Tampere University

MARCIN KOLASA

Magnetic Resonance Imaging Follow-up and JCV Serology in Multiple Sclerosis

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ACADEMIC DISSERTATION
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TO MY FAMILY

ABSTRACT

Currently, magnetic resonance imaging (MRI) plays an important role in the diagnostic process and monitoring of the disease course in multiple sclerosis (MS). However, routinely used conventional MRI (T1- and T2-weighted sequences) is not specific to underlying MS pathology, and correlations between radiological findings and clinical measures are only modest. Potent therapies for MS, such as natalizumab (NTZ), have been associated with progressive multifocal leukoencephalopathy (PML). PML is a severe demyelinating disease caused by the reactivation of neurotropic JC virus (JCV). Due to the risk of PML, the serological assessment of antibodies against JCV is performed before starting NTZ.

The main goal of this four-year follow-up study was to determine changes in the brain using nonconventional MRI techniques, such as volumetric measurements and diffusion tensor imaging (DTI), in clinically isolated syndrome (CIS) and MS. Whether volumetric and DTI-derived metrics could play prognostic roles in the prediction of the conversion of CIS to MS and whether these nonconventional measures correlate with disability progression expressed by an increase in the expanded disability status scale score in MS were also evaluated. In the third part of this thesis, the seroprevalence of the anti-JCV antibodies and temporal changes in JCV serostatus in a Finnish cohort of patients with CIS and MS were evaluated. The effect of demographic factors and MS therapies on JCV status was also determined.

In the first and second part of this study, the higher baseline volumes of focal brain lesions related to MS pathology were associated with the conversion of CIS to MS. In contrast, whole brain atrophy and volumes of focal lesions were not clearly correlated with disability progression over four years in MS. With regard to DTI, diffusivity changes in the brain were stronger in CIS and MS when compared to healthy controls. Moreover, the worsening of DTI metrics was primarily observed in the CIS group that converted to MS. However, a clear correlation between baseline DTI metrics and the conversion to MS was not found. In MS, a tendency for a correlation between the DTI metric in the corpus callosum (CC) and disability progression was observed.

The results suggest a potential role for DTI in monitoring disease activity in CIS and MS. Volumetric measurements seem to be helpful in evaluating disease

progression in CIS but not in MS. However, further studies with larger populations and longer follow-up times are required to confirm these results.

The third part of the thesis showed a high seroprevalence of anti-JCV antibodies (57%) in a cohort of CIS and MS patients. Moreover, marked temporal fluctuations in JCV serostatus were observed over four years. Demographics, such as higher age and male gender, were associated with anti-JCV antibody seropositivity. These observations are consistent with the reports from multinational studies and confirm high JCV seroprevalence in Finnish MS patients. Moreover, temporal changes in JCV serostatus should be considered in clinical practice.

TIIVISTELMÄ

Magneettikuvausta (MK) käytetään nykyään yleisesti multippeliskleroosin (MS) diagnostiikassa ja taudin seurannassa. Konventionaalinen MK (T1 ja T2 painotteiset sekvenssit) ei kuitenkaan ole spesifinen MS-taudin patologian suhteen. Lisäksi radiologiset löydökset korreloivat vain kohtalaisesti kliinisen kokonaistoimintakyvyn kanssa.

Tehokkaat MS-taudin lääkehoidot, kuten natalitsumabi (NTZ), lisäävät progressiivisen multifokaalisen leukoenkefalopatian (PML) riskiä. PML on vakava demyelinoiva keskushermoston sairaus, jonka aiheuttaa neurotrooppisen JC-viruksen reaktivaatio. PML:n riskin vuoksi MS-potilailta tutkitaan JC-virusvasta-aineet ennen NTZ:n lääkehoidon aloitusta.

Väitöskirjatutkimuksen päätavoitteena oli tutkia uusien MK:een perustuvien kuvantamismenetelmien, volumetrian ja diffuusiotensorikuvauksen (DTI) avulla aivoparenkyymissä tapahtuvia muutoksia MS-potilailla ja kliinisesti eriytyneessä oireyhtymässä (KEO) neljän vuoden seurantatutkimuksessa. Tavoitteena oli selvittää voidaanko volumetrialla tai DTI-muutosten arvioinnilla ennustaa progressiota KEO:sta MS-tautiin. MS-potilailla selvitettiin myös korreloivatko havaitut muutokset toimintakyvyn huononemiseen expanded disability status scale-asteikon avulla mitattuna. Kolmannessa osatyössä tutkittiin JC-virusvasta-aineiden seroprevalenssia suomalaisessa MS-potilasaineistossa sekä selvitettiin JC-virusvasta-aineiden stabiliteettia neljän vuoden seurannassa. Myös MS-taudin immunomoduloivan hoidon ja muiden kliinisten tekijöiden vaikutusta JC-viruksen esiintymiseen selvitettiin.

Tuloksien mukaan suurempi MS-plakkien tilavuus lähtötilanteessa on yhteydessä progressioon KEO:sta MS-tautiin. Sen sijaan MS-potilailla aivoatrofia tai plakkien volyymi eivät selkeästi korreloineet kokonaistoimintakyvyn huononemiseen neljän vuoden seurannassa. DTI:n avulla havaitut aivoparenkyymin muutokset olivat merkittävämmät KEO- ja MS- potilailla kuin terveillä verrokeilla. Lisäksi havaittiin, että DTI-arvojen huononeminen seurannassa oli yleisempää sellaisessa KEO-ryhmässä, joka eteni MS-tautiin. Lähtötilanteen DTI-muutokset eivät kuitenkaan ennustaneet konversiota. MS-potilailla corpus callosumin DTI-arvojen ja kokonaistoimintakyvyn huononemisen välillä oli heikko yhteys.

Kuvantamistulokset viittaavat siihen, että DTI:lla on potentiaalia taudin aktivisuuden monitoroinnissa sekä KEO:ssa että MS-taudissa. Volumetriassa havaitut muutokset olivat yhteydessä taudin progressiossa vain KEO:ssa. Tulosten varmentaminen edellyttää kuitenkin suurempaa potilasaineistoa ja pidempää seuranta-aikaa.

JC-virusvasta-aineiden merkitystä selvittävässä tutkimuksessa havaittiin korkea JC-virusvasta-aineiden seroprevalenssi sekä KEO- että MS-potilailla (57%). Seurannassa oli huomattavaa vasta-aineiden vaihtelua. Korkeampi ikä ja miessukupuoli olivat yhteydessä JC-virusvasta-ainepositiivisuuteen. Yhteenvetona todetaan, että JC-viruksen seroprevalenssi suomalaisilla MS potilailla on korkea kuten muissakin maissa. JC-virusvasta-ainestatuksen vaihtelu on syytä huomioida kliinisessä toiminnassa.

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ABBREVIATIONS

3D	Three-dimensional
9-HPT	Nine-hole peg test
AD	Axial diffusivity
BPF	Brain parenchymal fraction
CC	Corpus callosum
CIS	Clinically isolated syndrome
CNS	Central nervous system
CSF	Cerebral spinal fluid
DIR	Double inversion recovery
DIS	Dissemination in space
DIT	Dissemination in time
DTI	Diffusion tensor imaging
DWI	Diffusion weighted imaging
EBV	Epstein-Barr virus
EDSS	Expanded Disability Status Scale
ELISA	Enzyme-Linked Immunosorbent Assay
FA	Fractional anisotropy
FLAIR	Fluid attenuated inversion recovery
Gd	Gadolinium
IC	Internal capsule
IFN	Interferon
JCV	John Cunningham virus
MD	Mean diffusivity
MHC	Major histocompatibility complex
MPRAGE	Magnetisation prepared rapid gradient echo
MRI	Magnetic resonance imaging
MS	Multiple sclerosis
MSFC	Multiple Sclerosis Functional Composite
MTI	Magnetization transfer imaging

NABT	Normal-appearing brain tissue
NAGM	Normal-appearing grey matter
NAWM	Normal-appearing white matter
NEDA	No evidence of disease activity
NMO	Neuromyelitis optica
NTZ	Natalizumab
PASAT	Paced Auditory Serial Additions Test
PET	Positron emission tomography
PML	Progressive multifocal leukoencephalopathy
PPMS	Primary-progressive multiple sclerosis
PSIR	Phase-sensitive inversion recovery
RD	Radial diffusivity
RIS	Radiologically isolated syndrome
ROI	Region of interest
RRMS	Relapsing-remitting multiple sclerosis
RT	Repetition time
SPMS	Secondary-progressive multiple sclerosis
SWI	Susceptibility weighted imaging
T	Tesla
TBSS	Tract-based spatial statistics
TE	Echo time
TI	Inversion time
VBM	Voxel-based method

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following publications, which are referred in the text by Roman numerals I-III. The original publications have been reproduced with the permission of the copyright holders.

Publication I Kolasa M, Hakulinen U, Helminen M, Hagman S, Raunio M, Rossi M, Brander A, Dastidar P, Elovaara I. (2015). Longitudinal assessment of clinically isolated syndrome with diffusion tensor imaging and volumetric MRI. Clinical Imaging, 39(2), 207-212.

Publication II Kolasa M, Hakulinen U, Brander A, Hagman S, Dastidar P, Elovaara I, Sumelahti M-L. (2019). Diffusion tensor imaging and disability progression in multiple sclerosis: A 4-year follow-up study. Brain and Behavior, 9:e01194.

Publication III Kolasa M, Hagman S, Verkkoniemi-Ahola A, Airas L, Koivisto K, Elovaara I. (2016). Anti-JC virus seroprevalence in a Finnish MS cohort. Acta Neurologica Scandinavica, 133: 391-3.

1 INTRODUCTION

Multiple sclerosis (MS) is an inflammatory degenerative disease of the central nervous system (CNS) that affects young adults, leading to marked disability and cognitive impairment (Thompson et al., 2018). MS prevalence is high in northern countries, including Finland (Browne et al., 2014; Sumelahti et al., 2001). The pathogenesis of MS is not fully understood, but the autoimmune response of the T and B cells against antigens in the CNS is thought to be responsible for tissue damage (Kasper & Shoemaker, 2010; Schirmer et al., 2014). Histopathologically, MS is characterized by demyelination, axonal loss, and gliosis (Lassmann, 2018). Neurodegeneration observed in MS leads to brain atrophy, which appears early in disease (Uher et al., 2014) and progresses faster in MS than in healthy populations (Bermel & Bakshi, 2006).

The first clinical episode suggestive of inflammatory demyelination is called clinically isolated syndrome (CIS) (Miller, D. et al., 2005). The most common clinical phenotype of MS is relapsing-remitting MS (RRMS), which is characterized by episodes of relapses followed by recovery (Lublin, 2014). RRMS progresses to secondary-progressive MS (SPMS) in approximately 30-60% of cases within approximately 20 years after MS onset (Tutuncu et al., 2013). SPMS is characterized by gradual progression of disability with or without superimposed relapses (Lublin et al., 2014). MS patients with progressive onset of disease are categorized as primary-progressive MS (PPMS), and these individuals represent a minority of MS patients (Miller, D. H. & Leary, 2007).

Conventional magnetic resonance imaging (MRI), including T1- and T2-weighted sequences with and without gadolinium (Gd) contrast, is a main paraclinical tool used for diagnosing MS, monitoring disease course and treatment effect (Wattjes, Rovira et al., 2015). Diffusion tensor imaging (DTI) is a nonconventional MRI sequence in which image contrast depends on differences in water diffusion in different brain regions (Enzinger et al., 2015). Several DTI studies have revealed changes in brain regions that appear normal on conventional MRI in the so-called normal-appearing grey (NAGM) and white matter (NAWM) (Bozzali et al., 2002; Preziosa et al., 2011). In addition, brain atrophy measurements play promising roles

in disease monitoring, as brain volume loss is associated with clinical progression in MS (Jacobsen et al., 2014; Kalincik et al., 2012). As the extent of brain abnormalities observed on conventional MRI correlates only moderately with clinical measures in MS (Barkhof, 1999), there is still a need to search for imaging biomarkers that more specifically reflect disease-related pathology and correlate more accurately with clinical status.

Immunomodulatory MS therapies, such as natalizumab (NTZ), fingolimod, and dimethyl fumarate, are associated with severe adverse effects, such as progressive multifocal leukoencephalopathy (PML) (Berger et al., 2018; Linda et al., 2009; Rosenkranz et al., 2015). PML is caused by the reactivation of JC virus (JCV) and the subsequent lytic infection of the brain (Major et al., 2018). The quantification of antibodies against JCV was included in PML risk stratification for patients receiving NTZ (Plavina et al., 2014). Therefore, information on the JCV antibody status and the temporal stability of such antibodies is of great importance in MS patients.

2 REVIEW OF THE LITERATURE

2.1 Overview of MS

2.1.1 Epidemiology and risk factors

MS is an immune-mediated inflammatory demyelinating disease (Baecher-Allan et al., 2018; Lassmann, 2018) that typically affects young adults between the ages of 20 – 30 years (Kobelt et al., 2017; Noseworthy et al., 2000). The overall prevalence rate of MS in Europe is high, occurring in approximately 83/100,000 individuals (Kingwell et al., 2013; Pugliatti et al., 2006). Both the prevalence and incidence of MS have increased during recent decades, especially among women (Koch-Henriksen & Sorensen, 2010; Trojano et al., 2012). The prevalence of MS in Finland is high and increasing, with incidents ranging between 108 – 280/100,000 individuals (Sumelahti et al., 2000; Sumelahti et al., 2001; Sumelahti et al., 2003) and with marked differences between regions (Pirttisalo et al., 2019).

The aetiology of MS is unknown (Reich et al., 2018). Nonetheless, genetic and environmental factors play important roles in the development of MS (Ebers, 2008). Among genetic risk factors, first-degree relatives affected by MS (Nielsen, N. M. et al., 2005), female gender, Scandinavian and Caucasian ethnic origin (Kahana, 2000) have been linked to a higher risk of MS. The environmental risk factors for MS are as follows: higher geographical latitude, lower vitamin D levels probably related to decreased sunlight exposure, Epstein-Barr virus (EBV) infection in childhood, obesity and smoking (Olsson et al., 2017). Early exposure to environmental risk factors (before the age of 15) is crucial to the development of MS. Migration from the area with a high risk of MS to an area with a lower risk of MS early in life has been related to a reduction in the risk of MS development, indicating the relevant role of environmental factors in MS development (Ramagopalan et al., 2010).

2.1.2 Pathology of MS

The pathologic features of MS are demyelination and inflammatory infiltrates consisting of monocytes, microglial, T and B cells (Kuhlmann et al., 2017). Demyelinating inflammatory processes occur in the white and grey matter (Lassmann, 2018) and begin around small veins, which distinguishes MS from other demyelinating conditions, such as neuromyelitis optica (NMO) or PML (Bauer et al., 2015; Misu et al., 2013). Focal demyelinating lesions called plaques can be divided into two main groups, acute active and chronic. Acute active plaques are characterized by diffuse infiltration of macrophages and lymphocytes, and such plaques are frequently present in early stages of MS (Frischer et al., 2009). Inflammation at early stages of MS is responsible for blood-brain barrier damage (Gaitan et al., 2011; Hochmeister et al., 2006). Chronic lesions are predominantly observed in progressive MS and consist of a hypocellular demyelinated core surrounded by a rim of activated iron-rich microglia and macrophages (Frischer et al., 2015; Popescu, B. F. et al., 2013; Prineas et al., 2001). Axonal degeneration observed in the plaques is strongly associated with inflammation, and axonal density is profoundly decreased in chronic lesions (Frischer et al., 2015). Cortical demyelination appears early in the disease course (Lucchinetti et al., 2011) and increases in progressive MS (Choi et al., 2012; Magliozzi et al., 2007). Diffuse and perivascular meningeal inflammation seems to be responsible for cortical pathology in MS (Howell et al., 2011; Lucchinetti et al., 2011).

Diffuse abnormalities beyond demyelinating plaques in the NAWM and NAGM are also observed in MS. These anomalies represent inflammatory infiltration, oedema, microglia activation, axonal injury, and astrogliosis (Kutzelnigg et al., 2005). These changes are partially caused by focal lesions through Wallerian degeneration (Dziedzic et al., 2010), meningeal inflammation (Haider et al., 2016), and diffuse inflammatory processes (Lassmann, 2013).

Diffuse and focal pathology results in whole brain atrophy with grey matter atrophy progressing faster than white matter atrophy (Chard et al., 2004; Fisher et al., 2008). Both cortical atrophy and deep grey matter atrophy have been observed since the early stages of MS (Bergsland et al., 2012; Henry, R. G. et al., 2008; Tiberio et al., 2005), and these conditions are more prominent in progressive MS than in RRMS (Calabrese et al., 2013).

2.1.3 Diagnostic criteria of MS

MS diagnosis is based today on the clinical and MRI evidence of disease dissemination in space (DIS) and time (DIT) (Filippi et al., 2016; Polman et al., 2005; Polman et al., 2011; Thompson et al., 2018). Paraclinical tool, such as MRI has gained much attention in recent years as it allows the visualization of clinically silent lesions (Harris et al., 1991) that may confirm DIS and DIT and the early diagnosis of MS, even during the first clinical presentation (Brownlee et al., 2015; Polman et al., 2011). Diagnostic criteria for relapsing MS and their evolution through the ages are presented in Table 1. These criteria should be applied in the clinical context in patients with typical CIS and other diseases mimicking MS should be carefully excluded (Filippi et al., 2016).

The newest criteria for MS published in 2018 strengthen the role of MRI in the diagnosis of MS (Thompson et al., 2018). The main changes proposed in this revision are as follows: in patients presented with CIS with DIS, the presence of oligoclonal bands in the CSF may confirm DIT and thus the diagnosis of MS. Moreover, symptomatic lesions observed on MRI can confirm both DIS and DIT. Additionally, cortical lesions that are not clearly observed on conventional MRI can be used to demonstrate DIS, thereby possibly reinforcing the role of nonconventional MRI techniques, such as double inversion recovery (DIR) and phase-sensitive inversion recovery (PSIR), which are able to visualize cortical lesions (Favaretto et al., 2015; Filippi et al., 2010).

Radiologically isolated syndrome (RIS) is defined as incidental brain lesions consisting of demyelination observed on MRI in individuals without symptoms suggestive of MS (Okuda et al., 2009). In one study, MS developed in 30% of RIS within 5 years (D. T. Okuda et al., 2014). The radiological risk factors for the conversion of RIS to MS include Gd-enhancing lesions and spinal cord lesions (Lebrun et al., 2009; Okuda et al., 2011).

Table 1. Diagnostic criteria for MS

	2005 McDonald criteria	2010 McDonald criteria	2017 McDonald criteria
DIS	At least 3 out of 4: ≥1 Gd+ lesion or 9 T2 lesions if there is no Gd+ lesion ≥1 infratentorial lesion ≥1 juxtacortical lesion ≥3 periventricular lesions	At least 1 T2 lesion in ≥2 areas: periventricular juxtacortical infratentorial spinal cord	At least 1 T2 lesion in ≥2 areas: periventricular cortical or juxtacortical infratentorial spinal cord
	A spinal cord lesion can be considered equivalent to a brain infratentorial lesion: Gd+ spinal cord lesion is considered to be equivalent to Gd+ brain lesion, and individual spinal cord lesions can contribute together with brain lesions to reach the required number of T2 lesions	Exclusion of symptomatic lesions in brainstem and spinal cord syndromes	No distinction between symptomatic and asymptomatic MRI lesions
DIT	Gd+ lesion ≥3 months after onset of the initial clinical event at a site not implicated by the event	Simultaneous presence of asymptomatic nonenhancing and Gd+ lesions at any time	Simultaneous presence of nonenhancing and Gd+ lesions at any time, no distinction between symptomatic and asymptomatic lesions is required
	or a new T2 lesion occurring any time after 30 days from onset of the initial clinical event	or a new T2 and/or Gd+ lesion on follow-up MRI irrespective of timing of baseline scan	or a new T2 or Gd+ lesion on follow-up, irrespective of the timing of baseline scan or oligoclonal bands in CSF

DIS dissemination in space; DIT dissemination in time; Gd+ gadolinium-enhancing; CSF cerebral spinal fluid. The table is based on Thompson et al., 2018; Filippi et al., 2016; Polman et al., 2005; Polman et al., 2011; Swanton et al., 2007.

2.1.4 Clinical course and methods of quantifying disability in MS

The clinical course of MS is classified as RRMS and progressive MS. In 85% of patients, MS begins as CIS, which refers to a first clinical episode suggestive of inflammatory demyelination in the CNS (Confavreux & Vukusic, 2006; Scalfari et al., 2010). Typically, CIS presents with optic neuritis, brainstem or spinal cord symptoms that can be caused by a single lesion or lesions in more than one site in the CNS (Miller, D. H. et al., 2012).

The most common clinical phenotype of MS (85%) is RRMS (Confavreux & Vukusic, 2006). This condition is characterized by clinical exacerbations that last at least 24 hours with complete or partial recovery between relapses (Lublin et al., 2014). In approximately 65% of cases, RRMS is followed by a progressive phase in which continuous clinical worsening occurs with or without superimposing relapses (Compston & Coles, 2008; Lublin, 2014). Such a transition occurs gradually and begins at approximately 45 years (Soldan et al., 2015; Tutuncu et al., 2013), with a median time from MS onset to a progressive phase of approximately 19 years (Amato & Ponziani, 2000; Vukusic & Confavreux, 2003). The early conversion to SPMS has been associated with older age at MS onset and longer disease duration (Vukusic & Confavreux, 2003).

The least common clinical phenotype of MS is PPMS (15%). This phenotype is characterized by disability progression from the onset without preceding the relapsing-remitting stage (Miller, D. H. & Leary, 2007). In PPMS, spinal manifestation is common, disease onset is usually later, and the progression of irreversible disability is faster than that in RRMS (Confavreux & Vukusic, 2006; Rice et al., 2013). There are no reliable blood, cerebral spinal fluid (CSF), or imaging biomarkers distinguishing MS phenotypes (Lublin et al., 2014; Rice et al., 2013).

A widely used clinical scale to assess disability caused by MS and its progression is the Expanded Disability Status Scale (EDSS), which ranges from 0 (normal) to 10 (death due to MS) (Kurtzke, 1983). EDSS scores below 4.0 refer to patients who are fully ambulatory and EDSS scores of 4.0 or above refer to patients with impairment to ambulation. An EDSS score of 6 means intermittent or constant assistance required to walk 100 metres. The disease progression index is defined as EDSS divided by the disease duration. The Multiple Sclerosis Functional Composite

(MSFC) is a global measure that assesses walking speed using a timed 25-foot walk, arm and hand dexterity using a nine-hole peg test (9-HPT), and cognition function using the Paced Auditory Serial Additions Test (PASAT) (Cutter et al., 1999). The Multiple Sclerosis Severity Score (MSSS) relates the single EDSS score to the distribution of disability in patients with similar disease durations (Roxburgh et al., 2005).

2.1.5 Active MS and immunomodulatory treatment

From a clinical and pathological point of view, MS is classified into relapsing and progressive phenotypes, which represent predominant inflammatory and degenerative phases, respectively. These phenotypes can be further divided into active and nonactive subtypes (Lublin et al., 2014).

