.38±16.74		40.34±16.60			
Gender						
Men	24	(23.08)	240	(23.08)		
Women	80	(76.92)	800	(76.92)		
Median observation years (IQR)						
Prior to index date	2	(0-10)	5	(0-15)		
After index date	4	(2-7.5)	5	(2-8)		
BMI (kg/m^2)						
<18.5	37	(35.58)	323	(31.06)	0.94	(0.21-4.23)
18.5-24.9	2	(1.92)	18	(1.73)	Reference	
25.0-29.9	22	(21.15)	229	(22.02)	0.84	(0.48-1.47)
≥30	25	(24.04)	188	(18.08)	1.16	(0.67-2.01)
Unknown	18	(17.31)	282	(27.12)	0.50	(0.27-0.94)
Smoking						
Non-smoker	33	(31.73)	379	(36.44)	Reference	
Current smoker	29	(27.88)	161	(15.48)	2.08	(1.22-3.55)*
Ex-smoker	11	(10.58)	101	(9.71)	1.22	(0.57-2.63)
Unknown	31	(29.81)	399	(38.37)	0.83	(0.48-1.44)
Alcohol consumption (units/week)						
Never/Ex-drinker	14	(13.46)	123	(11.83)	Reference	
Current 1-9	43	(41.35)	336	(32.31)	1.09	(0.57-2.11)
Current ≥ 10	6	(5.77)	80	(7.69)	0.63	(0.23-1.77)
Unknown	41	(39.42)	501	(48.17)	0.65	(0.33-1.30)

Abbreviation: BMI, body mass index; IQR, interquartile range
†adjusted for age, gender, registered general practitioner and index year
*p<0.05

5.3.1 Retrospective observation

At index date, the proportion of people with at least one point of Charlson comorbidity was significantly higher in incident NMO/NMOSD patients (25.96%) than in matched controls (14.04%; p<0.0001). As Table 5-2 shows, incident NMO/NMOSD patients tended to have higher Charlson

comorbidity index than matched controls did before index date. Cardiovascular diseases were 2.6-fold more common in NMO/NMOSD patients one year before NMO/NMOSD diagnosis than in matched controls. Neurological and mental disorders were also higher in NMO/NMOSD 10 years or 1 year before disease diagnosis than in matched controls.

Table 5-2 Comorbidities present 10 years and 1 year before index date

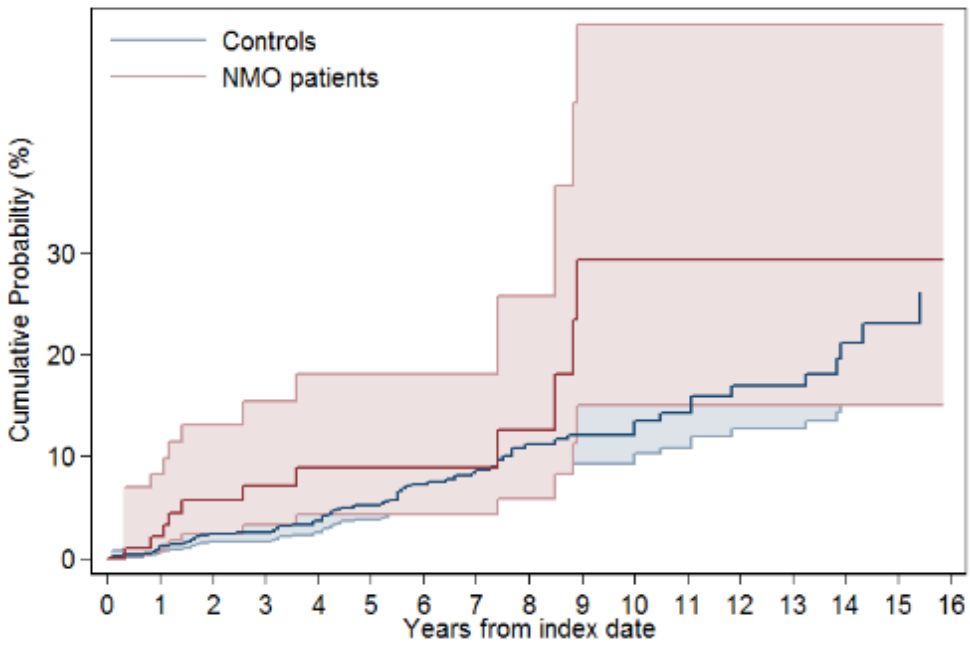
	10-year period before index date								1-year period before index date							
	NMO/NMOSD		Controls		Unadjusted OR		Adjusted OR†		NMO/NMOSD		Controls		Unadjusted OR		Adjusted OR†	
Charlson index																
0	77	(74.04)	894	(85.96)	Reference		Reference		92	(88.46)	970	(93.27)	Reference		Reference	
1-2	23	(22.12)	132	(12.69)	2.14	(1.27-3.58)*	1.83	(1.07-3.13)*	11	(10.58)	67	(6.44)	1.79	(0.90-3.56)	1.41	(0.69-2.90)
3-4	3	(2.88)	11	(1.06)	3.80	(0.97-14.9)	2.68	(0.66-10.9)	1	(0.96)	2	(0.19)	5.46	(0.49-60.7)	6.78	(0.59-77.6)
≥5	1	(0.96)	3	(0.29)	4.01	(0.41-38.9)	3.33	(0.33-33.9)	0	(0)	1	(0.10)	n.a.		n.a.	
Neoplasms	3	(2.88)	25	(2.40)	1.21	(0.36-4.11)	1.13	(0.33-3.89)	1	(0.96)	12	(1.15)	0.83	(0.11-6.48)	0.71	(0.09-5.70)
Solid malignancy, leukaemia and lymphoma	3	(2.88)	24	(2.31)	1.26	(0.37-4.31)	1.18	(0.34-4.08)	1	(0.96)	12	(1.15)	0.83	(0.11-6.48)	0.71	(0.09-5.70)
Metastatic solid tumours	0	(0)	1	(0.10)	n.a.		n.a.		0	(0)	1	(0.1)	n.a.		n.a.	
Cardiovascular diseases	14	(13.46)	94	(9.04)	1.72	(0.88-3.38)	1.32	(0.65-2.67)	8	(7.69)	27	(2.6)	3.31	(1.42-7.75)*	2.58	(1.07-6.20)*
Hypertension	12	(11.54)	83	(7.98)	1.62	(0.80-3.28)	1.19	(0.57-2.51)	7	(6.73)	24	(2.31)	3.21	(1.31-7.87)*	2.46	(0.97-6.26)
Cardiac arrhythmias	1	(0.96)	4	(0.38)	2.65	(0.27-26.3)	1.54	(0.15-15.9)	1	(0.96)	1	(0.1)	10	(0.63- 160)	12.31	(0.73- 209)
Cerebrovascular disease	1	(0.96)	10	(0.96)	1	(0.13-7.96)	0.92	(0.11-7.48)	0	(0)	2	(0.19)	n.a.		n.a.	
Congestive heart failure	1	(0.96)	0	(0)	n.a.		n.a.		1	(0.96)	0	(0)	n.a.		n.a.	
Myocardial infarction	0	(0)	3	(0.29)	n.a.		n.a.		0	(0)	0	(0)	n.a.		n.a.	
Peripheral vascular disease	1	(0.96)	0	(0)	n.a.		n.a.		0	(0)	0	(0)	n.a.		n.a.	
Valvular heart disease	0	(0)	1	(0.1)	n.a.		n.a.		0	(0)	0	(0)	n.a.		n.a.	
Genitourinary diseases	1	(0.96)	7	(0.67)	1.43	(0.18-11.6)	0.98	(0.11-8.43)	0	(0)	0	(0)	n.a.		n.a.	
Urolithiasis	0	(0)	6	(0.58)	n.a.		n.a.		0	(0)	0	(0)	n.a.		n.a.	
Renal diseases	1	(0.96)	1	(0.1)	10	(0.63- 160)	4.06	(0.24-68.9)	0	(0)	0	(0)	n.a.		n.a.	
Metabolic and endocrine diseases	12	(11.54)	65	(6.25)	2.11	(1.05-4.24)	1.94	(0.94-4.01)	5	(4.81)	29	(2.79)	1.82	(0.67-4.96)	1.52	(0.54-4.29)
Uncomplicated diabetes mellitus	7	(6.73)	28	(2.69)	2.82	(1.14-6.96)*	2.36	(0.92-6.08)	3	(2.88)	16	(1.54)	1.91	(0.54-6.70)	1.36	(0.37-4.99)
Diabetes mellitus with complications	2	(1.92)	8	(0.77)	2.82	(0.52-15.3)	1.92	(0.33-11.1)	1	(0.96)	8	(0.77)	1.27	(0.15-11.1)	0.90	(0.10-8.21)
Hyperlipidaemia	4	(3.85)	25	(2.4)	1.74	(0.54-5.60)	1.68	(0.50-5.57)	1	(0.96)	8	(0.77)	1.27	(0.15-11.1)	1.27	(0.14-11.8)
Hypothyroidism	3	(2.88)	19	(1.83)	1.6	(0.46-5.56)	1.70	(0.47-6.10)	1	(0.96)	5	(0.48)	2	(0.23-17.1)	2.77	(0.31-24.7)
Gastrointestinal and hepatic diseases	1	(0.96)	5	(0.48)	2	(0.23-17.1)	1.40	(0.16-12.6)	0	(0)	1	(0.1)	n.a.		n.a.	
Peptic ulcer disease	0	(0)	2	(0.19)	n.a.		n.a.		0	(0)	0	(0)	n.a.		n.a.	
Mild liver disease	1	(0.96)	3	(0.29)	3.33	(0.35-32.1)	2.07	(0.21-20.9)	0	(0)	0	(0)	n.a.		n.a.	
Moderate to severe liver disease	0	(0)	0	(0)	n.a.		n.a.		0	(0)	0	(0)	n.a.		n.a.	
Chronic pulmonary diseases	13	(12.5)	83	(7.98)	1.66	(0.89-3.10)	1.35	(0.70-2.57)	5	(4.81)	35	(3.37)	1.46	(0.55-3.88)	1.19	(0.44-3.23)
Musculoskeletal and connective tissue diseases	8	(7.69)	41	(3.94)	2.17	(0.94-5.01)	1.81	(0.76-4.33)	2	(1.92)	11	(1.06)	1.84	(0.40-8.46)	1.35	(0.28-6.42)
Osteoarthritis	2	(1.92)	23	(2.21)	0.86	(0.20-3.77)	0.82	(0.18-3.63)	1	(0.96)	8	(0.77)	1.25	(0.16-9.99)	0.98	(0.12-7.97)
Rheumatological disease	5	(4.81)	22	(2.12)	2.41	(0.87-6.66)	1.99	(0.69-5.73)	1	(0.96)	8	(0.77)	1.26	(0.15-10.3)	0.94	(0.11-7.93)
Gout	0	(0)	8	(0.77)	n.a.		n.a.		0	(0)	1	(0.1)	n.a.		n.a.	
Neurological and mental disorders	41	(39.42)	157	(15.1)	3.85	(2.47-6.00)*	3.41	(2.10-5.54)*	22	(21.15)	41	(3.94)	7.35	(4.01-13.5)*	6.55	(3.40-12.6)*
Dementia	0	(0)	1	(0.1)	n.a.		n.a.		0	(0)	0	(0)	n.a.		n.a.	
Other neurological diseases	18	(17.31)	12	(1.15)	18.52	(8.29-41.4)*	19.39	(8.26-45.5)*	10	(9.62)	0	(0)	n.a.		n.a.	
Psychosis	3	(2.88)	3	(0.29)	10.00	(2.02-49.6)*	12.10	(2.29-63.9)*	3	(2.88)	0	(0)	n.a.		n.a.	
Depression	25	(24.04)	143	(13.75)	2.05	(1.25-3.37)*	1.58	(0.92-2.70)	8	(7.69)	41	(3.94)	2.06	(0.93-4.58)	1.46	(0.63-3.38)
Other comorbidities	10	(9.62)	59	(5.67)	1.81	(0.88-3.70)	1.48	(0.70-3.10)	1	(0.96)	16	(1.54)	0.61	(0.08-4.77)	0.59	(0.07-4.67)
Anaemia	7	(6.73)	46	(4.42)	1.57	(0.69-3.62)	1.29	(0.55-3.05)	1	(0.96)	11	(1.06)	0.91	(0.11-7.25)	0.83	(0.10-6.69)
Psoriasis	3	(2.88)	13	(1.25)	2.41	(0.66-8.82)	1.95	(0.52-7.30)	0	(0)	5	(0.48)	n.a.		n.a.	
HIV infection	0	(0)	0	(0)	n.a.		n.a.		0	(0)	0	(0)	n.a.		n.a.	

Charlson index includes myocardial infarction, congestive heart failure, peripheral vascular disease, cerebrovascular disease, dementia, chronic pulmonary disease, rheumatologic disease, peptic ulcer disease, mild liver disease, moderate or severe liver disease, diabetes mellitus [DM], DM with chronic complications, renal diseases, any malignancy [including leukaemia and lymphoma], metastatic solid tumour and human immunodeficiency virus [HIV] infection. †adjusted for BMI class, smoking status and alcohol consumption. * $p<0.05$

5.3.2 Follow-up data after index date

Figure 5-4 shows the cumulative probabilities of having new Charlson comorbidity at 1, 2, 5 and 10 years from index date were 2.09%, 5.88%, 9.25% and 22.21% in incident NMO/NMOSD patients and 0.83%, 2.39%, 4.99% and 12.02% in matched controls, among people with a Charlson score of zero at index date (p=0.0939).

Figure 5-4 The cumulative probability of having new Charlson comorbidity in NMO/NMOSD patients (red) and matched controls (blue) with a Charlson score of zero at index date.



Among people with a Charlson score of zero at index date, the cumulative probabilities of having new Charlson comorbidity from index date in incident NMO/NMOSD patients were greater but not significantly different from those in matched controls (p=0.0939).

NMO/NMOSD was associated with an adjusted hazard ratio (HR; 95% CI) of 2.34 (1.10–4.97) for having a Charlson comorbidity index ≥ 1 after index date. Next we investigated the relative risk of

developing a pre-specified comorbidity after the index date. Table 5-3 shows NMO/NMOSD patients had a higher risk of developing incident comorbidity including psoriasis (adjusted HR 6.17), neurological and mental disorders (HR 5.43), anaemia (HR 5.34), and malignancy (HR 3.56). NMO/NMOSD also had a higher cumulative probability of having new malignancy (p=0.02) and peripheral vascular disease (p=0.04) (Table 5-4).

Table 5-3 Risk of incident comorbidity after index date

	At-risk NMO/NMOSD		At-risk controls		Unadjusted HRs		Adjusted HRs	
Charlson index ≥1	77	(14.29)	894	(8.28)	2.48	(1.35-4.58)*	2.34	(1.10-4.97)*
Any increase in Charlson index	104	(10.58)	1040	(7.12)	1.88	(0.96-3.70)	1.85	(0.90-3.79)
Neoplasms	101	(6.93)	1015	(2.46)	2.92	(1.28-6.67)*	3.27	(1.31-8.14)*
Solid malignancy, leukaemia and lymphoma	101	(6.93)	1015	(2.36)	3.07	(1.29-7.28)*	3.56	(1.37-9.22)*
Metastatic solid tumours	104	(0.96)	1039	(0.29)	2.13	(0.33-13.8)	n.a	
Cardiovascular diseases	90	(7.78)	946	(7.19)	1.52	(0.69-3.37)	1.59	(0.69-3.67)
Hypertension	92	(7.61)	957	(5.43)	2.02	(0.98-4.16)	2.08	(0.97-4.46)
Cardiac arrhythmias	103	(0)	1036	(0.97)	n.a		n.a	
Cerebrovascular disease	103	(0)	1030	(1.55)	n.a		n.a	
Congestive heart failure	103	(0)	1040	(0.29)	n.a		n.a	
Myocardial infarction	104	(0.96)	1037	(0.29)	4.11	(0.42-40.2)	n.a	
Peripheral vascular disease	103	(0)	1040	(0.1)	n.a		n.a	
Valvular heart disease	104	(0.96)	1039	(0.67)	4.3	(0.41-45.1)	5.19	(0.13- 210)
Genitourinary diseases	103	(1.94)	1033	(0.68)	3.07	(0.77-12.2)	2.56	(0.77-8.48)
Urolithiasis	104	(1.92)	1034	(0.39)	5.26	(1.33-20.9)*	2.56	(0.77-8.48)
Renal diseases	103	(0)	1039	(0.29)	n.a.		n.a	
Metabolic and endocrine diseases	92	(5.43)	975	(4.1)	1.42	(0.53-3.84)	1.32	(0.49-3.57)
Uncomplicated diabetes mellitus	97	(1.03)	1012	(1.88)	0.66	(0.08-5.45)	0.07	(0.00-3.59)
Diabetes mellitus with complications	102	(3.92)	1032	(2.71)	1.91	(0.61-5.97)	1.32	(0.32-5.47)
Hyperlipidaemia	100	(3)	1015	(1.77)	2.44	(0.44-13.6)	2.42	(0.36-16.0)
Hypothyroidism	101	(0.99)	1021	(1.47)	0.66	(0.08-5.47)	0.48	(0.05-5.01)
Gastrointestinal and hepatic diseases	103	(0)	1035	(0.1)	n.a		n.a	
Peptic ulcer disease	104	(0)	1038	(0.1)	n.a		n.a	
Mild liver disease	103	(0)	1037	(0)	n.a		n.a	
Moderate to severe liver disease	104	(0)	1040	(0.1)	n.a		n.a	
Chronic pulmonary diseases	91	(3.3)	957	(2.72)	1.19	(0.34-4.15)	0.79	(0.23-2.72)
Musculoskeletal and connective tissue diseases	96	(4.17)	999	(3.9)	1.48	(0.49-4.42)	1.04	(0.31-3.52)
Osteoarthritis	102	(0)	1017	(2.46)	n.a		n.a	
Rheumatological disease	99	(0)	1018	(1.87)	n.a		n.a	
Gout	104	(0.96)	1032	(0.87)	2.21	(0.23-21.1)	n.a	
Neurological and mental disorders	63	(28.57)	883	(9.06)	5.09	(2.56-10.1)*	5.43	(2.47-12.0)*
Dementia	104	(0)	1039	(0.38)	n.a		n.a	
Other neurological diseases	86	(18.6)	1028	(0.68)	74.1	(18.5- 296)*	368.86	(33.0-4126)*

Psychosis	101	(1.98)	1037	(0.19)	15.09	(1.24- 183)*	n.a	
Depression	79	(10.13)	897	(7.8)	1.18	(0.53-2.62)	1.08	(0.44-2.65)
Other comorbidities	94	(12.77)	981	(3.87)	4.36	(2.16-8.78)*	5.58	(2.45-12.7)*
Anaemia	97	(9.28)	995	(3.12)	4.36	(1.85-10.3)*	5.34	(1.87-15.2)*
Psoriasis	101	(3.96)	1027	(0.88)	6.15	(2.04-18.6)*	6.17	(2.31-16.5)*
HIV infection	104	(0)	1040	(0.1)	n.a		n.a	

Abbreviation: BMI, body mass index

Figures are number of at-risk population (percentage of at-risk population who developed incident comorbidity) and HRs (95% CIs)

†Adjusted for BMI class, smoking status and alcohol consumption

*$p<0.05$

Table 5-4 Cumulative probability of comorbidity after diagnosis of neuromyelitis optica

	Patients with incident NMO/NMOSD					Controls					
	At diagnosis	**1 year**	**2 years**	**5 years**	**10 years**	**At diagnosis**	**1 year**	**2 years**	**5 years**	**10 years**	**p Value**
Charlson index ≥1	25.96	29.44	33	36.08	47.97	14.04	15.78	17.31	19.86	26.73	<0.0001*
Any increase in Charlson index	0	2.09	5.88	9.25	22.21	0	0.83	2.39	4.99	12.02	0.0939
Neoplasms	2.88	3.11	5.56	8.79	18.23	2.4	2.8	3.4	4.77	6.85	0.0274*
Solid malignancy, leukaemia and lymphoma	2.88	3.11	5.56	8.79	18.23	2.31	2.7	3.18	4.55	6.62	0.0218*
Metastatic solid tumours	0	0	0	0	3.28	0.1	0.1	0.22	0.38	0.68	0.3706
Cardiovascular diseases	13.46	15.46	16.7	21.6	24.86	9.04	10.89	11.83	16.2	20.05	0.1543
Hypertension	11.54	13.47	14.72	21.28	24.56	7.98	9.37	10.2	13.52	17.1	0.0927
Cardiac arrhythmias	0.96	1.04	1.04	1.04	1.04	0.38	0.42	0.66	1.11	2.02	0.7877
Cerebrovascular disease	0.96	1.05	1.05	1.05	1.05	0.96	1.35	1.59	2.66	3.26	0.3552
Congestive heart failure	0.96	1.04	1.04	1.04	1.04	0	0	0	0.15	0.15	0.2046
Myocardial infarction	0	0	0	0	0	0.29	0.31	0.31	0.47	1.07	0.5790
Peripheral vascular disease	0.96	1.04	1.04	1.04	1.04	0	0	0	0	0.3	0.0400*
Valvular heart disease	0	0	0	1.68	1.68	0.1	0.21	0.21	0.36	0.66	0.6837
Genitourinary diseases	0.96	1.04	2.29	2.29	5.49	0.67	0.83	0.83	1.44	1.75	0.1983
Urolithiasis	0	0	1.26	1.26	4.5	0.58	0.73	0.73	1.03	1.34	0.3404
Renal diseases	0.96	1.04	1.04	1.04	1.04	0.1	0.1	0.1	0.41	0.41	0.3661
Metabolic and endocrine diseases	11.54	13.4	14.63	14.63	20.95	6.25	7.25	8.09	9.16	14.29	0.0384*
Uncomplicated diabetes mellitus	6.73	8.29	8.29	8.29	8.29	2.69	3.01	3.61	4.22	6.33	0.1282
Diabetes mellitus with complications	1.92	2.07	2.07	7.01	10.22	0.77	1.25	1.96	2.58	5.55	0.1809
Hyperlipidaemia	3.85	4.19	4.19	4.19	10.91	2.4	2.91	3.39	3.7	5.82	0.1768
Hypothyroidism	2.88	3.11	4.34	4.34	4.34	1.83	2.08	2.32	3.24	5.05	0.7262
Gastrointestinal and hepatic diseases	0.96	1.05	1.05	1.05	1.05	0.48	0.52	0.52	0.67	0.67	0.6253
Peptic ulcer disease	0	0	0	0	0	0.19	0.21	0.21	0.36	0.36	0.5881
Mild liver disease	0.96	1.05	1.05	1.05	1.05	0.29	0.31	0.31	0.31	0.31	0.2678
Moderate to severe liver disease	0	0	0	0	0	0	0	0	0	0	--
Chronic pulmonary diseases	12.5	13.33	15.76	15.76	19	7.98	9.07	9.31	10.37	13.04	0.1044
Musculoskeletal and connective tissue diseases	7.69	8.33	10.86	14.23	14.23	3.94	4.76	5.36	7.34	10.87	0.1093
Osteoarthritis	1.92	2.08	2.08	2.08	2.08	2.21	2.69	2.81	4.49	7.19	0.2237
Rheumatological disease	4.81	5.24	5.24	5.24	5.24	2.12	2.39	2.99	3.91	5.41	0.6121
Gout	0	0	1.27	1.27	1.27	0.77	0.83	0.83	1.59	2.49	0.6350
Neurological and mental disorders	39.42	50	52.38	57.14	69.39	15.1	17.4	18.92	22.75	28.38	<0.0001*
Dementia	0	0	0	0	0	0.1	0.1	0.22	0.22	0.22	0.5375
Other neurological diseases	17.31	29.9	29.9	35.15	41.98	1.15	1.25	1.49	1.95	2.55	<0.0001*
Psychosis	2.88	3.11	4.35	4.35	7.65	0.29	0.31	0.31	0.62	0.62	<0.0001*

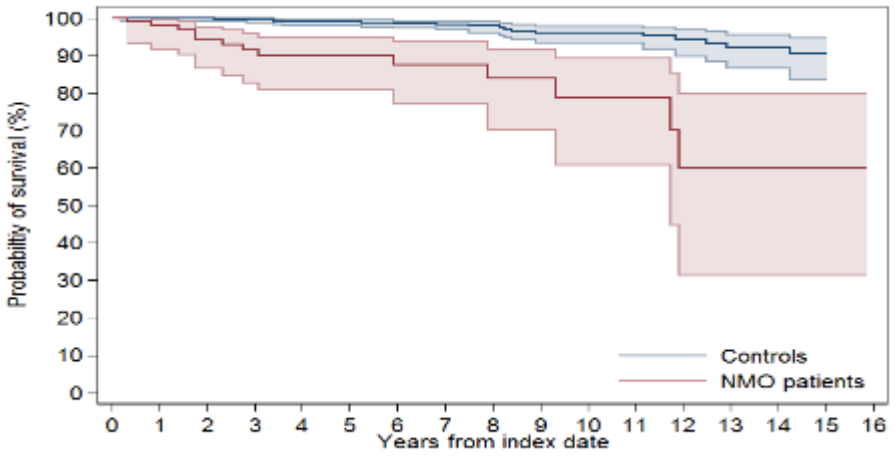
Depression	24.04	27.27	29.62	31.12	36.98	13.75	15.98	17.16	20.55	25.93	0.0051*
Other comorbidities	9.62	14.66	17.19	22.01	25.01	5.67	6.71	6.94	8.89	12.67	<0.0001*
Anaemia	6.73	9.42	10.69	15.52	21.45	4.42	5.06	5.18	6.82	10.03	0.0023*
Psoriasis	2.88	5.24	6.5	6.5	6.5	1.25	1.66	1.78	2.24	2.85	0.0031*
HIV infection	0	0	0	0	0	0	0	0	0	0.3	0.7547

Figures are probability of incident comorbidity (percentage)
* $p<0.05$

5.3.3 All-cause mortality between NMO/NMOSD and matched general population

Figure 5-5 shows the survival curves for NMO/NMOSD patients and matched controls. After index date, mortality at 5 years and 10 years from any cause was 10.36% and 18.90% in incident NMO/NMOSD patients, compared with 1.02% and 3.39% in matched controls (log-rank test, $p<0.0001$) (Table 5-5). NMO/NMOSD was associated a crude HR for death of 10.61 (95% CI, 3.29-34.26). The hazard ratio was further adjusted for Charlson comorbidity index at diagnosis as well as for BMI class, smoking status, and alcohol consumption in addition to the matching variables and NMO/NMOSD was associated with an HR for all-cause mortality of 11.19 (95% CI, 3.33-37.59; $p<0.05$) (Table 5-6).

Figure 5-5 Survival curve of incident NMO/NMOSD patients (red) and matched controls (blue).



After index date, mortality from any cause was higher in incident NMO/NMOSD patients than matched controls (log-rank test, p<0.0001).