The goal of immunomodulatory treatment in MS is the absence of disease activity defined clinically and by MRI (Comi et al., 2017). Clinical activity is demonstrated by relapses and disability progression, while MRI activity is expressed by Gd-enhancing lesions and new or enlarging T2 lesions (Giovannoni et al., 2017; Lublin et al., 2014; McNamara et al., 2017). There is no cure for MS, but therapy should be started early (Landfeldt et al., 2018), as immunomodulatory treatment has been shown to delay the conversion of CIS to MS (Comi et al., 2012; Jacobs et al., 2000; Kappos, Traboulsee et al., 2006; Miller, A. E. et al., 2014) and disease progression in MS (Trojano et al., 2009).

In the early 1990s, interferon (IFN) beta was the first drug approved for the treatment of MS (Jacobs et al., 1996). During the last 20 years, a large number of new immunomodulatory drugs have been introduced into MS treatment (Cohen et al., 2012; Comi et al., 2001; H. P. Hartung et al., 2002; Kappos et al., 2010; O'Connor et al., 2011; Polman et al., 2006). Current standard therapies approved in Finland for the treatment of RRMS are presented in Table 2.

The concepts related to treatment strategy in MS depend mainly on disease activity. Initial treatment with highly potential drugs (alemtuzumab, NTZ, cladribine) aims to minimize as much as possible inflammation-related neurodegeneration, preferably already at early stages of MS (Cree et al., 2019; Giovannoni, 2018). However, the highly potential drugs expose patients to drug-related risks and serious adverse effects, such as PML. Another, and more common approach, recommends the initiation of treatment with safe, moderately effective drugs (IFN, glatiramer

acetate, teriflunomide, dimethyl fumarate) and escalation to more potential treatments, when necessary (Comi et al., 2017).

Table 2. Current therapies for relapsing-remitting MS

Therapy	Mechanism	Efficacy	Possible adverse effects
Interferon-beta 1a and 1b	Anti-inflammatory cytokine, IM	Moderate	Flulike symptoms, increased liver enzymes, injection site reactions
Glatiramer acetate	Shifting immunoresponse from Th1 to Th2, IM	Moderate	Injection site reactions, flushing, lipoatrophy
Teriflunomide	Inhibition of T- and B-cells proliferation, IM	Moderate	Hair thinning, diarrhoea
Dimethyl fumarate	Shifting immunoresponse from Th1 to Th2, antioxidant response, IS	Moderate/high	Flushing, gastrointestinal reactions, lymphopenia, PML
Fingolimod	Retention of lymphocytes in the lymph nodes, IS	High	Bradycardia, lymphopenia, herpes encephalitis, macular oedema, PML
Cladribine	Inhibition of DNA synthesis, T- and B-cell depletion, IS	High	Lymphopaenia, herpes infections
Natalizumab	Anti-VLA4 mAb, blocking entry of leukocytes into CNS, IS	Very high	Infusion reactions, herpes infections, meningitis, PML
Alemtuzumab	Anti-CD52 mAb, prolonged T- and B-cell depletion, IS	Very high	Secondary autoimmunity, opportunistic infections
Mitoxantrone	Inhibition of topoisomerase results in severe immunosuppression	Very high	Nausea, alopecia, cardiomyopathy, leukaemia
Ocrelizumab	Anti-CD20 mAb, B-cell depletion, IS	Very high	Infusion reactions, herpes infections, malignancies

mAb, monoclonal antibody; IM, immunomodulation; IS, immunosuppressive effect; PML, progressive multifocal leukoencephalopathy; VLA4, very late antigene 4; Th T helper lymphocyte; Based on Finnish Neurological Society, 2019; Giovannoni, 2018; McNamara et al., 2017; Pardo & Jones, 2017.

2.2 Magnetic resonance imaging in MS

2.2.1 Conventional MRI

2.2.1.1 MRI findings in MS

Conventional MRI, including T1-weighted images with and without Gd, T2-weighted images including fluid attenuated inversion recovery (FLAIR) sequence, is a sensitive paraclinical tool for the detection of brain and spinal cord lesions suggestive of MS. However, MRI findings are not specific to MS (Absinta et al., 2012). Typical MS lesions are hyperintense on T2-weighted images, ovoid in shape and orientated around a central vein (known as "central vein" sign seen on susceptibility weighted imaging SWI). MS lesions are typically located juxtacortically, infratentorially, and periventricularly, including the corpus callosum (Solomon et al., 2015; Thompson et al., 2018). T2 lesions are pathologically nonspecific and represent acute inflammation, oedema, gliosis and axonal loss (Filippi et al., 2019; Markovic-Plese & McFarland, 2001). The majority of new T2 lesions decrease in size within a few months after their development due to the resorption of oedema and subsequent degeneration and repair processes (Rovira et al., 2013).

On non-contrast T1-weighted images, the majority of focal white matter MS lesions are isointense to white matter. A subgroup of T1 lesions (14%-41%) is hypointense compared to white matter, i.e., black holes. Approximately 20%-60% of newly formed black holes persist as hypointense on follow-up scans, and these anomalies represent lesions with significant demyelination and axonal loss (Bitsch et al., 2001; Sahraian et al., 2010; Walderveen et al., 1998). Gd-enhancing lesions on postcontrast T1-weighted images, i.e., acute lesions predominantly encountered at early stages of MS, indicate the disruption of the blood-brain barrier and active inflammation (Grossman et al., 1986; Hochmeister et al., 2006; Saade et al., 2018). In progressive MS, the hypointense rim observed in some focal lesions on 3T and 7T susceptibility images depicts chronic lesions with ongoing inflammatory activity, i.e., chronic active lesions (Absinta et al., 2016; Absinta et al., 2018; Kilsdonk et al., 2014). Leptomeningeal enhancement observed on postcontrast FLAIR images in 3T and 7T MRI (Harrison et al., 2017; Zivadinov et al., 2017) represents meningeal

inflammation and seems to be associated with the formation of cortical lesions and cortical atrophy in progressive MS (Absinta et al., 2015; Makshakov et al., 2017; Zurawski et al., 2017).

The brain atrophy rate, which indicates neurodegeneration and irreversible damage of brain tissue, is higher in patients with MS than in healthy controls, with 0.5-1.3% per year and 0.2-0.5% per year, respectively (Fjell et al., 2009; Vagberg et al., 2013). Brain atrophy already occurs in CIS (Dalton et al., 2002) and early RRMS (De Stefano et al., 2010; Fisher et al., 2008; Zivadinov et al., 2001). Moreover, grey matter atrophy, particularly in the thalamus, is more pronounced than white matter atrophy at the early stages of disease (Henry, R. G., Shieh, Okuda, Evangelista, Gorno-Tempini, & Pelletier, 2008b). There is no evident difference in the progression of brain atrophy between MS subtypes (De Stefano et al., 2010); however, the greater severity of brain (Lin, X. & Blumhardt, 2001; Pagani et al., 2005; Tedeschi et al., 2005) and spinal cord atrophy (Lin, X., Tench, Turner, Blumhardt, & Constantinescu, 2003) has been observed in SPMS than in RRMS. Brain atrophy is unspecific to underlying pathology, as this effect results from demyelination and axonal loss within plaques and normal-appearing brain tissue (NABT) (Chard et al., 2003; Kalkers, Vrenken, Uitdehaag, Polman, & Barkhof, 2002; Siffrin, Vogt, Radbruch, Nitsch, & Zipp, 2010). The underlying cause of deep grey matter atrophy seems to differ between MS phenotypes. Recent studies have shown that deep grey matter atrophy results from white matter lesions in RRMS, while in progressive MS mainly results from local microstructural damage (Pontillo et al., 2019).

There is no clear imaging distinction regarding brain MRI characteristics between patients with relapsing and progressive MS (Lublin et al., 2014); patients with RRMS and SPMS may share similar brain MRI characteristics with regard to cerebral T2 lesion volume and atrophy (Tauhid et al., 2014). Recently, it was shown that T2-weighted images without use of Gd-enhanced T1 sequences are sensitive enough to detect radiological activity in routine MRI follow-up in patients with MS (Eichinger et al., 2019). It is important as Gd retention in the dentate nucleus and globus pallidus has been observed and it was related to multiple administration of Gd-based contrast agents (Forslin et al., 2019).

2.2.1.2 MRI as a prognostic tool in MS

In MS, the correlations between clinical course and characteristics derived from conventional MRI are less evident than those in CIS. However, measures from conventional MRI obtained early in the clinical course of MS can add valuable prognostic information on clinical status (Rahn et al., 2019; Tintore et al., 2015).

In CIS, there is evidence that T2 lesion volume and lesion number, early increase in T2 lesion volume (Fisniku et al., 2008; Kuhle et al., 2015; Swanton et al., 2010; Tintore et al., 2015), infratentorial T2 lesions (Giorgio et al., 2013; Tintore et al., 2010) and non-enhancing T1 lesions (Mitjana et al., 2014) are associated with a higher risk of conversion from CIS to MS. Moreover, the number of T2 lesions and infratentorial focal lesions have been linked to long-term disability as measured by EDSS (Fisniku et al., 2008; Minneboo et al., 2004). A high rate of increase in T2 lesion volume has also been associated with future progression from CIS to SPMS (Fisniku et al., 2008).

In CIS, a higher degree of global brain atrophy after the first clinical presentation (Dalton et al., 2012; Filippo et al., 2010; Perez-Miralles et al., 2013), atrophy of the superior gyrus, thalamus, cerebellum (Calabrese, Rinaldi, Mattisi et al., 2011), and callosal atrophy (Kalincik et al., 2012; Odenthal et al., 2017) have been associated with early conversion to MS.

In MS, increased volume of brain T2 lesions (Barkhof, 2002; Mesaros et al., 2008; Mostert et al., 2010) and higher activity of new T2 lesion formation (Bermel et al., 2013) are only modestly correlated with disability progression. Additionally, lesion location is important in predicting disability progression, as supratentorial and periventricular brain lesions are associated with the increased accumulation of disability (Altermatt et al., 2018; Kincses et al., 2011; Vellinga et al., 2009). T1 brain lesion volume correlates better with clinical disability (expressed by EDSS) than T2 lesions (Caramanos et al., 2012; Giorgio et al., 2014; Lukas et al., 2013). The number of Gd-enhancing T1 lesions correlates only modestly with clinical disability accumulation (Kappos et al., 1999).

With regard to brain atrophy, volume loss of deep grey matter is mainly responsible for disability progression in MS (A. Eshaghi et al., 2018; Fisher et al., 2008; Roosendaal et al., 2011; Stefano et al., 2010). Higher amounts of whole brain atrophy (Khaleeli et al., 2008; Radue et al., 2015), grey matter atrophy (Filippi et al., 2013; Jacobsen et al., 2014) and cervical spinal cord atrophy (Lukas et al., 2015) were variably correlated with long-term physical disability progression in MS (RRMS and

PPMS). Moreover, cognitive impairment was associated with cortical atrophy in MS (Calabrese, Poretto et al., 2012).

The simultaneous use of multiple MRI markers derived from different MRI techniques (composite MRI Scores) improved the correlation between radiological findings and EDSS in MS (Poonawalla et al., 2010). In CIS, the risk stratification of the conversion of CIS to MS is improved when both MRI (MRI criteria for MS, number of T1 lesions) and clinical biomarkers (age at onset, presence of oligoclonal bands) are taken into consideration (Martinelli et al., 2017).

2.2.1.3 MRI in the assessment of treatment response in MS

MRI is more sensitive in the detection of disease activity than clinical evaluation, as the magnitude of MRI lesions is higher than clinical activity assessed by the number of relapses, i.e., clinically silent MRI lesions (Harris et al., 1991; Isaac et al., 1988). The MRI outcome measures used in the assessment of disease activity can be divided into markers of inflammation (Gd-enhancing T1 lesions, new or enlarging T2 lesions, i.e., active lesions) and markers of degeneration (brain atrophy and evolution of "black hole" T1 lesions).

Gd-enhancing T1 and active T2 lesions occurring after treatment initiation have been widely used in clinical trials to monitor the efficacy of immunomodulatory treatment in MS. MRI brain lesion load in treated MS patients was associated with future occurrence of relapses, and to a lesser extent, with worsening of disability at the group level (Sormani et al., 2009; Sormani et al., 2010). In RRMS patients on IFN therapy, Gd-enhancing lesions and new T2 lesions have been related to short-term and long-term disability progression (Bermel et al., 2013; Prosperini et al., 2009; Rio et al., 2009). Additionally, a decreased rate of conversion of Gd-enhancing T1 lesions to black holes was observed in IFN- and NTZ-treated patients (Bagnato et al., 2005; Dalton et al., 2004; Nagtegaal et al., 2014; Zivadinov et al., 2007), indicating a positive effect of treatment on MS-related neurodegeneration.

Reduced progression of whole brain and deep grey matter atrophy in MS patients receiving immunomodulatory treatment was observed in some clinical trials (Comi et al., 2013; Filippi et al., 2014; Miller, D. H. et al., 2007; Rudick et al., 1999). However, atrophy measurements in individual patients are not yet recommended mainly due to the inconsistent results of previous studies and the effect of confounding factors on brain atrophy (Azevedo & Pelletier, 2016; Stefano et al., 2014; Wattjes et al., 2015).

The value of pre-treatment MRI in the prediction of subsequent treatment response is limited in MS (Leocani et al., 2016). There is some evidence from previous studies in IFN-treated RRMS suggesting that higher T2 lesion load and the presence of Gd-enhancing lesions before treatment initiation (Barkhof et al., 2003; Kappos, Polman et al., 2006; Romeo et al., 2013; Tomassini et al., 2006) along with clinical measures such as older age, longer disease duration (Villoslada et al., 2004), higher pre-treatment relapse rate (Waubant et al., 2003) and higher baseline EDSS (Rio et al., 2006) are related to poorer response to MS therapy.

2.2.1.4 MRI and pharmacovigilance in MS

Radiological pharmacovigilance in MS includes the detection of paradoxical reactions, such as tumefactive demyelination (i.e., tumour-like lesions) occurring in patients treated with fingolimod (Visser et al., 2012), comorbidities (vascular, neoplastic) (Capkun et al., 2015; P. Tettey et al., 2014) and opportunistic infections (e.g., varicella zoster in patients treated with fingolimod (Kappos et al., 2014). PML, another opportunistic infection related to immunomodulatory treatment, has been observed in MS patients receiving NTZ (Linda et al., 2009), dimethyl fumarate (Linker & Haghikia, 2016) and fingolimod (Berger et al., 2018; Khatri, 2016). As the early detection of PML in the presymptomatic stage improves survival (Dong-Si et al., 2014; Dong-Si et al., 2015; Hoepner et al., 2017), regular MRI monitoring of patients receiving immunomodulatory and immunosuppressive drugs is of great importance. The frequency of screening MRI in NTZ-treated patients is based on clinical and serological risk stratification and varies from every 3-4 months in patients with high risk to every 1 year in patients with low risk (Wattjes et al., 2015).

2.2.2 Nonconventional MRI

2.2.2.1 Diffusion weighted and diffusion tensor imaging

Nonconventional MRI is represented by several quantitative MRI techniques, such as magnetization transfer imaging (MTI) and DTI. These imaging techniques reveal

abnormalities in the brain regions that appear normal on conventional MRI. Moreover, imaging characteristics derived from nonconventional MRI provide more specific correlation with underlying histopathology than findings revealed by conventional MRI (Enzinger et al., 2015).

Diffusion weighted imaging (DWI) is commonly used in clinical practice to demonstrate abnormal restriction of water diffusion in clinical conditions, such as cerebral ischaemia, cerebral abscess and neoplasms (Drake-Perez et al., 2018). In DWI, there is an assumption that water diffusivity is similar in every direction, i.e., isotropic. A degree of reduced diffusion in biological tissue is averaged to a single value called the apparent diffusion coefficient (ADC) (Alexander et al., 2007).

In contrast to DWI, DTI characterizes water diffusion in three-dimensional (3D) space. DTI allows measurements of the magnitude (as in DWI) and directionality of water diffusion (Mukherjee et al., 2008).

Brain white matter is highly anisotropic tissue due to axonal fibres. To describe the diffusivity of water molecules in 3D anisotropic tissue, the mathematical concept of tensor is used (Bihan et al., 2001). Tensor describes directionality (eigenvectors) and magnitude (described by eigenvalues) of diffusivities in three principal orthogonal axes (eigenvectors 1, 2, and 3). The largest eigenvector reflects the main direction of diffusivity and is called longitudinal or axial diffusivity (AD). The average of the two medium and minor eigenvectors that are perpendicular to axial diffusivity is called radial diffusivity (RD). The average value of all three principal eigenvalues is called mean diffusivity (MD) and describes the magnitude of water diffusivity in a voxel or anatomical region. Another measure calculated from eigenvalues is fractional anisotropy (FA), which reflects the degree of directionality along the axonal fibres. The values of FA vary between 0 and 1, indicating completely isotropic diffusion as in the CSF and completely anisotropic diffusion as in axonal fibres, respectively (Pierpaoli et al., 1996).

2.2.2.2 Methods for the quantitative analysis of DTI

Commonly used methods for brain DTI analysis are region of interest (ROI), voxel-wise, histogram, and tractography-based approaches.

ROI analysis is based on the manual delineation of the area in the brain structure of interest, usually on the non-diffusion-weighted b0 or FA maps, and then automatically transferred to the diffusion maps. The ROI can be geometrical in shape, e.g., circle, or can be defined according to the shape of the analysed structure,

i.e., freehand ROI with the latter method being less susceptible to partial volume effects (Snook et al., 2005). The ROI-based method is sensitive to small changes of parameters in the selected region and is suitable for investigating diffusivity indices in well-defined anatomical structures, such as the internal capsule (IC) and the CC (Snook et al., 2007). This approach is operator-dependent and thus susceptible to variability of intra- and inter-observer measurements (Ozturk et al., 2008). The highest agreement for repeatability measurements was observed in the CC and IC and lowest for corona radiata and centrum semiovale (Brander et al., 2010).

In contrast, histogram analysis is a fully automated method that allows extraction of DTI indices from the whole brain or separately for the whole white and grey matter. In this method, regional differences in diffusion are not obtained. Moreover, histogram variables are sensitive to partial volume effects related to brain atrophy (Heidi Johansen-Berg & Timothy E.J. Behrens, 2009).

The voxel-based method (VBM) allows regionally specific analysis of diffusivity in the whole brain in a fully automated way (Snook et al., 2007). This approach includes the registration of all diffusion maps for one subject onto a common normalized skeleton, followed by voxel-by-voxel comparisons between the studied groups (Ashburner & Friston, 2000). The limitations of VBM are related to the accuracy of alignment during registration and the number of smoothing images, which may cause post-processing calculation errors (Ridgway et al., 2008). The newer voxel-based method, Tract-Based Spatial Statistics (TBSS), partially overcomes these problems by using nonlinear registration (Smith et al., 2006).

Tractography allows the tracking of white matter fibres according to the dominant diffusion tensor of each voxels (Chung et al., 2011; Mukherjee et al., 2008).

2.2.2.3 Applications of DTI in clinical setting: correlations with histopathology and clinical observations

FA and MD are modulated by demyelination and axonal loss, as shown in studies that correlate MRI findings with histopathology (Mottershead et al., 2003; Schmierer, Wheeler-Kingshott et al., 2007). However, none of these parameters allow clear discrimination between axonal and myelin damage (Song et al., 2003; Tyszka et al., 2006). In mouse experimental models, increased RD has been consistently related to demyelination (Song et al., 2002; Song et al., 2005; Sun et al., 2006). Decreased AD has usually been associated with axonopathy (Song et al., 2003; Sun et al., 2006), but this correlation has not been clear in every study (Kronlage et al., 2017; Song et al.,

2002; Xie et al., 2010). Mathematical modelling revealed that a low signal-to-noise ratio, the presence of crossing fibres in brain tissue, and anisotropy caused by pathology may confound radiological-pathological correlations and lead to the misinterpretation of the results (Wheeler-Kingshott & Cercignani, 2009; Wheeler-Kingshott et al., 2012).

Increased MD and decreased FA have been typically observed in focal MS lesions (Bammer et al., 2000; Filippi et al., 2000), NAWM (Cercignani et al., 2001; Filippi et al., 2001; Guo et al., 2001), cortical and deep NAGM (Oreja-Guevara et al., 2005) in the brain tissue of patients with MS. The formulation of new different types of focal lesions is preceded by diffusivity abnormalities (Fox et al., 2011; Naismith et al., 2010; Werring et al., 2000). Diffusivity changes are already observed in CIS and become more evident in patients with MS (Braley et al., 2012; Preziosa et al., 2011; Roosendaal et al., 2009). DTI differentiates MS at early stages from healthy subjects (Henry, R. G. et al., 2009; Rashid et al., 2008; Raz et al., 2010a); however, such discrimination was not always obvious (Pulizzi et al., 2007).

DTI has been widely used in studies investigating correlations between diffusivity metrics and clinical aspects in various neurological disorders, such as amyotrophic lateral sclerosis, Parkinson's disease, Alzheimer's dementia, epilepsy, and traumatic brain injury (Tae et al., 2018). In MS, correlations between DTI metrics from the NABT and clinical manifestations, such as cognitive (Bodini et al., 2013; Llufriu et al., 2012; Schoonheim et al., 2015) and physical (Lin et al., 2007; Pokryszko-Dragan et al., 2018; Tortorella et al., 2014) impairment, have been observed. In CIS, diffusivity abnormalities were associated with disease severity and activity in some studies (Bester et al., 2008; Caramia et al., 2002; Pagani et al., 2005) but not in others (Gallo et al., 2005; Raz et al., 2010b; Rovaris et al., 2008; Vishwas et al., 2013). Changes in DTI metrics have also been related to immunomodulatory therapies (Fox et al., 2011; Zivadinov, Hagemeier et al., 2018; Zivadinov, Bergsland et al., 2018) and rehabilitation (Ibrahim et al., 2011) in MS patients, indicating the potential role of DTI in monitoring treatment effects. Additionally, more severe abnormalities of diffusivity markers were observed in MS when compared to acute disseminated encephalomyelitis and NMO (Kim et al., 2017; Tillema et al., 2012), indicating the potential of DTI in the diagnostic process.

2.2.3 Future perspectives of MRI in MS

2.2.3.1 MRI in the diagnosis and differential diagnosis of MS

Cortical demyelinating lesions can be detected by MRI in CIS and MS using DIR (Calabrese et al., 2007; Filippi et al., 2010) and PSIR (Favaretto et al., 2015) sequences. Since cortical lesions are not observed in other diseases resembling MS, such as NMO (Calabrese, Oh et al., 2012) and migraine (Absinta et al., 2012), their detection may be helpful in differential diagnosis. Moreover, a high proportion of white matter lesions with a central vein known as central vein sign identified on SWI at 3T and 7T is characteristic of MS and may differentiate MS from other imaging mimickers of MS, such as brain microangiopathy (Mistry et al., 2016), inflammatory vasculopathies (Maggi et al., 2018), Susac syndrome (Wuerfel et al., 2012), and migraine (Solomon et al., 2015). Therefore, cortical lesions and central vein sign may be useful in the diagnostic work-up of MS.

Cortical lesions have also been associated with a higher rate of conversion of CIS to MS (Filippi et al., 2018; Preziosa et al., 2018), faster progression of physical disability (Calabrese et al., 2012) and increased cognitive impairment (Calabrese, Rinaldi, Grossi et al., 2011; Muhlert et al., 2015; Nelson et al., 2011), making these anomalies useful in the assessment of the clinical course of MS.

The detection of cortical lesions and central vein sign has not yet been widely implemented in clinical practice due to a lack of standardized MRI protocol and unified criteria (Filippi et al., 2016; Sati et al., 2016).

2.2.3.2 MRI in disease monitoring

Advanced MRI techniques, such as positron emission tomography (PET)-MRI and MTI, are more specific to underlying MS-related pathophysiology than conventional MRI. These techniques may play a potential role in monitoring disease course and treatment outcome.

Increased microglial activation detected by PET-MRI has been observed in CIS and MS (Airas et al., 2017) and was related to the conversion of CIS to MS (Giannetti et al., 2015) and higher disability in MS (Rissanen et al., 2018).

The quantification of myelin content using MTI can be a marker of demyelination and remyelination (Schmierer, Tozer et al., 2007), and deteriorated MTI-derived parameters have been associated with cognitive impairment in long-term follow-up

studies (Deloire et al., 2011; Filippi et al., 2013). MTI-derived metrics indicating remyelination rate have been also proposed as an outcome measure in clinical trials (van den Elskamp et al., 2010).

2.2.3.3 MRI techniques in monitoring treatment effects

The development of more potent drugs in MS has caused the evolution of treatment goals in MS therapies and emphasized the role of MRI in treatment monitoring. No evidence of disease activity (NEDA) composite has been proposed as a new therapeutic goal in MS trials (Havrdova et al., 2009; Kappos et al., 2017). According to NEDA, evaluation of the treatment effect is based on a combined assessment of clinical activity (relapses, increased disability expresses by EDSS change) and radiological activity (new and/or enlarging T2 lesions or enhancing lesions) (Giovannoni et al., 2011). Brain atrophy measurement, which expresses neurodegeneration, has been recently added into NEDA as a new tool in monitoring treatment effects (Kappos et al., 2016).

2.3 JC virus

2.3.1 Epidemiology and biology of JCV

JCV is a human polyomavirus that was isolated for the first time in 1971 (Padgett et al., 1971). Approximately 50 -70% of immunocompetent adults are asymptomatically infected with JCV as detected by serum IgG antibodies against virus (Egli et al., 2009; Knowles et al., 2003; Stolt et al., 2003; Verbeeck et al., 2008). Primary infection with JCV occurs early in childhood (Kean et al., 2009; Stolt et al., 2003), and the rate of JCV seroprevalence increases with ageing (Antonsson et al., 2010; Kean et al., 2009; Knowles et al., 2003). JCV is an aetiologic factor of PML and the lytic brain infection of oligodendrocytes in immunocompromised individuals (Ferenczy et al., 2012). Moreover, the JCV infection of neurons is responsible for cerebellar granule cell neuropathy (Henry, C. et al., 2015).

JCV is transmitted from the environment into the blood through the respiratory and digestive tract. After primary infection, the virus persists in the kidneys (Degener et al., 1997; Randhawa et al., 2005), lymphoid tissue including bone marrow (Houff

et al., 1988; Tornatore et al., 1992), and the brain (Tan et al., 2010), resulting in latent infection. Virus crosses the blood-brain barrier as a free virus or inside B cells (Diotti et al., 2013). Rearrangements in the noncoding control region of the viral genome, which are believed to occur in lymphoid cells and bone marrow, are responsible for the transformation of latent virus into a neurovirulent form (Marzocchetti et al., 2008). It was suggested that recombination between JCV and EBV may be responsible for such a transition (Wortman et al., 2016).

2.3.2 JCV and PML

In cases of impaired immunosurveillance, neurovirulent JCV may reactivate and cross the blood-brain barrier, causing PML (Khalili et al., 2007). PML was initially observed in patients with primary immunodeficiency (Day-Williams et al., 2015; Zerbe et al., 2016) and in patients with AIDS (Eng et al., 2006). In AIDS, impaired T lymphocyte-mediated immunity (suppression of CD4+, CD8+) may be responsible for the development of PML in patients infected with JCV (Engsig et al., 2009; Misbah, 2017).

The second large group of patients predisposed to PML are those under monoclonal antibody therapy, including NTZ (Major, 2010). It was suggested that NTZ activates the migration of JCV-infected CD34+ and pre-B cells (Frohman et al., 2014) from the bone marrow to the blood, contributing to PML pathogenesis (Zohren et al., 2008). Natalizumab can also upregulate transcription factor SpiB, which may lead to increased JCV replication (Meira et al., 2016).

2.3.3 Diagnosis of PML and the management of patients at risk of PML

Diagnostic criteria for PML include clinical symptoms and MRI findings suggestive of PML, and the detection of JCV DNA in CSF by polymerase chain reaction (PCR) or brain biopsy (Berger et al., 2013). Clinical manifestations consistent with PML are cognitive impairment, motor deficits, speech problems, and visual defects (Berger, 2011). MRI may show brain lesions in the presymptomatic stage of PML (Langer-Gould et al., 2005; Linda et al., 2009). Typical radiological features in NTZ-associated PML include lesions that are hyperintense on T2-weighted images and DWI, hypointense on T1-weighted images with subcortical distribution involving U-fibres (Hodel et al., 2016; Yousry et al., 2012). Histopathologically, brain lesions in

PML demonstrate demyelination, bizarre astrocytes, and enlarged nuclei of oligodendrocytes (Berger, Aksamit et al., 2013).

The factors increasing the risk of PML in NTZ-treated patients are duration of NTZ treatment longer than two years, seropositivity for anti-JCV antibodies, and immunosuppressive therapy preceding NTZ treatment (Major et al., 2018). For patients having all three factors, the risk of PML is approximately 11/1000, while in patients without any risk factors, the risk is as low as 0.1/1000 (Bloomgren et al., 2012). For comparison, in patients treated with rituximab, the risk of PML is lower occurring approximately 1/30,000 individuals, depending on the treated disease (Amend et al., 2010; Zaheer & Berger, 2012) with case reports published in patients receiving alemtuzumab (Gerevini et al., 2019; Isidoro et al., 2014; Keene et al., 2011), infliximab (Sammut et al., 2016), dimethyl fumarate (Baharnoori et al., 2016), and fingolimod (Gyang et al., 2016).

Anti-JCV antibody screening indices are measured in the serum or plasma with a second-generation ELISA known as STRATIFY JCV ™ DxSelect ™ (Lee et al., 2013). Higher values of anti-JCV screening indices are associated with a higher risk of PML (Lee et al., 2013; Trampe et al., 2012). NTZ-treated patients with no prior immunosuppression in which the anti-JCV antibody screening index is greater than 1.5 have a higher risk of PML when compared to patients with a lower anti-JCV antibody index (Plavina et al., 2014). There are limitations of JCV serology, such as false-negative results of anti-JCV antibody measurements (Berger, Houff et al., 2013; Major et al., 2013) and changes in serostatus over time (i.e., seroconversion to JCV positivity and seroreversion to JCV negativity) (Cambron et al., 2017; Outteryck et al., 2013; Trampe et al., 2012), which complicates the interpretation of JCV status.

Recently, L-selectin (Basnyat et al., 2015; Schwab, Schneider-Hohendorf, Pignolet, Spadaro et al., 2016) and lipid-specific IgM bands (Villar et al., 2015) have been investigated and proposed as new serological biomarkers that may identify patients with a higher risk of NTZ-associated PML.

3 AIMS OF THE STUDY

The general aim of this study was to define the role of conventional MRI and DTI techniques in the prediction of clinical course and disease severity in patients with CIS and MS. Additionally, we aimed to establish the seroprevalence of JCV in Finnish patients with MS. The specific aims were as follows:

1. To determine whether DTI and brain volume measures could play prognostic roles in predicting the conversion of CIS to MS (Study I).

2. To determine whether DTI and brain volume measures could play prognostic roles in the prediction of disability progression in patients with MS (Study II).

3. To assess the anti-JCV antibody seroprevalence and the temporal change of the JCV seroprevalence in patients with MS and evaluate whether demographic factors and MS therapies have effect on the anti-JCV antibody status (Study III).

4 SUBJECTS AND METHODS

4.1 CIS and MS: a clinicoradiological follow-up (Studies I and II)

Twenty patients with CIS (Study I), 46 patients with MS (Study II), and 10 age-matched healthy controls (Studies I and II) were included in the four-year follow-up study at the Tampere University Hospital between December 2006 and September 2012. The clinical characteristics of the subjects in Studies I and II are displayed in Table 3. The diagnosis of MS was based on the 2005 McDonald criteria (Polman et al., 2005). CIS was defined as a first clinical episode suggestive of inflammatory demyelinating disease with no paraclinical evidence of dissemination in time (Miller, D. H. et al., 2012). The Lublin and Reingold criteria were used to evaluate the clinical course of MS (Lublin & Reingold, 1996; Lublin, 2014).

All CIS and MS patients were examined by the same neurologist yearly over the whole follow-up period (five clinical examinations all together) (Studies I and II). In CIS patients, DTI was performed at baseline, at one year, at two years, and at the end of the follow-up (four MRI examinations all together), and MRI volumetry was performed at baseline, at two years, and at the end of the follow-up (Study I). In MS patients, DTI was performed at baseline and at one year of the follow-up, and MRI volumetry at baseline (Study II). The same healthy control group was included in the cross-sectional analysis at baseline in both studies (Studies I and II).

The assessment of conversion of CIS to definite MS over the follow-up was based on clinical or radiological evidence of DIT or DIS (Study I). Clinical disability was evaluated by the EDSS (Kurtzke, 1983) (Study I and II). Progression of disability over the four-year follow-up was defined as an EDSS score increase ≥ 1.0 when the baseline EDSS was < 6.0 or an increase of EDSS ≥ 0.5 when the baseline EDSS ≥ 6 (Wingerchuk et al., 1997) (Study II).

4.2 CIS and MS: a JCV study (Study III)

In total, 503 patients with MS and CIS were included in the study. Patients from a cross-sectional study (n=406) were examined clinically (between January 2012 and February 2013) in four MS centres in Tampere (140 patients), Helsinki (114 patients), Seinäjoki (98 patients), and Turku (54 patients). Ninety-seven patients examined in the Tampere University Hospital were included in the four-year longitudinal study (between December 2006 and September 2012). The diagnosis of MS was based on the 2005 McDonald criteria and on the 2010 McDonald criteria (Polman et al., 2005; Polman et al., 2011). CIS was defined as a first clinical episode suggestive of inflammatory demyelinating disease with no paraclinical evidence of dissemination in time. The clinical characteristics of subjects from Study III are displayed in Table 3. In the longitudinal assessment, four serological measurements were performed yearly over the whole follow-up period.

Overall, 12/67 (18%) CIS patients from Study I and 42/67 (63%) MS patients (30 RRMS, 12 SPMS) from Study II were also included in the longitudinal analysis of Study III (67 patients in longitudinal analysis).

Studies I-III were approved by the Ethics Committee of Tampere University (ethical codes of the studies are R05157 and R11116), and all subjects provided written informed consent.

Table 3. Clinical characteristics of patients and controls at baseline and follow-up

	Patients		Controls
STUDY I (CIS patients)			
No. of subjects	20		10
Gender (M/F)[a]	2/18		5/5
Age (years)[b]	35.3	(21.8 - 52.5)	40 (26 - 61)
Time from first symptoms (years)	4.7	(0.5 - 8.8)	NA
Positive IgG status[a]	7	(35%)	NA
Positive oligoclonal band status[a]	15	(75%)	NA
Onset symptoms[a]			NA
Optic neuritis	14	(70%)	
Brainstem	2	(10%)	
Spinal cord	3	(15%)	
Brainstem and spinal cord	1	(5%)	
Conversion to MS over follow-up[a]	11	(55%)	NA
No. of relapses over follow-up (0 / 1)[a]	14/6		NA
EDSS at baseline[c]	0	(0 - 1)	NA
EDSS at the end of follow-up[c]	0	(0 - 1.5)	NA
STUDY II (MS patients)			
No. of subjects	46		10
Gender (M/F)[a]	15 / 31		5/5
Age (years)[b]	39.6	(18 - 61)	40 (26 - 61)
Clinical phenotype RRMS / SPMS[a]	33 / 13		NA
Disease duration (years)[c]	4.2	(0 - 31.2)	NA
No. of relapses 3 years before baseline (0 / 1-2 / 3-5)[a]	15 / 24 / 7		NA
No. of relapses over follow-up (0 / 1-2 / 3-6)[a]	24 / 12 / 10		NA
EDSS at baseline[c]	2	(0 - 7)	NA
EDSS at the end of follow-up[c]	2	(0 - 8)	NA
Treatment at baseline[a]	18	(39%)	NA
Duration of treatment (months)[c]	18.5	(1 - 122)	NA
STUDY III (JCV)			
Cross-sectional study			
No. of patients	406		-
Clinical phenotype CIS / RRMS / SPMS / PPMS[a]	9 / 350 / 39 / 8		-
Gender (M/F)[a]	101 / 305		-
Age (years)[b]	40.9	(19.3 - 71.7)	-
MS duration (years)[c]	6.9	(0 - 34.2)	-
EDSS[c]	2	(0 - 7)	-
No. of relapses 2 years before baseline (0 / 1 / 2-5)[a]	235 / 101 / 70		-
Prior and current natalizumab therapy[a]	92	(23%)	-
Duration of natalizumab therapy (months)[c]	25.5	(1 - 69)	-
Prior and current immunomodulator therapy[a]	348	(86%)	-
Prior and current immunosuppressant tharapy[a]	35	(9%)	-
Longitudinal study			
No. of patients	67		-

Clinical phenotype CIS / RRMS / SPMS / PPMS[a]	12 / 29 / 12 / 14		-
Gender (M/F)[a]	22 / 45		-
Age (years)[b]	41.9	(18 - 67)	-
MS duration (years)[c]	4.4	(0 - 31.2)	-
EDSS at baseline[c]	2	(0 - 7)	-
No. of relapses 2 years before baseline (0 / 1 / 2-5)[a]	16 / 21 / 16		-
Prior and current natalizumab therapy[a]	0		-
Duration of natalizumab therapy (months)[c]	NA		-
Prior and current immunomodulator therapy[a]	22	(33%)	-
Prior and current immunosuppressant therapy[a]	3	(4%)	-

[a] Number of subjects; [b] Mean (range); [c] Median (range); NA not applicable; EDSS expanded disability status scale; CIS clinically isolated syndrome, RRMS relapsing-remitting MS; SPMS secondary progressive MS; PPMS primary progressive MS; In Study III (longitudinal analysis), serological changes over the whole follow-up were obtained for 67 patients.

4.3 Magnetic resonance imaging (Studies I and II)

4.3.1 MRI protocol

Whole brain imaging was performed on a 1.5 T magnetic resonance scanner (Magnetom Avanto SQ, Siemens Medical Solutions, Erlangen, Germany). The MRI acquisition protocol was as follows: T1-weighted header followed by an axial 3D T1-weighted magnetisation prepared rapid gradient echo (MPRAGE), 3D T2-weighted turbo spin-echo, FLAIR, T1-weighted spin echo with magnetisation transfer contrasts, multi-directional diffusion-weighted echo planar imaging, and Gd-enhanced T1-weighted MPRAGE. Gadolinium dose was based on body weight. The DTI protocol consisted of a single-shot spin-echo-based echo-planar diffusion-weighted imaging with 3 averages, and 12 gradient encoding directions, with b values of 0 and 1000 s/mm^2.

For T1 MPRAGE, the imaging parameters were as follows: repetition time (TR) = 1160 ms; echo time (TE) = 4.2 ms; inversion time (TI) = 600 ms; slice thickness = 0.9 mm; interslice gap = 0 mm; matrix 256*256, in-plane resolution 0.45*0.45 mm. For FLAIR, the following parameters were used: TR = 8500 ms; TE = 100 ms; TI = 2500 ms; slice thickness = 5 mm; interslice gap = 0 mm; matrix 256*256; in-plane resolution 0.45*0.45 mm. Scanning parameters for T2-weighted sequence: TR = 750 ms; TE = 115 ms; slice thickness = 3 mm; in-plane resolution = 0.9*0.9 mm. DTI was performed with the following scanning parameters: TR = 3500 ms; TE = 96 ms;

slice thickness = 5 mm; interslice gap = 1.5 mm; matrix = 128*128 mm; in-plane resolution = 1.8*1.8 mm.

4.3.2 Volumetric analysis

Segmentation and volumetric measurements were performed using semiautomated Anatomatic software operating in a PC Windows environment (Heinonen et al., 1998). In brief, segmented region was defined at every slice containing ROI using appropriate threshold coefficient. Volumes of segmented images were presented automatically as voxels. Conversion to volumes was done by multiplying the results by voxel dimensions in the original MR images. The total brain volume of hypointense T1 and hyperintense FLAIR lesions from the grey and white matter was assessed. T1 lesions were defined as lesions with an intensity lower than that of NAWM, and both T1 Gd-enhancing and non-enhancing lesions were included (Filippi et al., 2011). T2 images were used to increase the confidence in lesion identification especially in the infratentorial brain. Relative brain atrophy was determined by brain parenchymal fraction (BPF), which was defined as the ratio of brain parenchymal volume to the total volume within the brain surface contour on the T1 images (Rudick et al., 1999).

4.3.3 DTI postprocessing

The DTI data were analysed with commercial Neuro 3D software (Siemens Healthcare, PA, USA) on an offline workstation. Multidirectional diffusion data were assessed visually for distortions and artefacts. Circular ROIs (Study I) and freehand ROIs (Study II) were manually placed on MD, FA, and non-diffusion-weighted b_0 maps. The ROIs were placed at the following anatomical regions: the cerebral peduncle, corona radiata posterior and anterior, centrum semiovale, thalamus, head of the caudate nucleus bilaterally (Study I), genu and splenium of the CC, IC (study I and II), and corpus of the CC bilaterally (Study II). For cases in which demyelinating plaque was defined in pre-defined ROI, the ROI was shifted to the closest area surrounding the pre-defined ROI. FA, MD (Study I and II), RD, and AD indices (Study II) were included in the DTI analysis.

4.4 Assessment of anti-JCV antibodies (Study III)

A confirmatory second-generation ELISA (STRATIFY JCV ™ DxSelect) was used to test the sera for anti-JCV antibodies at the Unilabs, Denmark. An anti-JCV screening index value of less than 0.2 was considered anti-JCV antibody negative, whereas a screening index value greater than 0.4 was considered anti-JCV antibody positive. The samples with a screening index between 0.2 and 0.4 were evaluated with a confirmation test and results greater than 45% were classified as anti-JCV antibody positive (Lee et al., 2013).

4.5 Statistical analysis (Studies I – III)

Statistical analyses were performed using SPSS Statistics version 20.0 and 22.0 for Windows. Baseline comparisons for radiological (Studies I and II), serological (Study III) and clinical parameters (Studies I – III) between two unpaired groups were performed using t tests for normally distributed data and the Mann-Whitney U test for skewed distributed data. A baseline comparison for anti-JCV antibody seropositivity in different age groups was calculated using one-way ANOVA (Study III). Comparisons between groups for categorical variables such as gender and disease types were calculated using the chi-square test (Studies I – III). Spearman's correlation coefficient was used to analyse the associations between clinical and MRI variables, and p-value < 0.05 was considered statistically significant (Study II). The Wilcoxon test was used for paired DTI (Study II) and anti-JCV screening indices (Study III) comparisons. Associations between baseline MRI markers and conversion to MS over four years were analysed using Cox regression analysis (Study I). Logistic regression models with multiple covariates were used to estimate associations between DTI metrics, volumetric measures, and disability progression over four years (Study II). Logistic regression adjusted for age and sex was used to assess associations between several clinical factors, including MS treatment and JCV seroprevalence (Study III). P-value < 0.05 was considered statistically significant (Studies I – III). In Study II, the Bonferroni-corrected p-value for six comparisons was also calculated in the analysis concerning DTI.

5 RESULTS

5.1 MRI in CIS and MS (Studies I and II)

5.1.1 Clinical activity of the study populations

In Study I, 11 out of 20 (55%) CIS patients converted to MS over approximately four years. DIT and DIS were confirmed clinically (i.e., second relapse) in four patients and radiologically (i.e., new or enlarging T2 lesions) in seven patients.

In Study II, 22 out of 46 (48%) MS patients presented an increase in EDSS scores over the four-year follow-up, confirming the progression of disability (median 3.0 (range 0 – 7) at baseline and 6.0 (1 – 8) at 4 years). Over the follow-up period, relapses were observed in 22 out of 46 (48%) MS patients (in 12 out of 24 (50%) whose EDSS was stable and in 10 out of 22 (45%) MS patients whose EDSS increased over the follow-up period.

5.1.2 Predictive value of brain volumetry in the assessment of disease activity in CIS and MS

In Study I, T1 and FLAIR brain lesion volumes were assessed at baseline, at two years, and at four years of follow-up (Table 4). The higher T1 and FLAIR lesion volumes at baseline were associated with the conversion of CIS to MS over four years (for T1 lesion volume hazard ratio 1.99, p=0.021 and for FLAIR lesion volume hazard ratio 1.24, p=0.015). The changes of volumetric values over the follow-up were unspecific.

Table 4. T1 and FLAIR brain lesion volume (mm^3) in CIS patients over the follow-up; median (range)

	Baseline				Year 2				Year 4				p-value
	Non-converting	n=9	Converting	n=11	Non-converting	n=9	Converting	n=10	Non-converting	n=6	Converting	n=10	Nonconverting vs converting
T1 lesion load	13	(0-186)	506	(125-1329)[a]	41	(2-41)	603	(123-1967)	45	(3-274)	613	(84-2011)	0.4
FLAIR lesion load	499	(3-1006)	1879	(379-8555)[b]	979	(134-1301)	3240	(1259-6271)	323	(39-921)	1658	(626-3914)	0.146

p-value for group-by-time interaction term from linear mixed-effect model

[a] Comparison between T1 lesion load in the nonconverting vs. converting group at baseline, p value=0.012, Mann-Whitney test

[b] Comparison between FLAIR lesion load in the nonconverting vs. converting group at baseline, p value=0.017, Mann-Whitney test. Reprinted with permission from Kolasa et al., 2015.

In Study II, the volume of demyelinated plaques on T1-weighted and FLAIR images and BPF were analysed at baseline in MS patients (Table 5). Only the FLAIR lesion volume was significantly higher ($p=0.03$) in a group of MS patients with disability progression (i.e., progression group) when compared to the stable group. Regression analysis did not reveal a clear association between baseline volumetric measurements and EDSS increase over four years (Table 5).