Table 5-5 Cumulative probability of all-cause mortality after diagnosis of NMO/NMOSD

	Incident NMO/NMOSD					Matched controls					
	At diagnosis	**1 year**	**2 years**	**5 years**	**10 years**	**At diagnosis**	**1 year**	**2 years**	**5 years**	**10 years**	**p Value**
Overall	0	2.07	5.72	10.36	18.9	0	0.1	0.1	1.02	3.39	<0.0001*
Gender											
Men	0	0	5.71	5.71	46.12	0	0.47	0.47	2.42	6.32	0.0007*
Women	0	2.67	5.76	11.59	11.59	0	0	0	0.6	2.52	<0.0001*

Figures are probability of mortality (percentage)
*p<0.05

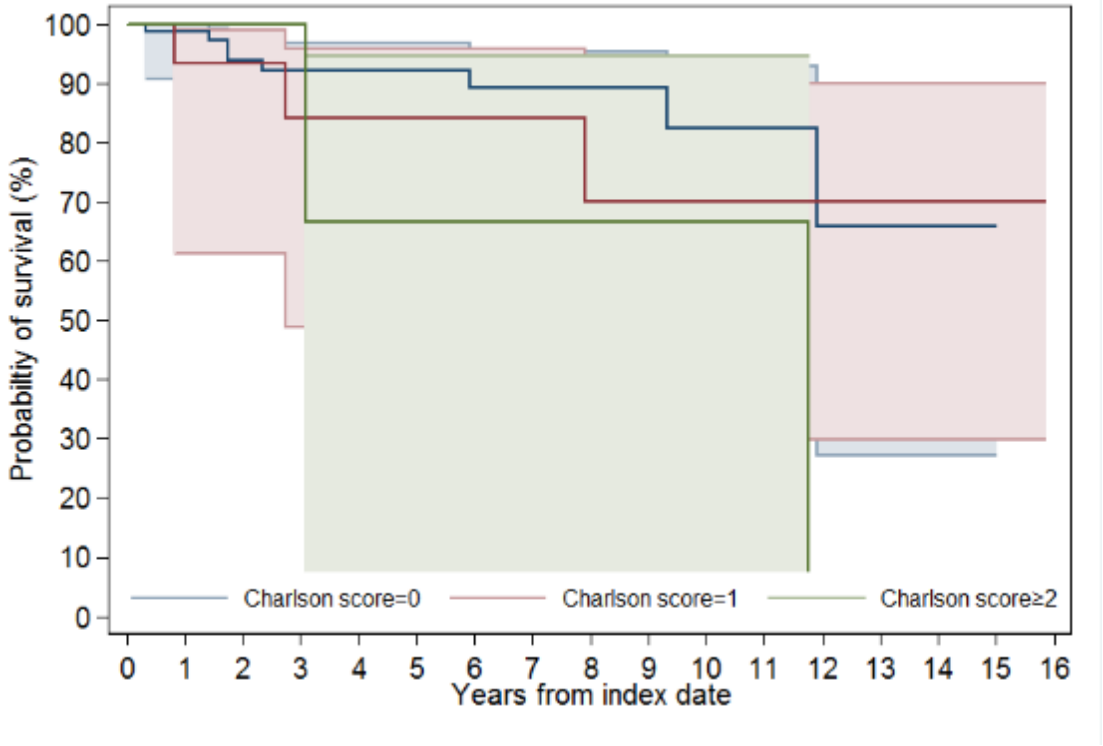
Table 5-6 Predictors for all-cause mortality for NMO/NMOSD to matched general population

	Hazard ratios (95% CI confidence intervals)		
Characteristics	**All patients**	**Men**	**Women**
Model 1: unadjusted	10.61 (3.29-34.26)*	5.75 (1.00-33.21)*	15.08 (3.57-70.04)*
Model 2: adjusted for BMI class, smoking status and alcohol consumption	14.01 (4.33-45.33)*	7.97 (0.09-677.65)	31.11 (8.20-118.04)*
Model 3: adjusted for BMI class, smoking status and alcohol consumption and all comorbidities at index date	11.19 (3.33-37.59)*	6.48 (0.01-3202.44)	31.60 (7.31-136.59)*

*p<0.05

To evaluate the impact of pre-existing comorbidities on survival of NMO/NMOSD patients since diagnosis, we further limited analyses within NMO/NMOSD patients and evaluated the association between baseline Charlson score and all-cause mortality. As shown in Figure 5-6, all-cause mortality in NMO/NMOSD patients at 5-years was 8.10%, 15.81% and 28.57% for those with a Charlson score of 0, 1 and ≥ 2 at index date, respectively (log-rank test, p=0.3323). Crude HRs (95% CI) for all-cause death associated with a Charlson score of 1 and ≥ 2 were 1.64 (0.41-6.52) and 2.98 (0.91-9.77), respectively. After the adjustment for BMI class, smoking status and alcohol consumption in addition to the matching variables at diagnosis, Charlson score of 1 and ≥ 2 were associated with adjusted HRs (95% CI) for all-cause death of 1.44 (0.19-10.84) and 2.52 (0.49-12.92), respectively.

Figure 5-6 Survival curve of incident NMO/NMOSD patients with a Charlson score of 0 (blue), 1(red), and ≥2 (green) at diagnosis.



After index date, cumulative mortality from any cause was slightly higher but not statistically significantly in incident NMO/NMOSD patients with pre-existing Charlson index 1 (red), or ≥ 2 (green) than those with 0 (blue) (log-rank test, p=0.3323).

5.3.4 All-cause mortality comparison between NMO/NMOSD and MS

There were 1,016 MS patients with similar age and gender distributions (Table 5-7). Pre-existing comorbidity at disease diagnosis were not different between NMO/NMOSD and MS (Table 5-8). After

diagnosis, patients with NMO/NMOSD had a greater risk to have anaemia, psoriasis, and any new Charlson comorbidity including malignancy than patients with MS did (Table 5-9 and Table 5-10). From date of disease diagnosis, the cumulative probabilities of having new Charlson comorbidity at 1, 2, 5 and 10 years between incident NMO/NMOSD patients and matched MS patients were marginally higher although the difference was not statistically significant (p=0.05) (Table 5-9).

Table 5-7 Baseline characteristics of patients with incident NMO/NMOSD and matched MS.

	Incident NMO/NMOSD (n=104)	**Matched MS (n=1,016)**	**Adjusted ORs† (95% CI)**
Age (years)	40.38±16.74	41.20±16.01	
Gender			
Men	24 (23.08)	240 (23.62)	
Women	80 (76.92)	776 (76.38)	
Median observation years (IQR)			
Prior to index date	2 (0-10.5)	3 (0-13)	
After index date	4 (2-7.5)	6 (3-11)	
BMI (kg/m^2)			
<18.5	37 (35.58)	382 (37.6)	0.71 (0.16-3.17)
18.5-24.9	2 (1.92)	36 (3.54)	Reference
25.0-29.9	22 (21.15)	241 (23.72)	0.92 (0.52-1.61)
≥30	25 (24.04)	194 (19.09)	1.28 (0.74-2.20)
Unknown	18 (17.31)	163 (16.04)	1.06 (0.56-1.98)
Smoking			
Non-smoker	33 (31.73)	289 (28.44)	Reference
Current smoker	29 (27.88)	215 (21.16)	1.34 (0.78-2.33)
Ex-smoker	11 (10.58)	161 (15.85)	0.98 (0.47-2.06)
Unknown	31 (29.81)	351 (34.55)	1.25 (0.71-2.20)
Alcohol consumption (units/week)			
Never/Ex-drinker	14 (13.46)	107 (10.53)	Reference
Current 1-9	43 (41.35)	386 (37.99)	0.84 (0.43-1.63)
Current ≥ 10	6 (5.77)	80 (7.87)	0.63 (0.23-1.75)
Unknown	41 (39.42)	443 (43.6)	0.93 (0.46-1.86)

Abbreviation: BMI, body mass index; IQR, interquartile range. †adjusted for age, gender and index year. *p<0.05

Table 5-8 Comorbidities present 10 years and 1 year before the diagnosis of NMO/NMOSD or matched MS

	10-year period before index date								1-year period before index date							
	NMO/NMOSD		Matched MS		Unadjusted OR		Adjusted OR†		NMO/NMOSD		Matched MS		Unadjusted OR		Adjusted OR†	
Charlson index																
0	77	(74.04)	823	(81.00)	Reference		Reference		92	(88.46)	920	(90.55)	Reference		Reference	
1	16	(15.38)	129	(12.70)	1.30	(0.73-2.32)	1.26	(0.70-2.25)	6	(5.77)	65	(6.40)	0.88	(0.37-2.13)	0.78	(0.32-1.92)
≥2	11	(10.58)	64	(6.30)	1.84	(0.90-3.78)	1.61	(0.78-3.35)	6	(5.77)	31	(3.05)	2.02	(0.80-5.07)	1.75	(0.68-4.48)
Neoplasms	3	(2.88)	17	(1.67)	1.78	(0.52-6.13)	1.53	(0.44-5.34)	1	(0.96)	5	(0.49)	2.00	(0.23-17.1)	1.72	(0.20-15.2)
Solid malignancy, leukaemia and lymphoma	3	(2.88)	16	(1.57)	1.89	(0.55-6.56)	1.63	(0.46-5.72)	1	(0.96)	5	(0.49)	2.00	(0.23-17.1)	1.72	(0.20-15.2)
Metastatic solid tumours	0	(0)	1	(0.1)	n.a.		n.a.		0	(0)	1	(0.1)	n.a.		n.a.	
Cardiovascular diseases	14	(13.46)	103	(10.14)	1.44	(0.74-2.81)	1.28	(0.65-2.55)	8	(7.69)	49	(4.82)	1.64	(0.71-3.80)	1.38	(0.58-3.26)
Hypertension	12	(11.54)	82	(8.07)	1.56	(0.76-3.21)	1.38	(0.66-2.90)	7	(6.73)	38	(3.74)	1.84	(0.75-4.51)	1.56	(0.62-3.93)
Cardiac arrhythmias	1	(0.96)	6	(0.59)	1.70	(0.19-14.8)	1.60	(0.18-14.3)	1	(0.96)	1	(0.1)	10.00	(0.63- 160)	9.86	(0.56- 173)
Cerebrovascular disease	1	(0.96)	22	(2.17)	0.45	(0.06-3.37)	0.37	(0.05-2.79)	0	(0)	11	(1.08)	n.a.		n.a.	
Congestive heart failure	1	(0.96)	4	(0.39)	2.50	(0.28-22.4)	2.23	(0.24-20.5)	1	(0.96)	1	(0.1)	10.00	(0.63- 160)	11.25	(0.70- 182)
Myocardial infarction	0	(0)	3	(0.3)	n.a.		n.a.		0	(0)	1	(0.1)	n.a.		n.a.	
Peripheral vascular disease	1	(0.96)	4	(0.39)	2.50	(0.28-22.4)	3.44	(0.36-32.8)	0	(0)	0	(0)	n.a.		n.a.	
Valvular heart disease	0	(0)	5	(0.49)	n.a.		n.a.		0	(0)	3	(0.3)	n.a.		n.a.	
Genitourinary diseases	1	(0.96)	11	(1.08)	0.90	(0.11-7.36)	0.90	(0.11-7.49)	0	(0)	4	(0.39)	n.a.		n.a.	
Urolithiasis	0	(0)	9	(0.89)	n.a.		n.a.		0	(0)	4	(0.39)	n.a.		n.a.	
Renal diseases	1	(0.96)	4	(0.39)	2.65	(0.27-26.3)	2.76	(0.27-28.4)	0	(0)	1	(0.1)	n.a.		n.a.	
Metabolic and endocrine diseases	12	(11.54)	79	(7.78)	1.55	(0.79-3.05)	1.43	(0.72-2.84)	5	(4.81)	32	(3.15)	1.62	(0.60-4.33)	1.37	(0.51-3.73)
Uncomplicated diabetes mellitus	7	(6.73)	33	(3.25)	2.05	(0.85-4.93)	1.81	(0.74-4.42)	3	(2.88)	19	(1.87)	1.62	(0.46-5.71)	1.37	(0.38-4.91)
Diabetes mellitus with complications	2	(1.92)	13	(1.28)	1.56	(0.34-7.12)	1.22	(0.26-5.67)	1	(0.96)	7	(0.69)	1.43	(0.18-11.6)	1.08	(0.13-8.97)
Hyperlipidaemia	4	(3.85)	27	(2.66)	1.53	(0.51-4.60)	1.48	(0.49-4.49)	1	(0.96)	6	(0.59)	1.77	(0.18-17.0)	1.54	(0.16-15.2)
Hypothyroidism	3	(2.88)	28	(2.76)	1.08	(0.32-3.64)	1.02	(0.30-3.49)	1	(0.96)	7	(0.69)	1.43	(0.18-11.6)	1.12	(0.14-9.27)
Gastrointestinal and hepatic diseases	1	(0.96)	6	(0.59)	1.70	(0.19-14.8)	1.40	(0.16-12.5)	0	(0)	1	(0.1)	n.a.		n.a.	
Peptic ulcer disease	0	(0)	5	(0.49)	n.a.		n.a.		0	(0)	1	(0.1)	n.a.		n.a.	
Mild liver disease	1	(0.96)	3	(0.3)	3.75	(0.33-43.1)	2.62	(0.23-30.1)	0	(0)	1	(0.1)	n.a.		n.a.	
Moderate to severe liver disease	0	(0)	1	(0.1)	n.a.		n.a.		0	(0)	1	(0.1)	n.a.		n.a.	
Chronic pulmonary diseases	13	(12.5)	111	(10.93)	1.10	(0.59-2.03)	1.06	(0.56-1.98)	5	(4.81)	47	(4.63)	0.97	(0.37-2.54)	0.88	(0.33-2.36)
Musculoskeletal and connective tissue diseases	8	(7.69)	37	(3.64)	2.23	(1.00-5.00)*	2.10	(0.92-4.76)	2	(1.92)	16	(1.57)	1.26	(0.28-5.65)	1.18	(0.26-5.40)
Osteoarthritis	2	(1.92)	31	(3.05)	0.63	(0.14-2.72)	0.63	(0.14-2.77)	1	(0.96)	7	(0.69)	1.43	(0.18-11.6)	1.63	(0.20-13.5)
Rheumatological disease	5	(4.81)	21	(2.07)	2.36	(0.86-6.49)	2.05	(0.73-5.74)	1	(0.96)	11	(1.08)	0.91	(0.12-7.14)	0.79	(0.10-6.33)
Gout	0	(0)	6	(0.59)	n.a.		n.a.		0	(0)	4	(0.39)	n.a.		n.a.	

Neurological and mental disorders[a]	34	(32.69)	321	(31.59)	1.07	(0.69-1.66)	1.00	(0.64-1.57)	17	(16.35)	150	(14.76)	1.12	(0.65-1.94)	1.07	(0.61-1.86)
Dementia	0	(0)	2	(0.2)	n.a.		n.a.		0	(0)	2	(0.2)	n.a.		n.a.	
Other neurological diseases	9	(8.65)	95	(9.35)	0.90	(0.44-1.83)	0.85	(0.42-1.74)	5	(4.81)	55	(5.41)	0.83	(0.32-2.13)	0.82	(0.32-2.09)
Psychosis	3	(2.88)	7	(0.69)	4.54	(1.12-18.4)*	4.50	(1.03-19.5)*	3	(2.88)	2	(0.2)	15.00	(2.51-89.8)*	14.06	(2.21-89.3)*
Depression	25	(24.04)	238	(23.43)	1.07	(0.66-1.74)	1.00	(0.60-1.64)	8	(7.69)	90	(8.86)	0.88	(0.41-1.88)	0.83	(0.39-1.80)
Other comorbidities	10	(9.62)	84	(8.27)	1.15	(0.57-2.30)	1.08	(0.53-2.19)	1	(0.96)	27	(2.66)	0.37	(0.05-2.71)	0.33	(0.04-2.52)
Anaemia	7	(6.73)	63	(6.2)	1.12	(0.50-2.50)	1.08	(0.48-2.43)	1	(0.96)	21	(2.07)	0.47	(0.06-3.54)	0.44	(0.06-3.35)
Psoriasis	3	(2.88)	20	(1.97)	1.53	(0.44-5.29)	1.40	(0.40-4.92)	0	(0)	6	(0.59)	n.a.		n.a.	
HIV infection	0	(0)	2	(0.2)	n.a.		n.a.		0	(0)	1	(0.1)	n.a.		n.a.	

Figures are patient number (percentage); Charlson index includes myocardial infarction, congestive heart failure, peripheral vascular disease, cerebrovascular disease, dementia, chronic pulmonary disease, rheumatologic disease, peptic ulcer disease, mild liver disease, moderate or severe liver disease, diabetes mellitus [DM], DM with chronic complications, renal diseases, any malignancy [including leukaemia and lymphoma], metastatic solid tumour and human immunodeficiency virus [HIV] infection. †adjusted for BMI class, smoking status and alcohol consumption. * $p<0.05$;
[a]Epilepsy is 0 at both 10 and 1 year before diagnosis of NMO/NMOSD

Table 5-9 Cumulative probability of comorbidity after diagnosis of NMO/NMOSD or matched MS

	Patients with incident NMO/NMOSD					Matched MS					
	At diagnosis	**1 year**	**2 years**	**5 years**	**10 years**	**At diagnosis**	**1 year**	**2 years**	**5 years**	**10 years**	**p Value**
Charlson index ≥1	25.96	29.44	33	36.08	47.97	19.00	21.79	23.25	27.84	36	0.0502
Any increase in Charlson index	0	2.09	5.88	9.25	22.21	0	2.07	3.54	8.25	16.69	0.4651
Neoplasms	2.88	3.11	5.56	8.79	18.23	1.67	2.79	3.13	4.09	7.49	0.0209*
Solid malignancy, leukaemia and lymphoma	2.88	3.11	5.56	8.79	18.23	1.57	2.68	3.14	4.1	7.5	0.0205*
Metastatic solid tumours	0	0	0	0	3.28	0.1	0.1	0.22	0.36	0.79	0.3500
Cardiovascular diseases	13.46	15.46	16.7	21.6	24.86	10.14	12.19	13.65	18.67	25.06	0.5175
Hypertension	11.54	13.47	14.72	21.28	24.56	8.07	9.54	10.44	13.99	19.03	0.1611
Cardiac arrhythmias	0.96	1.04	1.04	1.04	1.04	0.59	0.83	0.94	1.63	3.14	0.5582
Cerebrovascular disease	0.96	1.05	1.05	1.05	1.05	2.17	2.37	2.94	4.04	4.91	0.1546
Congestive heart failure	0.96	1.04	1.04	1.04	1.04	0.39	0.41	0.41	0.41	0.85	0.5866
Myocardial infarction	0	0	0	0	0	0.3	0.41	0.64	1.19	1.85	0.9484
Peripheral vascular disease	0.96	1.04	1.04	1.04	1.04	0.39	0.41	0.53	0.94	1.38	0.9350
Valvular heart disease	0	0	0	1.68	1.68	0.49	0.72	0.72	1	1.43	0.9770
Genitourinary diseases	0.96	1.04	2.29	2.29	5.49	1.08	1.14	1.36	2.05	2.48	0.3982
Urolithiasis	0	0	1.26	1.26	4.5	0.89	0.93	1.16	1.16	1.37	0.4317
Renal diseases	0.96	1.04	1.04	1.04	1.04	0.39	0.41	0.41	1.1	1.32	0.9018
Metabolic and endocrine diseases	11.54	13.4	14.63	14.63	20.95	7.78	9.04	9.83	12.96	16.91	0.2697
Uncomplicated diabetes mellitus	6.73	8.29	8.29	8.29	8.29	3.25	3.81	4.27	4.68	5.54	0.1725
Diabetes mellitus with complications	1.92	2.07	2.07	7.01	10.22	1.28	1.86	2.2	3.02	4.53	0.1218
Hyperlipidaemia	3.85	4.19	4.19	4.19	10.91	2.66	3.1	3.55	4.93	6.65	0.3407
Hypothyroidism	2.88	3.11	4.34	4.34	4.34	2.76	3.5	4.07	5.71	6.98	0.5513
Gastrointestinal and hepatic diseases	0.96	1.05	1.05	1.05	1.05	0.59	0.83	0.94	1.22	2.3	0.7421
Peptic ulcer disease	0	0	0	0	0	0.49	0.72	0.72	0.86	1.08	0.3589
Mild liver disease	0.96	1.05	1.05	1.05	1.05	0.3	0.31	0.42	0.56	1.43	0.8000
Moderate to severe liver disease	0	0	0	0	0	0.1	0.21	0.21	0.21	0.42	0.6209
Chronic pulmonary diseases	12.5	13.33	15.76	15.76	19	10.93	12.22	12.78	14.82	17.37	0.7500
Musculoskeletal and connective tissue diseases	7.69	8.33	10.86	14.23	14.23	3.64	4.84	5.86	8.99	12.98	0.3089
Osteoarthritis	1.92	2.08	2.08	2.08	2.08	3.05	3.61	3.83	4.38	6.1	0.2152
Rheumatological disease	4.81	5.24	5.24	5.24	5.24	2.07	2.89	3.22	4.32	5.39	0.6973
Gout	0	0	1.27	1.27	1.27	0.59	0.72	1.06	1.34	1.34	0.8939
Neurological and mental disorders[a]	32.69	40	42.33	43.83	52.7	31.59	37.47	40.54	46.91	56.21	0.6851
Dementia	0	0	0	0	0	0.2	0.31	0.31	0.45	0.88	0.4279
Other neurological diseases	8.65	13.47	13.47	16.74	19.94	9.35	11.89	12.33	13.83	15.53	0.5384
Psychosis	2.88	3.11	4.35	4.35	7.65	0.69	0.93	0.93	1.07	1.5	0.0016*

Depression	24.04	27.27	29.62	31.12	36.98	23.43	29.17	32.36	39.17	48.04	0.1875
Other comorbidities	9.62	14.66	17.19	22.01	25.01	8.27	9.59	10.39	13.27	19.15	0.0262*
Anaemia	6.73	9.42	10.69	15.52	21.45	6.2	7.33	7.78	10.26	15.11	0.1110
Psoriasis	2.88	5.24	6.5	6.5	6.5	1.97	2.27	2.61	3.3	5.46	0.0842
HIV infection	0	0	0	0	0	0.2	0.21	0.21	0.21	0.21	0.6508

Figures are probability of incident comorbidity (percentage); [a] Epilepsy was 0 at and after diagnosis in patients with NMO/NMOSD
*p<0.05

Table 5-10 Risk of incident comorbidity after diagnosis of NMO/NMOSD or matched MS

	At-risk NMO/NMOSD cases		**At-risk matched matched MS**		**Unadjusted HRs**		**Adjusted HRs**	
Charlson index ≥1	77	(14.29)	823	(15.43)	1.59	(0.93-2.71)	1.78	(1.02-3.11)*
Any increase in Charlson index	104	(10.58)	1016	(12.5)	1.53	(0.89-2.63)	1.65	(0.94-2.89)
Neoplasm	101	(6.93)	999	(4.6)	3.38	(1.76-6.50)*	3.61	(1.50-8.72)*
Solid malignancy, leukaemia and lymphoma	101	(6.93)	999	(4.6)	3.38	(1.76-6.50)*	3.61	(1.50-7.82)*
Metastatic solid tumours	104	(0.96)	1015	(0.39)	n.a.		n.a.	
Cardiovascular diseases	90	(7.78)	913	(12.16)	1.29	(0.67-2.48)	1.18	(0.60-2.32)
Hypertension	92	(7.61)	934	(8.46)	1.99	(1.01-3.91)*	1.75	(0.87-3.54)
Cardiac arrhythmias	103	(0)	1010	(1.58)	n.a.		n.a.	
Cerebrovascular disease	103	(0)	994	(2.01)	n.a.		n.a.	
Congestive heart failure	103	(0)	1012	(0.3)	n.a.		n.a.	
Myocardial infarction	104	(0.96)	1013	(0.99)	1.56	(0.25-9.72)	2.52	(0.34-14.73)
Peripheral vascular disease	103	(0)	1012	(0.79)	n.a.		n.a.	
Valvular heart disease	104	(0.96)	1011	(0.59)	2.54	(0.31-21.05)	n.a.	
Genitourinary diseases	103	(1.94)	1005	(0.9)	3.19	(0.60-16.94)	3.54	(0.85-14.73)
Urolithiasis	104	(1.92)	1007	(0.3)	7.71	(1.49-39.88)*	n.a.	
Renal diseases	103	(0)	1012	(0.59)	n.a.		n.a.	
Metabolic and endocrine diseases	92	(5.43)	937	(7.68)	1.19	(0.51-2.76)	1.14	(0.44-2.94)
Uncomplicated diabetes mellitus	97	(1.03)	983	(2.03)	1.05	(0.12-8.97)	1.01	(0.08-12.52)
Diabetes mellitus with complications	102	(3.92)	1003	(2.39)	2.97	(1.05-8.39)*	3.64	(1.48-8.91)*
Hyperlipidaemia	100	(3)	989	(3.24)	1.54	(0.49-4.83)	1.48	(0.43-5.10)
Hypothyroidism	101	(0.99)	988	(3.24)	0.36	(0.04-3.03)	0.30	(0.03-2.70)
Gastrointestinal and hepatic diseases	103	(0)	1010	(0.99)	n.a.		n.a.	
Peptic ulcer disease	104	(0)	1011	(0.4)	n.a.		n.a.	
Mild liver disease	103	(0)	1013	(0.59)	n.a.		n.a.	
Moderate to severe liver disease	104	(0)	1015	(0.2)	n.a.		n.a.	
Chronic pulmonary diseases	91	(3.3)	905	(5.19)	0.89	(0.31-2.60)	0.88	(0.28-2.70)
Musculoskeletal and connective tissue diseases	96	(4.17)	979	(8.27)	0.69	(0.25-1.95)	0.65	(0.23-1.87)
Osteoarthritis	102	(0)	985	(2.44)	n.a.		n.a.	

Rheumatological disease	99	(0)	995	(2.71)	n.a.		n.a.	
Gout	104	(0.96)	1010	(0.59)	2.76	(0.47-16.25)	n.a.	
Neurological and mental disorders	70	(17.14)	695	(27.34)	0.73	(0.39-1.35)	0.68	(0.35-1.32)
Dementia	104	(0)	1014	(0.79)	n.a.		n.a.	
Other neurological diseases	95	(7.37)	921	(5.21)	1.78	(0.72-4.36)	1.75	(0.68-4.50)
Psychosis	101	(1.98)	1009	(0.5)	2.33	(0.38-14.27)	21.03	(0.19-2264)
Depression	79	(10.13)	778	(24.16)	0.39	(0.18-0.87)	0.37	(0.16-0.84)
Other comorbidities	94	(12.77)	932	(8.37)	2.77	(1.50-5.12)*	3.12	(1.61-6.06)*
Anaemia	97	(9.28)	954	(6.71)	2.35	(1.17-4.72)*	2.62	(1.23-5.56)*
Psoriasis	101	(3.96)	996	(2.31)	4.62	(1.52-14.10)*	5.84	(1.22-27.98)*
HIV infection	104	(0)	1014	(0)	n.a.		n.a.	

Abbreviation: BMI, body mass index

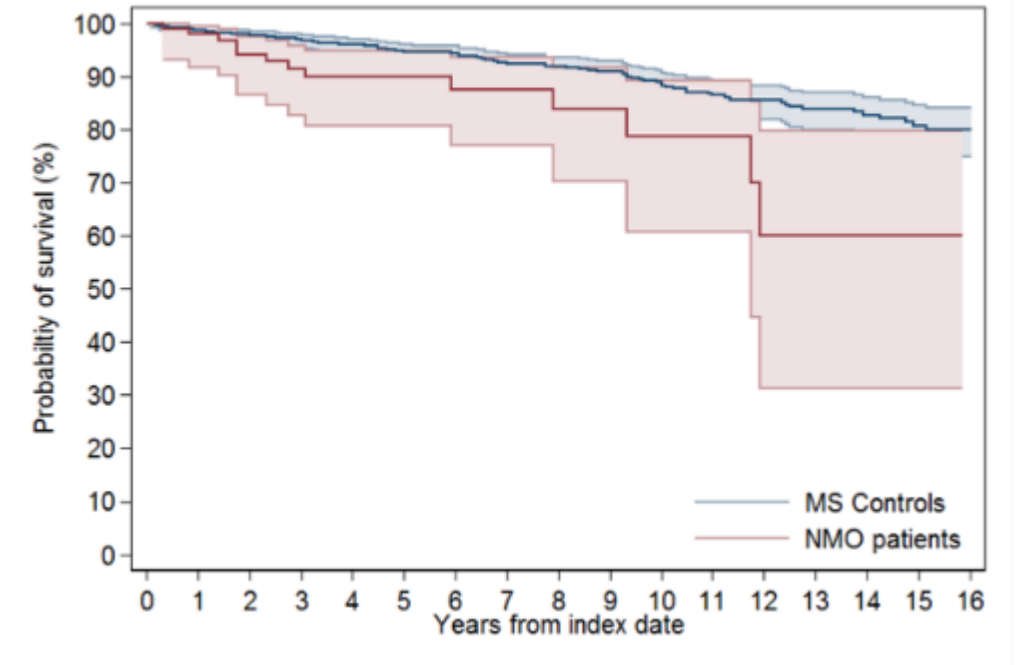
Figures are number of at-risk population (percentage of at-risk population who developed incident comorbidity) and HRs (95% CIs)

†Adjusted for BMI class, smoking status and alcohol consumption

*$p<0.05$

Figure 5-7 shows the survival curves for NMO/NMOSD patients and matched MS patients. After index date (the date of the first medical records for each disease), mortality rate at 1, 2, 5, or 10 years from any cause in incident NMO/NMOSD patients was significantly higher than that in matched MS patients, respectively (log-rank test, p=0.0093) (Table 5-11). NMO/NMOSD was associated an adjusted HR for death of 2.77 (95% CI, 1.43-5.36) (Table 5-12).

Figure 5-7 Survival curve of incident NMO/NMOSD patients (red) and matched MS patients (blue).



After diagnosis, cumulative mortality from any cause was higher in incident NMO/NMOSD patients than matched MS patients (log-rank test, p=0.0093).

Table 5-11 Cumulative probability of all-cause mortality after diagnosis of neuromyelitis optica

	Patients with incident NMO/NMOSD					Matched MS					
	At diagnosis	**1 year**	**2 years**	**5 years**	**10 years**	**At diagnosis**	**1 year**	**2 years**	**5 years**	**10 years**	**p Value**
Overall	0	2.07	5.72	10.36	18.9	0	1.33	2.11	5.18	10.78	0.0093*
Gender											
Men	0	0	5.71	5.71	46.12	0	1.74	2.68	7.22	11.54	0.0503 *
Women	0	2.67	5.76	11.59	11.59	0	1.21	1.94	4.55	10.54	0.0379*

Figures are probability of mortality (percentage)
*P<0.05

Table 5-12 Predictors for all-cause mortality

	Hazard ratios (95% CI confidence intervals)		
Characteristics	**All patients**	**Men**	**Women**
Model 1: unadjusted	2.87 (1.63-5.05)*	2.94 (1.20-7.24)*	2.84 (1.41-5.73)*
Model 2: adjusted for BMI class, smoking status and alcohol consumption	2.72 (1.39-5.31)*	3.62 (1.11-11.82)*	2.98 (1.21-7.36)*
Model 3: adjusted for BMI class, smoking status and alcohol consumption and all comorbidities at index date	2.77 (1.43-5.36)*	2.24 (0.54-9.23)	3.15 (1.36-7.31)*

*p<0.05

5.4 DISCUSSION

Using population-based general practice records, we identified 104 persons with firstly recorded NMO/NMOSD in the past 2 decades, and found that their mortality risk was 11 times greater than matched general population and 2.77 times greater than matched incident MS. As for comorbidity, approximately a quarter of NMO/NMOSD patients already had a Charlson comorbidity index score ≥ 1 at diagnosis comparing to only one-seventh of matched general population and one-fifth of matched MS. The burden of comorbidity is already higher at diagnosis of NMO/NMOSD, and the risk of having some new comorbid conditions in incident NMO/NMOSD patients is higher than matched general population as well as matched incident MS. The burden of comorbidity may partly explain the increased mortality, however the entity of NMO/NMOSD itself is associated with the increased risk of deaths. We conclude that NMO/NMOSD is a debilitating disease and patients' survival is worse than matched MS and the general population.

Our data also showed that female patients with NMO/NMOSD had a higher adjusted hazard risk for death comparing to matched general population, but male patients did not. The female gender effect on mortality risk is consistent to the finding in a cohort consisting of 80 pateints (Wingerchuk and Weinshenker 2003). Wingerchuk et al showed that rate ratio of female to male is 10 for mortality after a long-term follow up to 31 years (Wingerchuk and Weinshenker 2003). The 5-year mortality rate of the current study (10.36%) is lower than previous case-series in Caribbean database (15%) (Cabre, Gonzalez-Quevedo et al. 2009) and in Mayo clinic (32%) (Wingerchuk, Hogancamp et al. 1999). The difference may attribute to improvement of patient care during the past decade, the current data include more heterogenous phenotypes of NMO/NMOSD because the evolution of diagnostic criteria and treatment strategy since 1999.

When comparing to MS, NMO/NMOSD is more severe in terms of more prominent loss of ambulation, respiratory function and vision, and poor recovery of all functional levels (Wingerchuk, Hogancamp et al. 1999, Wingerchuk and Weinshenker 2003). The current study further shows a higher risk of mortality in NMO/NMOSD than MS, and the risk remains high in

females after adjusting various comorbidities. However, the burden of comorbidity did not significantly increase mortality in patients with comorbidity compared to those without. The findings support the initial hypothesis that NMO/NMOSD is more severe than MS in terms of survival of those affected.

The observation that NMO/NMOSD patients have a higher cumulative risk to develop any malignancy than matched general population and matched MS patients is intriguing. Paraneoplastic features have been reported in some patients who were seropositive for aquaporin 4 (AQP4) autoantibodies. AQP4 proteins are expressed at the end-feet of astrocytes in the central nervous system to maintain the integrity of the blood-brain-barriers, and loss of immunostaining of AQP4 proteins is a cardinal feature of lesions in NMO/NMOSD. The AQP4 autoantibodies are detectable in more than half of NMO/NMOSD patients. Paraneoplastic syndromes are specific neurological manifestations appearing within 5 years preceding the cancers (Graus, Delattre et al. 2004). Paraneoplastic NMO/NMOSD had been reported in association with breast cancers (Armagan, Tuzun et al. 2012), carcinoid tumours (co-existing with anaemia and neuroendocrine syndrome) (Figueroa, Guo et al. 2014), or thymoma (may co-exist with myasthenia gravis) (Chan, Kwan et al. 2010). Surgical removal of tumours is an integral part of treating associated neurological disease, but continued relapses of NMO/NMOSD were still observed (Yang, Woo et al. 2014). These tumour cells were shown to express aquaporin 4 proteins either on the cell membrane (Chan, Kwan et al. 2010) (Armagan, Tuzun et al. 2012) or in the cytoplasm (Figueroa, Guo et al. 2014). The onset age of paraneoplastic NMO/NMOSD tends to be older than 50 years (Figueroa, Guo et al. 2014, Cai, He et al. 2016), while the mean onset age of all NMO/NMOSD patients is 40 years. Another explanation of increased cancer risk in NMO/NMOSD patients primarily involves the possible role of AQP-4 in the tumour migration with recent evidence showing an increased expression of AQP-4 in tumour cells including glioblastoma (Ding, Ma et al. 2011, Papadopoulos and Saadoun 2015).

The co-morbid peripheral vascular diseases after diagnosis of NMO/NMOSD is consistent with one previous report, in which 6 of 30 hospitalized patients with NMO/NMOSD had venous

thromboembolism (Farber, Gross et al. 2016). Farber et al concluded that NMO/NMOSD per se explains venous thromboembolism after comparing the risk to MS patients with similar clinical ambulatory functions (Farber, Gross et al. 2016). Although it can be arguable that venous thromboembolism can be related to acute and the long-term use of steroids in NMO/NMOSD or comorbid lupus. In addition, animal models have demonstrated that decreased AQP4 expression in retina is accompanied with elevated expression of inflammatory cytokines. Fuma et al demonstrated that transient changes of decreased expression of AQP4 proteins, elevated expression of interleukin-6 (IL-6), monocyte chemoattractant Protein-1 (Mcp-1) and platelet derived growth factor alpha (PDGF-A) within 24 hours after vascular occlusion were associated with focal oedema of retina (Fuma, Nishinaka et al. 2017). In retina, AQP4 proteins are expressed in Müller cells (Nagelhus, Veruki et al. 1998, Motulsky, Koch et al. 2010), which are principal retinal glial cells with end-feet on the large retinal blood vessels forming blood-retina-barriers (Tout, Chan-Ling et al. 1993, Nagelhus, Veruki et al. 1998). The relationships between coexistence of index disease and comorbidity in the same individual are more complex than our current understanding of both diseases (Rhee, Hewitt et al. 2004, Valderas, Starfield et al. 2009). Another observation found AQP4 expression in placental trophoblast cells which when exposed to maternal AQP4 autoantibodies, complement deposition and local necrosis may occur. This may explain an increased miscarriage rate in NMO/NMOSD patients (Davoudi, Keyhanian et al. 2016).

The present work has at least three limitations to be acknowledged. Firstly, there may be misclassification bias because physician diagnosis, rather than according to accepted criteria were used to identify patients with NMO/NMOSD (Wingerchuk, Hogancamp et al. 1999, Wingerchuk, Lennon et al. 2006, Wingerchuk, Banwell et al. 2015) or MS (Poser, Paty et al. 1983, Polman, Reingold et al. 2005, Polman, Reingold et al. 2011). Although the codes for MS in the CPRD have been investigated and validated (Hernan, Jick et al. 2004), the codes for NMO/NMOSD have not. Similarly, there may have been some misclassification of comorbidities, however most of the comorbidities in this study have been validated previously (Herrett, Thomas et al. 2010). Since we used a unified case definition for comorbidities in NMO/NMOSD patients, matched general

population, and matched MS patients, the potential differential misclassification between each group is expected to be kept to a minimum. Also, the observation periods before and after the index date were similar for patients in each group. Secondly, some of potential confounders, such as race, education, annual household income, marital status, and treatment, were not included. However, this study was strengthened by considering the timing of disease onset and the burden of various comorbidities in the disease course of NMO/NMOSD, by comparing age- and gender-matched controls from both general population and MS patients for the robust risk estimation. Thirdly, we did not include a separate comorbidity category for autoimmune diseases due to the rarity of these conditions. Important systemic autoimmune diseases such as systemic lupus erythematosus and rheumatoid arthritis are included in the category of rheumatological diseases, identical to the definitions of the original Charlson comorbidity index. We did identify 5 patients who had these autoimmune diseases prior to the first diagnosis of NMO/NMOSD, however, none of the at-risk population develop systemic autoimmune diseases after NMO/NMOSD diagnosis. The results support hypotheses that NMO/NMOSD patients have more pre-existing comorbidity, a higher risk to have a new comorbidity, and an increased risk for mortality than matched controls or matched MS. However, the hypothesis that pre-existing comorbidity had a dose-effect on mortality is not fully supported in the current study.

5.5 CONCLUSIONS

In conclusion, all-cause mortality is higher in NMO/NMOSD patients than matched general population. The mortality risk is 2.7 times higher in NMO/NMOSD than in MS.

Chapter 6. THE CHANGES OF NORMAL APPEARING WHITE MATTER IN NEUROMYELITIS OPTICA MEASURED BY MRI AT 7T FIELD STRENGTH

6.1 INTRODUCTION

In previous chapters, we estimated the incidence and prevalence of NMO/NMOSD and analysed the associated comorbidity and mortality in NMO/NMOSD. NMO/NMOSD is a severe disease, but whether the disease entity of NMO/NMOSD involves NAWM or not has not been evaluated by MRI at 7Tesla field strength. In this chapter, we pioneer apply indices of two quantitative MRI techniques to quantify the NAWM in NMO/NMOSD compared to MS and healthy subjects.

NMO/NMOSD are rare but debilitating conditions with an estimated prevalence of 0.52–4.4 per 100,000 population (Marrie and Gryba 2013). NMO is characterized by optic neuritis and long-segment transverse myelitis occurring simultaneously or sequentially (Wingerchuk, Lennon et al. 2006). NMOSD include classic NMO and a range of conditions whose diagnosis can be supported by a positive serological test of autoantibodies against aquaporin-4 (AQP-4) (Chou, Whitehouse et al. 2014, Wingerchuk, Banwell et al. 2015). NMO/NMOSD are known to impact on the quality of life on those affected (Kanamori, Nakashima et al. 2011) and to be associated with an increased mortality compared to the general population (Wingerchuk and Weinshenker 2003).

In a recent cross-sectional study, two-thirds of NMO/NMOSD patients were shown to have white matter lesions in the brain MRI (Matthews, Marasco et al. 2013). Emerging evidence suggests that the brain white matter involvement in NMO/NMOSD is distinct from that in MS (Pittock, Weinshenker et al. 2006, Wingerchuk, Lennon et al. 2007, Matthews, Marasco et al. 2013). In the early clinical observation, about 10% of NMO patients had white matter lesions localizing to the hypothalamic and periependymal areas, known to be rich in AQP4. (Pittock, Weinshenker et al. 2006, Wingerchuk, Lennon et al. 2007) Later, collective studies of NMO/NMOSD revealed various T2 white matter lesions (Kim, Park et al. 2010, Kim, Paul et al. 2015). These include hemispheric extensive and confluent lesions (diameter > 3 mm), nonspecific punctate or small (< 3 mm) patches in deep white matter, contiguously longitudinal lesions along the corticospinal tracts, and “cloud-like” gadolinium-enhancing lesions (Ito, Mori et al. 2009, Kim, Park et al. 2010, Kim, Paul et al. 2015). In contrast, classical white matter lesions in MS are T2 hyperintense linear-shaped lesions in periventricular regions that are perpendicular to the long axis of the corpus

callosum (the so-called “Dawson finger”), persistent hypointense T1 lesions (so-called black holes), and multiple plaques disseminated in the white matter and each plaque may become confluent with another to form a sheet of hyperintense lesions. However, up to a quarter of NMO/NMOSD patients have brain white matter lesions indistinguishable from MS lesions morphologically and geographically using conventional MRI (Wingerchuk, Lennon et al. 2007, Matthews, Marasco et al. 2013). Further, the MS lesions had been quantified by quantitative MRI techniques with histopathological validation (Bitsch, Kuhlmann et al. 2001, Schmierer, Scaravilli et al. 2004), but detailed *in vivo* MRI quantitative studies of the damaged substrate within the white matter lesions in NMO/NMOSD are lacking.

The term normal-appearing white matter (NAWM) was previously defined in patients with MS as visually normal areas on macroscopic histopathological study (Allen and McKeown 1979) or conventional MRI (Trapp, Peterson et al. 1998, Evangelou, Esiri et al. 2000). These normal appearing areas have been shown to contain microscopic abnormalities such as demyelination, gliosis (Allen and McKeown 1979), axonal damage (Trapp, Peterson et al. 1998, Evangelou, Esiri et al. 2000), or evidence of blood-brain barrier disturbance (Plumb, McQuaid et al. 2002), which can also be quantified by MRI (Filippi, Rocca et al. 1999, Manfredonia, Ciccarelli et al. 2007). In MS, the abnormalities within the NAWM supplemental to T2-weighted white matter lesions are related to clinical disability (Bellmann-Strobl, Stiepani et al. 2009), cortical lesion load (Mistry, Abdel-Fahim et al. 2014), and cognitive decline (Dineen, Vilisaar et al. 2009).

In patients with NMO/NMOSD, thorough histopathology study of NAWM is lacking. The MRI analysis of NAWM did not differ from healthy subjects using the magnetisation transfer ratio (MTR) technique at 1.5T previously (Rocca, Agosta et al. 2004, Pichiecchio, Tavazzi et al. 2012). However, diffusion tensor imaging did show abnormalities confined to specific tracts within NAWM, including the optic tracts and corticospinal tracts at 1.5T and 3T (Yu, Lin et al. 2006, Yu, Lin et al. 2008, Pichiecchio, Tavazzi et al. 2012, Zhao, Zhou et al. 2012). Therefore, whether NAWM in NMO/NMOSD patients is indeed normal is still open to debate and a more sophisticated methodology such as using 7 T MRI for quantitative analysis is necessary to solve this question.

We hypothesised that (i) NMO/NMOSD lesions are less demyelinated than MS lesions and (ii) the integrity of NAWM in NMO/NMOSD is less compromised than in MS. Therefore, we acquired T1 relaxation time and MTR maps from the brains of patients with NMO/NMOSD, MS and healthy participants using high-resolution imaging at 7T to compare their intracranial lesions and NAWM.

6.2 METHODS

This study was approved by the Research Ethics Committee (REC), and by the relevant National Health Service (NHS) Research & Development (R&D) department (REC reference: 13/EM/0080).

6.2.1 Patients

Patients referred by neurologists during the period July 2013 to November 2014 were enrolled at the Sir Peter Mansfield Magnetic Resonance Centre (SPMMRC) at the University of Nottingham. Patients were eligible if (1) they had a diagnosis of NMO/NMOSD or MS according to the diagnostic consensus as assessed by the MS specialists, and (2) they were clinically stable, fully conscious and orientated. Age- and gender-matched healthy volunteers were recruited by posters displayed on the public areas and were interviewed by a neurologist to exclude any neurological or systemic inflammatory diseases. The exclusion criteria included claustrophobia, metal objects in their bodies (implanted devices such as vascular clips, pacemakers, certain artificial heart valves, artificial joints, or other metallic/electronic material), pregnancy or intention to become pregnant. All participants provided written informed consents. Patients were assessed with the Expanded Disability Status Scale (EDSS) (Kurtzke 1983), the Multiple Sclerosis Functional Composite (MSFC) (Cutter, Baier et al. 1999) and self-administered Fatigue Severity Scale (FSS) (Krupp, LaRocca et al. 1989). Healthy volunteers were only assessed with MSFC and FSS.

6.2.2 MR imaging acquisition

All participants underwent MRI scanning using a 7T Philips Achieva (Philips Medical Systems, Best, Netherlands) implemented with whole body gradients. The scanner is equipped with a 32-channel head-only parallel imaging Sensitivity Encoding technique (SENSE) receive coil and a head-only volume quadrature transmit radiofrequency (RF) coil (Nova Medical, Inc., Wilmington, MA). All participants completed an MRI safety questionnaire. The parameters of MRI sequences are detailed in Table 6-1 and the techniques are detailed previously (Mougin, Coxon et al. 2010, Mougin, Clemence et al. 2013, Al-Radaideh, Mougin et al. 2015, Mougin, Abdel-Fahim et al.

2016). In short, whole brain 3D Phase-sensitive inversion recovery (PSIR) images were acquired and segmented to obtain a white matter mask (Mougin, Abdel-Fahim et al. 2016). Magnetization-prepared rapid gradient echo (MPRAGE) sequences with seven inversion times were acquired and processed to produce quantitative measurement of T1 relaxation time (Brant-Zawadzki, Gillan et al. 1992, Al-Radaideh, Mougin et al. 2015). Magnetization transfer images were acquired for quantitative measurement of magnetization transfer ratio derived from two raw imaging–MT_{nosat} (mixed proton- and T2*-weighted imaging with no presaturation) and MT_{sat} (with presaturation to water) (Mougin, Coxon et al. 2010, Mougin, Clemence et al. 2013). In addition, MT_{sat} imaging was used for lesion identification, which have a compatible identifiability to 3T fluid-attenuated inversion recovery (FLAIR) imaging (appendix 2). The scanning protocol included a B1 map to correct the MT imaging for the effect of B1 inhomogeneity.

6.2.3 Post- processing

Image post-processing was performed on all subjects independently by I-J.C. and O.E.M., who were blinded to patient identification and clinical information. Firstly, we generated a lesion mask and a NAWM mask for each subject.

To obtain the lesion mask, the lesions were visually identified and outlined on the MT_{sat} using a semi-automated, seed-growing technique implemented in the in-house NeuRoi image analysis software (Dr Christopher R Tench; Clinical Neurology, University of Nottingham, UK; http://www.nottingham.ac.uk/scs/divisions/clinicalneurology/software/neuroi.aspx). The lesions were identified from all imaging slices covering the supratentorium, midbrain, rostral pons, and part of the cerebellum. Then, the total volume of these lesions was calculated and a binary lesion mask was generated using NeuRoi.

To obtain the NAWM mask, the white matter probability map was firstly segmented from PSIR imaging using automatic segmentation (Ashburner and Friston 2005) implemented in Statistical Parametric Mapping Software (SPM8, Wellcome Department of Imaging Neuroscience, London; http://www.fil.ion.ucl.ac.uk/spm/), with a threshold value of 0.95 to generate a binary WM mask.

Voxels were determined as white matter depending on which probability map showed the greatest probability at that voxel. Further, the lesion-free white matter mask was generated by applying the lesion masks and then eroded by three voxels to exclude any partial volume effects at the grey matter-white matter junctions, lesion-white matter or cerebrospinal fluid (CSF)-white matter borders (Al-Radaideh, Mougin et al. 2015).

Subsequently, a T1 map and a B1-corrected MTR map were calculated from raw images for each subject on a pixel-by-pixel basis. The T1 map was calculated from the formula (Kingsley 1999, Vaithianathar, Tench et al. 2002, Vaithianathar, Tench et al. 2003)

$$M_0 = M\left(1 - \alpha \exp\left(\frac{-TI}{T1}\right)\right)$$

The MTR map was calculated from the formula

$$MTR = \frac{(MTnosat - MTsat)}{MTnosat}$$

Finally, the T1 map and B1-corrected MTR map were combined with the lesion mask and the NAWM mask to yield a lesion map and a NAWM map for each subject (Figure 6-1). All voxels of NAWM with MTR less than 10% were excluded from analysis to avoid signals of image noise and CSF (Filippi and Rovaris 2000, Davies, Ramio-Torrenta et al. 2004). All the acquired images were registered to the same space using FLIRT in FSL (available at www.fmrib.ox.ac.uk/fsl) (Jenkinson et al., 2002).

We compared lesion properties using the mean values of the lesion maps. To detect subtle changes in NAWM, histogram metrics of the NAWM map derived from each subject were generated for statistical analysis. An NAWM histogram with a bin size of 1 millisecond for T1 relaxation time and of 0.1% for MTR was normalized to the total voxel count to eliminate the effect of differences

in NAWM volumes between subjects. Five parameters of the histogram were extracted: the height and position of the histogram peak and the positions corresponding to the 25th, 50th and 75th percentiles.

6.2.4 Statistical Analysis

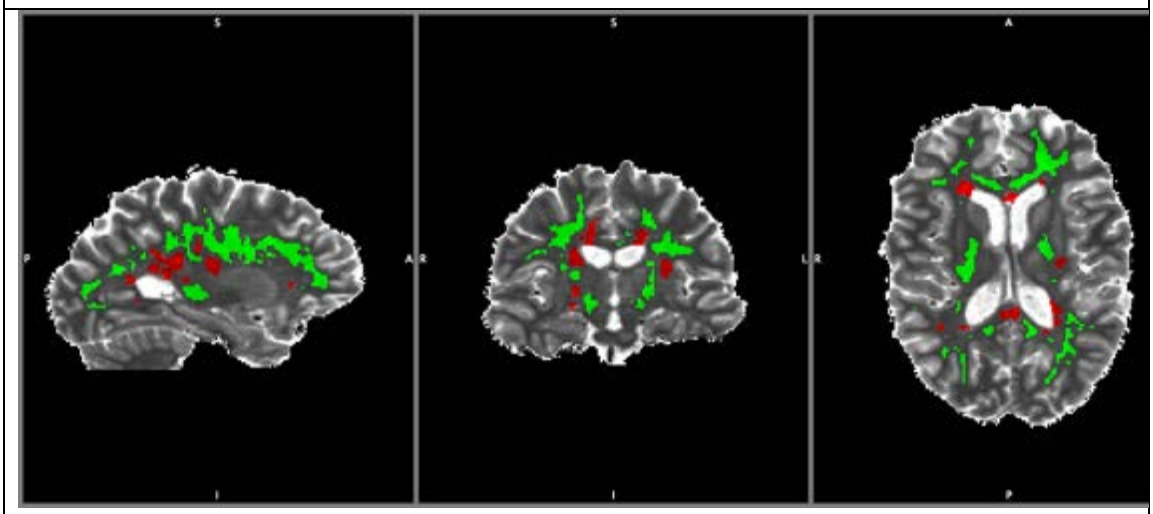
Comparisons between the subgroups were performed using the Fisher's exact test for dichotomised data, the Mann-Whitney U test for median value of continuous data and ordinal clinical rating scales, and the independent-samples T test for mean value of continuous data and histogram parameters. To assess the correlation between MRI-derived parameters, Spearman's rank correlation coefficient (rho) was used. A *p*-value of less than 0.05 was considered to be significant. All statistical tests were two-tailed. All calculations were performed by the IBM SPSS software package, version 22 (Chicago, Ill).

Table 6-1 Imaging sequences.

Parameters	Magnetization transfer imaging	T1-MPRAGE imaging	Phase-sensitive inversion recovery (PSIR) imaging
Data acquisition	3D, magnetisation transfer prepared turbo field echo (MT-TFE)	3D, Magnetisation Prepared (Inverted) - Turbo Field Echo (IR-TFE)	3D, Magnetisation Prepared (Inverted) - Turbo Field Echo (IR-TFE) with two readouts
Repetition time/Echo time (msec)	12/6.4	6.9/3.2	13/6
Inversion time (msec)		7 sets (153, 303, 503, 803, 1203, 1803, 2503)	2 sets (780 and 2380)
Shot-to-shot interval (sec)	10	8	5
TFE factor	450	240 per inversion	
Flip angle (degrees)	8	8	8
Field of view (mm^3)	192x180x120	200x180x120	200x181x120
Resolution (mm)	0.6x0.6x0.6	1.25x1.25x1.25	0.6x0.6x0.6
Matrix dimension	320x320x200	160x160x96	336x336x200
Additional information	Two images: (1) MTnosat was acquired with not presaturation), (2) MTsat was acquired with presaturation 20 off-resonance pulses (sinc pulses with bandwidth of 300 Hz and off-resonance by 1.0k Hz (-3.4 ppm), 20ms between each pulse. (Mougin, Coxon et al. 2010, Mougin, Clemence et al. 2013)	Seven images from the inversion recovery (Al-Radaideh, Mougin et al. 2015)	Two images from the inversion recovery reconstructed together (Mougin, Abdel-Fahim et al. 2016)
Acquisition time (min: sec)	12:55	13:53	11:35

Abbreviations: mm: millimeter; mm^3: cubic millimeter; ms: millisecond; sec: second.

Figure 6-1 A NAWM mask (green) and a lesion mask (red) of T1 map in a patient with multiple sclerosis.



Lesion mask (red) was visually identified and outlined using a semi-automated, seed-growing technique implemented in the in-house NeuRoi image analysis software. NWAM mask (green) was obtained from white matter mask segmenting lesions (red) out and then eroded by three voxels to exclude any partial volume effects.

6.3 RESULTS

6.3.1 Patient characteristics

We prospectively investigated 36 participants: 14 patients with NMO/NMOSD, 12 patients with MS and 10 healthy participants. All patients were in remission status. The date of the MRI scan was separated from a previous attack by at least 30 days. Ten patients (71%) with NMO presented with optic neuritis and transverse myelitis simultaneously or sequentially according to the revised diagnostic criteria (Wingerchuk, Lennon et al. 2006) and four (29%) had NMOSD (Wingerchuk and Lucchinetti 2007). Eight MS patients (67%) had relapsing-remitting MS, 2 (17%) had secondary progressive MS, and 2 (17%) had primary progressive MS.

Characteristics of subject groups are shown in Table 6-2 and clinical assessments are summarised in Table 6-3. The age and gender distribution was not different between all three groups. Between MS and NMO/NMOSD patients, the disease duration, EDSS, FSS, quantitative functional tests of leg function/ambulation (Timed 25-Foot Walk), and arm function (9-Hole Peg Test) were not different. However, the cognitive function (Paced Auditory Serial Addition Test with a 3-second interstimulus interval, PASAT-3'') was significantly lower in patients with NMO/NMOSD than those with MS (mean correction number 34.1 vs 47.6, respectively; p= 0.006). The MSFC score derived from a total of three aforementioned components was similar between NMO/NMOSD and MS group.

Between patients and healthy controls, measures were significantly worse in patients than in healthy controls except the cognitive function (PASAT-3'') was similar in MS patients and healthy controls.