Table 5. T1 and FLAIR brain lesion volumes and brain parenchymal fraction at baseline in MS patients

	Stable group n=23		Progression group n=19		p value	Odds ratio	95%CI	
	median	range	median	range				
T1 lesion load (cm^3)	1.0	(0.1 - 28.5)	2.2	(0.1 - 14.7)	0.9	1.01	0.9	1.1
FLAIR lesion load (cm^3)	2.8	(1 - 39)	8.2	(1 - 33)	0.4	1.03	0.96	1.1
Brain parenchymal fraction	0.73	(0.64 - 0.8)	0.68	(0.6 - 0.81)	0.1	0.0	0.0	28.3

p value for logistic regression adjusted for age and duration of symptoms for prediction of EDSS progression over 4 years; CI confidence interval. Reprinted and modified with permission from Kolasa et al., 2019.

5.1.3 DTI findings in CIS and MS: cross-sectional and longitudinal analysis

In the CIS group (Study I) that converted to MS, baseline MD was significantly higher than that in controls in the right IC (0.71 ± 0.03 vs 0.68 ± 0.01, respectively, $p<0.05/3$), left corona radiata anterior (0.69 ± 0.04 vs 0.65 ± 0.002, $p<0.05/3$), and right centrum semiovale (0.71 ± 0.05 vs 0.67 ± 0.03, $p<0.05/3$). Additionally, in the converted CIS group, baseline FA was lower than that in the controls in the right cerebral peduncle (0.76 ± 0.05 vs 0.82 ± 0.03, respectively, $p<0.05/3$) and the left caudate nucleus (0.18 ± 0.04 vs 0.24 ± 0.03, $p<0.05/3$).

In the stable CIS group, baseline MD was higher than that in the controls in the right (0.73 ± 0.03 vs 0.68 ± 0.01, $p<0.05/3$) and left IC (0.73 ± 0.05 vs 0.67 ± 0.02, $p<0.05/3$), right corona radiata anterior (0.69 ± 0.05 vs 0.64 ± 0.02, $p<0.05/3$), and right centrum semiovale (0.71 ± 0.03 vs 0.67 ± 0.03, $p<0.05/3$). Moreover, FA was lower in the right cerebral peduncle (0.74 ± 0.03 vs 0.82 ± 0.03, $p<0.05/3$). There were no significant differences regarding baseline DTI between the converting and stable CIS groups.

In Study I, annual rates of changes in regional DTI values were determined in converted CIS patients and in stable CIS patients (Table 6). In the converting group, a significant decrease in FA was observed in 3/9 brain regions (the corona radiata anterior and posterior, thalamus) and a significant increase in MD in 4/9 brain regions (the corona radiata anterior and posterior, centrum semiovale, splenium of the CC). In the stable CIS group, an increase in FA was observed only in the cerebral peduncle and an increase in MD was observed in the centrum semiovale.

Table 6. Percent of annual FA and MD change with relation to baseline in nonconverting and converting group of CIS patients over follow-up calculated from estimates of the group-wise linear mixed-effect model

		FA		MD	
Brain region	Side	Nonconverting	Converting	Nonconverting	Converting
Cerebral peduncle	R	**1.84%***	1.19 %	-1.15 %	-0.40 %
	L	1.18 %	-0.36 %	-0.61 %	-0.42 %
Internal capsule	R	0.55 %	-1.12 %	-0.19 %	0.63 %
	L	-0.10 %	-0.11 %	-0.35 %	0.86 %
Corona radiata anterior	R	-1.32 %	**-3.88%****	-0.31 %	**1.81%*****
	L	-1.42 %	-1.23 %	-0.05 %	**1.13%***
Corona radiata posterior	R	-1.92 %	**-3.09%****	-0.21 %	**1.09%***
	L	-0.34 %	**-2.07%****	0.13 %	**1.27%****
Centrum semiovale	R	-0.25 %	-2.68 %	0.76 %	**1.79%****
	L	0.62 %	-0.28 %	**1.37%***	**1.95%****
Corpus callosum. genu	NA	-0.35 %	-0.48 %	0.19 %	0.78 %
Corpus callosum. splenium	NA	-0.01 %	-0.12 %	0.26 %	**1.53%***
Thalamus	R	0.21 %	-1.44 %	-0.39 %	0.67 %
	L	-0.39 %	**-3.20%****	-0.12 %	0.54 %
Caudate nucleus	R	0.36 %	-2.11 %	0.14 %	0.88 %
	L	-2.68 %	-1.71 %	-0.07 %	0.49 %

FA fractional anisotropy; MD mean diffusivity (10^{-3} mm^2/s); R right hemisphere, L left hemisphere; p values for time from separate linear mixed-effect models for non-converting and converting groups; * $p<0.05$; ** $p<0.01$; *** $p<0.001$. Reprinted with permission from Kolasa et al., 2015.

In Study II, the differences in baseline DTI values between all MS patients and healthy controls were assessed and the strongest differences were observed in the CC for FA, MD, and RD indices (Figure 1).

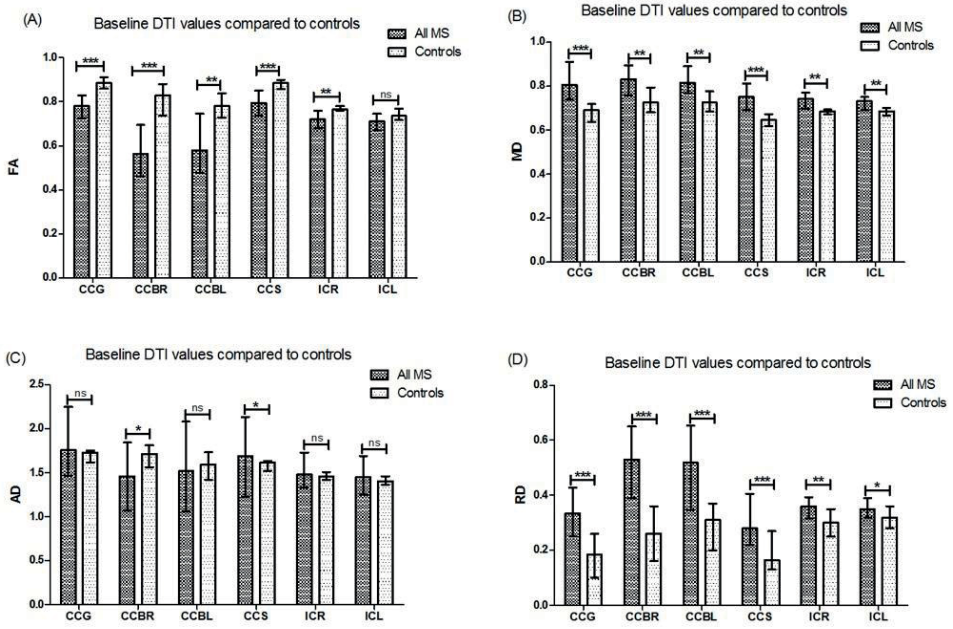

Figure 1. DTI metrics in MS patients and healthy controls at baseline. A DTI analysis was performed for 46 MS patients and 10 healthy controls. Values are expressed as the median ± range. Statistical analysis was carried out by Mann-Whitney U test; ∗p<0.05, ∗∗ p<0.01, ∗∗∗p<0.001, ns not significant. CCG genu of the corpus callosum, CCBR right body of the corpus callosum, CCBL left body of the corpus callosum, CCS splenium of the corpus callosum, ICR internal capsule right, ICL internal capsule left; DTI diffusion tensor imaging. Reprinted with permission from Kolasa et al., 2019.

Longitudinal changes in DTI over a one-year follow-up were studied in MS patients (Study II). During the follow-up, FA significantly ($p<0.05$) increased in 4/6 ROIs, and RD decreased in 4/6 ROIs (CC genu, body, and the CC splenium). AD showed a significant increase in 3/6 ROIs (the CC genu, CC body) (Table 7). The results remained significant except for RD in the CC genu and AD in the left CC body after the Bonferroni corrections ($p<0.008$). In the IC, the changes were non-significant (Table 7). We did not observe significant differences with regard to one-year DTI change between the disability progression and stable MS groups.

Table 7. DTI indices at baseline and after one year of follow-up in MS patients

	Relapsing-onset MS patients, n=46									
	DTI metrics	Baseline			Year 1			Annual change			p value
Corpus callosum genu	FA	0.78	0.48	0.88	0.81	0.48	0.93	0.03	-0.08	0.14	**<0.001**
	MD	0.80	0.62	1.31	0.82	0.67	1.07	-0.01	-0.34	0.20	0.891
	AD	1.73	1.47	2.25	1.84	1.40	2.23	0.10	-0.62	0.43	**0.006**
	RD	0.34	0.17	0.84	0.32	0.12	0.66	-0.04	-0.25	0.14	**0.009**
Corpus callosum body right	FA	0.56	0.30	0.87	0.68	0.32	0.89	0.06	-0.17	0.30	**<0.001**
	MD	0.83	0.58	1.08	0.81	0.70	1.11	0.01	-0.17	0.24	0.797
	AD	1.47	1.07	1.84	1.63	1.13	1.95	0.12	-0.37	0.65	**0.003**
	RD	0.53	0.20	0.92	0.44	0.20	0.75	-0.08	-0.33	0.11	**<0.001**
Corpus callosum body left	FA	0.58	0.29	0.85	0.68	0.37	0.88	0.10	-0.18	0.31	**0.001**
	MD	0.82	0.67	1.39	0.83	0.68	1.11	0.01	-0.42	0.24	0.589
	AD	1.46	1.06	2.08	1.64	1.08	2.00	0.12	-0.37	0.65	**0.027**
	RD	0.52	0.23	1.14	0.45	0.20	0.74	-0.09	-0.40	0.15	**<0.001**
Corpus callosum splenium	FA	0.79	0.52	0.94	0.82	0.58	0.94	0.02	-0.07	0.20	**0.004**
	MD	0.75	0.56	1.28	0.75	0.58	1.11	-0.02	-0.20	0.21	0.215
	AD	1.67	1.23	2.13	1.69	1.42	2.07	0.04	-0.27	0.30	0.157
	RD	0.28	0.11	0.86	0.25	0.09	0.69	-0.04	-0.31	0.14	**0.003**
Internal capsule right	FA	0.72	0.62	0.83	0.71	0.55	0.85	0.00	-0.16	0.07	0.304
	MD	0.74	0.66	0.79	0.74	0.67	0.83	0.01	-0.05	0.10	0.245
	AD	1.46	1.33	1.73	1.45	1.27	1.80	0.00	-0.13	0.14	0.743
	RD	0.36	0.24	0.47	0.37	0.23	0.52	0.00	-0.08	0.18	0.345
Internal capsule left	FA	0.71	0.47	0.80	0.71	0.49	0.83	0.01	-0.18	0.32	0.814
	MD	0.73	0.66	0.87	0.73	0.66	0.84	0.01	-0.06	0.07	0.092
	AD	1.46	1.25	1.69	1.48	1.25	1.83	0.03	-0.20	0.45	0.068
	RD	0.35	0.26	0.61	0.35	0.25	0.58	0.01	-0.27	0.15	0.566

Measurements expressed by median, range; p value for Wilcoxon test; in bold, $p<0.05$; FA fractional anisotropy; MD mean diffusivity ($x10^{-3}mm^2/s$); axial diffusivity ($x10^{-3}mm^2/s$); radial diffusivity ($x10^{-3}mm^2/s$). Reprinted with permission from Kolasa et al., 2019.

5.1.4 Predictive value of DTI in the assessment of disease activity in CIS and MS

The were no clear associations between baseline DTI indices and conversion of CIS to MS over a four-year period (Study I). In MS patients (Study II), a lower baseline FA and higher RD in the CC genu, right CC body, and the CC splenium were associated with disability progression measured by EDSS increase over four years ($p<0.05$) in the logistic regression analysis containing age and time from onset symptoms as covariates. Moreover, a higher baseline MD in the right CC body and higher MD and AD in the CC splenium were associated with disability progression (Table 8). The results did not remain significant after the Bonferroni's correction for multiple comparisons ($p<0.008$).

Table 8. Relationship of baseline DTI metrics with disability progression measured by EDSS increase over the four-year follow-up

		Stable group n=24			Progression group n=22						
	DTI metrics	median	min	max	median	min	max	p value	Odds ratio	95%CI	
Corpus callosum genu	FA	0.81	0.52	0.88	0.74	0.48	0.88	**0.04**	0.00	0.00	0.61
	MD	0.80	0.62	1.21	0.83	0.67	1.31	0.06	1.05	1.00	1.10
	AD	1.70	1.47	2.07	1.76	1.55	2.25	0.36	1.02	0.98	1.06
	RD	0.28	0.17	0.80	0.37	0.17	0.84	**0.04**	1.05	1.00	1.09
Corpus callosum body right	FA	0.67	0.40	0.87	0.52	0.30	0.77	**0.01**	0.00	0.00	0.24
	MD	0.80	0.58	1.07	0.87	0.69	1.08	**0.04**	1.08	1.00	1.15
	AD	1.51	1.07	1.79	1.39	1.18	1.84	0.25	0.98	0.95	1.01
	RD	0.46	0.20	0.73	0.62	0.30	0.92	**0.01**	1.07	1.02	1.12
Corpus callosum body left	FA	0.66	0.38	0.85	0.53	0.29	0.82	0.07	0.02	0.00	1.37
	MD	0.81	0.67	1.32	0.84	0.70	1.39	0.12	1.03	0.99	1.08
	AD	1.50	1.19	2.08	1.42	1.06	2.04	0.53	0.99	0.97	1.02
	RD	0.46	0.23	0.93	0.58	0.30	1.14	**0.04**	1.04	1.00	1.08
Corpus callosum splenium	FA	0.82	0.63	0.89	0.76	0.52	0.94	**0.04**	0.00	0.00	0.76
	MD	0.71	0.56	0.95	0.79	0.68	1.28	**0.01**	1.13	1.03	1.23
	AD	1.62	1.23	1.87	1.80	1.50	2.13	**0.01**	1.08	1.02	1.14
	RD	0.25	0.16	0.50	0.35	0.11	0.86	**0.02**	1.07	1.01	1.14
Internal capsule right	FA	0.72	0.62	0.78	0.72	0.62	0.83	0.89	1.01	0.89	1.14
	MD	0.72	0.66	0.79	0.75	0.66	0.78	0.14	1.13	0.96	1.33
	AD	1.45	1.33	1.61	1.49	1.34	1.73	0.16	1.05	0.98	1.12
	RD	0.35	0.29	0.47	0.36	0.24	0.46	0.78	1.02	0.90	1.15
Internal capsule left	FA	0.71	0.47	0.80	0.71	0.58	0.80	0.49	1.03	0.94	1.13
	MD	0.71	0.66	0.87	0.74	0.66	0.80	0.15	1.12	0.96	1.32
	AD	1.42	1.25	1.63	1.48	1.30	1.69	0.05	1.07	1.00	1.15
	RD	0.35	0.26	0.61	0.36	0.31	0.48	0.86	0.99	0.90	1.09

p value for logistic regression adjusted for age and duration of symptoms for prediction of EDSS progression over the 4-year follow-up; in bold, $p<0.05$; FA fractional anisotropy; MD mean diffusivity ($x10^{-3}mm^2/s$); axial diffusivity ($x10^{-3}mm^2/s$); radial diffusivity ($x10^{-3}mm^2/s$). Reprinted with permission from Kolasa et al., 2019.

In the logistic regression analysis containing baseline EDSS and number of relapses, associations between disability progression and DTI metrics remained significant for CC body and CC splenium. The majority of these associations disappeared after Bonferroni's correction ($p<0.008$).

Covariates, such as medication, disease duration, and sex, had no effect on disability progression in the regression analysis. The association between disability progression and DTI disappeared in the CC genu, and statistical power was slightly decreased in the other CC areas in the models of regression analysis containing FLAIR lesion volume and BPF. The T1 lesion volume had no effect in any regression model.

Longitudinal changes over 1 year after baseline were not associated with disability progression over four years in MS patients (Study II).

We observed moderate and strong ($r>0.4$) correlations between volumetric measurements and DTI indices at baseline (Study II). The strongest correlations were observed in the CC genu between the T1 brain lesion volume and FA ($p= 0.001$, $r= -0.48$), MD ($p<0.001$, $r= 0.52$), RD ($p<0.001$, $r= 0.52$) and between FLAIR lesion volume and FA ($p<0.001$, $r= -0.6$), MD ($p<0.001$, $r= 0.54$), and RD ($p<0.001$, $r= 0.6$). Regarding brain atrophy, the strongest correlations were found between BPF and RD ($p=0.002$, $r= -0.46$) in the right CC body, RD ($p=0.007$, $r= -0.41$) in the left CC body, MD ($p=0.004$, $r= -0.44$), AD ($p= 0.001$, $r= -0.49$) in the right IC, and MD ($p<0.001$, $r= -0.53$), AD ($p=0.002$, $r= -0.47$) in the left IC.

5.2 JCV serology in MS (Study III)

In Study III, the seroprevalence of anti-JCV antibody in the cohort of 406 MS patients was 57.4% (95% CI 52.6-62.2). The anti-JCV antibody seropositivity was higher in males (67%) than in females (54%) ($p=0.02$) and tended to increase with ageing (ANOVA, $p=0.08$). The use of different MS treatments did not influence anti JCV-serostatus; however, in patients treated with ongoing NTZ treatment ($n=72/406$), the screening-index values were lower than those in patients without such therapy (median 0.3 (range 0.1-3.12) vs 0.64 (0.1-3.12), $p=0.01$), respectively).

Longitudinal changes over approximately 4.5 years in anti-JCV antibody serostatus and screening index values were assessed in 67 patients. Seroconversion from anti-JCV antibody seronegativity to seropositivity was observed in 4/19 (21%,

approximately 4.7% per year) patients, and seroreversion from anti JCV antibody seropositivity to seronegativity was observed in 4/48 (8.3%, approximately 1.8% per year) patients. Stable seronegativity over the whole follow-up was observed in 15/19 (79%) patients, and stable seropositivity was observed in 44/48 (91.7%) patients. Moreover, marked interindividual variation in the magnitude of screening-index values was observed over the follow-up period.

6 DISCUSSION

6.1 MRI in MS (Studies I and II)

Conventional MRI (i.e., T1-weighted images with and without Gd contrast and T2-weighted images) is a well-established tool in the diagnostic process and monitoring of disease course in patients with CIS and MS (Reich et al., 2018; Wattjes, Steenwijk et al., 2015). This result is mainly related to the sensitivity (and to a lesser degree to specificity) of conventional MRI for detecting white-matter focal lesions (Geraldes et al., 2018) and to long experience with this imaging technique (McDonald et al., 2001; Thompson et al., 2018). However, only modest correlations have been observed between clinical measures and findings derived from conventional MRI, which is known as clinicoradiological paradox (Filippi et al., 2002). To fill this gap, several novel MRI techniques and postprocessing methods have been investigated within the last decade, such as volumetric measures of brain atrophy and focal lesions and DTI (Enzinger et al., 2015; Louapre et al., 2017). The use of nonconventional MRI techniques is not widely incorporated into clinical practice, mainly due to the conflicting results of previous studies, the lack of the well-established specificity of measurements and difficulties with reproducibility and interpretation of the results in real-life situations (Wattjes et al., 2015).

In this context, the main focus of this thesis was to investigate whether brain volumetric measurements and DTI-derived indices from the NABT can predict clinical activity and the severity of disease in CIS and MS patients.

6.1.1 MRI and conversion of CIS to MS (Study I)

In Study I, we observed significant differences in diffusivity in the NAWM between CIS and healthy controls at baseline. Moreover, we demonstrated that the worsening of DTI parameters over four years is more prominent in CIS patients progressing to

MS than in individuals in the stable CIS group. However, the only baseline parameter that correlated with the conversion of CIS to MS was brain T1 and FLAIR lesion load but not diffusivity parameters.

Several previous longitudinal studies have investigated the association between conventional MRI markers and the conversion of CIS to MS. The importance of T2-FLAIR lesions in the assessment of MS risk in CIS patients observed in the present study was also confirmed by the results of large four-year (Gaetani et al., 2017; Kuhle et al., 2015) and seven-year (Tintore et al., 2015) follow-up studies that demonstrated a strong correlation between the number of brain T2 lesions on baseline MRIs and the development of MS. In contrast, multivariate analysis performed by Paolillo et al. did not revealed any value of measurements of brain T1 or T2 lesion volumes in the prediction of the conversion of CIS to MS, most likely due to short follow-up (18 months) (Paolillo et al., 2004).

CIS populations are heterogeneous in terms of different extents of brain MRI abnormalities at symptom onset (Odenthal & Coulthard, 2015). Additionally, some patients, especially those with normal brain MRIs, may not develop MS over the study period or even during the patient's lifetime (Brodsky et al., 2008; Fisniku et al., 2008; Tintore et al., 2006). Moreover, the location of demyelinating plaques is important in the context of conversion to MS. Patients with infratentorial lesions (Tintore et al., 2010) and lesions within large supratentorial white-matter tracts (Giorgio et al., 2013) have a higher risk of developing MS. Additionally, MRI-visible lesion load in CIS patients with monofocal rather than multifocal symptoms is a predictor for the conversion to MS (Nielsen, J. M. et al., 2009) emphasizing the importance of clinical symptomatology features in the development of MS.

Relatively high volumes of T1 lesions (as well as T2 lesions) were observed in our cohort of CIS patients (Kalincik et al., 2012). As non-enhancing T1 lesions (black holes) depict severe structural damage in brain tissue, this may partially explain the correlation between T1 plaques and the conversion to MS observed herein. Consistent with our observations, non-enhancing T1 lesions were associated with conversion to MS; however, this correlation was not independent of T2 lesions in one study (Mitjana et al., 2014). We have not observed Gd-enhancing lesions in our CIS population, but this kind of lesions has also been associated with MS risk in previous studies (Nielsen, J. M. et al., 2009; Rovira et al., 2009). Importantly, the duration of lesion enhancement ranges typically between four and eight weeks, which may hypothetically influence the incidence of Gd-enhancing lesions observed

in previous studies (Filippi et al., 1997). Moreover, higher doses of contrast agent, longer delay between contrast injection and scanning as well as higher magnetic field strength are associated with increased detection of active MS lesions (Poloni et al., 2011).

The identification of new or enlarging T2 lesions over the follow-up period is of great importance in CIS and MS. Possible errors in the detection of T2 lesions related to interobserver variability on serial MRI examinations (Altay et al., 2013) might be minimized using image subtraction methods and automated quantification of the lesions (Battaglini et al., 2014). Moreover, a standardized protocol (adequate repositioning and the same MRI system) on serial scanning is also important (Vrenken et al., 2013; Wattjes et al., 2006; Wattjes et al., 2015).

Taken together, based on the results of this and previous studies, we can conclude that T2 lesions and, to a lesser degree, T1 lesions (Mitjana et al., 2014) are associated with MS risk in CIS patients (Martinelli et al., 2017).