6.3.2 MRI

6.3.2.1 Lesion

As shown in Table 6-4, lesion count and volume were significantly higher in MS patients than NMO patients and healthy controls. A few incidental lesions (small and nonspecific hyperintensity

on MT images) were identified in 4 healthy controls. Lesions in MS patients had significantly higher T1 relaxation times than lesions in NMO/NMOSD patients or healthy controls. Similarly, MS lesions had the lowest mean lesion MTR value, followed by NMO/NMOSD lesions. There was a correlation between mean T1 and mean MTR of lesions in NMO/NMOSD (Spearman's rho = -0.835, p <0.001) and in MS (-0.825, p=0.001), but there was no such correlation in the few lesions found in healthy participants (p=0.200).

6.3.2.2 NAWM

The histogram metrics of T1 relaxation time and MTR in the NAWM of the groups are shown in Table 6-5 and averaged histograms are shown on Figure 6-2. The heights of peak in the histogram of both T1 relaxation time and MTR values in the NAWM of NMO/NMOSD patients were significantly lower than those in healthy subjects and not different from those in MS patients, reflecting the width of these relative frequency histograms. The peak position was not statistically different between the three groups, but in MS patients, the 75th percentile for T1 was larger and 25th percentile for MTR was lower than those in healthy participants.

Table 6-2 Comparisons of demographics and clinical characteristics between patients with NMO/NMOSD or MS, and healthy controls.

	NMO/NMOSD (n = 14)	**MS[a] (n = 12)**	**Controls (n = 10)**	**P_{NMO-MS}**	**$P_{NMO-control}$**	**$P_{MS-control}$**
Median years of age (IQR)	54.0 (37.0–63.0)	55.5 (40.5–63.5)	44.5 (24–57)	0.631	0.709	0.228
Female (n, %)	10 (71)	7 (58)	7 (70)	0.683	1.000	0.675
Presence of serum Ab (n, %)	NMO-IgG/AQP4: 8 (57) MOG: 1 (7)	n/a	n/a	n/a	n/a	n/a
Median years of disease duration (IQR)	8 (3.0–11.0)	13 (7.5–20.5)	n/a	0.123	n/a	n/a
Receiving immunosuppressants or immunomodulatory agents (n, %)	14 (100)[b]	5 (42)[c]	n/a	0.001*	n/a	n/a

Abbreviations: Ab, antibody; AQP4, Aquaporin 4; IgG: immunoglobulin G; IQR, interquartile range; MOG, Myelin Oligodendrocyte Glycoprotein; MS, multiple sclerosis; NMO, neuromyelitis optica; NMOSD, neuromyelitis optica spectrum disorders; n/a: not applicable or not available; SD, standard deviation.
*A p-value of less than 0.05 was considered to be significant. All statistical tests were two-tailed. Analyses included Mann-Whitney U test of median and $\chi 2$ test, as appropriate.
MS patients included 8 (67%) relapsing remitting MS, 2 (17%) secondary progressive MS, and 2 (17%) primary progressive MS
NMO patients received long-term immunosuppresants (5 with azathioprine, 5 with rituximab, 3 mycophenolate mofetil, and of them 3 combined with low dose corticosteroid) and one patient was treated by corticosteroid alone.
Four MS patients received long-term immunomodulatory agents (1 with interferon beta-a, 1 glatiramer acetate, 1 Natalizumab, and 1 dimethyl fumarate) and one patient just tapered steroid before the scan.

Table 6-3 Comparison of EDSS, FSS, and MSFC between 14 patients with NMO/NMOSD, 12 patients with MS, and 10 healthy subjects.

	NMO/NMOSD (n = 14)	MS (n = 12)	Controls (n = 10)	P_{NMO-MS}	$P_{NMO-control}$	$P_{MS-control}$
Median EDSS and FSS (IQR)						
EDSS	5.25 (4.0–6.0) n=12	6.0 (4.0–6.5) n=12	n/a n=10	0.742	n/a	n/a
FSS	5.6 (5.3–6.3)	6.0 (4.9–6.4)	2.1 (1.2–2.6)	0.630	<0.001*	<0.001*
Mean MSFC raw score and z-score[a] (± SD)						
T25-FW [b]	n=11	n=12	n=10			
Raw score, sec	8.56 ± 2.46	9.98 ± 4.14	4.59 ± 0.30	0.392	<0.001*	0.001*
Z-score	-13.40 ± 8.31	-17.69 ± 14.56	0 ± 1	0.392	<0.001*	0.001*
9HPT	n= 14	n=12	n=10			
Raw score dominant hand, sec	34.83 ± 24.79	27.83 ± 4.80	17.60 ± 1.93	0.318	0.022*	<0.001*
Raw score nondominant hand[c], sec	86.48 ± 199.24	31.85 ± 12.10	19.95 ± 2.75	0.324	0.234	0.006*
Z-score of both hands	-3.89 ± 2.00	-3.42 ± 1.20	0 ± 1	0.471	<0.001*	<0.001*
PASAT-3'' [d]	n=13	n=10	n=10			
Raw score (correct number/60)	34.15 ± 10.58	47.60 ± 10.29	52.00 ± 6.68	0.006*	<0.001*	0.274
Z-score	-2.67 ± 1.58	-0.66 ± 1.54	0 ± 1	0.006*	<0.001*	0.274
MSFC sum score	n=10	n=10	n=10			
Z-score[e]	-6.72 ± 2.84	-6.05 ± 4.83	0 ± 0.57	0.588	<0.001*	0.003*

Abbreviations: EDSS, Expanded Disability Status Scale; FSS, Fatigue Severity Scale; IQR, interquartile range; MSFC, Multiple Sclerosis Functional Composite; MS, multiple sclerosis; n/a: not applicable or not available; NMO, neuromyelitis optica; NMOSD, neuromyelitis optica spectrum disorders; PASAT-3'', Paced Auditory Serial Addition Test with a 3-second interstimulus interval; SD, standard deviation; sec, second; T25-FW, Timed 25-Foot Walk test; 9HPT-dominant, 9-Hole Peg Test of dominant hand.

*A p-value of less than 0.05 was considered to be significant. All statistical tests were two-tailed. Analyses included Mann-Whitney U test of median and t-test for mean, as appropriate.

A z-score is a standardized number representing how close a test result is to the mean of 10 healthy controls in this study and is expressed in units of standard deviation. A negative z-score indicates the test result was worse than the mean performance of healthy controls by how many standard deviation units.

The T25-FW was unable to be performed in 2 NMO patients because of limited physical ability, and was not completed in 1 NMO patient because of physical fatigue.

One subject with NMO was unable to perform the test on non-dominant hand because of limited physical ability, a value of 777 seconds was applied as suggested by MSFC scoring manual.

PASAT-3'' test was refused to be performed in 1 NMO patient and 2 MS patients because of they think they could not comprehend the principle of test.

MSFC sum Z score was calculated for subjects who completed all tests in combining the 3 components into a single z score.

Table 6-4 Comparisons of lesion load and lesion T1 relaxation time and MTR between NMO/NMOSD, MS and healthy controls.

	NMO/NMOSD (n = 14)	**MS (n = 12)**	**Controls (n = 10)**	**P_{NMO-MS}**	**$P_{NMO-control}$**	**$P_{MS-control}$**
Lesion count (median, IQR)	11 (2.0–18.0)	74 (33.5–112.5)	0 (0.0–3.0) [a]	0.001*	0.001*	<0.001*
Lesion volume (mm^3) (median, IQR)	242.4 (60.7–696.0)	5217.0 (2356.8–9488.2)	0 (0–27.2) [a]	<0.001*	<0.001*	<0.001*
T1 relaxation time (msec) mean ±SD	1869±367	2163±268	1511±117	0.033*	0.080	<0.001*
MTR (%) mean ±SD	38.8±6.5	32.0±4.6	46.5±3.8	0.006*	0.042*	<0.001*

Abbreviations: IQR, interquartile range; mm^3, cubic millimeter; MS, multiple sclerosis; msec, millisecond; MTR, magnetization transfer ratio; NMO, neuromyelitis optica; NMOSD, neuromyelitis optica spectrum disorders; SD, standard deviation;

[a] Among 10 healthy controls, 4 had incidental findings of at least one nonspecific white matter lesion.

*A *p*-value of less than 0.05 was considered to be significant. All statistical tests were two-tailed. Analyses included Mann-Whitney U test of median and t-test of mean, as appropriate.

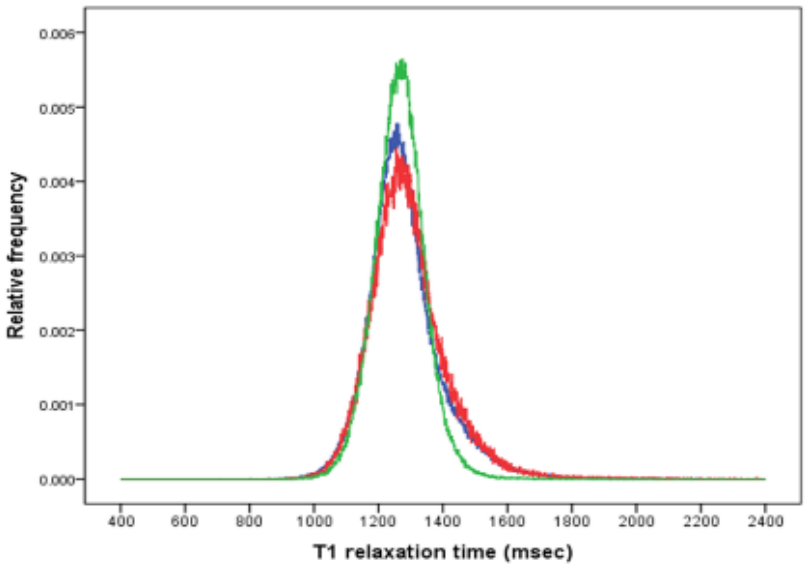
Table 6-5 Comparisons of histogram metrics of NAWM measured by T1 relaxation time or by MTR between NMO/NMOSD, MS, and healthy controls.

	NMO/NMOSD (n = 14)	**MS (n = 12)**	**Controls (n = 10)**	**P_{NMO-MS}**	**$P_{NMO-control}$**	**$P_{MS-control}$**
T1 relaxation time (mean ± SD)						
Peak height (10^{-3} pixels)	5.6±1.5	5.6±1.2	6.8±1.0	0.987	**0.035***	**0.026***
Peak position (msec)	1259±43	1265±47	1264±25	0.739	0.732	0.969
25th percentile (msec)	1216±40	1217±31	1218±27	0.949	0.891	0.931
50th percentile (msec)	1275±49	1282±37	1265±23	0.695	0.550	0.225
75th percentile (msec)	1339±64	1353±55	1312±22	0.560	0.166	**0.033***
MTR (mean ± SD)						
Peak heights (10^{-3}, pixels)	7.6±1.5	6.6±1.3	8.9±1.0	0.098	**0.038***	**<0.001***
Peak position (%)	55.3±3.4	54.2±3.2	56.2±3.8	0.367	0.563	0.182
25th percentile (%)	51.0±2.9	49.3±3.3	52.5±3.5	0.183	0.260	**0.039***
50th percentile (%)	54.9±3.1	53.9±2.9	55.7±3.6	0.395	0.551	0.195
75th percentile (%)	58.7±3.3	58.4±3.0	58.9±3.8	0.804	0.871	0.705

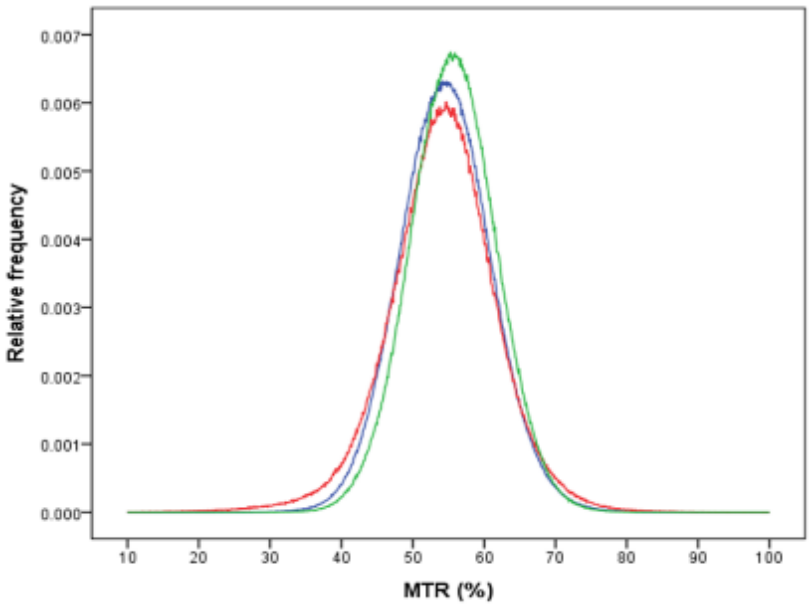
Mean values of five parameters derived from each group were used for analysis: the heights and positions of the histogram peak and the positions corresponding to the 25th, 50th and 75th percentiles of the histogram.
Abbreviations: NMO, neuromyelitis optica; NMOSD, neuromyelitis optica spectrum disorders; MS, multiple sclerosis; msec, millisecond; MTR, magnetization transfer ratio; SD, standard deviation.
*A p-value of less than 0.05 was considered to be significant. All statistical tests were two-tailed using t-test of mean.

Figure 6-2 The averaged histograms of NAWM (a) T1 relaxation time maps (b) magnetization transfer ratio maps in patients with NMO/NMOSD (blue), MS (red) and healthy subjects (green).

(a)



(b)



(a) The average height of peak in the histogram of T1 relaxation time values in the NAWM of NMO/NMOSD patients was significantly lower than that in healthy subjects and not different from that in MS patients, reflecting the wider width of the histograms in NMO/NMOSD and in MS. The peak position was not statistically different between the three groups, but in MS patients, the 75th percentile for T1 was larger than

that in healthy participants. (b) The average height of peak in the histogram of MTR values in the NAWM of NMO/NMOSD patients was significantly lower than that in healthy subjects and not different from that in MS patients, reflecting the width of these relative frequency histograms. The peak position was not statistically different between the three groups, but in MS patients, the 25th percentile for MTR was lower than that in healthy participants.

6.4 DISCUSSION

This study used high resolution 7T quantitative MRI to quantify the T1 relaxation time and MTR measures of intracranial lesions and NAWM in NMO/NMOSD patients compared to MS and healthy controls. For lesions, NMO/NMOSD have abnormal mean MTR values, and both T1 relaxation time and MTR values are different from those in MS. Our findings indicate that NMO/NMOSD lesions contain abnormal myelin but relatively similar levels of mobile water to healthy controls. One possible hypothesis is that the NMO/NMOSD lesions could be possibly destroyed in a way that preserves T1. When compared to MS lesions, NMO/NMOSD lesions have a different composition and lesions in NMO/NMOSD apparently had less change in the tissue. In addition, the standard deviation of lesions measured by both techniques in NMO/NMOSD was as wide as in MS, suggesting that lesion heterogeneity, which has been reported in MS, also exists in NMO/NMOSD. In the NAWM, NMO/NMOSD had a lower histogram peak height measured of both the T1 relaxation time and MTR than in healthy controls, but all these histogram metrics were not significantly different from those in MS. However, there was a trend for NMO/NMOSD measures to be less different from controls than in the MS patients, similar to the changes seen in lesions. The decreased peak heights in NAWM in both MS and NMO/NMOSD patients indicate that at least some of the NAWM in patients with NMO/NMOSD is abnormal, as has been shown in MS. Overall, our study suggests that NMO/NMOSD lesions had less myelin damage than MS lesions, and as in MS, NAWM in NMO/NMOSD is abnormal.

T1 relaxation time is an intrinsic biophysical property of the tissue and is determined primarily by water binding to macromolecules including myelin, and iron content in brain tissue. It has previously been shown that the increased T1 relaxation time found in the white matter of MS patients, compared to intact white matter was highly associated with damaged structural integrity of the white matter (Ormerod, Bronstein et al. 1986, Schmierer, Scaravilli et al. 2004). In MS, elevated T1 relaxation time is correlated with loss of myelin and axons (Bitsch, Kuhlmann et al. 2001), and also increased free water that to some extent reflects gliosis by virtue of the increase in water protons (Ormerod, Miller et al. 1987). MT imaging measures the proton exchange between

free protons and macromolecular bound protons and is a relatively specific measurement of the myelin content of white matter (Wolff and Balaban 1989, Schmierer, Scaravilli et al. 2004). The decrease in MTR reflects decreased myelin content, which can be due to demyelination or incomplete remyelination (Schmierer, Scaravilli et al. 2004). Our results in MS lesions were similar to previous studies, with significantly increased mean T1 and decreased mean MTR compared to visible white matter hyperintensities in healthy controls.

Our study shows abnormal mean T1 relaxation time and MTR in lesions of NMO/NMOSD patients compared to the hyperintensities of healthy controls but the deviation from normal was smaller in NMO/NMOSD than that in MS patients. Our findings support the first hypothesis and seem to be compatible with the known pathological characteristics of NMO/NMOSD and MS lesions. The histopathological hallmark of NMO/NMOSD lesions in active lesion areas is a loss or decreased stain of AQP4 protein and absence of astrocyte or loss of astrocyte distal processes (Parratt and Prineas 2010, Lucchinetti, Guo et al. 2014, Popescu, Guo et al. 2015). Loss of AQP4 proteins located at the end-feet processes of astrocytes causes disruption of the interface between brain parenchyma and perivascular space, and between brain and cerebrospinal fluid (Nicchia, Nico et al. 2004). Previous studies generally indicated a relative preservation of myelin within the early active lesions in NMO/NMOSD patients compared to MS lesions (Lucchinetti, Mandler et al. 2002, Bruck, Popescu et al. 2012, Misu, Hoftberger et al. 2013, Lucchinetti, Guo et al. 2014). MS lesions are characterised by perivenular inflammation with demyelination and incomplete remyelination, and confluent sheets of macrophage infiltration admixed with reactive astrocytes in completely demyelinated regions (Lucchinetti, Parisi et al. 2005, Young, Weinshenker et al. 2010).

In contrast to previous histopathological findings of focal necrosis or cystic formation in autopsied patients with fulminant NMO/NMOSD, the patients in the current study had overall fewer destructive lesions than MS patients. One possible explanation is that patients with NMO/NMOSD in this study survived with effective acute immunotherapy including plasma exchange and are kept on immunosuppressants or B cell depletion therapy. Another possibility is lesion heterogeneity. Many other forms of lesions were found in NMO/NMOSD, including blood vessel wall thickening and hyalinization, rim or rosette of immunoglobulin and complement, infiltration of eosinophils

or granulocytes (Roemer, Parisi et al. 2007, Misu, Hoftberger et al. 2013, Guo, Lennon et al. 2014). However, these changes may not contribute enough water to account for the observed changes measured by T1 relaxation time. Furthermore, patients with MS in the study had relatively longer disease courses than patients with NMO/NMOSD did, and as such the tissue destruction of the chronic lesions may be more obvious in MS lesions.

Previous studies showed that abnormalities in NAWM are subtype dependent (Vrenken, Rombouts et al. 2006): secondary progressive MS (SPMS) shows changes in both peak position and height of NAWM histogram but RRMS and primary progressive MS (PPMS) show decreased height only (Vrenken, Geurts et al. 2006, Manfredonia, Ciccarelli et al. 2007, Vrenken, Pouwels et al. 2007, Papadopoulos, Tozer et al. 2010). The change in NAWM of SPMS has been linked to microglia-mediated neuroinflammation (Moll, Rietsch et al. 2011), which has been shown to be associated with cognitive dysfunction and brain atrophy (Vrenken, Pouwels et al. 2007). In addition, axonal degeneration (i.e. axonal swellings and axonal end bulbs) (Giovannoni, Cutter et al. 2017, Petrova, Carassiti et al. 2017) of NAWM was shown to be more frequent in SPMS and PPMS than in RRMS (Kutzelnigg, Lucchinetti et al. 2005). It is of note that the axonopathy of NAWM could develop independently from the white matter lesions (Pelletier, Nelson et al. 2003, Kutzelnigg, Lucchinetti et al. 2005). Since our study included more RRMS and PPMS, the change in NAWM peak location was not observed. However, our study found that MS patients had a decrease in the peak height of NAWM histogram for both T1 and MTR suggesting a decreased proportion of normal white matter.

This study also compared histogram metrics of NAWM between NMO/NMOSD and S patients. We found a decreased proportion of normal white matter in NMO/NMOSD compared to those in healthy controls but the metrics of the histogram were indistinguishable statistically to those in MS, rejecting the second testing hypothesis. The lack of difference of the peak height of NAWM between NMO/NMOSD and MS (p=0.098) may be attributed to the small sample size. However, we also observed that both NMO/NMOSD and MS patients have a certain degree of cognitive dysfunction as measured by PASAT-3'' test. In MS, Audoin et al. showed that tissue disorganisation within the normal appearing white matter measured by MTR were likely associated

with aberrant cortical neural networks subserving attention and speed of information processing to execute PASAT-3'' by a functional MRI study (Audoin, Au Duong et al. 2005). Summers et al observed that the MTR changes of NAWM at early relapsing remitting MS is predictive to PASAT-3" performance at five years later (Summers, Fisniku et al. 2008). The histopathological changes of NAWM in MS include variation of myelin density, presence of activated microglia, oedema, axonal swelling and decreased density, and alteration of blood-brain-barriers (Allen and McKeown 1979, Trapp, Peterson et al. 1998, Evangelou, Esiri et al. 2000, Plumb, McQuaid et al. 2002). Cortical activation and volume are both related to PASAT-3" performance in the priori MS studies (Audoin, Au Duong et al. 2005, Summers, Fisniku et al. 2008). Furthermore, the observation of a relatively preserved capacity of cognition and hand function compared to ambulatory function in MS patients in our MS patient cohort is in line with central length-dependent axonopathy hypothesis. It hypothesizes that clinical observable neurodegeneration (e.g. loss of axons in NAWM or cortex in MS) is severer in long fibre tracts than short tracts because the long tract fibres is more frequently attacked by random focal inflammation than short tract fibres (Giovannoni, Cutter et al. 2017). Although the axons in NAWM of NMO/NMOSD seemed to be preserved as measured by MR spectroscopy at 1.5T or 3T field strength in previous studies (Bichuetti, Rivero et al. 2008, Aboul-Enein, Krssak et al. 2010, de Seze, Blanc et al. 2010, Pichiecchio, Tavazzi et al. 2012), there has been as yet no comparative MRI and histological studies on NAWM in NMO/NMOSD. In addition to NAWM, the decreased performance of PASAT-3 in NMO/NMOSD may also attribute to cortical pathology, such as a neuronal loss in cortical layers II, III and IV (Saji, Arakawa et al. 2013) and loss of AQP4 proteins at pial surfaces that cause disruption of the brain-blood barriers (Guo, Weigand et al. 2017).

These abnormal findings in NAWM of NMO/NMOSD appear to be robust because they were disclosed by two different MRI techniques based on different physical principles. Previous studies using diffusion tensor imaging (DTI), a sensitive tool to the microstructural diffusivity of white matter tracts, also found abnormalities in optic tracts, corpus callosum, medulla oblongata, and corticospinal tracts in NMO/NMOSD patients (Yu, Lin et al. 2006, Yu, Lin et al. 2008, Pichiecchio,

Tavazzi et al. 2012, Zhao, Zhou et al. 2012). Using MTR quantitative imaging at 1.5T field strength to evaluate NAWM of NMO/NMOSD, two earlier studies did not find altered MTR mean values or peak heights (Rocca, Agosta et al. 2004, Pichiecchio, Tavazzi et al. 2012). However, using MR imaging at ultra-high-field, we found evidence suggesting abnormal MTR in 'normal' appearing white matter. We assume that we were able to detect MTR change at ultrahigh field. It is possible that the difference in spatial resolution between our study and the lower field study makes it possible to reveal smaller changes in NAWM. This question could be answered by repeating the 7T scanning at the spatial resolution used previously at lower field.

Several limitations of the current study deserve discussion. Firstly, the temporal lobes were incompletely included because the signal loss caused by inhomogeneity in the applied transmit radiofrequency (RF) field at high field is more pronounced in these regions. Secondly, the NAWM map included supratentorial, basal ganglia and infratentorial regions. The pathology in the basal ganglia and infratentorial brain might be different from the supratentorial areas and this needs further evaluation. Thirdly, the lesion mask was applied on the MTR imaging which might miss approximate 1.5% FLAIR detectable small lesions (i.e. less than 3 mm in diameter) in our previous experience (appendix 2). Furthermore, the study comprises a heterogeneous clinical phenotype of MS (i.e. RRMS, SPMS, PPMS) and NMO/NMOSD (i.e. AQP4 or MOG antibodies positive or double negative patients). The difference between homogenous phenotypes of NMO/NMOSD and MS is still elusive. Nevertheless, the current study is strengthened by piloting two quantitative methods using 7T MRI and comparing two groups of patients who were matched for demographic characteristics and levels of disability.

6.5 CONCLUSIONS

The analysis of T1 relaxation time and MTR values suggests that NMO/NMOSD lesions had decreased myelin content but the changes in the lesions were less pronounced than in MS lesions, suggesting that they are less destructive. The 7T MRI demonstrated a decreased peak height of NAWM histograms in patients with NMO/NMOSD compared to those of healthy controls but the metrics of the histogram were indistinguishable statistically to those in MS, suggesting that at least part of the NAWM in patients with NMO/NMOSD is indeed abnormal, which has not been observable using MRI at a lower field strength.

Chapter 7. CONCLUSIONS AND FUTURE RESEARCH

7.1 SUMMARY

During my period of research as demonstrated by this thesis I have been able to more clearly describe the epidemiology of NMO/NMOSD or MS in the UK, including its comorbidity and associated mortality, and to show that the normal appearing white matter is at some degree abnormal in NMO/NMOSD as well as MS patients.

The main findings are:

1. **NMO/NMOSD is very rare in England and the prevalence of MS is 70 times higher than that of NMO/NMOSD.**

   The estimation was based on CPRD and HES linked dataset as patients with NMO/NMOSD may need hospitalization for disease diagnosis and treatment. The estimate of patient number of NMO/NMOSD is slightly higher than the testing hypothesis based on local experience at the Queen's Medical Centre in Nottinghamshire (NMO/NMOSD: MS about 1:100). This discrepancy may be explained by the fact that the increased recognition of disease entity of NMO/NMOSD and the availability of laboratory tests for antibodies against APQ4 and MOG through the Oxford Neuroimmunology Service in the past decade. Patients who were diagnosed to be MS a decade ago may be classified as NMO/NMOSD using the updated consensus criteria and laboratory tests. Nevertheless, population-based estimate from CPRD confirms the rarity of NMO/NMOSD in England, which is consistent with local empirical experience.

2. **Patients with NMO/NMOSD have a higher risk of mortality than the matched general population, and the matched MS patients.**

   The findings support the testing hypothesis. Since NMO/NMOSD patients have a higher disease severity and mortality, they are more likely to need hospitalization and specialist care. Therefore, incidence and prevalence of NMO/NMOSD are underestimated with a

greater extent than MS when using CPRD main (primary care) database alone compared to using CPRD with linked HES dataset.

3. **The comorbidity is relevant to premature mortality in patients with MS and NMO/NMOSD. Patients with NMO/NMOSD have relatively higher comorbidity at disease diagnosis and all-cause mortality than patients with MS.**

   Population-based estimates from CPRD support our testing hypothesis that patients with MS and NMO/NMOSD have a higher comorbidity burden and risk for premature mortality. Comorbidities in patients with MS are important consideration in clinical care for these patients because the burden of comorbidity at MS diagnosis is already higher than that in the matched controls, which is negatively impacted on patient survival in a dose dependent effect. Similar to MS, NMO/NMOSD has a similar trend on the dose effect of comorbidity on all-cause mortality despite not statistically significant. The comorbidity at disease diagnosis is higher in NMO/NMOSD than in MS, and the all-cause mortality is also higher in NMO/NMOSD when compared to the age- and gender-matched MS. However, the cumulative new comorbidity in MS or NMO/NMOSD after disease diagnosis is not different from that in matched controls, and this rejects the initial hypothesis that patients with MS and NMO/NMOSD cumulate comorbidities faster than controls.