In Study I, we performed a longitudinal analysis with four DTI examinations over the four-year follow-up in patients with CIS. Our results suggest that ROI-based DTI analysis can detect changes in water diffusivity in CIS when compared to healthy subjects. This difference was observed for both MD and FA metrics in several brain regions. Previous results are inconsistent, and one main reason for this discrepancy could be the different methods of DTI data analysis (ROI, histogram, TBSS, tractography) (Urger et al., 2013). Studies using histogram analysis (Yu et al., 2008), tractography (Pagani et al., 2005), and TBSS (Preziosa et al., 2011) have found differences in MD in NABT between CIS and healthy subjects. One study using TBSS found lower FA in the NAWM of CIS than in healthy controls (Raz et al., 2010a). In contrast, a ROI-based study, including younger and slightly more disabled patients than ours, did not show differences between CIS and healthy subjects at baseline (Caramia et al., 2002). Additionally, the histogram-based approach failed to show differences in DTI metrics between CIS and healthy subjects (Pulizzi et al., 2007).

We observed that worsening of diffusivity metrics in CIS converting to MS occurs in a higher number of ROIs than in stable CIS. However, clear correlations between DTI metrics and conversion to MS were not revealed. A few longitudinal studies have investigated the role of DTI in the prediction of MS development in CIS. The observation periods in these studies were up to approximately three years,

and the number of DTI examinations was between 1 and 2 (Caramia et al., 2002; Gallo et al., 2005; Raz et al., 2010b; Rovaris et al., 2008), with one study including four DTI examinations, as in our study (Bester et al., 2008).

The one-year follow-up study of Gallo et al. in CIS patients with a relatively high focal brain lesion load revealed diffusivity abnormalities in the NAWM detected by histogram-based DTI. Similar to our observations, such diffusivity changes have not been associated with progression to MS (Gallo et al., 2005). The same results were also observed in the paediatric population of CIS (Vishwas et al., 2013). In contrast, a three-year follow-up study by Bester et al. using ROI methodology revealed a correlation between decreased FA in the CC splenium and the development of MS in patients with optic neuritis (Bester et al., 2008). Since the rate of conversion from CIS to MS increases with longer observation times (Brex et al., 2002), different follow-up durations across the studies may explain the inconsistent results. Additionally, different clinical characteristics of the study population may influence the results since younger age and different types of clinical symptoms at CIS onset contribute to the risk of MS (Gaetani et al., 2017; Spelman et al., 2017; Tintore et al., 2015).

The identification of CIS patients with a high risk of developing of MS is important, as the initialization of immunomodulatory therapy has been shown to reduce the rate of conversion to MS (Beck et al., 2002; Comi et al., 2001; Comi et al., 2009; Kappos et al., 2009; Spelman et al., 2017). Therefore, disease activity expressed by dynamic changes in diffusivity indices together with measurements of brain lesion volume may play a role in the detection of patients with active ongoing processes related to MS pathology.

Although our results suggest diffusivity abnormalities in CIS, a firm conclusion on the value of DTI use in individual patients in clinical practice cannot be made. The limitation of Study I is the small number of CIS patients and the variable time between study entry and the initial symptoms. The same image protocol and the MRI scanner over the whole follow-up is a strength of our study; however, a higher number of gradient encoding directions in DTI (i.e., high-angular-resolution diffusion imaging) could improve the characterization of water diffusion in brain tissue (Jones, 2004; Mukherjee et al., 2008). DTI-derived biomarkers need to be optimally reproducible and reliable for incorporation into clinical practice. Inter-scanner variability related to magnetic field strength, gradient strength and even different software versions can affect the results obtained in DTI studies and thus

reproducibility (Bisdas et al., 2008; Pagani et al., 2005; Takao et al., 2012). Differences in DTI measurements related to inter- and intra-site variability can be at least partially minimized by using several postprocessing statistical models (Mirzaalian et al., 2015; Pagani et al., 2010; Venkatraman et al., 2015).

6.1.2 MRI and disability progression in MS (Study II)

In Study II, whole brain atrophy at baseline did not clearly predict disability progression over the four years. However, brain atrophy modified correlations between DTI indices and disability progression, indicating its influence on disability accumulation in our study population. Brain atrophy is a well-established marker of neurodegeneration in MS. Previously, greater whole brain atrophy and localized atrophy in specific brain regions have been correlated with disability worsening in cross-sectional and longitudinal studies (D. H. Miller et al., 2018; Ghione et al., 2018; Hanninen et al., 2019; Horakova et al., 2009; Jacobsen et al., 2014; Popescu, V. et al., 2013; Steenwijk et al., 2016; Zivadinov et al., 2013). Confounding factors, such as image contrast and resolution, pseudoatrophy related to cessation of oedema due to MS treatment, age and comorbidities must be taken into account when analysing brain atrophy, as these factors influence brain atrophy measurements (Khoury & Bakshi, 2010; Stefano et al., 2014). Moreover, the software used in brain atrophy analysis differs with regard to reproducibility and repeatability (Storelli et al., 2018).

Concerning the prognostic value of brain lesion volume, we did not observe a clear association between T1/T2 plaque volume and disability progression in our MS study cohort. Our results confirm previous observations indicating no (Gumberz et al., 2016; Jacobsen et al., 2014) or moderate correlation (Gauthier et al., 2007; Minneboo et al., 2008) between clinical parameters and T2 brain lesion load in MS, with better correlation observed in long-term studies (Fisniku et al., 2008; Popescu, V. et al., 2013). The majority of previous studies revealed a significant correlation between higher levels of T1 "black holes" in the brain and worse EDSS values (Caramanos et al., 2012; Giorgio et al., 2014; Lukas et al., 2013; Minneboo et al., 2009; Thaler et al., 2015), which can be explained by the close association of T1 lesions to profound, irreversible tissue damage in MS (Kutzelnigg & Lassmann, 2014).

The lack of a clear association between volumetric measurements (whole brain volume, T1/T2 brain lesion volumes) and clinical worsening in our study could be partially explained by the small study group and the fairly gross nature of total EDSS. Another possible explanation might be that disability accumulation in MS is not only related to brain pathology but also to spinal cord atrophy, as observed in previous cross-sectional and longitudinal studies (Daams et al., 2015; Lukas et al., 2015; Rocca et al., 2011; Valsasina et al., 2015).

Another observation from Study II suggests that diffusion abnormalities detected by DTI in the NAWM can distinguish MS from healthy controls. Diffusivity alterations in the NAWM of the CC and pyramidal tract of MS patients have also been previously observed (Banaszek et al., 2015; Lin et al., 2007; Ozturk et al., 2010; Pokryszko-Dragan et al., 2018; Roosendaal et al., 2009; Sigal et al., 2012; Wilson et al., 2003), and such differences were more pronounced in SPMS than in RRMS and CIS (Andersen et al., 2018; Braley et al., 2012; Preziosa et al., 2011). In our study cohort, the same trend was demonstrated as patients with SPMS had significantly worse diffusivity parameters in the body of the CC when compared to RRMS (data not shown). The diffusivity differences between our real-life MS cohort and healthy subjects were strong (even after Bonferroni's correction for multiple comparisons), especially for FA, MD, and RD metrics in the CC. Based on this result, we can speculate that demyelination in the CC represented here by increased RD (Song et al., 2003) is still occurring in our MS group. This finding may indicate the need for continuing immunomodulatory MS treatment, which is targeted at the cessation of inflammatory demyelination and degeneration.

We observed a tendency for an association between baseline DTI parameters in the CC and EDSS score increase over four years. Our results confirm previous observations from cross-sectional studies that reported correlations between EDSS and diffusivity metrics in the supratentorial NAWM, including CC (Barone et al., 2018; Ciccarelli et al., 2001; Giorgio et al., 2010; Harrison et al., 2013; Sigal et al., 2012). In contrast, no such correlation was demonstrated in some other studies (Fink et al., 2010; Llufriu et al., 2012). Another two longitudinal studies with baseline DTI performed in early RRMS (Kern et al., 2011) and PPMS (Bodini et al., 2013) also revealed that splenial FA and RD metrics are associated with worsening of motor dysfunction measured by EDSS and 9-HPT. We did not observe any association between EDSS and DTI metrics in the pyramidal tract represented here by the

internal capsule, which is consistent with some tractography-based DTI studies (Lin et al., 2007; Tortorella et al., 2014) but not with other studies (Giorgio et al., 2010; Tovar-Moll et al., 2015; Wilson et al., 2003). The weak correlation between EDSS and DTI in the CC and the inconsistent results of previous studies can be at least partially explained by the global nature of EDSS. Indeed, regional (corticospinal tract, CC, cerebellar peduncle) diffusivity abnormalities in the NAWM have been previously intercorrelated with specific clinical features, such as walking ability and fine hand motor skills (Klineova et al., 2016; Ozturk et al., 2010; Pokryszko-Dragan et al., 2018).

In Study II, we performed DTI analysis twice, at baseline and at one year of the follow-up. To our knowledge, there are few studies with longitudinal DTI measurements aiming to determine the predictive value of DTI in the assessment of disability progression in MS. Similar to our results, a longitudinal study by Samann et al. (Samann et al., 2012), including 55 MS patients with DTI histogram analysis performed at study entry and after one year, revealed that baseline ADC predicts clinical deterioration (measured by EDSS and MSFC). Consistent with our results, these authors also observed significant annual changes in ADC. Contrary to our predictions, we observed an increase rather than a decrease in FA and AD and a decrease rather than an increase in RD in the CC over one year. The counterintuitive direction of diffusivity changes in our study may be explained by only two measurements performed with a one-year interval, which does not allow us to observe sustained changes in DTI metrics. Moreover, the confounding effect of immunomodulatory treatment may influence temporal changes in diffusivity, as suggested by others (Ontaneda et al., 2017).

Taken together, these results and the results of previous studies (Cassol et al., 2004; Harrison et al., 2011) suggest that DTI is sensitive to temporal changes in water diffusivity and may serve as a tool in monitoring disease activity in MS. Moreover, DTI can distinguish patients with MS from healthy subjects on a group-level, suggesting the specificity of DTI to MS-related pathology. Diffusivity metrics may also play a role as a predictor of disability accumulation; however, validation of the measurements is needed. In contrast, volumetric measurements were not predictive of disability progression over four years.

6.2 JCV seroprevalence in MS (Study III)

We evaluated for the first time a seroprevalence of anti-JCV antibodies and anti-JCV screening indices in a large Finnish cohort of patients with MS and CIS. In our study population, overall anti-JCV antibody seropositivity was observed in 57% of patients. To date, there is only one study analysing anti-JCV antibodies in a Finnish population that included 150 pregnant women (Stolt et al., 2003). Consistent with our results, these authors found a high seroprevalence of anti-JCV antibodies, which increases with age. In contrast to our observations, no cases of anti-JCV antibody seroconversion or seroreversion were observed over the five-year follow-up.

The anti-JCV seroprevalence reported by us is also consistent with the results of a recent systematic review that reported a prevalence of positivity for anti-JCV antibodies in MS ranging between 40% and 69%; however, the values varied geographically (Paz et al., 2018). Consistent with previous results, male gender (Correia et al., 2017; Olsson et al., 2013; Trampe et al., 2012) and higher age (Dominguez-Mozo et al., 2017; Olsson et al., 2013; Salmen et al., 2016) were also related to higher seroprevalence.

Our longitudinal analysis revealed conversion from anti-JCV antibody seronegativity to seropositivity of approximately 4.7% per year, which is rather small compared with previous studies in multinational MS groups. Previous studies using the second-generation ELISA STRATIFY test have reported seroconversion rates ranging between 6% per year and 29% per year (Delbue et al., 2015; Plavina et al., 2014; Raffel et al., 2015; Schwab, Schneider-Hohendorf, Pignolet, Breuer et al., 2016; Vennegoor et al., 2016). We reported reversion from anti-JCV antibody seropositivity to seronegativity over the follow-up period at approximately 1.8% per year, which was also observed in previous studies (Hegen et al., 2017; Raffel et al., 2015; Schwab et al., 2016). The variable temporal change in JCV status observed in previous studies could be explained by the duration of follow-up since a longer observation period increases the likelihood of seroconversion (Schwab et al., 2018). Moreover, MS treatment may influence serostatus, as observed by some investigators (Outteryck et al., 2013; Raffel et al., 2015) but not by other researchers (Hegen et al., 2018; Trampe et al., 2012). Genetical variability of study individuals may also influence JCV serostatus, as major histocompatibility complex (MHC) genes are responsible for the different production of anti-JCV antibodies (Dominguez-Mozo

et al., 2017; Sundqvist et al., 2014). Interestingly, we observed marked interindividual variations in the magnitude of screening indices even in patients who remained anti-JCV antibody seropositive over the whole follow-up period. Similar observations were also made in a recent study with MS patients receiving disease-modifying treatment and without such therapy (Hegen et al., 2018).

In the cross-sectional study, we observed decreased levels of the anti-JCV antibody screening index in patients on NTZ therapy with no impact on anti-JCV seroprevalence. With regard to other MS therapies, we have not revealed any impact on anti-JCV seroprevalence, which is consistent with previous results from large multinational studies (Bozic et al., 2014; Olsson et al., 2013). The initialization of NTZ treatment was previously related to a decrease in screening indices, which confirmed our results and suggests some immunosuppressive effect of NTZ (Warnke et al., 2013). In contrast to these results, recent cross-sectional (Trampe et al., 2012; van Kempen et al., 2017) and longitudinal (Hegen et al., 2018) studies have not observed any relationship between NTZ and JCV status. Interestingly, an increase in anti-JCV antibody index levels in NTZ-treated patients has also been reported in previous studies (Raffel et al., 2015; Schwab et al., 2016), which could be partially explained by the effect of age (Raffel et al., 2015). Since we did not have any patients receiving NTZ therapy in our longitudinal cohort, we cannot assess the actual impact of this medication on JCV seroprevalence or index levels. Samples before and after treatment initialization would allow a more reliable assessment of NTZ-related fluctuations in the levels of anti-JCV antibody screening indices.

Based on our results, we confirmed the high prevalence of antibodies against JCV in a Finnish cohort of MS patients. Moreover, JCV serostatus expressed by anti-JCV positivity or negativity and levels of anti-JCV antibody screening indices undergo marked fluctuations over time, confirming the need for repeated measurements to stratify the risk of NTZ-related PML. Our observations have clinical implications, as increasing anti-JCV antibody screening indices over time have been related to a higher risk of NTZ-related PML (Plavina et al., 2014). Therefore, this information is important because it influences treatment planning in patients with MS (Finnish Neurological Society, 2019).

7 SUMMARY AND CONCLUSIONS

Based on the results of this thesis, the following conclusions may be drawn:

1. Increased volumes of T1 and FLAIR brain lesions are linked to the conversion of CIS to MS, confirming the predictive role of volumetric measures in the assessment of the clinical course of disease in CIS.

2. Diffusivity abnormalities in the NABT detected by ROI-based DTI analysis were observed in a group of patients with CIS and MS when compared to healthy controls.

3. DTI abnormalities in the NABT are not clearly associated with the conversion of CIS to MS. However, in CIS progressing to MS, worsening of DTI indices was observed in a higher number of ROIs than in nonconverting CIS patients. These results suggest diffusivity changes primarily in CIS patients developing MS; however, the influence of these changes on disease progression could not be confirmed.

4. Baseline diffusivity abnormalities in the normal-appearing CC were found among MS patients with disability progression when compared to stable MS. Moreover, temporal changes of DTI metrics were revealed in the CC in the whole MS group. These results indicate the potential of DTI in disease monitoring and prediction of disability progression. However, further serial DTI studies in a larger group of patients and with more frequent scanning are needed to confirm the stability and reproducibility of DTI measurements. Brain lesion volume and brain atrophy were not clearly associated with disability progression over four years in this MS cohort.

5. The seroprevalence of anti-JCV antibodies in a Finnish cohort of patients with CIS and MS is high. Older age and male sex but not immunomodulatory therapies are associated with anti-JCV antibody seropositivity. Temporal changes in anti-JCV serostatus occur.

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10 ORIGINAL PUBLICATIONS

PUBLICATION I

Longitudinal assessment of clinically isolated syndrome with diffusion tensor imaging and volumetric MRI

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Longitudinal assessment of clinically isolated syndrome with diffusion tensor imaging and volumetric MRI☆

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ABSTRACT

The potential of diffusion tensor imaging (DTI) indices and volumes of focal lesions on conventional magnetic resonance imaging to predict conversion to multiple sclerosis (MS) was analyzed in subjects with clinically isolated syndrome (CIS) over 4 years. Twenty patients with CIS and 10 healthy controls were included in the study. The data showed an association between the volumes of T1 and fluid-attenuated inversion recovery (FLAIR) lesions and conversion to MS (T1: $P = .02$; FLAIR: $P = .02$). The worsening of DTI indices (mean diffusivity and fractional anisotropy) was primarily seen in patients progressing to MS, but clear-cut association with conversion could not be detected.

1. Introduction

In the majority of patients, multiple sclerosis (MS) presents as a subacute, monophasic neurological episode suggestive of demyelination, i.e., clinically isolated syndrome (CIS) [1]. Currently, the diagnosis of MS is based on the revised McDonald criteria that emphasize the role of magnetic resonance imaging (MRI), which allows the diagnosis of MS at the time of the first demyelinating event [2]. According to the current evidence, the burden of lesions on the MRI scans as well as the presence of oligoclonal bands in the cerebrospinal fluid contributes to the risk of conversion to clinically definite MS [1]. Follow-up studies on CIS patients have demonstrated that clinically definite MS develops in 55% to 80% of patients with an abnormal initial MRI [3]. However, only a modest correlation has been found between the volume of plaques and clinical disability [4]. This may be explained by inability of conventional MRI (cMRI) to detect occult histopathological abnormalities in the normal-appearing white and gray matter (NAWM and NAGM) [4]. Nonconventional techniques, including diffusion tensor imaging (DTI), can detect the alterations outside the lesions visible on cMRI [4]. DTI is a quantitative MRI technique that measures water diffusion differences in the living tissues. Of DTI-derived metrics, mean diffusivity (MD) describes the magnitude of water diffusion in particular tissue, whereas fractional anisotropy (FA) represents the directionality of diffusion and reflects alignment of the tissue [5]. These parameters may indirectly reflect the state of axonal and myelin integrity [6,7]. Radial and axial diffusivities are thought to represent myelin content and axonal integrity, respectively, and appear to be more specific markers of the pathological substrate than mean diffusivity [8].

In cross-sectional studies, increased MD and reduced FA values within both demyelinated plaques and normal-appearing brain tissue (NABT) have typically been detected in patients with CIS and MS [9–12]. The short-term follow-up studies (1–3 years) performed on CIS patients have reported DTI changes concerning NAGM [13] and NAWM [14,15], but other authors have not been able to confirm these results [16]. Thus, there is still need of investigation for reliable surrogate markers for conversion of CIS to MS. In this study, we aimed to assess the potential of DTI and volumetric MRI measurements in the prediction of conversion of CIS to definite MS. The identification of CIS patients with an aggressive disease course is extremely important when considering whether to begin immunomodulatory treatment and when selecting the appropriate drug. Early treatment has been demonstrated to modify the course of the disease favorably in CIS [17].

☆ Conflict of interest: None of the authors has any conflict of interest to disclose.

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2. Materials and methods

2.1. Patients

Demographic and clinical data are shown in Table 1. Twenty patients with CIS (18 females, 2 males) attending the MS Outpatient Clinic of the Tampere University Hospital were consecutively enrolled into this 4-year follow-up study (between December 2006 and September 2012). All subjects provided informed written consent. The study was approved by the local ethics committee.

CIS was defined as a first episode suggestive of inflammatory demyelinating disease with no paraclinical evidence of dissemination in time [1]. Diagnosis of MS was based on the revised McDonald criteria and included MRI and clinical evidence of dissemination in time and space [18]. The control group consisted of 10 age-matched healthy subjects (5 females, 5 males) that were included in the cross-sectional DTI analysis at baseline. The age of subjects and the time between first symptoms and baseline examination in patients that converted to MS over the follow-up (converting group) and those subjects that remained CIS (nonconverting group) were not significantly different ($P > .05$). The inclusion criteria were diagnosis of CIS and no steroid treatment for at least 8 weeks before clinical and radiological assessment. The exclusion criteria included contraindication to MRI and other neuroimmunological, vascular, or metabolic diseases. Patients underwent annual neurological examination at baseline ($n = 20$ patients), 1 year ($n = 20$), 2 years ($n = 19$), 3 years ($n = 18$), and 4 years ($n = 16$) after the baseline visit followed by radiological examination on the same day except for the third year when MRI assessment was not performed. The same neurologist examined the patients over the whole observation period (M.R).

2.2. MR image acquisition

The following full-brain coverage sequences were obtained during imaging session by using a 1.5-T MR scanner (Magnetom Avanto SQ; Siemens Medical Solutions, Erlangen, Germany): T1-weighted header followed by an axial three dimensional (3D) T1-weighted magnetization prepared rapid gradient echo (MPRAGE), 3D T2-weighted turbo spin-echo, fluid-attenuated inversion recovery (FLAIR), T1-weighted spin echo with magnetization transfer contrasts, multidirectional diffusion-weighted echo planar imaging, and gadolinium enhanced T1-weighted MPRAGE. The DTI protocol consisted of a single-shot spin-echo-based echo planar diffusion-weighted imaging with 3 averages and 12 gradient encoding directions, with b values of 0 and 1000 s/mm^2. The imaging parameters are presented in Table 2.

Table 1
Demographic and clinical data

	Nonconverting[a] $n=9$	Converting[b] $n=11$	Controls $n=10$
Sex (female/male)[c]	8/1	10/1	5/5
Mean age at baseline, years (range)	37 (26–52)	34 (22–51)	40 (26–61)
Positive oligoclonal bands status[c]	4	11	NA
Positive IgG index status[c,d]	2	5	NA
Time since first symptoms to baseline [years, mean (range)]	2.1 (0.7–8.8)	2.7 (0.5–7.8)	NA
Time since first symptoms to diagnosis of MS [years, mean (range)]	NA	4.7 (1.5–8.8)	NA
EDSS [median (range)]			NA
Baseline	0 (0–1)	0 (0–1)	
1 year	0 (0–1)	0 (0–1)	
2 years	0 (0–1)	0 (0–1)	
3 years	0 (0–1.5)	0 (0–1)	
4 years	0 (0–1.5)	0 (0–1)	
Number of relapses over follow-up			NA
0	9	5	
1	0	6	
Onset symptoms[c]			NA
Optic neuritis	7	7	
Brainstem	0	2	
Spinal cord	1	2	
Brainstem and spinal cord symptoms simultaneously	1	0	

NA, not applicable.
[a] Nonconverting: patients with CIS that did not convert to MS over follow-up.
[b] Converting: patients with CIS that converted to MS over follow-up.
[c] Number of patients.
[d] IgG index status considered as positive when value >0.7.