4. **Patients with MS have a higher risk of epilepsy either before or after the diagnosis of MS.**

   Comparing to age, gender, and general practice matched controls, epilepsy is more commonly seen in MS patients at MS diagnosis and after the diagnosis after further adjusting for Charlson comorbidity index, life modifiable factors (body mass index, alcohol, tobacco smoking). The findings support the testing hypothesis.

5. **The NAWM of NMO/NMOSD at 7T MRI is not normal, and the quantitative features measured by T1 relaxation time and MTR are indistinguishable from those in a mixed population of MS subtypes.**

   There is at least a small proportion of intracranial NAWM in patients with NMO/NMOSD having subtle changes that were detectable in vivo using T1 relaxation time and MTR

imaging at 7T MRI. The histogram method to quantify NAWM cannot fully differentiate NMO/NMOSD from MS in the current study and therefore the results do not support the concept that the integrity of NAWM in NMO/NMOSD is less compromised than that in MS.

7.2 LIMITATIONS AND STRENGTH OF THE WORK

Several limitations should be noted. Firstly, the case definition of CNS inflammatory syndromes in this study is not based on published classification criteria but upon physician diagnosis. Prevalence and incidence estimates could potentially be affected by misclassification. However, at least two studies have validated the diagnostic codes of MS or the documented accuracy of MS diagnosis in primary care. Misclassification could also affect relative measures to a lesser degree since extensive differential misclassification is less likely to occur.

Secondly, it is desirable to use an age-appropriate comorbidity score to estimate comorbidity burden in paediatric population as comorbidities impact on mortality for both paediatric and adult patients. However in order to describe the whole MS population the analysis in the thesis used Charlson comorbidity index. This is because paediatric patients compose less than 5% of all MS population. Furthermore, two comorbidity indices which were developed for the paediatric population have not been validated (Tai, Dick et al. 2006, Rhee, Salazar et al. 2013). The first one is a surgical risk score and the highest score is achieved by those who had cardiac arrest, brain hypoxia, and intracranial injuries (Rhee, Salazar et al. 2013). The second one is for the prediction of mortality 1 year after hospital discharge and the authors stated that the most significant factors are brain cancer and diabetes insipidus (Tai, Dick et al. 2006).

Thirdly, temporal regions cannot be completely studied using the sequence of 7T MRI in this study because inhomogeneity in the applied transmit radiofrequency (RF) field is more pronounced in these regions. The imaging quality of temporal regions depends on the participant head size and receiver coils. Therefore, the volume estimation of the white matter was also not feasible in this study.

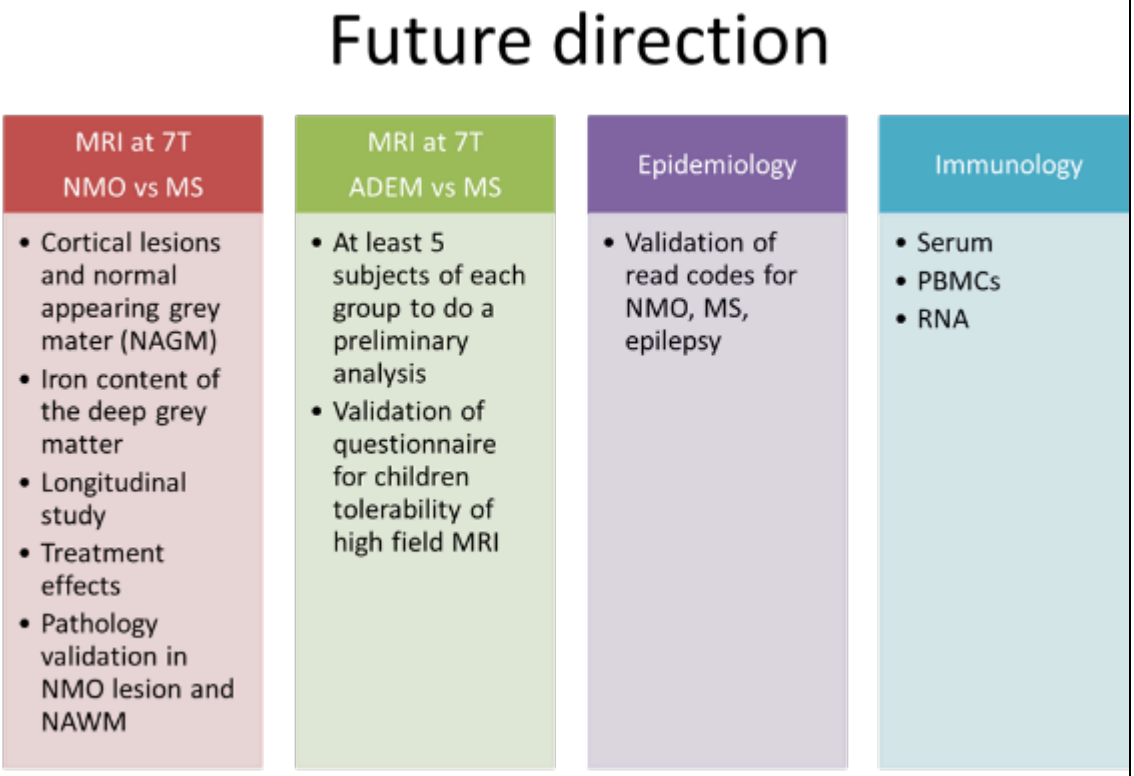
There are several strengths to the work in this thesis. Firstly, this thesis used novel high-resolution quantitative imaging at 7T to capture subtle white matter changes and therefore more accurately measure NAWM than imaging using MRI at lower field strength. This thesis provides evidence for abnormality in the NAWM in patients with NMO/NMOSD as well as MS. Secondly, this thesis

used large databases that are representative of the general population of the UK with selection bias being expected to be minimal. This thesis also applied several novel epidemiological methods and arrived at the conclusions with a cautious interpretation. The CPRD was used to address several important epidemiological and clinical questions in CNS inflammatory syndromes. The findings in this thesis lay the foundation for future research in NMO/NMOSD using high-resolution quantitative MRI and electronic databases.

7.3 RECOMMENDATIONS FOR FUTURE RESEARCH

There are many aspects for future exploration in extending this work (Figure 7-1).

Figure 7-1 Potential avenues for further research



Several interesting avenues can be explored as an extension of the current work, in aspects of high-field MRI, histopathology, and epidemiology. The blood samples collected in parallel with patient recruitment in the current study include serum, peripheral blood mononuclear cell (PBMC), and ribonucleic acid (RNA) for future comparative immunological analysis.

Epidemiology

The thesis explored the utility of CPRD for research regarding the epidemiology of CNS inflammatory syndromes, especially in MS, and demonstrated that the CPRD database is an ideal resource for epidemiological research. Nevertheless, validation of both the Read and ICD-10 codes for CNS inflammatory syndromes could greatly improve the reliability of CPRD in CNS inflammatory syndromes research. The CPRD has been validated for MS diagnosis, but not for

NMO/NMOSD, optic neuritis, or transverse myelitis diagnosis. Further studies that provide better information on the validity of the entered diagnosis of NMO/NMOSD in CPRD are warranted.

The results also indicate that proper epidemiological study focusing on paediatric CNS inflammatory diseases is probably not feasible using large population-based database such as CPRD. This is because the rarity of the disease exceeds the power of CPRD to identify enough number of patients with a sampling ratio of approximately 8% for the UK population. Therefore, a registry-based study may be more suitable to investigate the epidemiology of paediatric MS or NMO/NMOSD.

MRI vs histopathology correlation study in NMO/NMOSD is lacking

Although a histological analysis of various MR imaging findings has been done in MS, the histopathology-neuroimaging correlative studies are lacking on the lesions or NAWM in NMO/NMOSD. Further histological studies focusing on T1 relaxation time and MTR in NMO/NMOSD will help validate the findings in the current thesis. In addition, because it lacks an appropriate lesion detection imaging technique using MRI at 7T, the thesis pioneer evaluates the feasibility of applying magnetization transfer imaging at 7T as a proxy for lesion detection as it is derived from proton imaging and the lesion detection rate is compatible with conventional T2-weighted fluid attenuation inversion recovery (FLAIR) imaging at 3T (for details please refer to appendix 2). Future work to prospectively evaluate lesions in MS using similar spatial resolutions at 3T FLAIR imaging and 7T magnetization transfer imaging is needed to validate the current observation.

Questions remain to be clarified

Some important questions have been addressed in this thesis but others remain to be clarified. Firstly, the intracranial white matter lesions and NAWM may contribute to cognition decline in patients with MS and NMO/NMOSD. But cortical lesions, the changes in normal appearing grey matter (NAGM), or functional alterations of blood-brain-barriers on the pia surrounding the cerebral cortex (Guo, Weigand et al. 2017) might also play an important role in cognition.

Volumetric studies at 3T have shown differences of regional cortex thinning between MS and NMO/NMOSD (Liu, Xie et al. 2014). Nevertheless, the NAGM changes using high-resolution 7T imaging to measure cortex thickness with precision and to exclude MR imaging visible cortical lesions have not been done to compare MS and NMO/NMOSD.

Secondly, the paediatric population with demyelinating syndromes needs to be further investigated. For the neuroimaging study, paediatric onset CNS inflammatory syndromes usually present as ADEM and are indistinguishable from MS before the onset of third attacks. It is desirable to have a biomarker to differentiate the disease entity before the second or third relapses, and in vivo 7T quantitative MR imaging may have this potential. Nevertheless, the preparation of the ethical approval and inter-institutional cooperation was a time-consuming process, and the patient recruitment is very slow due to the rarity of the disease. To have a meaningful analysis, a minimum of 5 age- and gender-matched subjects per group (MS, ADEM, healthy controls) are needed. In addition, the questionnaire study (appendix 1) shows the tolerability of paediatric population underwent high-field MRI but the validation of these questionnaires remains to be performed.

To investigate the imaging properties of rare CNS demyelinating diseases, such as paediatric MS or NMO/NMOSD, high-field MRI may have its unique strength to explore. Firstly, the imaging quality of 7T MRI is substantially better than lower-field modalities. This is because of the small voxel size achievable using 7T MRI. Therefore, qualitative studies may be facilitated by the greater imaging quality. Secondly, the inherently shorter examination time acquired using 7T MRI is an important property to enhance enrolment of paediatric patients since they are less tolerant of lengthy examination. Therefore, 7T MRI can be an important imaging modality to visualise and investigate rare CNS inflammatory diseases.

The safety of 7T MRI in paediatric patients should be the main barrier to wider utilisation of the imaging modality. In this study, we used a field survey as a pre-protocol exercise to determine the tolerability of 3T and possibly 7T MRI in paediatric patients (appendix 1). Our results indicate that patients aged 12 years or above may well-tolerate 7T MRI. For younger patients, we should consider the balance of benefits (shorter duration of examination and greater image quality) and

shortcomings (noisier environment and theoretically potential harm by strong magnetic field) before commencing 7T MRI studies in younger patients. A more detailed safety assessment should be done. During my PhD study, only 3 ADEM and 1 MS paediatric patients were successfully enrolled. The rarity of the disease prevented a greater number of enrolled young subjects. Therefore, this thesis focuses on adults. A longer enrolment period and collaboration with paediatric neurologists in other areas for referrals of suitable patients are necessary to reach minimal enrolment threshold for a meaningful study.

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APPENDIX 1: SUBJECTIVE DISCOMFORT IN CHILDREN RECEIVING 3T MAGNETIC RESONANCE IMAGING AND EXPERIENCED ADULTS' PERSPECTIVE ON CHILDREN'S TOLERABILITY OF 7T: A CROSS-SECTIONAL QUESTIONNAIRE SURVEY

Introduction

Magnetic resonance imaging (MRI) depends on a static magnetic field to generate detectable and decipherable signals. Higher field strength potentially improves signal-to-noise and contrast-to-noise ratio, leading to images with a better spectral and spatial resolution. Currently 1.5 Tesla (T) MRI is the routine for clinical use and 3T MRI in a clinical context is becoming more common. With clear depiction of submillimetre anatomical details and better imaging contrast, 7T MRI may become an ancillary diagnostic tool for brain and musculoskeletal imaging in the near future.

Despite many technical improvements, obtaining optimal images relies on how well an individual tolerates lying in the MRI scanner, the surrounding environment, and attendant procedures. Factors which may reduce the subjects' tolerance for all types of MRI regardless of field strength include prolonged scanning time, environmental temperature, noisiness, uncomfortable position or padding, and the individual's level of anxiety (Marshall, Smith et al. 1995, Tyc, Fairclough et al. 1995, Haddad, Platt et al. 2013). Discomfort that is more commonly experienced in MRI systems operating at 3T or higher magnetic field strengths than that at 1.5T includes dizziness, vertigo, nausea, peripheral nerve stimulation, and the perception of a metallic taste (Glover, Cavin et al. 2007, Weintraub, Khoury et al. 2007, Theysohn, Maderwald et al. 2008, Glover 2009, Heilmaier, Theysohn et al. 2011, Versluis, Teeuwisse et al. 2013). These subjective perceptions have been described in adults. No study has been conducted to survey tolerability of 3T or 7T MRI in children despite increasing use of 3T MRI and a small number of children involved in 7T clinical functional MRI (Beisteiner, Robinson et al. 2011). In addition, ethical considerations in involving children in 7T MRI research have not been tackled.

To explore the possible discomfort perceived by children participating in 7T MRI research, and the age range in which children are most likely to tolerate it well, we launched this pre-protocol public and patient involvement survey. To do this we 1) documented the sources of discomfort reported by children (stratified by age group) undergoing clinical 3T MRI studies; 2) documented the sources of discomfort reported by adults undergoing 7T MRI studies; 3) compared the reported rates of

discomfort in a cohort of adults who underwent both 7T and 3T MRI, and 4) asked the adult participants who underwent 7T and 3T MRI scans to predict how well children (stratified by age group) would tolerate each system.

Materials and Methods

This public and patient involvement, which is in line with current guidance regarding service evaluation in the UK (Cavet and Sloper 2004, Nilsen, Myrhaug et al. 2006), was part of the successful research ethics committee application (13/EM/0080). Anonymized data from children undergoing clinical 3T MRI scans came from an ongoing service evaluation exercise performed in the MRI Department at the Nottingham University Hospitals NHS Trust, for which the ethics committee approved as a pre-protocol exercise. Data from the adult participants came from research studies approved by the local Research Ethics Committee (07/H0408/115, 05/Q2404/44).

Patient and public engagement

We invited a convenience sample of non-sedated children and young people under 18 years of age who underwent 3T clinical MRI for brain or musculoskeletal scans, and their parents / carers to complete an anonymized questionnaire on the day of the scan. Adult volunteers attending our 7T MRI research facility were also asked to help by completing an anonymized questionnaire. Some of the adults had undergone both 3T and 7T scans; they completed identical questionnaires for each scanner.

MRI

The characteristics of MRI scanners are shown in Table 2-4; the sequences and duration of scans are shown in Table 2-5. Adult participants underwent the MRI scans at a university MRI research facility using a 3T Philips Achieva (Philips Medical Systems, Best, Netherlands) with a bore diameter of 60 cm and a 7T Philips Achieva (Philips Medical Systems, Best, Netherlands) with a bore diameter of 58 cm. Children undergoing a clinical 3T scan were imaged using an identical Philips Achieva system in the MRI department of a tertiary referral teaching hospital. Earplugs and headphones were provided

in both 3T systems, while earplugs and acoustic foam pads were used in the 7T system because of the smaller size of transmit-receive head coil.

Table 1. A comparison between the 7T and 3T MRI scanners for brain images.

Scanner	7T MRI	3T MRI
Manufacturer	Philips, Achieva, Best, the Netherlands	Philips, Achieva, Best, the Netherlands
Gradient system	whole-body	whole-body
Radiofrequency transmitter coils	head-only	whole-body
Radiofrequency Receiver coils	head-only, 16 or 32 channel parallel imaging SENSE (sensitivity encoding) receive coil	head-only, 8-channel parallel imaging SENSE receive coil
Dimensions of the bore (diameter, length)	58 cm, 340cm	60cm, 180cm
Height of the bed	106 cm; fixed	54cm (lowest level); automatically adjustable table
Room temperature	central controlled	central controlled
Humidity setting	central controlled	central controlled
Noise protection	acoustic foam pads plus earplugs	headphones plus earplugs

Table 2. Characteristics of respondents to questionnaires on high-field MRI.

	Adults (n=58)		Children (n=31)
	7T (n=58)	**7T and 3T (n=26)**	**3T**
Age (years; mean, range)	41.5 (19-60)	39.7 (27-58)	12.7 (7-17)
Female	37 (65%)	15 (58%)	14 (45%)
Diagnosis			
Healthy (n, %)	10 (17%)	5 (19%)	0
MS/CIS (n, %)	48 (83%)	21 (81%)	0
Other (n, %)	0	0	31 (100%)
Body part scanned			
Brain (n, %)	58 (100%)	26 (100%)	70%[#]
Musculoskeletal (n, %)	0	0	30%[#]
Sequences	7T: 3D MPRAGE (± Gd), FLAIR,T2*, B1 map, T1 map, MT imaging, LLTFE	3T: FLAIR, T2, DTI	T1(± Gd), T2, FLAIR, ADC map, DWI
Duration of scan	55-75 minutes	7T: 55-75 minutes 3T: 20 minutes	20-60 minutes

ADC= apparent diffusion coefficient; CIS= clinically isolated syndrome; DTI= diffusion tensor imaging; DWI= diffusion-weighted imaging; FLAIR= fluid attenuated inversion recovery; Gd= gadolinium contrast agent; LLTFE= Look-Locker turbo field-echo; 3D MPRAGE= three-dimensional Magnetization-Prepared Rapid Acquisition and multiple Gradient Echoes imaging; MS= multiple sclerosis; MT= magnetization transfer. [#]Value estimated

Questionnaires

We designed age-appropriate and self-administered questionnaires to measure the degree of discomfort during MRI, which were designed by a paediatric neurologist (WPW) and an adult neurologist (CSC); both have many years' experience of designing and using questionnaires in service evaluation, clinical audit, and clinical trials. Six subjective measures were surveyed for all ages: general discomfort, dizziness, noisiness, claustrophobia, and feelings of cold or warm. These were chosen because they were the most common symptoms of discomfort reported in the high-field MRI literature.(Weintraub, Khoury et al. 2007, Theysohn, Maderwald et al. 2008, Heilmaier, Theysohn et al. 2011, Versluis, Teeuwisse et al. 2013) The less frequently occurring perception of metallic taste or sensation of twitches that could be related to peripheral nerve stimulation were also included in the questionnaires for children aged 8-11 years and 12-17 years, but not for children aged 5-7 years, considering their ability to understand these concepts and read lengthy text. We also asked participants aged 12-17 years whether having a blood test or being given contrast agents would affect their decision to be involved in future MRI research.

Separate questionnaires were designed for children aged 5-7 years, 8-11 years, 12-17 years, and adults. The questions for patients aged 5-7 years were illustrated with pictures and asked for "yes" or "no" answers. (For details please refer to section 1.1.6) Patients aged 8-11 years, 12-17 years, and adults were given a 5-point modified Likert scale: "yes very" (score 5), "yes a little" (score 4), "so-so/not sure" (score 3), "no not really" (score 2), "no not at all" (score 1). Children and adolescents were also asked if they would mind having the MRI again in one year's time.

To understand public opinion on the suitability of involvement of children and young people into high-field MRI research, we designed questionnaires for adults who had experienced 7T, and some of them also 3T, MRI and asked their opinion on how they thought children and young people of different ages (8-9, 10-11, 12-15 or 16-17 years) would tolerate 7T and 3T MRI. We also used the 5-point modified Likert scale as "yes definitely" (score 5), "yes probably" (score 4), "not sure" (score 3), "no probably not" (score 2), "no definitely not" (score 1).

A separate questionnaire was designed and given to parents or carers of involved children, which contained open questions to collect their views on the tolerability of 3T clinical MRI for their children. The questionnaires used are included in the section 2.2.6.

Image quality assessment

To test the possible relationship between the tolerability measured by the questionnaire and the quality of acquired images, the 7T research brain images of adult patients were evaluated by I-J Chou who was blinded to the results of the questionnaire. The image quality was classified as excellent, good, or blurred. The aspects of image quality such as lesion detection, motion artefacts, and sharpness were all considered.

Statistical analyses

Likert scale data were analysed as ordinal variables. We also dichotomised the data into "agree" (score 4 or 5) and "disagree" (score 1 to 3). Summary statistics were expressed as percentages for categorical data and mean ± standard deviation (SD) for approximately normally distributed continuous variables. Differences in the baseline characteristics and response of each question between the subgroups were tested using the Fisher's exact test for dichotomised data. Differences in the Likert scaled data were tested using the Mann–Whitney U-test. Differences among the paired groups were tested using the McNemar's chi-squared test for dichotomised data. Two-tailed tests were used and a p value less than 0.05 was considered statistically significant. All analyses were performed using IBM SPSS Statistics, version 21 (SPSS Inc., IBM Corp., Chicago, IL, USA).

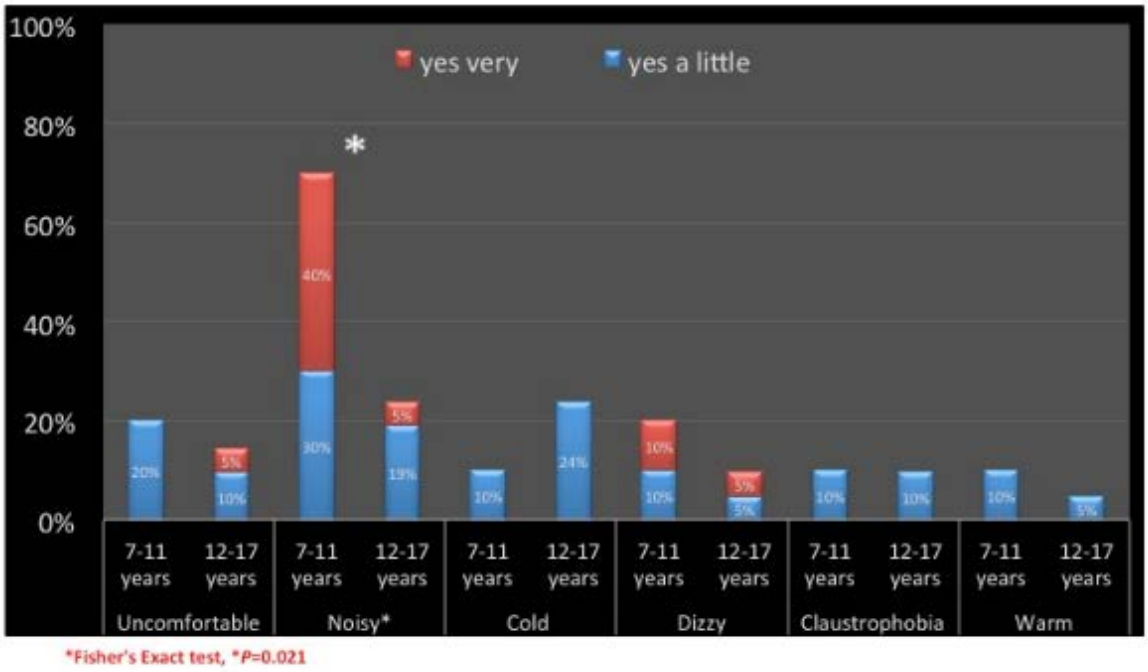
Results

Eighty-three percent (89/107) of involved individuals returned questionnaires (response rate of children: 78%; of adults: 87%). Questionnaires from 3 children (aged 9, 14, and 16 years) were partially completed. Twenty-six out of 58 adult respondents (45%) experienced both 3T and 7T research MRI. Children were aged 7-17 (mean 12.7, standard deviation (Whurr, Bhatia et al.) 3.0) years and adults 19-60 (mean 41.5, SD 9.9) years (Table 2-5).

Questionnaires

The most common discomfort in children and young people undergoing 3T MRI was "noisiness", as reported by 39% (12/31) of them. Discomfort was reported by 19% (6/31) for "cold", 16% (5/31) for "general discomfort", 13% (4/31) for "dizziness", and 10% (3/31) for claustrophobia ("being closed-in"). Only one of 31 children (3%) rated the experience as very uncomfortable. Additionally, one of 26 children (4%) reported a metallic taste ("funny" or "strange" taste), while 6 of them (23%) had a feeling consistent with possible peripheral nerve stimulation ("funny" or "strange" movements, or "twitches"). There was neither an age trend nor significant gender difference in their answers. Seventy-nine percent (22 out of 28) children were willing to have another MRI scan in a year's time. Figure 1 compares the proportion of subjective discomfort between children and young people older than 12 years and those younger. Only noisiness was reported more frequently in younger than in older children (p=0.021). Twenty-seven percent (7 out of 26) parents/carers reported that their children's discomfort was related to the hard mattress and lying too still for too long.

Figure 1 The subjective discomforts reported by 31 children who revceived 3T clinical MRI.



*The subjective discomforts reported by children aged 7-11 years (n = 10) and 12-17 years (n=21) uhndergoing 3T clinical MRI. 31 children reported discomfort in 6 domains using a 5-likert scale: "yes very"; "yes a little"; "so-so"; "no not really"; "no not at all". Histograms show % reporting discomfort as "yes very" (red) and "yes a little" (blue). *younger children reported noise discomfort significantly more often than older children, p=0.021.*

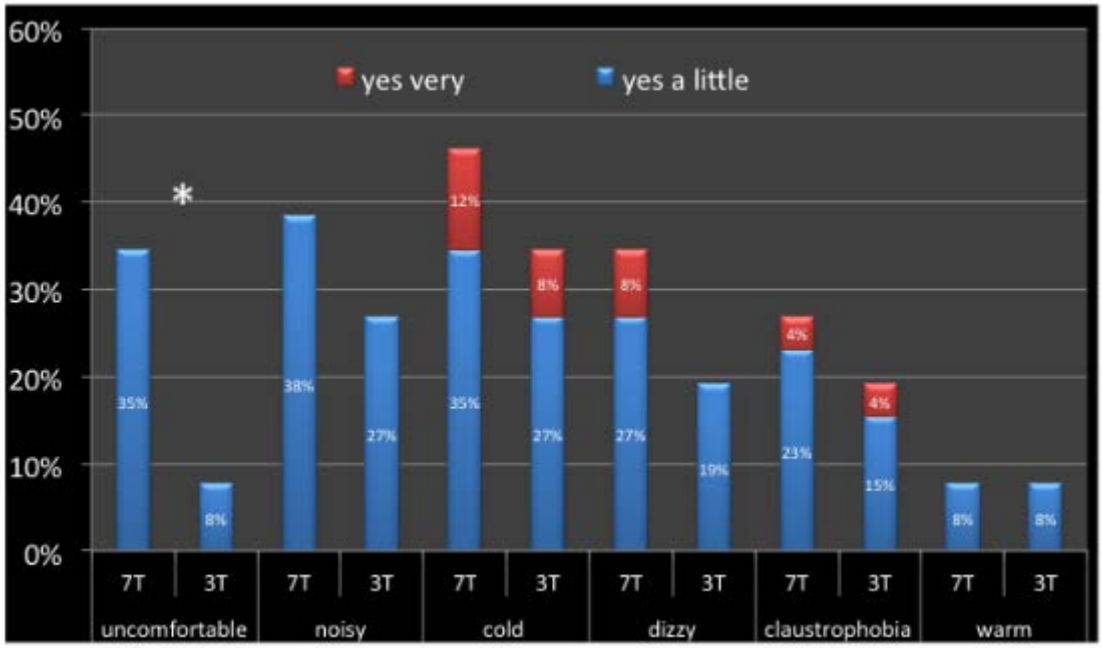
Of 58 adult respondents who underwent 7T MRI scans, noisiness was the most frequent discomfort 25/58 (43%), followed by cold 20/58 (34%), general discomfort 18/58 (31%), claustrophobia 18/58 (31%) and dizziness 17/58 (29%). Women reported higher "general discomfort" Likert scale scores than men (p = 0.042).

In 26 adults who underwent both 3T and 7T MRI scans, there was a higher frequency of general discomfort during 7T than 3T scans (McNemar test, p=0.031) (Figure 2). There was a higher frequency of noisiness, dizziness, or claustrophobia during 7T MRI scans than during 3T MRI scans although it did not reach statistical significance.

More than 85% of adults thought children aged 12-17 years would tolerate both 3T and 7T scans well, but only 35% and 15% thought children aged 10-11 years and 8-9 years would (Figure 3). The sum of scores for the 6 discomfort domains was negatively correlated with the sum of the scores of

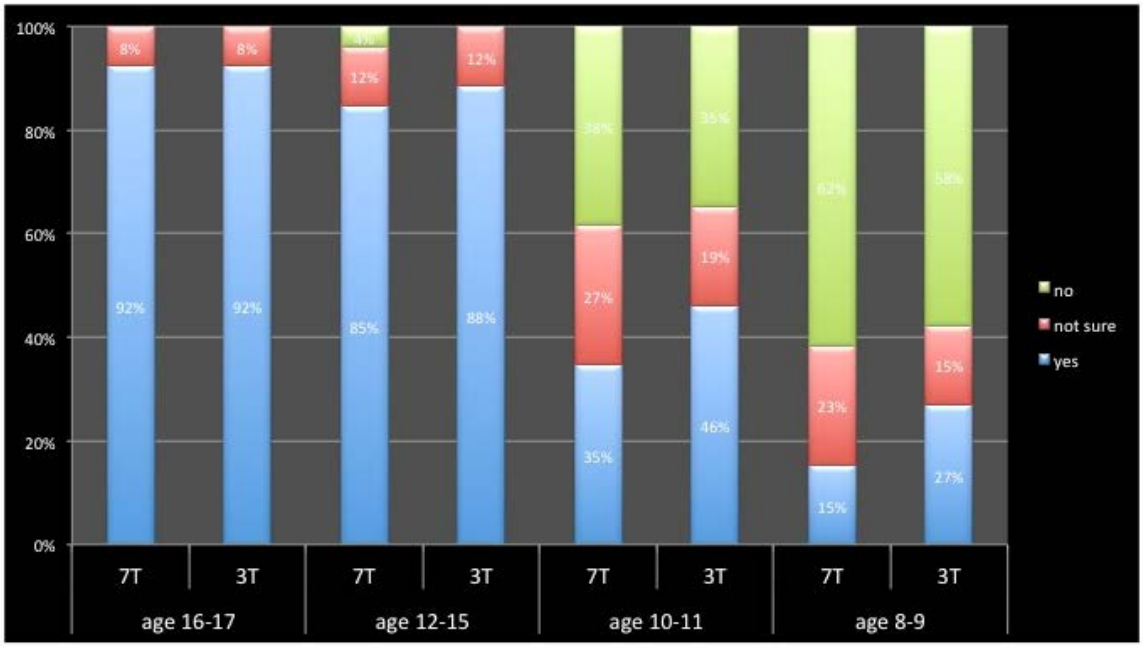
the adult's opinions of the children's probable ability to tolerate 7T MRI (Spearman's correlation = -0.330, $p = 0.011$).

Figure 2 The subjective discomforts reported by adults who underwent both 3T and 7T research MRI.



*26 adults reported discomfort in 6 domains using a 5-likert scale: "yes very"; "yes a little"; "not sure"; "no not really"; "no not at all". Histograms show % reporting discomfort as "yes very" (red) and "yes a little" (Blue). *adults found the 7T MRI generally more uncomfortable than the 3T MRI, McNemar's test p=0.031*

Figure 3 The opinions of experienced adults on whether children "would feel OK" undergoing 7T MRI.



26 adults who had undergone both 3T and 7T research MRI reported how, in their opinion, children, aged 16-17 years, 12-15 years, 10-11 years, and 8-9 years, would feel undergoing 7T research MRI. Histograms show % reporting the views as "yes definitely" or "yes probably" (blue), "not sure" (red), and "no probably not" or "no definitely not" (green).

Among adolescents aged 12-17 years, 8/19 (42%) of them agreed that having a blood test would "put them and other young people off" volunteering for a MRI research examination, while only 12/58 (21%) adults did (p=0.077). Further, 8/19 (42%) adolescents agreed that having a contrast agent injection during a scan would "put them and other young people off" volunteering for a research scan.

Image quality

In total, linked anonymized brain images of 29 adult patients were available to be examined retrospectively. Nineteen of 29 (65%) images were classified as excellent, 8/29 (28%) good, and 2/29

(7%) blurred. Both patients with blurred MRI images did not find 7T MRI uncomfortable. There was no statistical difference in the image quality between patients who reported discomfort and those who did not (p =1.000).

Discussion

This pre-protocol public and patient involvement found that only a small proportion (3%) of child respondents reported a "very uncomfortable" experience of 3T MRI scan and three quarters were willing to participate again in one year. It seems that these subjective discomforts irritate but do not preclude their willingness to undergo a clinical MRI again. Noise is the most common discomfort reported by children and young people undergoing 3T MRI; in particular, 70% of children younger than 12 years indicated noise as a cause of discomfort during the procedure. In contrast, only 25% of adults and older children found the 3T scanner noise uncomfortable. Adults found the 7T MRI more uncomfortable than the 3T MRI in general and noise was still the most common discomfort during 7T MRI, as reported by 40% of adult respondents. As for involvement of children and young people in 7T MRI research, a higher percentage of adults reported subjective discomfort indicating the possibility of even greater risk for subjective discomfort in children undergoing 7T MRI, particularly younger children who are more vulnerable to 3T discomfort. This view echoed the finding that most adult participants, based on their personal experiences, did not think children younger than 12 years would tolerate 7T MRI well. This survey supports the case for cautious enrolment of children and young people of 12 years or more of age in 7T MRI studies. However, we would caution against involving children under 12 years in 7T MRI studies, until more experience with the older children is accrued, or new techniques to further mitigate the discomfort are developed.

Our study collated the subjective discomfort of both 3T and 7T MRI scanning in adults who had experienced both procedures. Their experiences may serve as a proxy for potential discomfort that would occur in child participants. A higher proportion of adult respondents found the noise uncomfortable in the 7T MRI scanner than during 3T MRI. We would expect an even higher proportion of child participants will find the noise at 7T MRI uncomfortable, particularly those younger than 12 years old. These subjective perceptions reflect objective evidence that MRI-related

acoustic noise is field strength dependent. The peak sound pressure levels among the loudest sequences at 1.5T MRI system were measured at a range of 101 to 117 A-weighted decibels [dB(A)] (Counter, Olofsson et al. 1997, Shellock, Ziarati et al. 1998, Price, De Wilde et al. 2001), and at 3T MRI system were as high as 122 to 131 dB(A) (Foster, Hall et al. 2000, Hattori, Fukatsu et al. 2007). The audiometry measurement at 7T system has not been reported because of the difficulty of locating the metallic apparatus directly within its bore, where the magnetic field is intense. Note that the threshold for instantaneously acquired acoustic trauma is 140 dB(A), and for discomfort for normal individuals is 120 dB(A). Therefore, our current policy is to use double hearing protection (Hattori, Fukatsu et al. 2007) in both 3T and 7T MRI systems. Sound attenuation of 36 dB(A) is offered by earplugs (3M, E-A-R soft™ Yellow Neon EarPlugs); along with headphones or acoustic foam pads, the subjective sound pressure is expected to be decreased further to below the level 99 dB(A) as regulated by the International Electrotechnical Commission (IEC) (Commission). This sound pressure level is equivalent to listening to a single trumpet at a 25cm distance (Plontke and Zenner 2004). Despite hearing protective devices being used, there was still a high percentage of respondents who found both 3T and 7T scans uncomfortably noisy.

Our finding was consistent with previous studies in that the noise was a common discomfort perceived by children undergoing MRI. Marshall et al. (Marshall, Smith et al. 1995) reported that 16% of 80 children aged 10-18 years rated the noise as the most annoying part of scans. Tyc et al. (Tyc, Fairclough et al. 1995) asked 55 paediatric oncology patients aged 8 to 22 years, and their parents, to select which bothered the patient most about the MRI from among eight choices: size of machine, noise, darkness, small space, IV insertion, lying still, head restraint, and other. Noise was rated the fourth most distressful in 22% of patients, following IV injection (33%), confined space (25%), and lying still (24%). However, these two studies did not mention the magnetic field of the scanners. Since both studies were conducted in 1995, the magnetic field of the scanner was more likely to be at 1.5T or less. In our study, a higher proportion of children (39%) found the noise of a 3T scanner uncomfortable. MRI-related acoustic noise has been linked to a temporary shift in hearing thresholds, brief hearing loss (Brummett, Talbot et al. 1988) and a decrease in otoacoustic emissions

even with earplugs (Radomskij, Schmidt et al. 2002). Therefore, for children who are involved in 3T or 7T MRI examinations, it is very important to take all steps to reduce potential harm from noise-related hearing impairment. To better protect children and young people, age-appropriate hearing protection is essential to reduce the risk of discomfort as well as noise-induced hearing impairment and procedural discomfort. Furthermore, careful fitting of earplugs is essential as research showed that training in earplug insertion and visual evaluation of the earplug fit were important to achieve good attenuation (Toivonen, Paakkonen et al. 2002).

Other discomfort during MRI was reported less often in our survey. Only less than 20% of children undergoing 3T MRI complained of general discomfort, feeling of cold, claustrophobia or dizziness. Uncomfortable padding and long duration of scan were reported as sources of discomfort by their parents or carers. MRI-related discomfort or anxiety in children will not only come from the MRI itself but also from related factors such as the site of scanning, waiting time and cannulation. Tyc et al. (Tyc, Fairclough et al. 1995) showed intravenous cannulation performed prior to MRI to allow contrast agent injections can arouse anxiety in one-third of children. Our study revealed MRI scans which involved contrast agent administration or blood tests may impair recruitment of adolescents to research. A further study is needed to understand the contribution of these other factors to children's overall experience of different MRI scans.

Our study of user experiences of 7T and 3T MRI showed that the 7T was generally more uncomfortable as scanning time of 7T research scans was double or triple that of 3T research scans. The difference of subjective perceptions of the two systems has rarely been reported. Heilmaier et al. (Heilmaier, Theysohn et al. 2011) found more people complained about noisiness and dizziness in the 7T MRI than in the 1.5T MRI with the perception being more pronounced when subjects were positioned head-first into the bore compared to feet-first. Weintraub et al. (Weintraub, Khoury et al. 2007) showed that post-scan vertigo was more commonly encountered in patients undergoing 3T (5.6%) MRI than 1.5T (1.9%) or 0.6T (0%) MRI. Other less frequent field-strength related adverse feelings in both systems include metallic taste, peripheral nerve stimulation, and a feeling of warmth (Glover, Cavin et al. 2007, Versluis, Teeuwisse et al. 2013). It is therefore particularly important to

consider these factors when enrolling young children in 7T MRI research since they may be more sensitive and susceptible to discomfort related to high field strength.

Other subjective discomforts during scanning at 3T and 7T MRI are manageable. Experience of coldness will depend on the ambient temperature in the scanner room, which is often deliberately made low to assist the subject in dissipating the heat deposited in their body by the radiofrequency (RF) electromagnetic fields. Specific absorption rate (SAR) limits on RF power assumes temperatures in the MR examination room of less than 25 °C, good bore air-flow and the patient being lightly clothed. However, in practice, coldness is a common discomfort after a long duration of scan. A blanket may be needed to prevent the subject getting cold if heat loss mechanisms are not compromised. A leg support can decrease the discomfort caused by lying flat for too long and also reduce the risk of RF burns arising from crossing the legs. Slow movements inside the scanner hall, including slow bed movement can reduce the perception of dizziness and metallic taste. Claustrophobia, the most common cause of premature termination of scans, can be prevented by increasing lighting and air flow through the bore and using a mirror or prism glasses to allow the subject to see out of the bore. Finally, good verbal communication with the subject over the intercom is especially important in this vulnerable population.

A number of caveats regarding this work merit discussion. Firstly, the sample size was small and the adult research volunteers may have relatively positive views about MRI, making generalization to a younger population possibly biased. Secondly, there have been no validated questionnaires across all ages to evaluate subjective perceptions on MRI environment. Thirdly, adult respondents with or without children may have different viewpoints on the tolerability of MRI in children and young people. However, we did not collect information on parenthood. Fourthly, although the questionnaire is designed to be self-administered for each age range of the paediatric population, we cannot be certain whether the filling of the questionnaire is done by the patients themselves if the questionnaires were filled at home and then mailed to us, especially for those aged 5-7 yeas and those having brain tumours. Furthermore, a small proportion of children were positioned differently, not the head at the centre of the magnet bore and without a head coil. Therefore, these children may not perceive dizziness or claustrophobia, which might be more frequently perceived by those receiving a brain

scan that requires a head coil. However, this was not a controlled experiment, but rather a way of giving a voice to parents and children who had experienced routine clinical MRIs and to volunteers who had experienced research MRIs: to explore previously hidden experiences of children and adults undergoing 3T or 7T MRI.

To the best of our knowledge, this is the first account of children's tolerability during 3T clinical MRI and of adults' views on enrolment of children in 7T MRI research. Our findings can inform clinicians and professionals on the potential sources of discomfort that may arise during high-field MRI scanning and facilitate development of strategies to minimise discomfort. This report also provides useful data for Research Ethics Committees who are evaluating protocols for 7T MRI research involving children.

Conclusions

In general, 3T MRI is well tolerated by children aged 7-17 years. Noisiness was the most common discomfort across all ages in 3T and 7T scanners. Although general discomfort was more common during 7T than 3T scans in adults, most adults thought children aged 12 years or more would tolerate 7T MRI well. Further work to validate the preliminary questionnaires, and to assess how children actually tolerate 7T MRI is needed.

Age-appropriate questionnaires to measure the degree of discomfort during MRI

Consumer Involvement for MRI studies in children aged 5-7 years old

3 Tesla MRI in children

We want to know what it was like for you in the scanner. We would like to know what it felt like and if it was uncomfortable or scary.

Age: ☐

Boy ☐ Girl ☐

Date of scan: ☐

Please tick the answer

1. How was the scan?


☐


☐


☐



2. Did you feel dizzy in the scanner?

Yes ☐ No ☐



3. Was it too noisy?

Yes ☐ No ☐



4. Was it too cold?

Yes ☐ No ☐



5. Was it too hot?

Yes ☐ No ☐



6. Were you frightened of being closed-in?

Yes ☐ No ☐

7. Would you mind having another scan, say in a 1year?

I don't want one thanks ☐ I don't mind having another ☐

Thank you so much for helping us!

Prof Cris Constantinescu	Dr William Whitehouse	Dr I-Jun Chou
Professor Clinical Neurology University of Nottingham	Paediatric Neurologist	Paediatric Neurologist

Consumer Involvement for MRI studies in children aged 8-11 years old

3 Tesla MRI in children

We would really like your help to plan a research study. We are asking you as you have been in the scanner. We would like to know what it feels like and if it was uncomfortable or scary for you.

If you can fill the questions, that will help us a lot.

Age : ☐

Boy ☐ Girl ☐

Date of scan: ☐

Please tick the answer that you agree with most:

1. Was it uncomfortable?

Yes very ☐ ; Yes a little ☐ ; so-so ☐ ; No not really ☐ ; No not at all ☐

2. Did you feel dizzy during the exam?

Yes very ☐ ; Yes a little ☐ ; so-so ☐ ; No not really ☐ ; No not at all ☐

3. Was it noisy?

Yes very ☐ ; Yes a little ☐ ; so-so ☐ ; No not really ☐ ; No not at all ☐

4. Did you feel cold during the exam?

Yes very ☐ ; Yes a little ☐ ; so-so ☐ ; No not really ☐ ; No not at all ☐

5. Did you feel warm (or hot) during the exam?

Yes very ☐ ; Yes a little ☐ ; so-so ☐ ; No not really ☐ ; No not at all ☐

6. Were you frightened of being closed-in during the scan?

Yes very ☐ ; Yes a little ☐ ; so-so ☐ ; No not really ☐ ; No not at all

7. Did you feel that lying still inside the scanner was uncomfortable?

Yes very ☐ ; Yes a little ☐ ; so-so ☐ ; No not really ☐ ; No not at all ☐

8. Did you feel a funny taste in your mouth in the scanner?

Yes very ☐ ; Yes a little ☐ ; so-so ☐ ; No not really ☐ ; No not at all ☐

9. Did you feel any funny movements of your body or twitches in the scanner?

Yes very ☐ ; Yes a little ☐ ; so-so ☐ ; No not really ☐ ; No not at all ☐

10. How can we make the scan more comfortable?

11. Would you be willing to another scan in the future, say in about 1 year?

Yes definitely ☐ ; Yes probably ☐ ; No probably not ☐ ; No definitely not ☐ ; Not sure ☐

If you would like us to contact you for further help in the future, please write your name and contact details here:

Thank you so much for taking the time to look at this, and for helping us!

Prof Cris Constantinescu	Dr William Whitehouse	Dr I-Jun Chou
Professor Clinical Neurology University of Nottingham	Paediatric Neurologist	Paediatric Neurologist

Consumer Involvement for MRI studies in young people aged 12-17 years old

3 Tesla MRI in children

We would really like your help to plan a research study using MRI in children and young people. We are asking your advice as you have recently been in the 3T MRI scanner. We would like to know what it feels like and if it was uncomfortable or scary for you.

If you can spare a few minutes to answer some questions, it would mean a lot to us.

Your age: ☐ years

Are you male ☐ or female ☐ ?

Date of scan: ☐

Please tick the answer that you agree with most.

1. Was having the MRI uncomfortable?

Yes very ☐ ; Yes a little ☐ ; so-so ☐ ; No not really ☐ ; No not at all ☐

2. Did you feel dizzy in the MRI scanner?

Yes very ☐ ; Yes a little ☐ ; so-so ☐ ; No not really ☐ ; No not at all ☐

3. Was the noise uncomfortable in the MRI scanner?

Yes very ☐ ; Yes a little ☐ ; so-so ☐ ; No not really ☐ ; No not at all ☐

4. Did you feel cold in the MRI scanner?

Yes very ☐ ; Yes a little ☐ ; so-so ☐ ; No not really ☐ ; No not at all ☐

5. Did you feel warm (or hot) in the MRI scanner?

Yes very ☐ ; Yes a little ☐ ; so-so ☐ ; No not really ☐ ; No not at all ☐

6. Did you have claustrophobia: fear of being closed-in or being in a small space?

Yes very ☐ ; Yes a little ☐ ; so-so ☐ ; No not really ☐ ; No not at all ☐

7. Was having little contact with other people during the scan uncomfortable?

Yes very ☐ ; Yes a little ☐ ; so-so ☐ ; No not really ☐ ; No not at all ☐

8. Was the time in the scanner too long?

Yes very ☐ ; Yes a little ☐ ; so-so ☐ ; No not really ☐ ; No not at all ☐

9. Did you feel lying still inside the scanner uncomfortable?

Yes very ; Yes a little ; so-so ; No not really ; No not at all

10. Did you feel the position/padding inside the scanner uncomfortable?

Yes very ☐ ; Yes a little ☐ ; so-so ☐ ; No not really ☐ ; No not at all ☐

11. Did you feel any strange taste (or metallic taste) in your mouth during the scan?

Yes very ☐ ; Yes a little ☐ ; so-so ☐ ; No not really ☐ ; No not at all ☐

12. Did your body do any strange movements or twitches during the scan?

Yes very ☐ ; Yes a little ☐ ; so-so ☐ ; No not really ☐ ; No not at all ☐

13. Would you be willing to have a scan again in the future, in about 1 year?

Yes definitely ☐ ; Yes probably ☐ ; No probably not ☐ ; No definitely not ☐ ; Not sure ☐

14. Do you think having a contrast agent (dye) injection in the arm to get more information from the MRI would put you or other young people off having a scan for medical research?

Yes very much ☐ ; Yes a little ☐ ; No not really ☐ ; No not at all ☐ ; Not sure ☐

15. Do you think having a blood test would put you or other young people off agreeing to help with medical research into brain diseases?

Yes very much ☐ ; Yes a little ☐ ; No not really ☐ ; No not at all ☐ ; Not sure ☐

16. Can you make any suggestions to make the MRI scanner more comfortable?

If you would like us to contact you for more help in the future, please write your name and contact details here:

Thank you so much for taking the time to look at this, and for helping us!

Prof Cris Constantinescu
Professor Clinical Neurology
University of Nottingham

Dr William Whitehouse
Paediatric Neurologist

Dr I-Jun Chou
Paediatric Neurologist

Consumer Involvement for MRI studies in children and young people

3 Tesla MRI in children

We would really like your help to plan a research study using MRI in children and young people. We are asking your advice as your child has recently been in the 3T MRI scanner. We would like to know what it felt like and if it was uncomfortable or scary for them.

If you can spare a few minutes to answer some questions, and help your child with their questionnaire, it would mean a lot to us.

Your child's age: ☐ years

Are they male ☐ or female ☐ ?

Date of scan: ☐

Please tick the answer that you agree with most.

1. Was having the MRI uncomfortable for them?

Yes very ☐ ; Yes a little ☐ ; so-so ☐ ; No not really ☐ ; No not at all ☐ Don't know ☐

2. Was the time in the scanner too long?

Yes very much so ☐ ; Yes a little ☐ ; so-so ☐ ; No not really ☐ ; No not at all ☐

3. Did they complain of anything? No ☐ ; Yes ☐ Not sure ☐

4. What did they find uncomfortable?

5. Were there any aspects they liked?

6. Would they be happy to have a scan again in the future, in about 1 year?

Yes definitely ☐ ; Yes probably ☐ ; No probably not ☐ ; No definitely not ☐ ; Not sure ☐

7. Do you think having a contrast agent (dye) injection in the arm to get more information from the MRI would put your child off having a scan for medical research?

Yes very much so ☐ ; Yes a little ☐ ; No not really ☐ ; No not at all ☐ ; Not sure ☐

8. Do you think having a blood test would put your child off agreeing to help with medical research into brain diseases?

Yes very much so ☐ ; Yes a little ☐ ; No not really ☐ ; No not at all ☐ ; Not sure ☐

9. Can you make any suggestions to make the MRI scanner more comfortable?

10. Any other comments?

If you would like us to contact you for more help in the future, please write your name and contact details here:

Thank you so much for taking the time to look at this, and for helping us!

Prof Cris Constantinescu	Dr William Whitehouse	Dr I-Jun Chou
Professor Clinical Neurology University of Nottingham	Paediatric Neurologist	Paediatric Neurologist

Consumer Involvement for MRI studies in children and young people

3 Tesla MRI in children

We would really appreciate your help and advice about a study we are planning using the 3 Tesla research MRI machine. We are asking your advice as you have been in this scanner and know what it's like. We would like to know what it feels like and if it was uncomfortable or scary for you, and whether you think teenagers and primary school children will find it ok.

If you can spare a few minutes to answer some questions, that would be great. If you cannot just now, that is ok too. We don't need your name, but if you would like to be involved in this kind of consumer involvement in the future, please let us know and leave your name and contact details at the end of the questionnaire!

Gender Male ☐Female ☐ **; Age**

Please tick the answer that you agree with most.

1. Was having the 3T MRI uncomfortable?

Yes very ☐ ; Yes a little ☐ ; No not really ☐ ; No not at all ☐ ; Not sure ☐

2. Did you feel dizzy during 3T MRI exam?

Yes very ☐ ; Yes a little ☐ ; No not really ☐ ; No not at all ☐ ; Not sure ☐

3. Was the noise uncomfortable during the 3T MRI exam?

Yes very ☐ ; Yes a little ☐ ; No not really ☐ ; No not at all ☐ ; Not sure ☐

4. Did you feel cold during the 3T MRI exam?

Yes very ☐ ; Yes a little ☐ ; No not really ☐ ; No not at all ☐ ; Not sure ☐

5. Did you feel warm during the 3T MRI exam?

Yes very ☐ ; Yes a little☐; No not really☐; No not at all☐; Not sure ☐

6. Did you have claustrophobia (i.e., an *abnormal* fear of being closed-in or being in a confined space) during the 3T MRI exam?

Yes very much so ☐ ; Yes a little ☐ ; No not really ☐ ; No not at all ☐ ; Not sure☐

7. Did you have an ordinary MRI scan?

Yes ☐ ; No ☐ ;

If No, please skip questions 8 and 9.

8. **If yes**, was 3T scanner *more* uncomfortable than the ordinary MRI scanner?

Yes very much ☐ ; Yes a little ☐ ; No not really ☐ ; No not at all ☐ ;

9. **If yes**, why was it *more* uncomfortable than the ordinary MRI scanner?

It made you feel more (please tick one or more answers)

Dizzy ☐ ; noisy ☐ ; cold ☐ ; warm ☐ ; claustrophobia (i.e., closed-in) ☐;

Other ☐ : _____ .

10. Do you think teenagers 16 or 17 years old would feel ok in the 3T MRI scanner?

Yes definitely ☐ ;Yes probably ☐ ;No probably not ☐ ;No definitely not ☐ ; Not sure ☐

11. Do you think teenagers 12 to 15 years old would feel ok in the 3T MRI scanner?

Yes definitely☐ ; Yes probably ☐ ;No probably not ☐ ; No definitely not ☐ ; Not sure ☐

12. Do you think children 10 or 11 years old would feel ok in the 3T MRI scanner?

Yes definitely ☐ ; Yes probably☐; No probably not ☐ ;No definitely not☐;Not sure☐

13. Do you think children 8 or 9 years old would feel ok in the 3T MRI scanner?

Yes definitely ☐ ; Yes probably☐;No probably not ☐;No definitely not ☐ ; Not sure☐

14. Can you make any suggestions to make the 3T scanner more comfortable for children?

15. Have you any other comments about the 3T scanner?

16. Do you think having a blood test would put the patient and family off volunteering for a research study into brain demyelination disease?

Yes very much ☐ ; Yes a little ☐ ; No not really ☐ ; No not at all ☐ ; Not sure ☐

Thank you so much for taking the time to look at this, and for helping us!

Prof Cris Constantinescu	Dr William Whitehouse	Dr I-Jun Chou
Professor Clinical Neurology University of Nottingham	Paediatric Neurologist	Paediatric Neurologist

Consumer Involvement for MRI studies in children and young people

7 Tesla MRI in children

We would really appreciate your help and advice about a study we are planning using the 7 Tesla research MRI machine. We are asking your advice as you have been in this scanner and know what it's like. We would like to know what it feels like and if it was uncomfortable or scary for you, and whether you think teenagers and primary school children will find it ok.

If you can spare a few minutes to answer some questions, that would be great. If you cannot just now, that is ok too. We don't need your name, but if you would like to be involved in this kind of consumer involvement in the future, please let us know and leave your name and contact details at the end of the questionnaire!

Gender Male ☐Female ☐ **; Age**

Please tick the answer that you agree with most.

1. Was having the 7T MRI uncomfortable?

Yes very ☐ ; Yes a little☐ ; No not really ☐ ; No not at all ☐ ; Not sure ☐

2. Did you feel dizzy during 7T MRI exam?

Yes very ☐ ; Yes a little ☐ ; No not really☐; No not at all☐; Not sure ☐

3. Was the noise uncomfortable during the 7T MRI exam?

Yes very☐; Yes a little ☐ ; No not really☐; No not at all ☐ ; Not sure ☐

4. Did you feel cold during the 7T MRI exam?

Yes very ☐ ; Yes a little ☐ ; No not really ☐ ; No not at all ☐ ; Not sure ☐

5. Did you feel warm during the 7T MRI exam?

Yes very ☐ ; Yes a little ☐ ; No not really ☐ ; No not at all ☐ ; Not sure ☐

6. Did you have claustrophobia (i.e., an *abnormal* fear of being closed-in or being in a confined space) during the 7T MRI exam?

Yes very much so ☐ ; Yes a little ☐ ; No not really ☐; No not at all ☐ ; Not sure ☐

7. Did you ever have an ordinary MRI scan?

Yes ☐ ; No ☐ ;

If No, please skip questions 8 and 9.