2.3. MR imaging postprocessing

The DTI data were analyzed with commercial Neuro 3D software (Siemens Healthcare, Malvern, PA, USA) on an offline workstation. The FA and MD values at baseline and yearly up to 4-year visit for all patients and at baseline for controls were measured by a physicist with long experience with brain DTI measurements (U.H.). Multidirectional diffusion data were assessed visually for the presence of distortions and artifacts. There were no significant eddy current distortions due to diffusion gradients. Circular regions of interest (ROIs) were manually placed on MD, FA, and nondiffusion b_0 maps. The same anatomic areas had always equal-sized ROIs over both hemispheres and over all patients at every time point. The ROIs were positioned symmetrically (except for the corpus callosum) at the following anatomical locations: the cerebral peduncle, posterior limb of the internal capsule, posterior corona radiata posterior and anterior, centrum semiovale, genu and splenium of the corpus callosum, thalamus, and head of the caudate nucleus. The ROIs were centered in the structure of interest in the most homogeneous area, avoiding border areas to exclude partial volume effect. For cases in which demyelinated plaque was identified in predefined ROI, the ROI was shifted to the closest area surrounding the predefined ROI. As the centrum semiovale is a large anatomical area, the ROIs were outlined symmetrically on the first slice above the dorsal part of the trunk of the corpus callosum. The ROI in the corona radiata region was contoured at the level of body of the corpus callosum (Fig. 1). As regional brain asymmetry in DTI measurements was previously observed [19], we analyzed both hemispheres separately.

In patients with CIS, the total brain volume of T1 hypointense and FLAIR hyperintense lesions from both gray and white matter was assessed blindly using the semiautomatic segmentation software Anatomatic 2.23 [20] by the same reader (M.K.) at baseline, 2 years, and 4 years.

To assess the intraobserver repeatability of the DTI measurements, the coefficients of variation (CV%s) were calculated for baseline DTI values in controls. The same observer (U.H.) repeated the measurements with a time interval of 3 months. In all ROIs, the CV% for MD was lower than 2.5%, and that for FA was lower than 5.3% except for the FA in the left centrum semiovale (11.5%).

2.4. Statistical analysis

Statistical analysis was performed using PASW Statistics for Windows Version 20 (SPSS Inc., Chicago, IL, USA). Comparisons between the subgroups for demographic data were performed by t test (normally distributed data) and Mann–Whitney test (non-normal

Table 2
Imaging parameters

	Axial T1WI	Axial FLAIR	Axial DTI
Slice thickness (mm)	0.9	5	5
Interslice gap (mm)	0	0	1.5
Field of view (mm)	230*230	230*230	230*230
Matrix	256*256	256*256	128*128
Echo time (ms)	4,2	100	96
Repetition time (ms)	1160	8500	3500
Inversion time (ms)	600	2500	

T1WI T1-weighted imaging; FLAIR fluid attenuated inversion recovery; DTI diffusion tensor imaging.

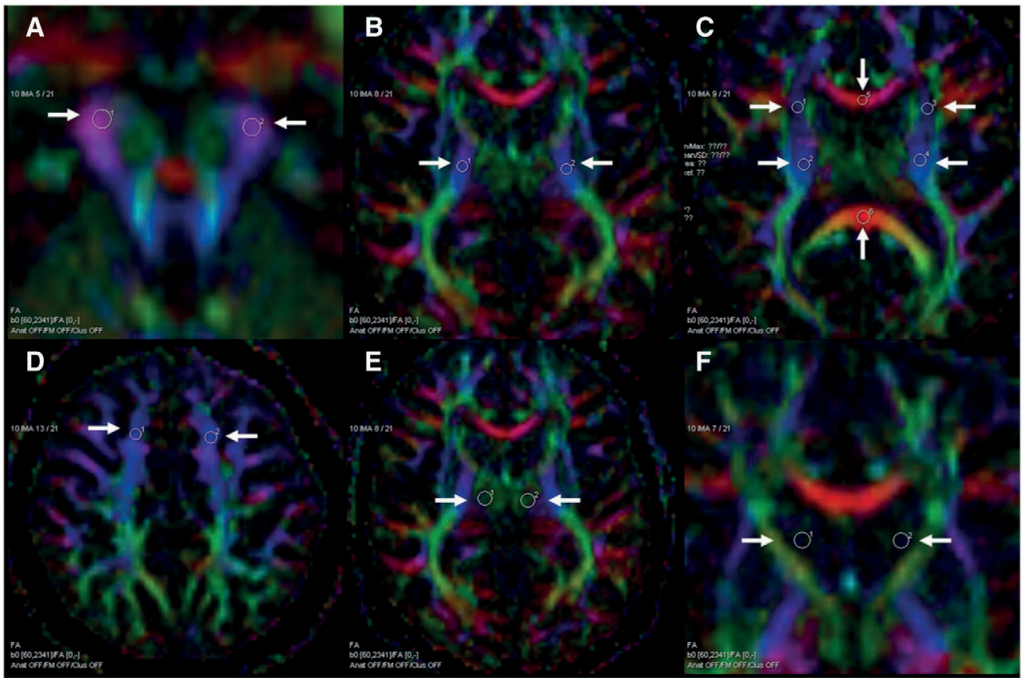

Fig. 1. ROI placement on the color-coded FA axial maps. (A) Cerebral peduncle (1, 2) (the size of ROIs 4 pixels); (B) posterior limb of the internal capsule (1, 2) (6 pixels); (C) posterior corona radiata anterior (1, 3) and posterior (2, 4) (6 pixels) and genu (5) (4 pixels) and splenium (6) (6 pixels) of the corpus callosum; (D) anterior part of the centrum semiovale (1, 2) (9 pixels); (E) thalamus (1, 2) (8 pixels); (F) head of the caudate nucleus (1, 2) (4 pixels). Pixel size 1.8*1.8 mm^2.

data). A *P* value <.05 was considered significant. In addition, the differences between patients and controls in DTI measurements at baseline were evaluated using the Mann–Whitney test followed by Bonferroni correction for three different comparisons. A Mann–Whitney test was run to determine the differences in volumetric measurements at baseline. Cox regression analysis was conducted to assess the associations between baseline MRI markers (FA, MD, and T1 and FLAIR lesion load) and the development of MS over 4 years. A linear mixed-effect model with T1, FLAIR, or one of the DTI parameters as a dependent variable was fitted using the function lme in R (Software environment for statistical computing and graphics, version 2.13.0, The R Foundation for Statistical Computing). The distribution of T1 and FLAIR values was considerably skewed, and therefore, logarithm transformation was used. Before this, a few zero values in some of the T1 and FLAIR volumetric measurements had to be transformed to "one." The binary variable for the conversion of the group, time from onset of symptoms to start of the study, and age (both continuous) were used as independent variables together with the time points (years) from the repeated measurements (years 0, 1, 2, and 4 for DTI and years 0, 2, and 4 for T1 and FLAIR). Possible group-by-time interaction was also assessed using an interaction variable. Random intercept was used together with independent random errors. The model estimates were used for calculation of the annual rate of change for all dependent variables, separately for the converting and nonconverting groups.

3. Results

3.1. Clinical and demographic data

The mean follow-up was 3.8 years (range 1.2–4.6). The main demographic and clinical characteristics are summarized in Table 1. At baseline, five patients presented MRI changes suggesting dissemination in space, and there were no gadolinium-enhancing lesions. Over the follow-up period, 11 (55%) patients (1 man and 10 women) presented either clinical (4 patients) or radiological (7 patients) evidence of dissemination in time or space, thus confirming diagnosis of MS. In our cohort, the mean time from baseline to MS diagnosis was 2 years (range 0.9–4.4). In two patients from the nonconverting group EDSS (Expanded Disability Status Scale) increased from 0 to 1 and from 1 to 1.5, respectively. In four patients from the converting group, EDSS worsened from 0 to 1. Over the follow-up period, four patients discontinued the study because they were not willing to continue. After MS diagnosis, two patients were treated with interferon β-1b and one patient with glatiramer acetate, whereas the other patients who had only mild symptoms preferred to stay under surveillance.

3.2. Baseline values of DTI and lesion volumes in CIS groups and controls

Comparisons between baseline DTI indices in different brain regions in the CIS groups and controls revealed significantly higher MD values in three out of nine regions in the converting group. These regions included the right internal capsule, left corona radiata anterior, and right centrum semiovale. It is noteworthy that, in comparison with controls, the nonconverting patients also had higher MD values in three out of nine regions, i.e., the right and left internal capsule, right corona radiata anterior, and right centrum semiovale.

Regarding FA indices, a comparison between the converting group and controls indicated lower values in the right cerebral peduncle and left caudate nucleus in converting patients. The nonconverting group had lower FA values in the right cerebral peduncle than controls. No significant differences were found for any DTI measurements between the converting and stable CIS groups (Supplementary Table 1).

Comparisons between baseline lesion volumes in the CIS groups revealed significantly higher T1 and FLAIR lesion load in the converting group (Table 3).

3.3. Changes of DTI indices and lesion volumes over the follow-up period

To assess changes over the follow-up period, the model estimates from linear mixed models were calculated and used for counting the annual rates of change (% per year) in both CIS groups. A significant annual increase of MD in four out of nine brain regions was found in the converting group (the left and right corona radiata anterior and posterior, left and right centrum semiovale, splenium of the corpus callosum), but such a change was found in only one region (left centrum semiovale) in nonconverting subjects. With respect to FA, the converting group displayed a significant annual decrease in three out of nine regions (the left corona radiata anterior, left and right corona radiata posterior, left thalamus), whereas the nonconverting group displayed an increase of FA in only one region (right cerebral peduncle) (Supplementary Table 2). Comparisons of the respective annual rate of change of DTI between the CIS groups revealed a significant difference in only the MD of the corona radiata anterior, which had an elevated value at the end of the follow-up in the converting group and no change in the nonconverting group ($P = .009$) (Supplementary Tables 3 and 4). With respect to volumetric measurements, the T1 lesion volume significantly increased at a rate of 7.9% per year only in the converting group ($P < .01$). The annual changes in FLAIR measurements over the follow-up period were not significant in any group of patients. The T1 and FLAIR brain lesion volume was not significantly different between the nonconverting and converting group in the longitudinal analysis (Table 3).

3.4. Associations between MRI values and the development of MS

The higher T1 and FLAIR lesion load at baseline was associated with the conversion to MS over 4 years (for T1-weighted hazard ratio 1.99, $P = .021$; for FLAIR hazard ratio 1.24, $P = .015$). These associations remained after adjustment for age and time from onset symptoms to baseline. No significant associations were found between baseline DTI values and conversion to MS, although DTI alterations were already present at baseline, and they tended to progress more rapidly in those patients who converted to definite MS.

4. Discussion

In this study addressing the potential of DTI and plaque volume assessment to predict conversion from CIS to MS, we present the 4-year follow-up data of annual imaging measurements and the neurological evaluation of patients with CIS.

It appeared that, compared with controls, DTI abnormalities were already present in patients with CIS, which confirm the results from several studies [10,11]. On the other hand, contrasting data have been reported by Pulizzi et al. [21]. In our study, the DTI measurements at baseline did not differ between converting and nonconverting patients, and none of the baseline DTI indices in the NABT were predictive of conversion of CIS to definite MS. This observation is consistent with earlier studies in pediatric and adult CIS populations with mean observation times of up to 2.4 years [11,22]. In addition, studies using magnetization transfer MRI and spectroscopy MRI [23,24] did not find a predictive value for these nonconventional techniques. Interestingly, an ROI-based DTI study by Bester et al. revealed that significant worsening of FA in the splenium of the corpus callosum is observed in patients with optic neuritis developing MS [15].

In our study, patients progressing to definite MS had higher FLAIR and T1 brain lesion volume at baseline, thus confirming the potential of cMRI in the prediction of MS. Similar results have been reported in previous short-term [25,26] and long-term studies [27,28]. On the contrary, Gallo et al. did not find any predictive value of volumetric MRI estimation over a 1-year follow-up [11].

Our results suggest that focal changes detected by cMRI, but not diffuse abnormalities of NABT, studied on DTI are potentially associated with progression to definite MS. Moreover, the burden of pathology in our two CIS subgroups detected by single DTI at baseline was similar, suggesting that this methodology may not be optimal in distinguishing the patients with different clinical outcomes. The radiological–pathological postmortem study suggests that MD strongly depends on the changes in the extracellular space, whereas FA appears to reflect axonal density [6]. Based on our data, we assume that water diffusion abnormalities in NABT at early stages of MS indicate both diffuse inflammatory demyelination and edema expressed by increased MD as well as degenerative changes represented by reduced FA.

Regarding the rate of progression, our data indicate that, in comparison to stable CIS subjects, the CIS patients developing MS displayed more rapid deterioration of DTI abnormalities in NAWM (the corona radiata, centrum semiovale, splenium of the corpus callosum) and NAGM regions (the thalamus). Previously, cMRI-visible lesions in the same NAWM regions were found to be predictive of clinical impairment in CIS [29,30]. Moreover, in cross-sectional studies in MS patients using the DTI approach, corresponding sites were affected by microscopic alterations [31–34]. In one follow-up study in CIS patients, the increase of MD in NAWM became visible after 1 year when approximately 85% of patients developed MS [14]. It is likely that the 4-year follow-up in our study, as well as the different clinical characteristics of studied populations, enabled us to observe these brain-region-specific DTI changes in CIS, especially in those patients that progressed to definite MS. Another study in patients with optic neuritis revealed a decrease of FA in both the splenium and genu of the corpus callosum over approximately 3 years [15]. We found that only MD in the splenium of corpus callosum increases in converting patients over 4 years. As myelin content is believed to have at least moderate impact on MD measures [6], our observations might indicate that the process of inflammatory demyelination might play a potential role in the pathology of the normal-appearing corpus callosum in CIS. On the other hand, Tract-Based Spatial Statistics (TBSS) and histogram-based studies in CIS and RRMS patients have not revealed any DTI changes over 2 years of observation [16,35,36], which is in contrast to our data.

With regard to NAGM, the abnormalities represented by the decreased FA in the caudate nucleus at baseline and temporal deterioration of FA in the thalamus were only observed in the converting group. Atrophic changes in the thalamus, hypothalamus, and striatum

Table 3
T1 and FLAIR lesion load (ml) in nonconverting and converting groups of patients over the follow-up period [median (percentiles)]

	Baseline		Year 2		Year 4		*P* value nonconverting vs. converting
	Nonconverting $n=9$	Converting $n=11$	Nonconverting $n=9$	Converting $n=10$	Nonconverting $n=6$	Converting $n=10$	
T1 lesion load	13 (0–186)	506 (125–1329)[a]	41 (2–41)	603 (123–1967)	45 (3–274)	613 (84–2011)	.4
FLAIR lesion load	499 (3–1006)	1879 (379–8555)[b]	979 (134–1301)	3240 (1259–6271)	323 (39–921)	1658 (626–3914)	.146

P value for group-by-time interaction term from linear mixed-effect model.
[a] Comparison between T1 lesion load in nonconverting vs. T1 lesion load in converting group at baseline, $P = .012$, Mann–Whitney test.
[b] Comparison between FLAIR lesion load in nonconverting vs. FLAIR lesion load in converting group at baseline, $P = .017$, Mann–Whitney test.

have been previously observed in CIS patients [37], and progression of overall gray matter atrophy has been associated with conversion to MS [38]. Earlier histogram-based DTI analysis in CIS patients indicated significant increases of MD in NAGM over a 3-year period, although it was not correlated with clinical activity [13]. We suggest that a combination of gliosis (which lowers both MD and FA) and loss of axons (which reduces FA and increases MD) may result in the pattern observed by us in the NAGM (reduced FA and unchanged MD). In earlier studies on patients with MS, elevated FA values were found in the thalamus and caudate nucleus [39,40]. The differences between CIS and MS may be explained by younger age of subjects with CIS and age-related increase of alterations of DTI parameters in the deep NAGM in healthy adults [41]. The annual FA increase in the cerebral peduncle in nonconverting patients reported by us may reflect an unsteady balance between the pathological and subsequent reparative processes in the fiber tract that occur in the initial stages of MS [36].

Methodological differences between DTI-analysis methods, such as the ROI, histogram, and newer TBSS, may explain some of the inconsistent results from studies performed until now. The ROI analysis used by us can miss significant abnormalities in regions that are not selected. The regional differences in reproducibility of DTI measurements, which are relatively low for the centrum semiovale and corona radiata [42], should also be taken into account. As reproducibility for DTI measurements in ROIs was at an acceptable level in our study center [19], the results reported by us seem to indicate that water diffusion abnormalities vary regionally. In addition to the technical aspects, the accumulation of subtle water diffusion alterations in NABT that reach a certain critical threshold may exceed the time of follow-up, resulting in the lack of predictive value for DTI.

To summarize, our 4-year study suggests that abnormalities in the NABT detected by repeated DTI are already observed in CIS patients. There appears to be a higher rate of changes in DTI parameters in patients who develop definite MS than in patients who maintain CIS over time. Moreover, only macroscopic brain lesion load and not diffuse “occult” damage detected by DTI appears to be associated with an increased risk of conversion to definite MS.

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Appendix A. Supplementary data

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PUBLICATION II

Diffusion tensor imaging and disability progression in multiple sclerosis: A 4-year follow-up study.

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Diffusion tensor imaging and disability progression in multiple sclerosis: A 4-year follow-up study

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Abstract

Objectives: Diffusion tensor imaging (DTI) is sensitive technique to detect widespread changes in water diffusivity in the normal-appearing white matter (NAWM) that appears unaffected in conventional magnetic resonance imaging. We aimed to investigate the prognostic value and stability of DTI indices in the NAWM of the brain in an assessment of disability progression in patients with a relapsing-onset multiple sclerosis (MS).

Methods: Forty-six MS patients were studied for DTI indices (fractional anisotropy (FA), mean diffusivity (MD), radial (RD), and axial (AD) diffusivity) in the NAWM of the corpus callosum (CC) and the internal capsule at baseline and at 1 year after. DTI analysis for 10 healthy controls was also performed at baseline. Simultaneously, focal brain lesion volume and atrophy measurements were done at baseline for MS patients. Associations between DTI indices, volumetric measurements, and disability progression over 4 years were studied by multivariate logistic regression analysis.

Results: At baseline, most DTI metrics differed significantly between MS patients and healthy controls. There was tendency for associations between baseline DTI indices in the CC and disability progression ($p < 0.05$). Changes in DTI indices over 1 year were observed only in the CC ($p < 0.008$), and those changes were not found to predict clinical worsening over 4 years. Clear-cut association with disability progression was not detected for baseline volumetric measurements.

Conclusion: Aberrant diffusivity measures in the NAWM of the CC may provide additional information for individual disability progression over 4 years in MS with the relapsing-onset disease. CC may be a good target for DTI measurements in monitoring disease activity in MS, and more studies are needed to assess the related prognostic potential.

KEYWORDS
diffusion tensor imaging, longitudinal study, multiple sclerosis

1 | INTRODUCTION

In multiple sclerosis (MS), demyelination and axonal injury in the central nervous system are responsible for neurological disability. Conventional magnetic resonance imaging (MRI) detecting T1 and T2 focal brain lesions is not specific to the underlying pathology, and it lacks sensitivity to the microstructural diffuse damage in the normal-appearing white matter (NAWM) (Filippi, Absinta, & Rocca, 2013). Conventional MRI markers correlate only moderately with clinical disability (Tintore et al., 2015), and their prognostic value in the assessment of disability progression in definite MS is limited (Filippi et al., 2013). Consequently, brain atrophy that has been related to long-term disability in MS (De Stefano et al., 2016) expresses the underlying pathological processes only nonspecifically. Confounding factors, such as disease-modifying therapies and causes unrelated to MS, complicate interpretation of MRI markers and atrophy in clinical practice (Kaunzner & Gauthier, 2017; Wattjes et al., 2015).

Diffusion tensor imaging (DTI) quantifies the magnitude and direction of water diffusion, and it is sensitive to diffuse microstructural abnormalities in the brain that appears unaffected on conventional MRIs (Rovaris et al., 2005). DTI-derived metrics, including fractional anisotropy (FA), mean diffusivity (MD), radial (RD), and axial (AD) diffusivities, seem to provide a better specificity to demyelination and axonal injury than conventional MRIs (Sun et al., 2006). Increased MD and decreased FA in the NAWM of different brain regions, including the corpus callosum (CC), have been typically detected in MS (Banaszek, Bladowska, Pokryszko-Dragan, Podemski, & Sasiadek, 2015; Preziosa et al., 2011; Sigal, Shmuel, Mark, Gil, & Anat, 2012). However, inconsistent results regarding the correlation between disability and DTI indices in the CC and the pyramidal tract have been reported in cross-sectional studies using different methods of DTI analysis and clinical scales of disability (Lin, Yu, Jiang, Li, & Chan, 2007; Llufriu et al., 2012; Pokryszko-Dragan et al., 2018; Roosendaal et al., 2009; Tortorella et al., 2014). The correlation between RD and secondary progression in MS has been observed in a 50-year clinical follow-up study indicating the potential role of DTI in the prediction of outcomes in MS (Andersen et al., 2018).

Previously, decreased FA and increased RD mostly in the CC of MS were observed in a 2-year longitudinal study (Harrison et al., 2011). In contrast, no changes in diffusivity were observed in the NAWM of MS over 2–4 years (Ontaneda et al., 2017; Rashid et al., 2008). Moreover, few studies with a short (1–2 years) follow-up have applied a regional and whole-brain DTI analysis to longitudinal measurements of diffusivity aiming to evaluate the prognostic value of DTI in the assessment of disability progression in MS (Rashid et al., 2008; Samann et al., 2012; Schmierer et al., 2004). In one of these studies, the increase of MD in the white matter of frontal lobe over 1 year was associated with clinical impairment in primary-progressive MS (Schmierer et al., 2004), while in another study, in early relapsing-remitting MS, no diffusivity changes were detected over 2 years (Rashid et al., 2008).

The investigation of the prognostic value of DTI in this cross-sectional and 4-year longitudinal study aims to assess white matter diffusion change and its stability in the relapsing-onset MS cohort considering the variable rate of disease progression.

2 | MATERIALS AND METHODS

The study was approved by the local ethics committee in the Hospital District of Pirkanmaa (R05157). All subjects provided informed written consent.

2.1 | Subjects

In total, 56 individuals, 46 patients with relapsing-onset MS, and 10 healthy subjects were enrolled in this 4-year follow-up study (between 2006 and 2012) at the Tampere University Hospital, Finland. The MS diagnosis was based on the revised McDonald criteria from 2005 (Polman et al., 2005) and the disease course classification on Lublin and Reingold criteria (Lublin et al., 2014). The inclusion criteria were a diagnosis of relapsing-remitting MS (RRMS) or secondary-progressive MS (SPMS), no steroid treatment at least 8 weeks before clinical and radiological assessments, and an Expanded Disability Status Scale (EDSS) score both at the study entry and after 4 years. Healthy subjects consisted of five females and five males, and the mean age of the subjects was 39.7 years (range 26–61). Healthy subjects were recruited from the hospital staff or their relatives with no history of neurological or psychiatric illness.

During the follow-up, MS patients underwent a clinical examination by the same neurologist at baseline and annually for 4 years (in total five examinations). Clinical progression was determined as the difference between the baseline EDSS and EDSS 4 years after the baseline. Progression of disability during the follow-up was defined as an EDSS score increase ≥1.0 when the baseline EDSS was <6.0 or an increase of EDSS ≥ 0.5 when the baseline EDSS ≥ 6.0, and these subjects were assigned to a progression group (Rovaris et al., 2003). All the other patients were included in the stable group.