8. **If yes**, was 7T scanner *more* uncomfortable than the ordinary MRI scanner?

Yes very much ☐ ; Yes a little ☐ ; No not really ☐ ; No not at all ☐ ;

9. **If yes**, why was it *more* uncomfortable than the ordinary MRI scanner?

Did it make you feel more (please tick one or more answers)

Dizzy ☐ ; noisy ☐ ; cold ☐; warm☐ ; claustrophobia (i.e., closed-in) ☐;

Other ☐ : _____ .

10. Do you think teenagers 16 or 17 years old would feel ok in the 7T MRI scanner?

Yes definitely ☐ ; Yes probably ☐ ; No probably not ☐ ;No definitely not ☐ ;Not sure☐

11. Do you think teenagers 12 to 15 years old would feel ok in the 7T MRI scanner?

Yes definitely ☐ ; Yes probably ☐ ; No probably not ☐ ; No definitely not ☐; Not sure☐

12. Do you think children 10 or 11 years old would feel ok in the 7T MRI scanner?

Yes definitely ☐ ; Yes probably ☐ ; No probably not ☐; No definitely not☐; Not sure☐

13. Do you think children 8 or 9 years old would feel ok in the 7T MRI scanner?

Yes definitely ☐ ; Yes probably☐ ; No probably not ☐ ; No definitely not ☐ ;Not sure ☐

14. Can you make any suggestions to make the 7T scanner more comfortable for children?

15. Have you any other comments about the 7T scanner?

16. Do you think having a blood test would put the patient and family off volunteering for a research study into brain demyelination disease?

Yes very much☐ ; Yes a little ☐ ; No not really ☐ ; No not at all ☐ ; Not sure☐

Thank you so much for taking the time to look at this, and for helping us!

Prof Cris Constantinescu Dr William Whitehouse Dr I-Jun Chou

Professor Clinical Neurology Paediatric Neurologist Paediatric Neurologist
University of Nottingham

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APPENDIX 2: MAGNETISATION TRANSFER WEIGHTED SEQUENCES AT 7T FIELD STRENGTH IN THE DETECTION OF WHITE MATTER LESIONS OF CLINICALLY ISOLATED SYNDROME AND MULTIPLE SCLEROSIS

Introduction

Multiple sclerosis (MS) is a chronic debilitating disease with a prevalence of 203 cases per 100,000 population in the UK (Mackenzie, Morant et al. 2014). After the first attack (clinically isolated syndrome (Tartaglia, Narayanan et al.), more than 85% of adult-onset patients experience a relapsing-remitting course (RRMS) and 10% have a primary progressive onset with no or only a single acute event (Thompson, Polman et al. 1997). In patients with CIS or MS, magnetic resonance imaging (MRI) is regarded as an essential tool for new lesion identification and for diagnosis (Polman, Reingold et al. 2011), estimation of lesion load and disease activity (Popescu, Agosta et al. 2013), and prediction of disease outcomes (Fisniku, Brex et al. 2008). Proton-density weighted imaging has been a traditional sequence to detect lesions in multiple sclerosis. Magnetization transfer ratio (MTR), comprising proton-density images without and with application the radio-frequency pulses, is a technique to measure the proton exchange between free protons and macromolecular bound protons (Grossman, Gomori et al. 1994). MTR is a measure of the integrity of the white matter, where the large macromolecule is mainly myelin (Wolff and Balaban 1989, Schmierer, Scaravilli et al. 2004). Theoretically proton-density imaging with magnetization transfer sequences (so called magnetization transfer weighted [MT_w] imging) amplifies the tissue contrast by reducing the signal, and therefore abnormal tissue with low myelination is visualised as hyperintensity to the surrounding intact white matter tissue. Dousset et al applied MT_w imaging to characterize white matter lesions in animal models and in humans with MS (Dousset, Grossman et al. 1992). Pathological correlation studies in animal models and human beings evidenced that the decrease in MTR of MT_w detectable lesions reflects decreased myelin content, which can be due to demyelination or incomplete remyelination (Brochet and Dousset 1999)(Schmierer, Scaravilli et al. 2004). With the specificity of myelin changes, Waesberghe et al further used MT_w at 1.5 Tesla magnetic field strength to detect white matter lesions in MS patients comparing to traditional proton-density images and found better lesion delineation of MT_w imaging despite the fact that the number of lesions visualised was equal (van Waesberghe, Castelijns et al. 1998). Magnetization transfer imaging has the potential to monitor the

evolution of both demyelination and remyelination of the white matter in patients with MS (Schmierer, Tozer et al. 2007).

T2 weighted techniques are sensitive for detecting inflammatory demyelinating lesions because water appears to be bright and in theory pathological processes containing increased tissue free water appear to be bright. T2 Fluid-attenuated inversion recovery (FLAIR) imaging, a special inversion recovery sequence with long T1 to remove the effects of free water (i.e. free water appears to be dark), is superior to T2 weighted imaging for better delineation of the interface between cerebrospinal fluid (free water in the brain) and the normal brain tissue (De Coene, Hajnal et al. 1992). It is highly sensitive to detect all kinds of abnormal tissues containing inflammation, oedema, demyelination, axonal loss, and gliosis (Kates, Atkinson et al. 1996). However, the detection of MS lesions located in the brainstem is inferior to T2-weighted imaging because the imaging is subjective to cerebrospinal fluid flow artefacts over the infratentorial region (Okuda, Korogi et al. 1999). Current clinical guidelines recommend to use T2 FLAIR imaging at 1.5 T or 3T to detect lesions in MS patients, especially in the supratentorial region (Simon, Li et al. 2006).

In comparison with conventional field strength scanners, MTR at 7T with a submillilitre imaging resolution enables detection of cortical lesions in multiple sclerosis (Mougin, Coxon et al. 2010)(Abdel-Fahim, Mistry et al. 2014). It also improves quantitative assessment of subtle changes in the non-lesional “normal appearing” brain tissue (Mistry, Abdel-Fahim et al. 2014). Therefore magnetization transfer at 7T provides relatively specific information on the integrity of myelin with high resolution (Mougin, Coxon et al. 2010, Abdel-Fahim, Mistry et al. 2014).

Currently, the use of T2-weighted FLAIR imaging remains limited at 7T and there is no proxy imaging at 7T for lesion detection for MS patient volunteers recruited for research MRI. Therefore, patients with various functional disabilities were asked to undergo imaging on both 7T scanner and 3T scanner for having at least a 3T FLAIR imaging for classic lesion detection and lesion load estimation, ideally on the same day. The aim of this study was to assess the value of MT imaging in white matter lesion detection by comparing the sensitivity of MT images at 7T to FLAIR images at

3T, obtained on the same day. The hypothesis was that the lesion detection rate of supratentorial white matter in patients with MS is equal between MT_w at 7T and FLAIR at 3T.

Methods

Patients

Fifteen patients with CIS, 6 with MS and 10 healthy participants were recruited. CIS and MS patients were assessed with the Expanded Disability Status Scale (EDSS) (Kurtzke 1983), Fatigue Severity Scale (FSS) (Krupp, LaRocca et al. 1989), and Expanded Disability Status Scale (MSFC) (Fischer, Rudick et al. 1999) by a neurologist (SYL). All patients were selected from the MS Clinic, Division of Clinical Neurology, University Hospitals Nottingham NHS Trust, Queen's Medical Centre. This study was approved by the local Research Ethics Committee (REC reference 07/H0408/115). All participants gave written informed consent before the examination.

MR imaging

The MR imaging was performed by both 3T and 7T MR systems. All participants underwent the MRI scans in the Sir Peter Mansfield Magnetic Resonance Centre (SPMMRC) using a 7T Philips Achieva scanner (Philips Medical Systems, Best, Netherlands). The scanner is equipped with whole body gradients, a 16-channel head-only receive coil and a head-only volume quadrature transmit radiofrequency (RF) coil (Nova Medical, Inc., Wilmington, MA). On the same day, MR imaging was also performed using a 3T whole-body MR system (Achieva 3.0 T; Philips Healthcare, Best, Netherlands) with an eight-channel head coil for reception (SENSE) and body coil for transmission.

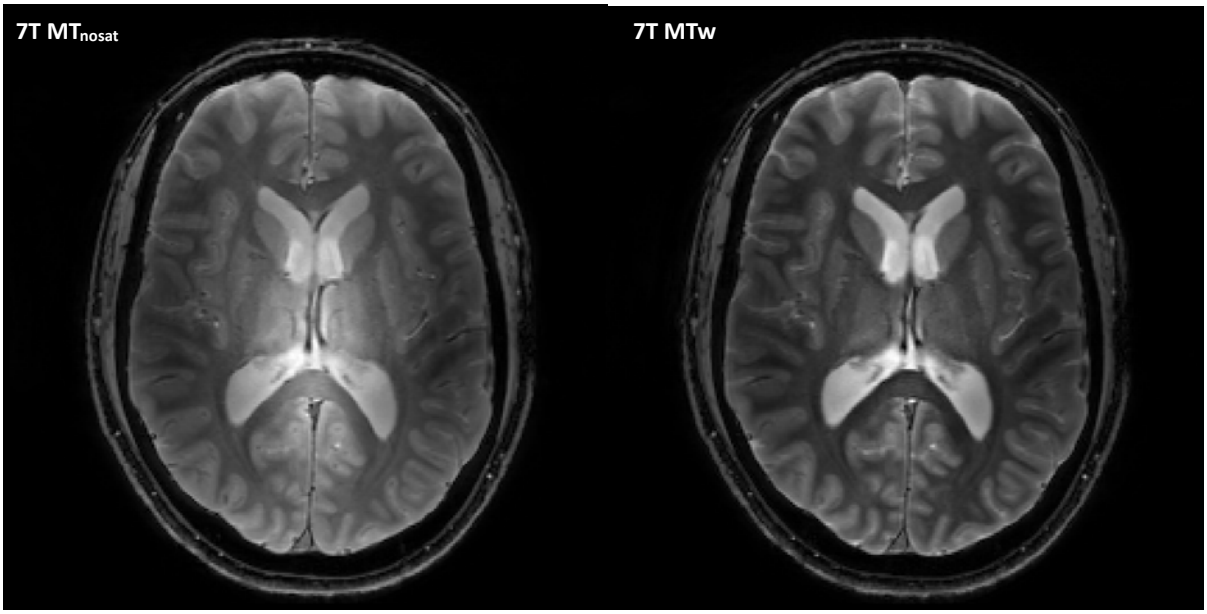
The area of interest was 15 mm above and below the longitudinal axis of the corpus callosum. Magnetization transfer images were acquired using a 3-dimensional (3D) Magnetisation Transfer prepared-Turbo Field Echo (MT-TFE) sequence in 2 volumes (Mougin, Coxon et al. 2010, Mougin, Clemence et al. 2013): the first with no saturation pulse (MT_{nosat}) and the second with off resonance saturation pulses at 1.05 kHz off resonance (MT_w; signal weighted by the magnetization transfer effect). The pulsed saturation was applied as a train of 20 off-resonance pulses (13.5 μT Gaussian-windowed, sinc pulses with a bandwidth of 300 Hz and off-resonance by ±1.05 kHz [3.5 ppm], with 55 ms between each pulse). The acquisition parameters were: field of view = 220 x 220 x 30 mm^3;

spatial resolution = 0.86 × 0.86 ×1.5 mm^3; Echo Time (TE) = 5.7 ms; Repetition Time (TR) = 9.8 ms; 20 slices; total acquisition time 8:22 minutes. Figure 1 shows image examples of MT images in a healthy participant and a patient with multiple sclerosis. This study used MT_w images (rather than MTR maps) as they had higher signal to noise ratio.

The acquisition parameters for T2-weighted 2D FLAIR sequence were: 256x256 matrix; 50 interleave slices; spatial resolution 1x1x3 mm^3; TE = 125 ms; inversion time (TI) = 2800 ms; TR = 11000 ms; total acquisition time 5:52 min. The scanning protocol included 3-D T1-weighted MPRAGE imaging for the co-registration of 3T-2D-FLAIR and 7T-3D-MT_w images. The acquisition parameters were TE = 3.2 ms; TR=6.9 ms; TI= 800 ms; flip angle of the TFE readout pulse = 8°; TFE factor = 240; shot-to-shot interval = 8 seconds; spatial resolution = 1.25×1.25×1.25 mm^3; Field of View = 200×200×72.5 mm; reconstruction matrix = 160×160×58; total acquisition time 2 min.

Figure 1 Axial 7T 3-dimensional MR imaging examples of MT_{nosat} and MTw in (a) a healthy subject and (b) a 27-year-old male patient with MS (EDSS 2.0). MTw images shows a better contrast of the grey matter to the white matter, and also a better contrast of white matter lesions to the normal appearing white matter than MT_{nosat} images.

a



b

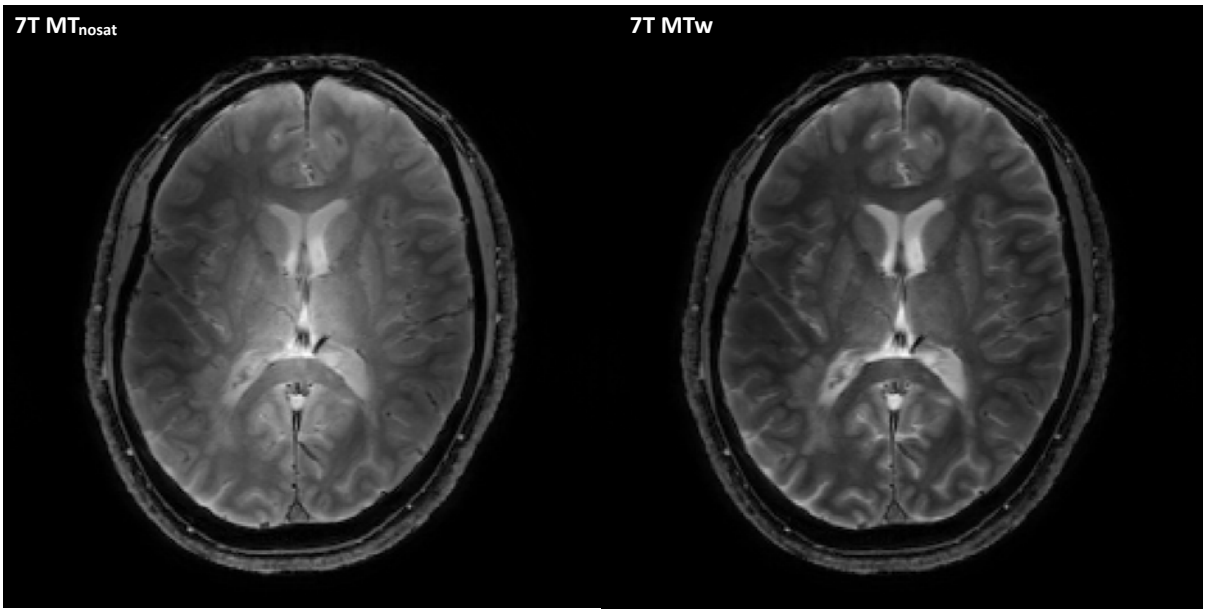


Image Analysis

Definition of lesion

White matter lesions were defined as high signal intensity regions compared to background white matter tissue not attributable to normal anatomical structures. Lesions were identified and marked using a consensus approach by two neurologists (IJC and RT) with experience in MR image analysis. They were blinded to patient identification and clinical information. The following were considered normal findings on both the FLAIR and MT_w images: increased signal in a region of width ≤ 2 mm at the anterior and posterior pole of the lateral ventricles, increased signal in a strip of width ≤ 2 mm at the remainder of the lateral and third ventricles, symmetrical areas of increased signal in the posterior limbs of the internal capsule, and any symmetric hyperintensity suggestive a vessel, artefact, or anatomical structure (Molyneux, Tubridy et al. 1998, Neema, Guss et al. 2009). If there was a disagreement, a third researcher (CSC) was consulted and made the decision.

Imaging post-processing

All acquired images were co-registered to the 3D MPRAGE image space using FLIRT linear rigid-body image registration algorithm from the FSL platform (FMRIB) (available at www.fmrib.ox.ac.uk/fsl) (Jenkinson, Bannister et al. 2002) for a side-by-side comparison of demarcated hyperintensities. Because of the difference in contrast between the original 7T-3D-MTw and 3T-2D-FLAIR scans and the reference scan, the cost function was the "mutual information" with a low number (100) of bins.

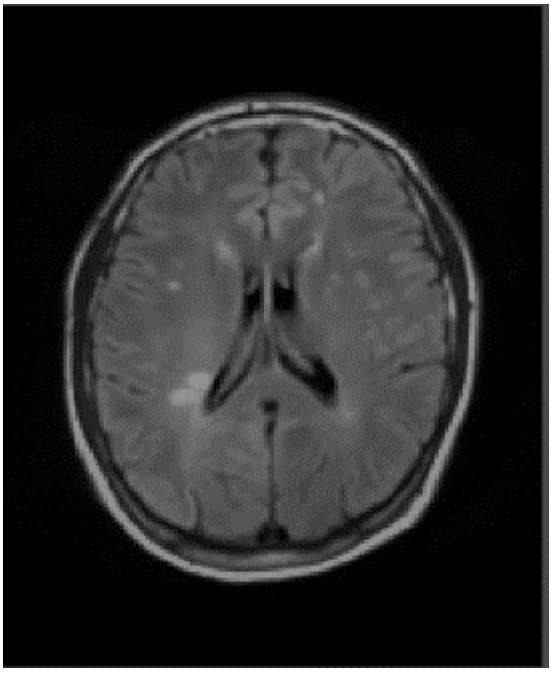
Qualitative assessment: lesion count

To determine the detection rate of white matter lesions, which were localised in the same neuroanatomical region, was analysed using 7T-3D-MT_w images compared to 3T-2D-FLAIR images on a side-by-side basis manually by a single researcher (IJC). Figure 2 illustrates the images of the two-step analysis: firstly, we did a prospective comparison on two images that were co-registered to the 3-D MPRAGE space. This step helped to quickly recognise the lesions on the same

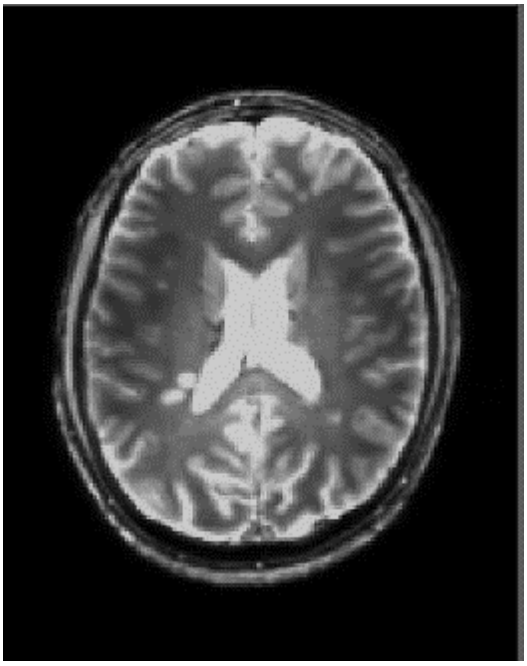
neuroanatomical region on both images. However, the co-registered images were not perfectly in alignment and were down-sampled (Figure 2a). We secondly performed a retrospective comparison on original 3T-2D-FLAIR and 7T-3D-MT_w images (Figure 2b).

Figure. 2 The two-step qualitative analysis of the white matter lesions was performed on a side-by-side basis by a single neurologist.

(a) Step 1. Prospective assessment was done after two sequences were co-registered to the same 3-D MPRAGE space

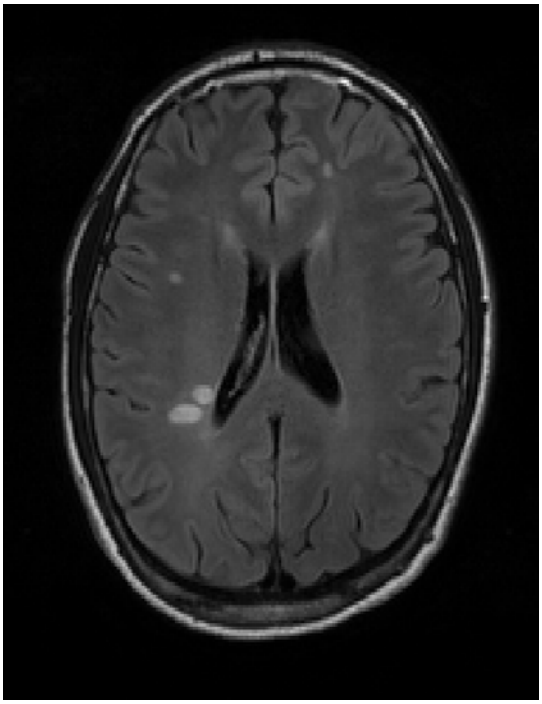


Registered 3T-2D-FLAIR
dimension: 1.25×1.25×1.25 mm3

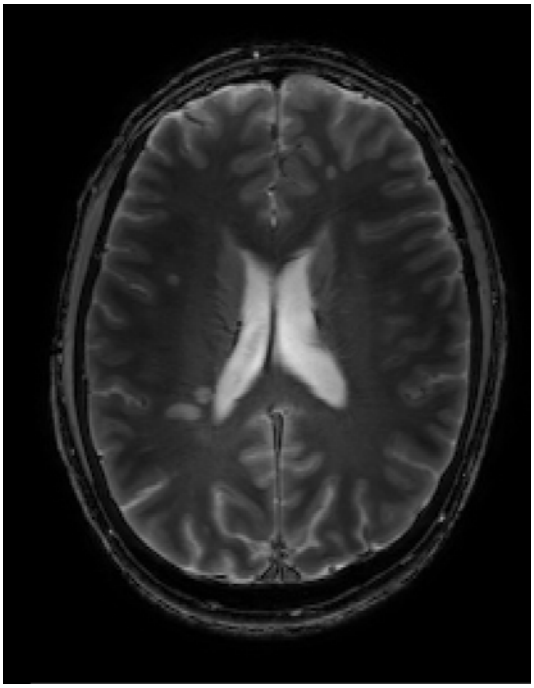


Registered 7T-3D-MTw
dimension: 1.25×1.25×1.25 mm3

(b) Step 2. Retrospective assessment was performed on the original images



Original 3T-2D-FLAIR
pixel dimension: 1x1 mm2, thickness of 3 mm



Original 7T-3D-MTw
voxel dimension: 0.86 × 0.86 ×1.5 mm3

Quantitative assessment: lesion load

The images of two sequences were presented in random order to the aforementioned two neurologists independently to determine inter-rater agreement. The lesions were outlined using a semi-automated, seed-growing technique using the in-house NeuRoi image analysis software (Dr. CR Tench; Clinical Neurology, University of Nottingham, UK; available at http://www.nottingham.ac.uk/scs/divisions/clinicalneurology/software/neuroi.aspx). The total volume of these lesions was calculated after manual segmentation.

To calculate intra-rater reliability, all images were reassessed with a delay of at least one week to minimise the potential for any memory of the images (Molyneux, Tofts et al. 1998). An averaged lesion load derived from a total of three assessments per subject was used for statistical analysis.

Global Image quality assessment

All images were evaluated by IJC. The image quality issues such as lesion detection, motion artefacts and sharpness of all slices were all considered and rated from 0 to 100, where 0 was perfect and 100 indicated the needed to re-do the scanning. The image quality was then classified as excellent (0-30), good (30-70) or blurred (70-100).

The contrast for lesions to normal appearing white matter

A total of 50 lesions were arbitrarily selected from different regions for estimation of the contrast ratio between signal intensity (SI) of lesions and of adjacent or contralateral normal-appearing white matter (NAWM). The percentage contrast of lesions to NAWM was calculated according to the following formula: Contrast = $(SI_{lesion} - SI_{NAWM})/ (SI_{lesion} + SI_{NAWM})$ x100 (Sati, George et al. 2012).

Statistical Analysis

Comparison between the subgroups was performed using the Fisher's exact test for dichotomised data and the Mann-Whitney U test for continuous data or ordinal clinical rating scales. The analysis of brain lesions on the different sequences was performed patient-wise. The relative comparison of the numbers of lesions on 7T-3D-MT_w versus 3T-2D-FLAIR was expressed as percentage gain or a

loss in the number of detected brain lesions. The inter-observer agreement in measuring lesion volumes from both sequences was calculated using intra-class correlation coefficient (ICC) and its two-sided 95% confidence interval (CI). Spearman Rank correlation was used for correlations of lesion load between 3T-2D-FLAIR and 7T-3D-MT_w and for correlations of clinical assessment (EDSS, FSS, or MSFC) and imaging lesion load. The difference in lesion CNR between the sequences was investigated using Student's t test for paired data. The two-tailed p-value ≤ 0.05 was considered as statistically significant. All calculations were performed by the SPSS software package (SPSS, Chicago, Ill).

Results

Patient characteristics

We included 15 CIS, 6 MS and 10 healthy volunteers. Characteristics of these subjects are shown in Table 1. Overall, the mean age was 36 ±10 years and 55% of the patients were female. The age and gender were not statistically different between subgroups. The median EDSS score of the patients was 2.5 (interquartile range [IQR], 1.5 to 3.0), median FSS 5.0 (IQR, 2.7 to 5.9), median MSFC sum score 0.11 (IQR, -0.36 to 0.42).

Table 1. Baseline characteristics of participants

	CIS patients[a] (n = 15)	**MS patients (n = 6)**	**Healthy participants (n = 10)**
Median age (IQR) (year)	39 (28–43)	34 (27–54)	35 (25–38)
Female gender (%)	10 (67%)	4 (67%)	3 (30%)
Median months of disease duration (IQR)	15 (8–24)	65 (28–168)*	n.a.
Median EDSS (IQR)	1.5 (1.0–3.0)	3.0 (3.0–3.5)*	n.a.
Median FSS (IQR)	4.2 (2.6–5.9)	5.3 (3.6–5.8)	n.a.
Median MSFC sum score (IQR)	-0.12 (-0.34 to 0.41)	0.23 (-0.42 to 0.43)	n.a.

Abbreviations: CIS, clinically isolated syndrome; EDSS, Expanded Disability Status Scale; FSS, Fatigue Severity Scale; IQR, interquartile range; MSFC, Multiple Sclerosis Functional Composite; MS, multiple sclerosis; n.a., not applicable.
a. Presenting event of CIS patients were unilateral optic neuritis (6/15), brainstem or cerebellar syndrome (3/15), spinal cord syndrome (3/15), and supratentorial involvement (5/15).
*Statistical significance ($p<0.05$) between patients with CIS and MS

MRI findings in healthy controls

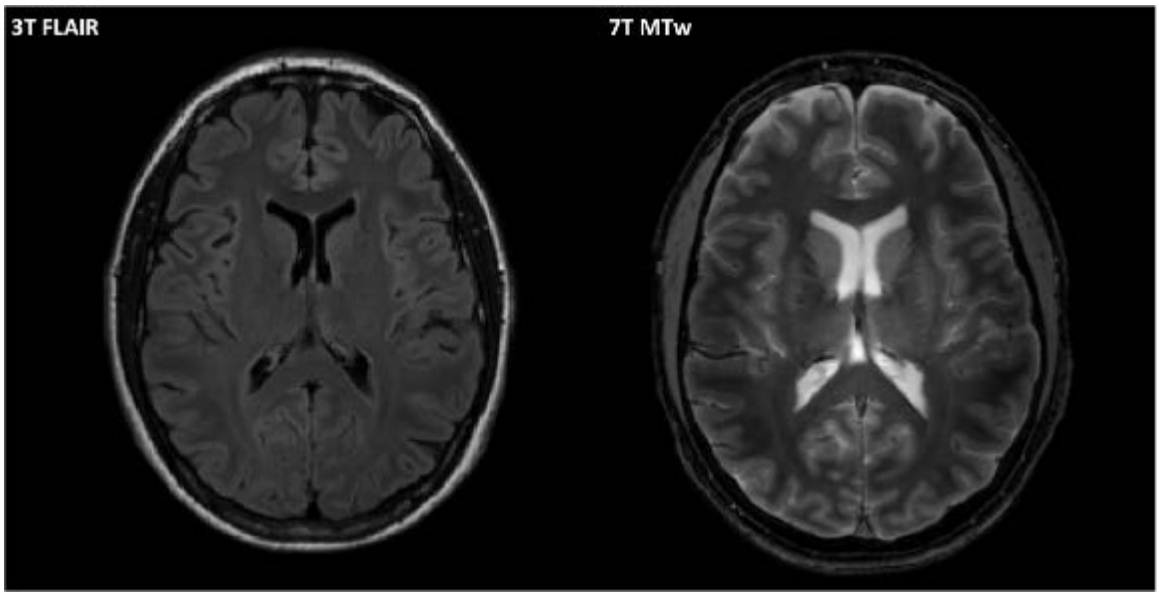
Figure 3a shows an example of 3T-2D-FLAIR and 7T-3D-MT_w images obtained in a healthy control participant. No white matter hyperintensity was identified in two healthy controls, whilst in 8 healthy participants non-specific discrete hyperintensities of the white matter were observed in periventricular and deep white matter regions in 3T-2D-FLAIR or 7T-3D-MT_w images. No diffuse periventricular hyperintensity or juxtacortical hyperintensity was found in any of the healthy volunteers.

Sensitivity of white matter lesion detection using 7T MT_w images

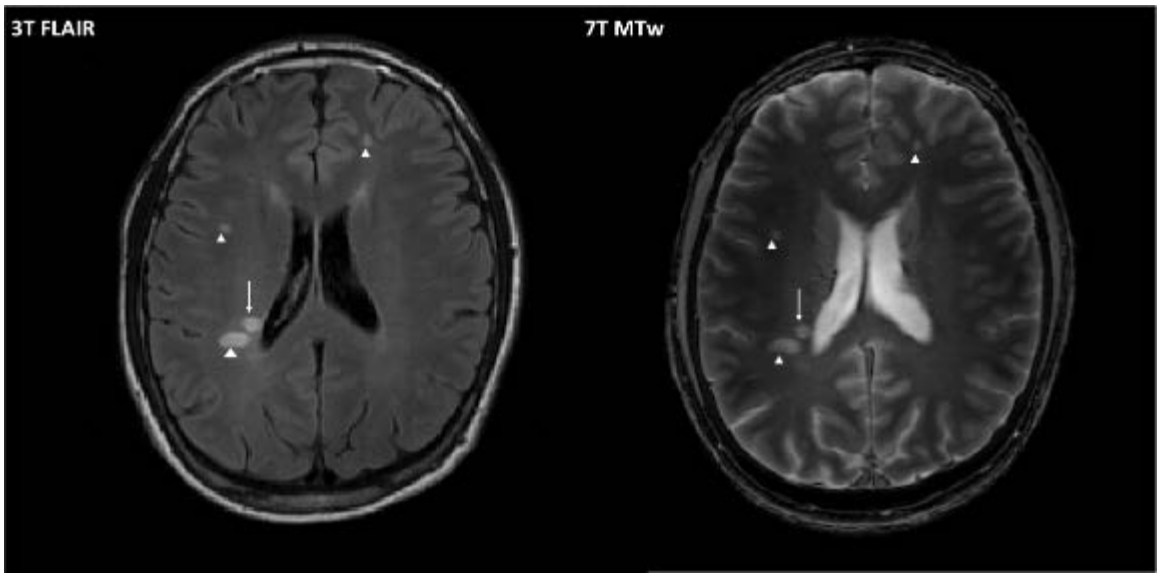
Figure 3 shows examples of original 3T-2D-FLAIR and 7T-3D-MT_w images from a healthy participant and a patient with CIS or MS. After both images were registered to the same 3-D T1-weighted MPRAGE imaging space, corresponding lesions were slightly larger in 3T-2D-FLAIR images than 7T-3D-MT_w images. However, the overall anatomical definition between grey and white matter is better in 7T MT_w images than the FLAIR images.

Figure 3 Axial 3T-2D-FLAIR images and 7T-3D-MTw images in (a) a healthy participant (b) a 40-year-old male CIS patient (EDSS 1.0) with discrete hyperintensities in the deep white matter (arrowhead) and periventricular white matter (arrow); (c) a 56-year-old female MS patient (EDSS 6.5) with diffuse periventricular hyperintensities (arrowhead) and juxtacortical hyperintensities (arrow).

(a)



(b)



(c)

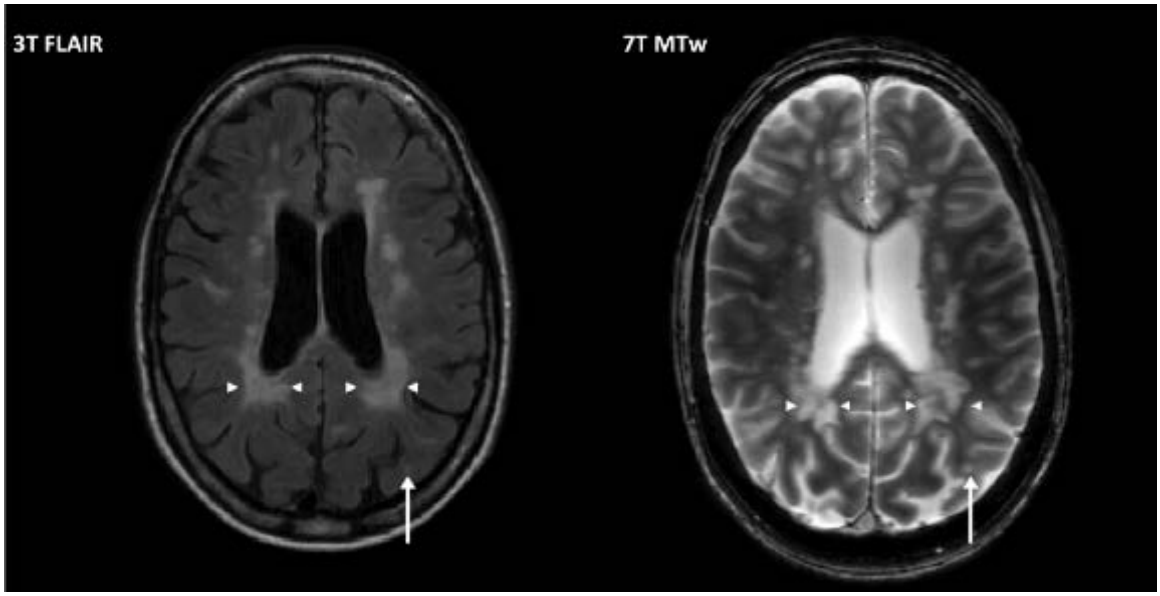


Table 2 shows the sensitivity of lesion detection rate of 7T-3D-MT_w compared to 3T-2D-FLAIR and retrospective assessment enhanced the sensitivity of the prospective one. Overall, a total of 662 lesions were identified on original 3T-2D-FLAIR images, and a total of 669 lesions were observed on original 7T-3D-MT_w images. However 10 of 662 hyperintensities (1.5%) found in 3T-2D-FLAIR images could not retrospectively be found in the corresponding regions on 7T-3D-MT_w images and 17 of 669 hyperintensities (2.5%) seen on 7T-3D-MT_w images could not be identified on the 3T-2D-FLAIR images. Thus taking the 3T-2D-FLAIR images as the gold standard scan we found a sensitivity of 98% (95% CI: 97% –99%) for detecting lesions on the 7T-3D-MT_w images. The sensitivity for detecting lesions on 7T-3D-MT_w was 98 % (95% CI: 96 % – 99 %) for CIS patients (318/325 lesions) and 99% (95% CI: 98% – 100%) for MS patients (327/329 lesions).

Table 2. The lesion counts and sensitivity of the two-step qualitative analysis for the corresponding white matter lesions on 3T-2D-FLAIR and 7T-3D-MTw images.

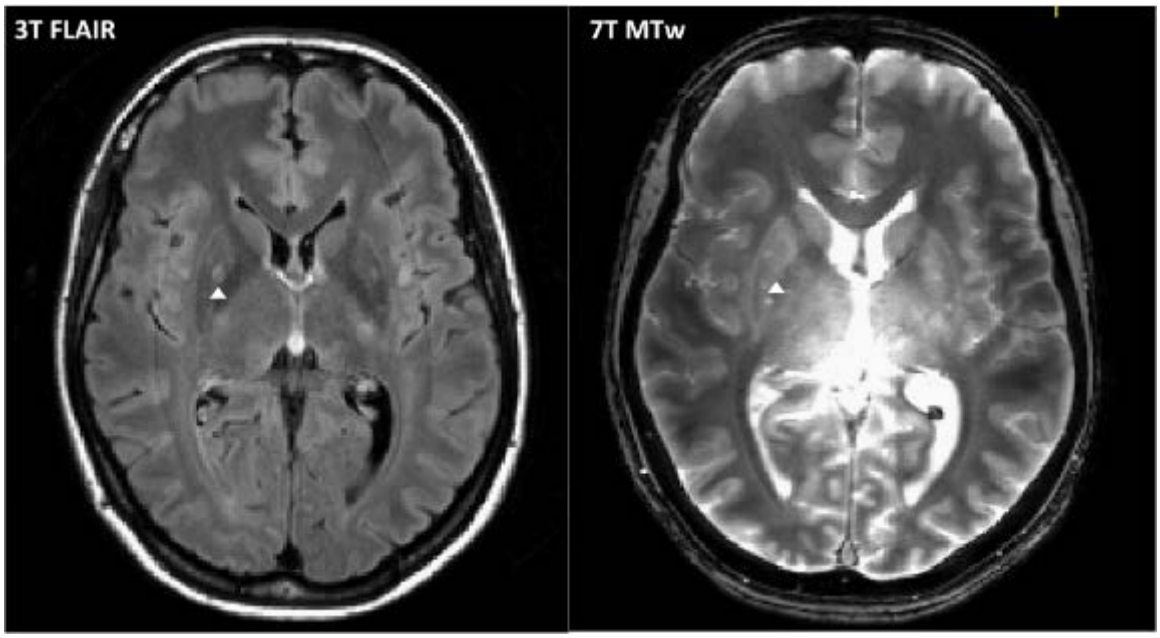
Two-steps	**Prospective analysis on registered images**				**Retrospective analysis on original images**			
Subjects	All	CIS patients	MS patients	Healthy participants	All	CIS patients	MS patients	Healthy participants
3T-2D-FLAIR (lesion count)	662	306	320	8	662	325	329	8
7T-3D-MTw (lesion count)	669	274	258	6	669	329	327	13
Seen on the same anatomical location (lesion count)	652	250	249	6	652	318	327	7
Sensitivity (%) of 7T-3D-MTw using 3T-2D-FLAIR as the gold standard (95% confidence interval)	98 (97–99)	82 (77-86)	78 (73-82)	75 (35–97)	98 (97–99)	98 (96–99)	99 (98-100)	88 (47-100)

Abbreviations: CIS, clinically isolated syndrome; D, dimensional; FLAIR, fluid-attenuated inversion recovery; MS, multiple sclerosis; MTw, magnetization transfer weighted; T, tesla.

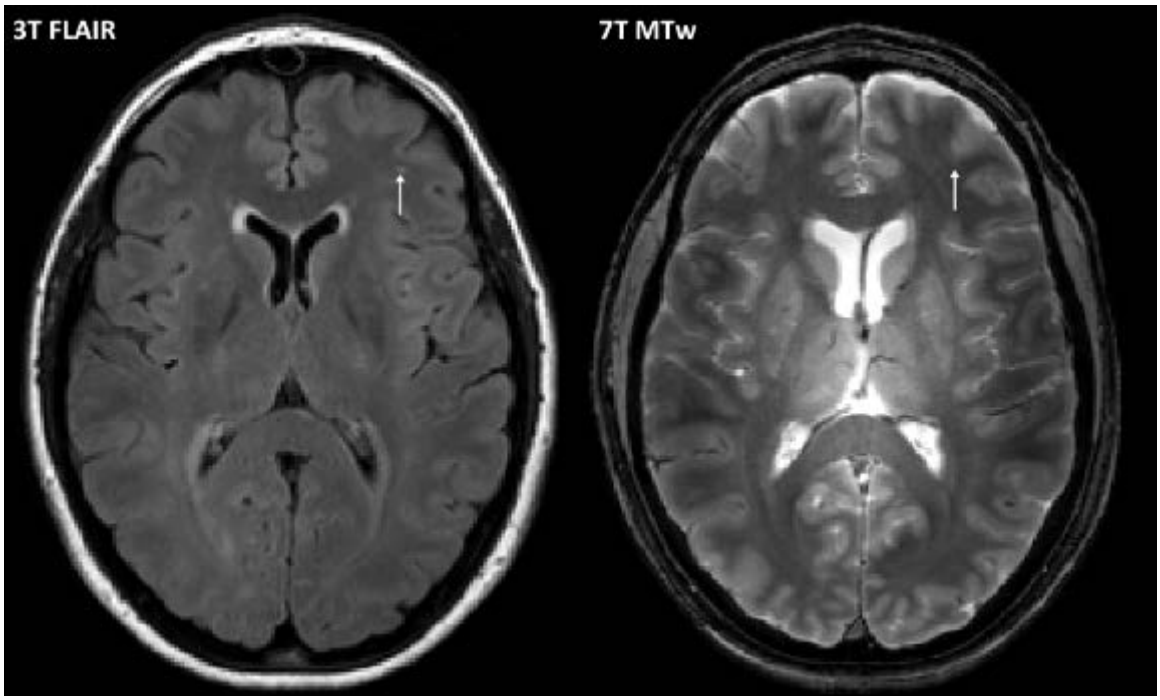
The 27 lesions found by one scan type but not by the other were relatively small with a maximum diameter of less than 3 mm (Figure 4). In general, hyperintensities observed in the basal ganglia on 3T-2D-FLAIR images were less distinguishable on the corresponding 7T-3D-MT_w images (Figure 4a); most of the hyperintensities observed only on 7T-3D-MT_w images were elongated in the through plane direction, and possibly related to perivenous spaces, namely Virchow-Robin spaces.

Figure 4 Comparison of discrete hyperintensity at the same neuroanatomical regions on axial 3T-2D-FLAIR images and 7T-3D-MTw images, (a) observable lesion in the basal ganglia (arrowhead) on 3T-2D-FLAIR only; (b) observable lesion in the deep white matter on 3T-2D-FLAIR only (arrow); (c) observable lesion (arrow) in the deep white matter on 7T-3D-MTw only.

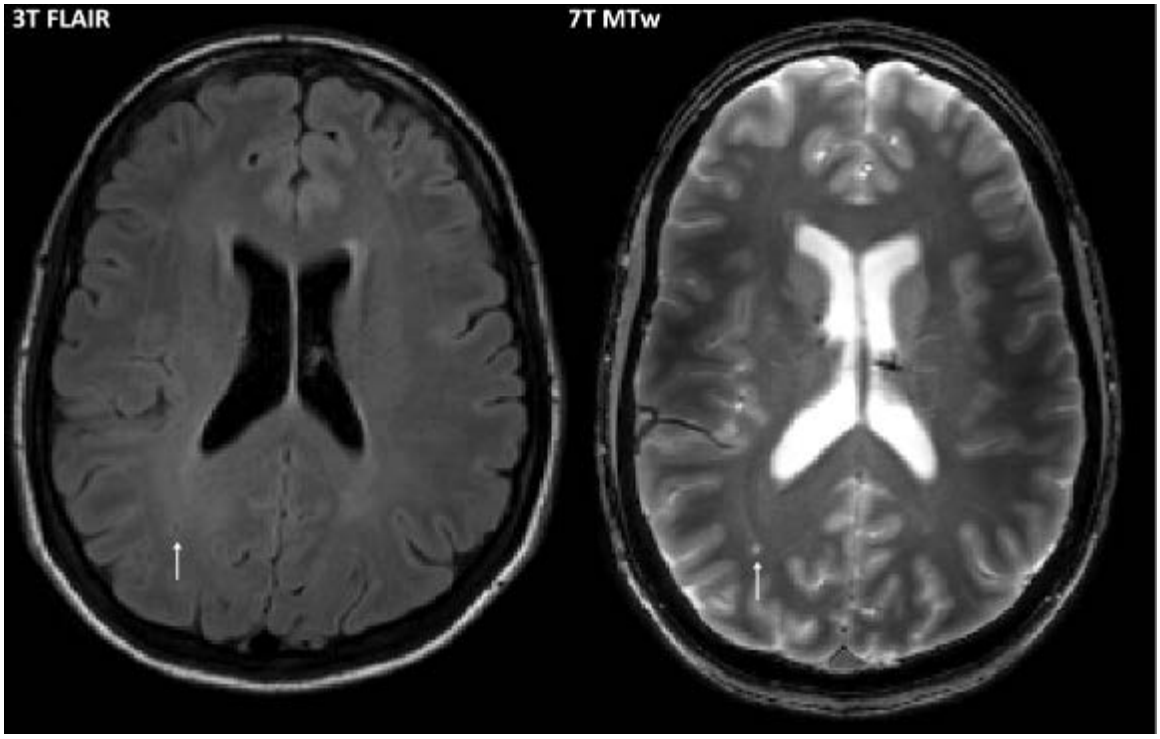
(a)



(b)



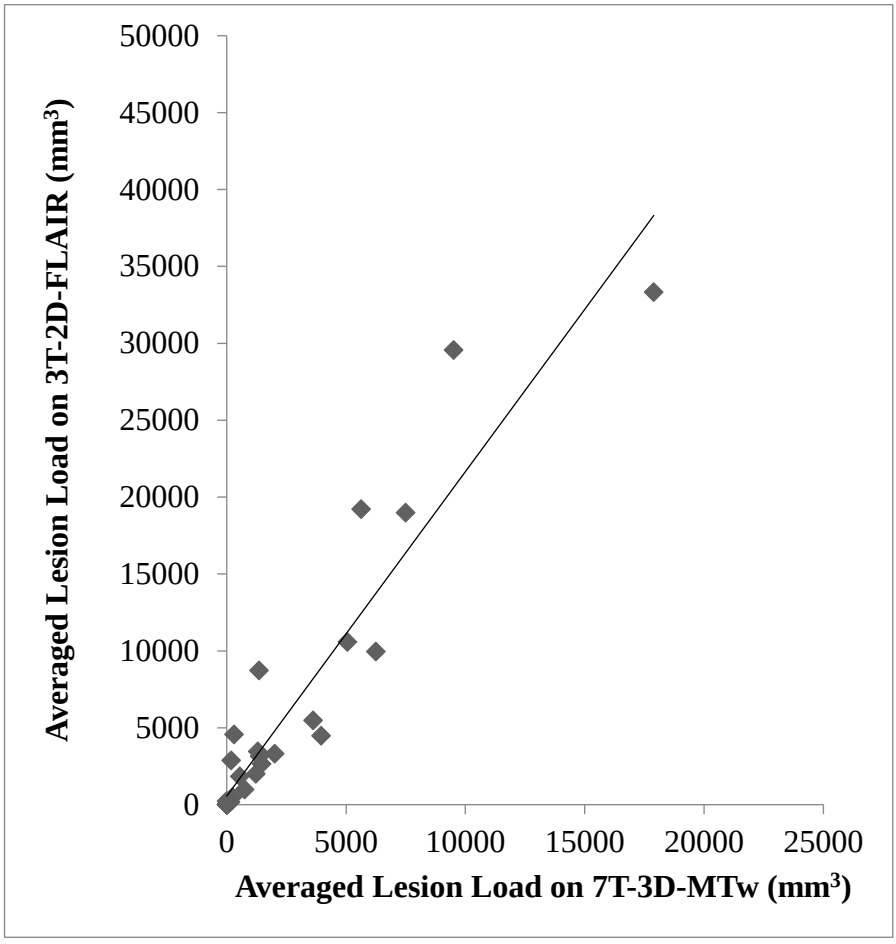
(c)



Intra-rater and inter-rater reliability and comparison of lesion loads

The mean lesion load in healthy participants, CIS and MS patients were 49, 4996, 15102 mm^3 for 3T-2D-FLAIR and 24, 2056, 6573 mm^3 for 7T-3D-MT_w respectively. The intra-rater reliability was excellent with an ICC of 96% (95% CI: 92%–98%) for 3T-2D-FLAIR and 98% (95% CI: 97%–99%) for 7T-3D-MT_w. The correlation between two neurologists showed strong agreement with an ICC of 98% (95% CI: 93%–98%) for 3T-2D-FLAIR and 82% (95% CI: 66%–91%) for 7T-3D-MT_w. The lesion load measured in the different sequences was highly correlated (Spearman Rank correlation =0.910, p<0.001) (Figure 5). When excluding healthy volunteers, the correlation coefficient was 0.875 (p<0.001).

Figure. 5 The strength of association between lesion loads estimated by 3T-2D-FLAIR and by 7T-3D-MTw images is measured by Spearman Rank correlation (rho=0.910, p<0.001).



MR imaging quality and contrast ratio between lesions and NAWM

The quality of images was rated as excellent (n=29, 94%) and good (n=2, 6%) for both sequences. None was classified as blurred. The Lesion-to-NAWM contrast for 7T-3D-MT_w images was not inferior to that for 3T-2D-FLAIR images (mean ± standard deviation, 27 ± 9% versus 26 ± 5%).

Clinical correlation

For the patients (n=21) EDSS score was correlated with the lesion load of 7T-3D-MT_w images (rho = 0.519, p = 0.016) and 3T-2D-FLAIR images (rho = 0.496, p = 0.022) and FSS score was correlated with the lesion load of 7T-3D-MT_w images (rho = 0.506, p = 0.019), and 3T-2D-FLAIR images (rho = 0.437, p = 0.048). No correlation was found between MSFC sum score and lesion load (7T-3D-MTw images, rho= -0.375, p= 0.094; 3T-2D-FLAIR images, rho= -0.412, p= 0.064).

Discussion

This study assessed the performance of 7T-3D-MT_w MR imaging in detecting white matter demyelinating lesions seen on 3T-2D-FLAIR in patients with CIS or MS. Defining 3T-2D-FLAIR images as gold-standard, we found that 7T-3D-MT_w imaging could detect WM lesions with 98% sensitivity. In addition, the correlation between the measured lesion loads and clinical parameters (disability status and fatigue) was similar using 7T-3D-MT_w and 3T-2D-FLAIR images. Therefore our study supports a potential role of 7T MT_w images for lesion detection in the supratentorial white matter of patients with inflammatory demyelinating conditions.

7T MR imaging of patients with MS or CIS has previously been demonstrated to be safe and well tolerated (Chou, Tench et al. 2014), and to provide high-resolution anatomical images allowing visualization and quantification of disease specific abnormalities of myelin content (Tallantyre, Morgan et al. 2010, Sinnecker, Mittelstaedt et al. 2012, Al-Radaideh, Wharton et al. 2013, Mistry, Dixon et al. 2013, Abdel-Fahim, Mistry et al. 2014, Mackenzie, Morant et al. 2014, Blazejewska, Al-Radaideh et al. 2015, Yao, Ikonomidou et al. 2015). In particular MTR maps and MT_w imaging at 7T have been shown to be useful for detecting intracortical lesions compared to the commonly used double inversion recovery (DIR) sequence at 3T (Abdel-Fahim, Mistry et al. 2014). The current study supports the use of 7T MT_w images for lesion detection with a high sensitivity of visualised lesions on the 3T FLAIR images and MTR images can particularly quantify the lesion myelin content by calculating the MTR at the same scan session.

Ultrahigh field 7T MRI provides higher sensitivity and contrast compared to lower field, and has been shown to generate superior anatomical imaging and disease-specific assessment in patients with MS or CIS compared to 3T (Tallantyre, Morgan et al. 2009, Tallantyre, Morgan et al. 2010). However, 7T MRI still presents some challenges, and so whereas T1- or T_2*-weighted scans are now widely used at 7T, the use of T2-weighted FLAIR imaging remains limited because of the effect of RF inhomogeneity and increased specific absorption rate (SAR), and the lengthened T1 relaxation times of GM and WM (so that signals are not fully recovered at the inversion time when CSF signal is nulled). Although efforts have been made to overcome these issues, current FLAIR imaging at 7T

shows variations in the transmit field causing hypointensity particularly in the temporal lobes (Zwanenburg, Hendrikse et al. 2010), heterogeneous CSF suppression (de Graaf, Zwanenburg et al. 2012), and long acquisition times (Saranathan, Tourdias et al. 2014). Therefore, 3D MT_w imaging at 7T is technically more straightforward and shows a similar white matter lesion detection rate to 3T-2D-FLAIR imaging, and therefore has the potential to be a useful research tool in inflammatory demyelinating diseases. Moreover, the magnetization imaging can also be used to produce the Magnetization Transfer Ratio (MTR), a marker related closely to the myelin content of the tissue (Schmierer, Tozer et al. 2007), which provides quantitative information useful for longitudinal or cross-sectional studies in MS. However, MT_w imaging does not detect 1.5% of hyperintensity on 3T FLAIR images and vice versa. These minor differences may be attributed to the signal density represented of the different imaging phenomena acquiring by each technique.

Eight of 10 healthy controls had unexpected nonspecific hyperintensity in the deep white matter. After carefully comparing different sequences by three neurologists, the hyperintensity was classified to be of no clinical significance. The rate of incidental findings here is higher than other reports done at 3T and a lower field strength (Cordonnier, Al-Shahi Salman et al. 2007)(Vermeer, Longstreth et al. 2007)(Morris, Whiteley et al. 2009)(Gur, Kaltman et al. 2013), which may be attributed to both higher field strength and smaller in-plane imaging resolution. Incidental findings in healthy volunteers are not uncommon in adults and children. Common well-known incidental findings that increase with age are white matter hyperintensities, followed by microbleeds, (Cordonnier, Al-Shahi Salman et al. 2007) and silent infarcts (Vermeer, Longstreth et al. 2007). Excluding the aforementioned abnormalities, the prevalence of neoplastic incidental findings was 0.70%, and non-neoplastic ones was 2.0% with a 1.5T or lower field strength MRI (Morris, Whiteley et al. 2009). The entity of definite demyelination was 0.06% (Morris, Whiteley et al. 2009). The rate of incidental findings was reported to be 10.6% at 3 Tesla MRI among 1,400 healthy volunteers aged 8 to 21 years (Gur, Kaltman et al. 2013), and vascular abnormalities (2.57%) or pineal cyst (2.43%) are the most common two findings. When the incidental lesions are thought to be not clinical significant, it is an ethic issue to decide to tell the volunteers or not (Illes, Rosen et al. 2004, Kumra, Ashtari et al. 2006).

There is no general policy for the insignificant incidental findings globally including UK (Booth, Waldman et al. 2012).

Some limitations of the current study deserve discussion. Firstly, we only included supratentorial regions. Demyelinating lesions in patients with MS or CIS occur most frequently in the periventricular regions, and thus were captured in our study. The visibility of lesions in the temporal lobes and the cerebellum needs further evaluation because the inhomogeneity in the applied transmit RF field is more pronounced in these regions. Secondly, caution is needed in directly comparing the estimates of lesion volumes on 3T-2D-FLAIR and 7T-3D-MT_w images as the image acquisition and resolution were different. However, the results showed that a very similar number of lesions were detected on both sequences at the same neuroanatomical regions. Also, the measured lesion load in 7T-3D-MT_w images correlated with the disability status and with fatigue to a similar extent to that in 3T-2D-FLAIR images, which is remarkable for this small number of patients. Thirdly, as mentioned, the sample size was relatively small and most patients enrolled in this study had short disease duration. However, for the purposes of validating 7T-3D-MT_w for lesion detection and delineation, this is an advantage as fewer lesions are confluent and the subtle abnormalities in the normal appearing brain tissue are easier to study.

Conclusions

MT_w imaging at 7T allows the evaluation and assessment of supratentorial lesions with similar lesion detection to 3T FLAIR images in patients with CIS or MS. The results support the application of MT_w imaging at 7T in the detection and volume estimation of demyelinating lesions as a reliable substitute for FLAIR imaging. However, a prospective study using same imaging resolution and dimension is needed to validate the current finding.

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