2.2 | MR imaging acquisition

MRI volumetry included T1 and FLAIR brain lesion volume and brain atrophy measurements, and it was carried out at baseline for 42 MS patients. DTI in 46 cases was performed at baseline and 1 year after the baseline visit. Healthy controls were assessed with DTI at baseline.

The patients underwent MRI on the same day as a clinical examination. The patients and controls underwent a whole-brain imaging by using a 1.5-Tesla MR scanner (Magnetom Avanto SQ, Siemens Medical Solutions, Erlangen, Germany), and the MRI acquisition and protocol were as follows: T1-weighted header followed by an axial three-dimensional (3D) T1-weighted magnetization prepared rapid gradient echo (MPRAGE), 3D T2-weighted turbo spin echo, fluid-attenuated inversion recovery (FLAIR), T1-weighted spin echo with magnetization transfer contrasts, multidirectional diffusion-weighted echo-planar imaging, and gadolinium-enhanced

T1-weighted MPRAGE when needed. The DTI protocol consisted of a single-shot spin-echo-based echo-planar diffusion-weighted imaging with three averages and 12 gradient encoding directions, with b values of 0 and 1,000 s/mm^2. The imaging parameters are presented in Table 1.

2.3 | MR imaging postprocessing

The DTI data were analyzed with commercial Neuro 3D software (Siemens Healthcare, Malvern, USA) at an offline workstation. Multidirectional diffusion data were assessed visually for the presence of distortions and artifacts. There were no significant eddy current distortions due to the diffusion gradients. Six freehand regions of interest (ROI) of approximately 26–48 mm^2 (depending on the anatomical region) were positioned on the left and right posterior limbs of the internal capsule (IC), CC genu, left and right CC body, and CC splenium (Figure 1). The ROIs were manually placed exactly the same way at both time points on axial images of the color-coded FA maps and were automatically transferred on the MD, eigenvalues, and non-diffusion-weighted b_0 maps. The ROIs were centered on the anatomical structure in the most homogeneous area, with guidance from conventional T2 images to exclude focal lesions from the ROI and partial volume effect from border areas. The size of ROI was reduced if a focal lesion was identified in the ROI. The difference in ROI size between the baseline and 1-year follow-up was very small, <9% (range 1.8%–8.8%) in all ROIs. The values of the following DTI parameters were obtained: FA, MD, AD, and RD.

The whole brain volume of the T1 hypointense, FLAIR hyperintense lesions, and brain parenchymal fraction (BPF) were assessed blindly using the semi-automatic segmentation software Anatomatic™ 2.23 (Heinonen et al., 1998) by the same reader. BPF was defined as a ratio of brain parenchymal volume to the total volume within the brain surface contour (Rudick, Fisher, Lee, Simon, & Jacobs, 1999).

2.4 | Statistical analysis

Means and standard deviations were given for normally distributed variables and medians and ranges for skewed distributed data. For the demographic and volumetric data, groups were compared using independent sample *t* tests for normally distributed continuous variables and Mann–Whitney *U* tests for skewed distributed continuous variables. Spearman's rank correlations were determined for correlations between clinical and MRI parameters. The Wilcoxon test was used to perform comparisons between DTI values at baseline and 1 year. To investigate association between DTI metrics, volumetric measurements, and disability progression over 4 years, a series of logistic regression models were created. The presence or absence of disability progression was used as a dependent variable in all models. In logistic regression Model 1, the age and time from the onset (first symptoms) to baseline were set as covariates. In Model 2, the covariates were as follows: sex, disease duration (time from MS diagnosis to baseline), baseline EDSS, number of relapses up to 3 years preceding the baseline, immunomodulatory medication status, and volumetric measurements (T1/FLAIR lesion volume, BPF). A resulting odds ratio (OR) is given with 95% confidence interval (CI), and the *p*-value <0.05 was considered statistically significant. The Bonferroni-corrected *p*-values for six comparisons ($p < 0.008$) were also investigated in the analyses concerning DTI. A statistical analysis was performed using SPSS Statistics for Windows version 22 (IBM Corp., Armonk, NY, USA).

TABLE 1 Imaging parameters

	Axial T1WI	Axial FLAIR	Axial DTI
Slice thickness (mm)	0.9	5	5
Interslice gap (mm)	0	0	1.5
Field of view (mm)	230 × 230	230 × 230	230 × 230
Matrix	256 × 256	256 × 256	128 × 128
Echo time (ms)	4.2	100	96
Repetition time (ms)	1,160	8,500	3,500
Inversion time (ms)	600	2,500	

Note. DTI: diffusion tensor imaging; FLAIR: fluid-attenuated inversion recovery; T1WI: T1-weighted imaging.

3 | RESULTS

3.1 | Clinical and radiological assessment at baseline and over the follow-up

In total, 22 of 46 (48%) patients showed disability progression over 4 years. The mean age of patients at baseline was 39.6 years (range 18–61). The demographic and clinical characteristics are summarized in Table 2. Seven patients had one demyelinating plaque in the IC, four patients had one demyelinating plaque in the CC, and one patient had several plaques in the CC.

Incidental findings in the brain white matter were found in four healthy subjects from control group; three subjects had one to two punctate white matter hyperintensities, one subject had several punctate white matter hyperintensities, and none of healthy subjects presented clinical signs of demyelinating disease.

In MS, compared to healthy subjects, the strongest differences ($p < 0.001$) were found in the CC for FA, MD, and RD (Supporting Information Figure S1).

The FLAIR lesion volumes were significantly higher ($p < 0.05$) in the disability progression group compared to the stable disability group (Table 2). No significant correlations were found between baseline DTI and age, disease duration, baseline EDSS, and number of relapses before baseline (data not shown).

At baseline, significant correlations ($p < 0.008$, $r > 0.4$) were found between MRI volumetric measurements and DTI indices. The strongest correlations were found in the CC genu between the T1 brain lesion volume and FA ($p = 0.001$, $r = -0.48$), MD ($p < 0.001$, $r = 0.52$), RD ($p < 0.001$, $r = 0.52$) and between FLAIR lesion volume and FA ($p < 0.001$, $r = -0.6$), MD ($p < 0.001$, $r = 0.54$), and RD

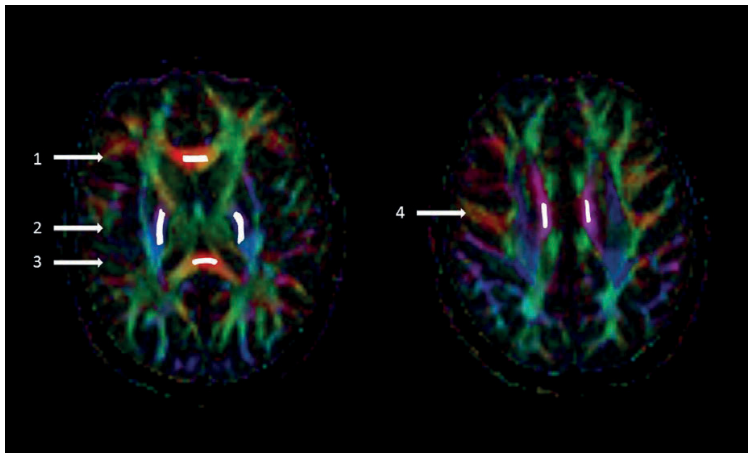

FIGURE 1 Freehand ROI placement on the color-coded fractional anisotropy axial maps. (1) Genu of the corpus callosum (size of ROI means 26 mm^2, range 13–71), (2) posterior limb of the internal capsule (48 mm^2, 13–81), (3) splenium of the corpus callosum (32 mm^2, 13–81), (4) body of the corpus callosum (26 mm^2, 19–84). Pixel size 1.8 × 1.8 mm

($p < 0.001$, $r = 0.6$). Regarding brain atrophy, the strongest correlations were found between BPF and RD ($p = 0.002$, $r = -0.46$) in the right CC body, RD ($p = 0.007$, $r = -0.41$) in the left CC body, MD ($p = 0.004$, $r = -0.44$) and AD ($p = 0.001$, $r = -0.49$) in the right IC, and MD ($p < 0.001$, $r = -0.53$) and AD ($p = 0.002$, $r = -0.47$) in the left IC (Supporting Information Table S1).

During the 1-year follow-up, FA significantly ($p < 0.05$) increased in 4/6 ROIs, and RD decreased in 4/6 ROIs (CC genu, body, and the CC splenium). AD showed a significant increase in 3/6 ROIs (the CC genu, CC body). The results remained significant except for RD in the CC genu and AD in left CC body after the Bonferroni corrections ($p < 0.008$). In the IC, the changes were nonsignificant (Table 3).

No group differences existed regarding DTI change over 1 year in any ROIs between disability progression and stable groups (data not shown).

To assess the intra-observer repeatability of DTI measurements, the intraclass correlations (ICCs) were calculated for 20 patients. The same observer (U.H.) repeated the measurements for the same scans with a time interval of approximately 3 months. In all ROIs, the ICCs were good and excellent and were 0.77–0.98 (mean 0.91) for FA, 0.75–0.96 (mean 0.84) for MD, 0.64–0.93 (mean 0.85) for AD, and 0.85–0.95 (mean 0.91) for RD.

3.2 | Association between MRI markers and disability progression

In logistic regression Model 1 with covariates of age and time from the onset to baseline (Table 4), a lower baseline FA and higher RD in the CC genu, right CC body, and the CC splenium were associated with disability progression ($p < 0.05$). Moreover, a higher baseline MD in the right CC body and higher MD and AD in the CC splenium were associated with disability progression. The results did not remain significant after the Bonferroni corrections. There were no significant associations between baseline DTI indices in the IC and disability progression over the follow-up. The age and symptom time had no effect in any of the analyzed ROIs.

In Model 2, which contained baseline EDSS and relapse number before baseline, an association between DTI and disability progression disappeared in the CC genu, body, and the splenium regrading several diffusivity parameters; however, none of these explanatory variables reached statistical significance (Supporting Information Tables S2 and S3). Medication, disease duration, and sex had no effect on disability progression (data not shown). T1, FLAIR, and BPF were not explanatory for disability progression (Supporting Information Table S4). However, the association between disability progression and DTI disappeared in the CC genu, and statistical power slightly decreased in the other CC areas in the models, including FLAIR lesion volume and BPF. The T1 lesion volume had no effect in any regression model (data not shown).

DTI change over 1 year did not relate to disability progression over 4 years in any ROIs (data not shown).

4 | DISCUSSION

The prognostic assessment of clinical disability accumulation by using conventional MRI is still suboptimal (Filippi et al., 2013), where additional challenges concern the individual and heterogenous disability progression. In the present study, the relapsing-onset MS patient cohort showed altered DTI indices at baseline compared to healthy controls, especially in the CC and to a lesser degree in the IC. The anatomical location of the observed differences may indicate that DTI is sensitive to microstructural abnormalities occurring in the NAWM tracts responsible for cognitive and locomotor functions. Our finding corroborates other reports showing that AD is less affected when compared to RD in the CC and the pyramidal tract,

TABLE 2 Demographic, clinical and radiological data for MS patients

	Whole group	Stable group	Progression group	*p*-Value[a]
No. of patients	46	24	22	
Female:male	31:15	17:7	14:8	0.6
Mean age at baseline, years, mean (range)	39.6 (18–61)	39.1 (20–61)	40.2 (18–58)	0.3
Median time from onset symptom to baseline, years (range)	9 (0.7–32.2)	7.6 (1.4–32.2)	12.3 (0.7–31.2)	0.6
Median disease duration, years (range)	4.2 (0–31.2)	2.3 (0–27.2)	5.9 (0–31.2)	0.1
EDSS, median (range)				
Baseline	2 (0–7)	1.5 (0–6)	3.0 (0–7)	0.2
Year 1	2 (0–7.5)	1.5 (0–6)	3.5 (0–7.5)	
Year 2	2.5 (0–8)	1.5 (0–6)	5.5 (0–8)	
Year 3	2 (0–8)	1.5 (0–6)	5.5 (0–8)	
Year 4	2 (0–8)	1.5 (0–6)	6.0 (1–8)	**<0.001**
Difference between EDSS over 4 years, median (range)	0.5 (−1.5 to 4)	0 (0.5 to −1.5)	1.5 (0.5–4)	
No. of relapses up to three years before baseline, no. of patients (%)				
0	15 (33)	5 (21)	10 (45)	0.07
1–2	24 (52)	14 (58)	10 (45)	
3–5	7 (15)	5 (21)	2 (10)	
No. of relapses during the follow-up, no. of patients (%)				
0	24 (52.2)	12 (50)	12 (54.5)	1.00
1–2	12 (26.1)	7 (29.2)	5 (22.7)	
3–6	10 (21.7)	5 (20.8)	5 (22.7)	
Duration of treatment at baseline, months, median (range)	18.5 (1–122)	18.5 (1–70)	15.5 (1–122)	0.9
Treatment at baseline, no. of patients (%)[b]	18 (39)	12 (50)	6 (27)	0.2
Treatment at the end of the follow-up, no. of patients (%)[b]	20 (43.4)	12 (50)	8 (36)	0.3
T1 brain lesion load at baseline cm^3, median (range)[c]	1.7 (0.1–28.5)	1 (0.1–28.5)	2.2 (0.1–14.7)	0.1
FLAIR brain lesion load at baseline cm^3, median (range)[c]	5.8 (1–39)	2.8 (1–39)	8.2 (1–33)	**0.03**
Brain parenchymal fraction at baseline, median (range)[c]	0.72 (0.6–0.81)	0.73 (0.64–0.8)	0.68 (0.6–0.81)	0.2

Note. EDSS, Expanded Disability Status Scale; Range was defined as minimum and maximum values.
[a]Comparison between stable versus progression groups, Mann-Whitney *U* test for median values, *t* test for mean values, and chi-square test for descriptive data; in bold, $p < 0.05$. [b]First-line treatment (beta-interferon, glatiramer acetate). [c]Values calculated for 42 MS patients (23 patients in stable group, 19 patients in progression group); there were no significant differences regarding clinical and demographic data between group of patients with DTI ($n = 46$) and the volumetric analysis.

including IC (Henry, Oh, Nelson, & Pelletier, 2003; Lin et al., 2007; Roosendaal et al., 2009).

In the NAWM of MS, FA is typically decreased, whereas MD is increased, expressing the loss of white matter tracts directionality and the increase in overall water diffusivity, respectively (Alexander, Lee, Lazar, & Field, 2007). Increased RD, a measure of perpendicular diffusivity to the fibers, is usually linked to demyelination (Fink et al., 2010). Diffusion parallel to the fibers, that is, AD, a marker of axonal integrity, is typically decreased and correlates clearly with axonal damage at the early stages of MS. At the chronic stage of MS, AD may conversely increase, representing the confounding effect of reparative processes, such as gliosis and cellular infiltration (Aung, Mar, & Benzinger, 2013). In this context, the nonsignificant difference between healthy controls and MS patients in our study may result from different directions of change in AD representing competing pathological processes at different progression stages in MS. The correlation between baseline brain lesion volume, brain atrophy, and DTI measurements in our MS group suggests that diffusivity abnormalities may be secondary to progression, both Wallerian degeneration of the axons passing through remote macroscopic brain

TABLE 3 DTI indices at baseline and after 1 year of the follow-up in MS patients

Relapsing-onset MS patients, *n* = 46										
	Baseline			Year 1			Annual change			
DTI metrics	Median	Min	Max	Median	Min	Max	Median	Min	Max	*p*-Value[a]
Corpus callosum genu										
FA	0.78	0.48	0.88	0.81	0.48	0.93	0.03	−0.08	0.14	**<0.001**
MD	0.80	0.62	1.31	0.82	0.67	1.07	−0.01	−0.34	0.20	0.891
AD	1.73	1.47	2.25	1.84	1.40	2.23	0.10	−0.62	0.43	**0.006**
RD	0.34	0.17	0.84	0.32	0.12	0.66	−0.04	−0.25	0.14	**0.009**
Corpus callosum body right										
FA	0.56	0.30	0.87	0.68	0.32	0.89	0.06	−0.17	0.30	**<0.001**
MD	0.83	0.58	1.08	0.81	0.70	1.11	0.01	−0.17	0.24	0.797
AD	1.47	1.07	1.84	1.63	1.13	1.95	0.12	−0.37	0.65	**0.003**
RD	0.53	0.20	0.92	0.44	0.20	0.75	−0.08	−0.33	0.11	**<0.001**
Corpus callosum body left										
FA	0.58	0.29	0.85	0.68	0.37	0.88	0.10	−0.18	0.31	**0.001**
MD	0.82	0.67	1.39	0.83	0.68	1.11	0.01	−0.42	0.24	0.589
AD	1.46	1.06	2.08	1.64	1.08	2.00	0.12	−0.37	0.65	**0.027**
RD	0.52	0.23	1.14	0.45	0.20	0.74	−0.09	−0.40	0.15	**<0.001**
Corpus callosum splenium										
FA	0.79	0.52	0.94	0.82	0.58	0.94	0.02	−0.07	0.20	**0.004**
MD	0.75	0.56	1.28	0.75	0.58	1.11	−0.02	−0.20	0.21	0.215
AD	1.67	1.23	2.13	1.69	1.42	2.07	0.04	−0.27	0.30	0.157
RD	0.28	0.11	0.86	0.25	0.09	0.69	−0.04	−0.31	0.14	**0.003**
Internal capsule right										
FA	0.72	0.62	0.83	0.71	0.55	0.85	0.00	−0.16	0.07	0.304
MD	0.74	0.66	0.79	0.74	0.67	0.83	0.01	−0.05	0.10	0.245
AD	1.46	1.33	1.73	1.45	1.27	1.80	0.00	−0.13	0.14	0.743
RD	0.36	0.24	0.47	0.37	0.23	0.52	0.00	−0.08	0.18	0.345
Internal capsule left										
FA	0.71	0.47	0.80	0.71	0.49	0.83	0.01	−0.18	0.32	0.814
MD	0.73	0.66	0.87	0.73	0.66	0.84	0.01	−0.06	0.07	0.092
AD	1.46	1.25	1.69	1.48	1.25	1.83	0.03	−0.20	0.45	0.068
RD	0.35	0.26	0.61	0.35	0.25	0.58	0.01	−0.27	0.15	0.566

Note. Annual change is defined as difference between median DTI value at 1 year and median DTI value at baseline.
DTI: diffusion tensor imaging; FA: fractional anisotropy; MD: mean diffusivity ($\times 10^{-3}$ mm^2/s); axial diffusivity ($\times 10^{-3}$ mm^2/s); radial diffusivity ($\times 10^{-3}$ mm^2/s).
[a]*p*-Value for Wilcoxon test; in bold, $p < 0.05$.

lesions (Ge et al., 2004; Lin et al., 2007) and brain atrophy due to the partial volume effect within voxels (Roosendaal et al., 2009).

The main observation in our study is the tendency for baseline DTI metrics' association in the CC with disability progression over 4 years with the most consistent and stable correlation observed in the CC splenium. We observed an increased baseline AD and RD, indirectly representing axonal integrity and demyelination, which is associated with disability progression even after correcting for focal lesion volume. This result corroborates observations in a previous study analyzing only FA maps where decreased FA in the CC splenium in primary-progressive MS (Bodini et al., 2013) was associated with EDSS progression over 5 years; however, longitudinal stability of DTI indices has not been analyzed in this study. As we investigated longitudinal changes in both AD and RD indices, which are more specifically related to MS pathology, we can speculate that inflammatory activity and axonal degeneration are responsible for clinical worsening in our MS cohort. Similar to our results, increased RD in the CC body has been associated with motor impairment expressed by the 9-hole peg test (NHPT) in a 1-year follow-up study with a small number (*n* = 22) of patients with RRMS (Kern, Sarcona,

TABLE 4 Relationship of baseline DTI metrics with disability progression measured by EDSS increase over the 4-year follow-up

	Stable group *n* = 24			Progression group *n* = 22						
DTI metrics	**Median**	**Min**	**Max**	**Median**	**Min**	**Max**	***p*-Value[a]**	**Odds ratio**	**95% CI**	
Corpus callosum genu										
FA	0.81	0.52	0.88	0.74	0.48	0.88	**0.04**	0.00	0.00	0.61
MD	0.80	0.62	1.21	0.83	0.67	1.31	0.06	1.05	1.00	1.10
AD	1.70	1.47	2.07	1.76	1.55	2.25	0.36	1.02	0.98	1.06
RD	0.28	0.17	0.80	0.37	0.17	0.84	**0.04**	1.05	1.00	1.09
Corpus callosum body right										
FA	0.67	0.40	0.87	0.52	0.30	0.77	**0.01**	0.00	0.00	0.24
MD	0.80	0.58	1.07	0.87	0.69	1.08	**0.04**	1.08	1.00	1.15
AD	1.51	1.07	1.79	1.39	1.18	1.84	0.25	0.98	0.95	1.01
RD	0.46	0.20	0.73	0.62	0.30	0.92	**0.01**	1.07	1.02	1.12
Corpus callosum body left										
FA	0.66	0.38	0.85	0.53	0.29	0.82	0.07	0.02	0.00	1.37
MD	0.81	0.67	1.32	0.84	0.70	1.39	0.12	1.03	0.99	1.08
AD	1.50	1.19	2.08	1.42	1.06	2.04	0.53	0.99	0.97	1.02
RD	0.46	0.23	0.93	0.58	0.30	1.14	**0.04**	1.04	1.00	1.08
Corpus callosum splenium										
FA	0.82	0.63	0.89	0.76	0.52	0.94	**0.04**	0.00	0.00	0.76
MD	0.71	0.56	0.95	0.79	0.68	1.28	**0.01**	1.13	1.03	1.23
AD	1.62	1.23	1.87	1.80	1.50	2.13	**0.01**	1.08	1.02	1.14
RD	0.25	0.16	0.50	0.35	0.11	0.86	**0.02**	1.07	1.01	1.14
Internal capsule right										
FA	0.72	0.62	0.78	0.72	0.62	0.83	0.89	1.01	0.89	1.14
MD	0.72	0.66	0.79	0.75	0.66	0.78	0.14	1.13	0.96	1.33
AD	1.45	1.33	1.61	1.49	1.34	1.73	0.16	1.05	0.98	1.12
RD	0.35	0.29	0.47	0.36	0.24	0.46	0.78	1.02	0.90	1.15
Internal capsule left										
FA	0.71	0.47	0.80	0.71	0.58	0.80	0.49	1.03	0.94	1.13
MD	0.71	0.66	0.87	0.74	0.66	0.80	0.15	1.12	0.96	1.32
AD	1.42	1.25	1.63	1.48	1.30	1.69	0.05	1.07	1.00	1.15
RD	0.35	0.26	0.61	0.36	0.31	0.48	0.86	0.99	0.90	1.09

Note. DTI: diffusion tensor imaging; FA: fractional anisotropy; MD: mean diffusivity ($\times 10^{-3}$ mm^2/s); axial diffusivity ($\times 10^{-3}$ mm^2/s); radial diffusivity ($\times 10^{-3}$ mm^2/s); EDSS: Expanded Disability Status Scale.

[a]*p*-Value for logistic regression adjusted for age and duration of symptoms for prediction of EDSS progression over the 4-year follow-up; in bold, $p < 0.05$.

Montag, Giesser, & Sicotte, 2011). Moreover, a histogram-based analysis revealed a correlation between whole-brain diffusivity alterations and disability progression expressed by the MS Functional Composite Scale over 1 year (Samann et al., 2012). The significance of our observation is strengthened by the fact that axonal degeneration, represented here by increased AD, is mainly responsible for sustained disability in MS (Tallantyre et al., 2010). The reason why the most stable correlation between DTI and disability progression was observed in the CC splenium of our study cohort might be related to thin axons that are densest in the splenium and their preferential susceptibility to injury in MS, as also suggested by others (Ciccarelli et al., 2003). As the CC body is a thin anatomical structure, the partial volume effect from cerebrospinal fluid may influence the results of DTI measurements in the region we observed. ROI-based methodology is sensitive to the change in DTI parameters, avoids postprocessing calculation errors, and is suitable for investigating well-defined brain structures such as CC and IC (Snook, Plewes, & Beaulieu, 2007). Good reproducibility of DTI measurements in our present and previous studies (Brander et al., 2010; Hakulinen et al., 2012; Kolasa et al., 2015), along with coherent fibers in the white matter tracts of the IC and the CC, suggests that diffusivity abnormalities, as observed here, may be related to white matter pathology

rather than method-based variability or crossing fibers within a voxel (Wheeler-Kingshott & Cercignani, 2009).

The 1-year longitudinal DTI analysis revealed a significant change of DTI metrics in the CC but not in the IC. In this cohort with active MS (Lublin et al., 2014), we observed an increase instead of the expected decrease of FA in the CC. This increase was driven by increased AD and decreased RD in the CC genu and the body and decreased RD in the CC splenium. Due to a short radiological follow-up with only two MRI examinations, we cannot fully determine the sustained changes in DTI parameters. A longitudinal DTI study with shorter interval MRI examinations would be more appropriate to evaluate the temporal changes in diffusivity (Tian et al., 2012). Moreover, without healthy controls in the longitudinal analysis, we cannot clearly assess pathophysiological processes involved in temporal DTI changes observed here in the CC. Similar to our observation, serial DTI study using tractography showed significant longitudinal change in DTI metrics in the supratentorial brain and the CC of the MS cohort with different disease phenotypes (Harrison et al., 2011). However, such temporal DTI evolution was not observed in a recent ROI-based MS study including natalizumab-treated patients (Ontaneda et al., 2017). In another study in early RRMS with a 2-year follow-up, the rate of change in diffusivity characteristics assessed by a histogram-based whole-brain analysis did not correlate with disability progression expressed by an EDSS increase, which confirms our results (Rashid et al., 2008). Conversely, the association between diffusivity in the frontal NAWM and disability as measured by the MS Functional Composite Scale has been found in primary-progressive MS (Schmierer et al., 2004). Thus, inconsistent results observed in previous studies may relate to technical differences, intrinsic heterogeneity of MS (Barone et al., 2018) and MS cohorts, and different clinical scales used in disability evaluation in MS.

Altogether, the results of our longitudinal study suggest that DTI is a sensitive tool in monitoring diffuse abnormalities responsible for disability accumulation, and CC may be a good target for DTI analysis. We believe that an assessment of the prognostic value of DTI in an MS cohort with variable clinical characteristics such as ours which is typically encountered in everyday practice has practical value, as suggested by others (Harrison et al., 2011). Moreover, changes in RD observed here may play an important role in monitoring immunomodulatory treatment effects because the attenuation of inflammatory demyelination is the main target of current MS therapies. This statement is supported by the results of the study by Fox et al., where DTI abnormalities indicating remyelination have been observed after starting natalizumab treatment (Fox et al., 2011).

We did not observe any associations in the IC between baseline DTI and disability progression. Moreover, no longitudinal changes in DTI metrics were observed in the IC, although significant differences related to DTI between healthy controls and the MS group were already observed. This result indicates that diffusivity abnormalities may already exist in the IC, but they progress at different rates, and image disability progression distinctly than in the CC (Ge et al., 2004). Our finding is supported by studies where no correlation between DTI indices in the corticospinal tract and disability progression expressed by an EDSS increase has been observed (Fritz, Keller, Calabresi, & Zackowski, 2017; Lin et al., 2007). Conversely, such correlation between DTI parameters and EDSS has been previously reported in cross-sectional studies (Daams et al., 2015; Tovar-Moll et al., 2015).

Our results corroborate the observed lack of clear-cut association between the T1/T2 brain lesion load, brain atrophy, and disability progression expressed by EDSS change in other follow-up studies for over 2 years in relapsing MS (Enzinger et al., 2011; Tiberio et al., 2005). Although volumetric measurements did not clearly correlate with disability progression in our study, the FLAIR lesion volume and BPF showed some effect and modified the correlation between DTI and disability progression. In contrast to our results, association between short-term physical worsening, T2 brain lesion load (Gauthier et al., 2007; Moodie et al., 2012), and brain atrophy has been reported elsewhere (Minneboo et al., 2008; Samann et al., 2012). These discordant results suggest that the focal brain lesion load and brain atrophy may have additional impact on disability accumulation in relapsing-onset MS. The lack of significant correlation here may be limited by a small number of cases in the study cohort, where disease activity and disability progression were variable. Other limitations in our inferences may result from the fairly gross nature of total EDSS in a situation where there is a need to evaluate subtle changes in motor functions during a short observation period.

In conclusion, our results among others suggest that diffusivity abnormalities exist in relapsing-onset MS patients; however, their dynamic change over time is different with respect to anatomical location. Additionally, diffusivity metrics in the normal-appearing CC may be associated with disability accumulation in relapsing-onset MS and suggest the crucial role of the CC in monitoring disease progression. Given its high sensitivity in detecting diffuse brain abnormalities, DTI indices may serve as a potential biomarker of disease progression; however, method standardization is needed. Moreover, stability and sensitivity to underlying pathology of DTI metrics have to be confirmed in longitudinal studies (Wattjes et al., 2015). A combination of diffusion measures with other findings from conventional MRI may provide complementary information on different types of pathological damage in MS.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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SUPPORTING INFORMATION

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PUBLICATION III

Anti-JC virus seroprevalence in a Finnish MS cohort

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Anti-JC virus seroprevalence in a Finnish MS cohort

Kolasa M, Hagman S, Verkkoniemi-Ahola A, Airas L, Koivisto K, Elovaara I. Anti-JC virus seroprevalence in a Finnish MS cohort. Acta Neurol Scand 2016: 133: 391–397.

Background – The risk of progressive multifocal leukoencephalopathy (PML) caused by the JC virus (JCV) is increased in patients with multiple sclerosis receiving biological therapies. *Objectives* – To determine the seroprevalence of anti-JCV antibodies in Finnish patients with multiple sclerosis (MS) and clinically isolated syndrome and to assess the clinical risk factors for JCV seropositivity. *Methods* – The JCV seroprevalence was analyzed in 503 patients using a second-generation two-step ELISA. Sixty-seven patients underwent longitudinal serological evaluation over 4.5 years. *Results* – The overall seroprevalence of JCV was 57.4%. The seropositivity was higher in men than in women, tended to increase with age, and was not affected by different immunomodulatory therapies. However, in patients with ongoing natalizumab treatment (n = 72), the anti-JCV antibody screening index was lower than in patients without such therapy [median 0.3 (range 0.1–3.1) vs 0.6 (0.1–3.1), respectively, P = 0.01]. Over 4.5 years, 4/19 (21%) initially seronegative patients converted to seropositivity, whereas 4/48 (8.3%) initially seropositive patients reverted to seronegativity. Fluctuations in serostatus were observed in 3/67 patients. *Conclusion* – The study confirmed a high anti-JCV antibody prevalence in patients with MS and its association with age and male gender but not with disease-modifying therapies. Our data suggest that therapy with natalizumab may cause a decrease in anti-JCV antibody levels, suggesting an immunosuppressive effect of natalizumab without an impact on JCV seroprevalence. The results of studies performed until now confirm the predictive value of anti-JCV antibody measurement in the assessment of PML risk; however, changes in serostatus need to be considered.

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Key words: multiple sclerosis; JC virus; anti-JCV antibodies; screening index

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Introduction

The John Cunningham virus (JCV) is an etiologic agent of progressive multifocal leukoencephalopathy (PML), an opportunistic infection of the brain that develops in immunocompromised subjects (1). The increasing use of biological therapies for the treatment of multiple sclerosis (MS) and other diseases such as rheumatic diseases, psoriasis, Crohn's disease, and non-Hodgkin's lymphoma has drawn attention to the significance of JCV due to its association with PML (2). The primary exposure to JCV occurs in early childhood, and the anti-JCV seroprevalence increases with age (3, 4). Approximately 50–60% of the adult population is infected, as determined by the presence of anti-JCV IgG (5, 6). After the spreading HIV epidemic in the 1980s, the incidence of PML increased up to 1-3/1000/year (7). The risk of PML in natalizumab-treated patients with MS depends on positive JCV serostatus, prior immunosuppressant use, and duration of natalizumab therapy reaching value of 11/1000 (8). In this study, we evaluated for the first time the prevalence of anti-JCV antibodies in a cohort of Finnish patients with MS and assessed whether demographic factors and MS therapies have an effect on the anti-JCV antibody status. The rate of JCV seroconversion and seroreversion over the 4.5-year follow-up period was also analyzed.

Materials and methods

In this prospective study, sera and clinical and demographic data were collected from 503 patients including 474 patients with MS and 29 with clinically isolated syndrome (CIS). The patients were enrolled consecutively from four Finnish MS centers (Tampere, 140 patients; Helsinki, 114 patients; Seinäjoki, 98 patients; and Turku, 54 patients) between January 2012 and February 2013. To assess longitudinal changes in anti-JCV antibody levels, a subset of samples from 97 patients with CIS ($n = 20$) and MS ($n = 77$) was collected annually over approximately 4.5 years (between December 2006 and September 2012). These samples, as well as clinical and demographic data, were collected at the Tampere University Hospital as part of a prospective 5-year study aimed at identifying candidate biomarkers for MS. CIS was defined as the first episode suggestive of inflammatory demyelinating disease with no paraclinical evidence of dissemination over time (9). The diagnosis of MS was based on the 2005 McDonald criteria (10) for the patients included in the longitudinal study and on the 2010 McDonald criteria (11) for the patients included in the cross-sectional analysis. Before JCV antibody testing, all samples were stored at −80°C and analyzed at Unilabs, Denmark. A confirmatory second-generation ELISA (STRATIFY JCV™ DxSelect, Focus Diagnostics, Cypress, CA, USA) was used for testing the sera for anti-JCV antibodies (12). In brief, the assay consisted of a screening enzyme-linked immunosorbent assay (ELISA) and a supplemental confirmation test. A screening index value of <0.2 was considered anti-JCV antibody negative, whereas a screening index value >0.4 was considered anti-JCV antibody positive. The samples with a screening index between 0.2 and 0.4 were evaluated with a confirmation test in which results greater that 45% were classified as anti-JCV antibody positive (13). The screening index values between 0.2 and 0.4 were also included in the calculations of median and range. This study was approved by the local ethics committee, and all patients provided written informed consent.

The valid percent with 95% confidence intervals (CI) was used to assess JCV seroprevalence. Chi-square or Fisher's exact tests for categorical data and *t*-tests or Wilcoxon's rank test for continuous data were used to analyze the associations among the demographic factors, MS treatment, and JCV seroprevalence. Logistic regression was used for the multivariate analysis, and the odds ratio and 95%CI were calculated. A P value <0.05 was considered statistically significant.

Results

The primary demographic and clinical characteristics are summarized in Table 1.

Cross-sectional study

Overall, the seroprevalence of anti-JCV antibodies in the cohort of 406 was 57.4% (95%CI 52.6–62.2). In JCV seronegative patients, the median value of the anti-JCV antibody screening index was 0.19 (range 0.1–0.39), while in seropositive patients, it was 1.64 (range 0.26–3.12). The rate of seropositivity was higher in men than in women (67% vs 54%) ($P = 0.02$) and tended to increase with age (Fig. 1). A longer duration of MS and the time from the initial symptoms were linked with a higher seroprevalence. However, after adjusting for age and gender, the associations between JCV seroprevalence, disease duration, and time from the onset of symptoms appeared

Table 1 Patient clinical and demographic characteristics

	Cross-sectional study	Longitudinal study
Number of patients	406	67
Mean age, years (SD)	40.9 (10.4)	41.9 (12)
Sex (males/females, n)	101/305	22/45
MS subtypes, n		
CIS	9	12
RRMS	350	29
SPMS	39	12
PPMS	8	14
Time from first symptoms, years*	9.7 (0–40.9)	10.6 (0.5–42)
MS duration, years*	6.9 (0–34.2)	4.4 (0–31.2)
EDSS*	2 (0–7)	2 (0–7)
Relapses 2 years preceding study, *n*		
0	235	16
1	101	21
2–5	70	16
Prior and current natalizumab therapy, *n*	92	0
Duration of natalizumab therapy (prior and current), months*	25.5 (1–69)	–
Prior and current immunomodulator therapy, *n*[1]	348	22
Prior and current immunosuppressant therapy, *n*	35[2]	3[3]

CIS, clinically isolated syndrome; RRMS, relapsing–remitting multiple sclerosis; SPMS, secondary progressive multiple sclerosis; PPMS, primary progressive multiple sclerosis; EDSS, expanded disability status scale.
*Median (range), n number of patients.
[1]Interferons, immunoglobulins i.v., or glatiramer acetate.
[2]Mitoxantrone, azathioprine, or fingolimod.
[3]Mitoxantrone.

non-significant (Table 2). Interestingly, the number of relapses in the 2 years preceding the study was associated with the anti-JCV seroprevalence ($P = 0.009$). After adjusting for age and gender, this association still remained significant ($P = 0.016$) (Table 2). The majority of patients (72.2%) were treated with MS drugs at the study entry. No association was observed between JCV positivity and other demographic parameters, such as the MS subtype or EDSS score.

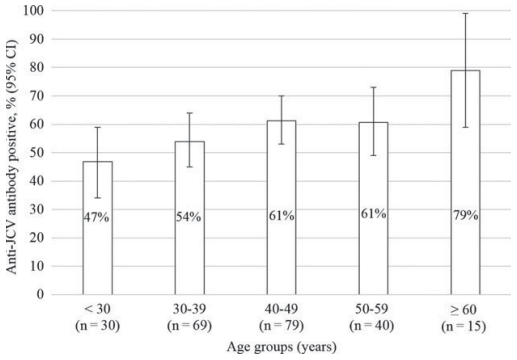

Figure 1. Anti-JCV antibody seropositivity according to age group (ANOVA, $P = 0.08$).

Furthermore, the prevalence was similar regardless of prior and ongoing MS treatment, including immunomodulators, natalizumab, immunosuppressants, and corticosteroids (Table 2). It is noteworthy that the screening index value of the 72 patients with ongoing natalizumab therapy (median time of treatment 31 months, range 1–69) was lower than in the 334 patients without current natalizumab use (median screening index 0.3 (range 0.1–3.12) vs 0.64 (0.1–3.12), respectively, $P = 0.01$) (Fig. 2). In contrast, there was no difference in anti-JCV antibody prevalence by current treatment with natalizumab (logistic regression adjusted for age and sex, $P = 0.2$).

Longitudinal study

This study included 97 patients. For 67 of the patients, test results were obtained both at baseline and at the end of the observation period, thus allowing the assessment of serological changes over the entire follow-up. The reasons for the incomplete data for 30 patients (CIS $n = 8$ and MS $n = 22$) were as follows: 24 patients declined to continue participation in the study, and in six patients, the quality of the samples was suboptimal. At baseline, 19/67 patients

Table 2 Anti-JCV antibody seroprevalence in subgroups; cross-sectional study

	JCV-positive	JCV-negative	*P*-value**
Mean age, years (SD)	42.1 (10.2)	39.4 (10.6)	**0.01**[1]
MS types, *n* (%)			
CIS	7 (77.8)	2 (22.2)	0.2[2]
RRMS	198 (56.6)	152 (43.4)	
SPMS	21 (53.8)	18 (46.2)	
PPMS	7 (87.5)	1 (12.5)	
Time from first symptoms, years*	10.7 (0–40.9)	8.7 (0.4–35.8)	0.03[3]/0.5[4]
MS duration, years*	7.6 (0–34.2)	5.3 (0–32.5)	0.02[3]/0.5[4]
EDSS*	2 (0–7)	2 (0–7)	–
Relapses 2 yrs preceding study, *n* (%)			
0	136 (57.9)	99 (42.1)	**0.016**[4]
1	67 (66.3)	34 (33.7)	
2–5	30 (42.9)	40 (57.1)	
Prior and current natalizumab therapy, *n* (%)			
No	181 (57.6)	133 (42.4)	0.7[4]
Yes	52 (56.5)	40 (43.5)	
Duration of natalizumab therapy (prior and current), months*	23 (1–64)	32.5 (2–69)	0.4[3]
Prior and current immunomodulators (i.v. immunoglobulins, interferon, glatiramer acetate), *n* (%)			
No	36 (62.1)	22 (37.9)	0.6[4]
Yes	197 (56.6)	151 (43.4)	
Prior and current immunosuppressant therapy (mitoxantrone, azathioprine, fingolimod), *n* (%)			
No	215 (58)	156 (42)	0.4[4]
Yes	18 (51.4)	17 (48.6)	
Prior steroid therapy, *n* (%)			
No	66 (60)	44 (40)	0.5[4]
Yes	167 (56.4)	129 (43.6)	

[1]*P* value of the unpaired *t*-test; [2]*P* value of the chi-square test; [3]*P* value of the 2-sided Mann–Whitney *U*-test; [4]*P* value of the logistic regression adjusted for age and sex; *Median (range); **P values in bold are accepted as significant; n number of patients.

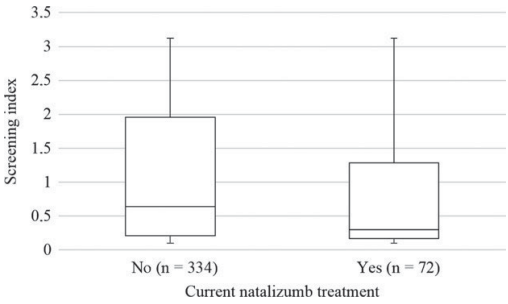

Figure 2. Anti-JCV antibody screening index levels according to current natalizumab use (horizontal line, median; box, interquartile range; horizontal bars, range; 2-sided Mann–Whitney *U*-test, $P = 0.01$).

were JCV seronegative, four of whom became anti-JCV antibody positive (21%), and the remaining 15 patients maintained seronegativity (79%) over 4.5 years (SD 0.3). Among the patients who were JCV seropositive at baseline (48/67 subjects), four subjects (8.3%) reverted to an anti-JCV antibody-negative status and 44 remained seropositive (91.7%) over 4.4 years (SD 0.3). In the patients who converted to seropositivity, there was an evident increase in the screening index values between the baseline [median 0.18 (range 0.16–0.34)] and the final analysis [1.7 (0.6–2.8)], although statistical significance was not reached, most likely because of the small number of patients ($P = 0.1$). In the four patients who reverted to JCV seronegativity, the screening indices preceding such change were near the positive cut-off values of the assay [mean 0.4, range 0.3–0.49]. Only one patient with a low screening index (0.16) after seroreversion showed high antibody levels [mean 2.9, range 2.6–3.1] before the change. In the patients who maintained JCV seropositivity during the 4.5 years, the anti-JCV antibody screening indices were stable in spite of marked interindividual variation in the magnitude of indices (at 1st measurement: median 2.4 (range 0.4–2.8), 2nd: 2 .3 (0.1–3.1), 3rd: 2.4 (0.3–2.9), 4th: 2.4 (0.1–3), and 5th: 2.5 (0.4–3.2)).

As we performed serological measurements annually over the follow-up period, we could evaluate the intermittent changes in the anti-JCV antibody status. During the follow-up period, intermittent conversion to seronegativity at one time point was observed in 3 of the 67 patients (4.5%); in two of them, the screening indices fluctuated around the positive cut-off point of the assay [mean 0.47 (range 0.11–0.7)], while the third patient showed marked variation [median 2 (range 0.13–2.53)].

During the follow-up period, three patients received natalizumab (the duration of the treatment was 11, 16, and 18 months). These patients were anti-JCV antibody positive for the entire follow-up period. In addition, three patients received mitoxantrone; one of them converted to JCV seropositivity, another one was persistently seronegative, and the remaining patient seroreverted to JCV negativity.

Discussion

The purpose of this study was to assess the prevalence and stability of anti-JCV antibodies in a Finnish cohort including patients with MS and CIS.

The overall anti-JCV antibody seroprevalence was 57%, which is consistent with previous reports from multinational populations of patients with MS (5, 14). Moreover, our cross-sectional results confirm earlier observations that both male gender and a higher age are associated with more frequent JCV positivity (5, 15–18). Notably, a longer duration of MS and time from the onset of symptoms were not related to a higher JCV seroprevalence in the multivariate analysis. The increasing seroprevalence of JCV over time confirms the moderate temporal stability of the anti-JCV antibody status. Unlike the polyomavirus BK, which already has a high seroprevalence in children and is stable over time, the JCV infection rate increases throughout life, reaching its highest levels in the aging population (6, 17, 19).

Interestingly, the patients with ongoing natalizumab therapy had lower anti-JCV antibody levels than the patients without such therapy. However, no difference was found in the magnitude of the JCV seropositivity. The lack of an association between anti-JCV antibody prevalence and MS treatment has been previously reported in other studies (5, 16, 20). In contrast, Outteryck et al. showed that the duration of natalizumab exposure is a risk factor for JCV seropositivity that might be related to the asymptomatic reactivation of JCV in natalizumab-treated patients (21). The lower levels of anti-JCV antibodies in our natalizumab-treated patients are in line with the results reported by Warnke et al. where the initiation of natalizumab was associated with a decrease in anti-JCV and antivaricella-zoster antibody levels (22). Moreover, natalizumab treatment leads to decrease in serum IgM and IgG levels in patients with MS as reported by Selter et al. (23). These results suggest the immunosuppressive effect of natalizumab, but the mechanism

of such an effect is not fully understood. It has been proposed that natalizumab impairs the homing of B cells to their niches, which might lead to a loss of pro-survival stimuli relevant to the persistence and quality of the antibody response to recall antigens (22). The other investigators suggested that natalizumab inhibits $\alpha 4\beta 1$-integrin preferentially on B cells (24) and increases the levels of circulating lymphocytes, preferentially of immature pre-B cells (25). Moreover, natalizumab treatment was associated with decreased levels of plasmacytoid dendritic cells, which display an activated phenotype (26). The recent study by Plavina et al. emphasized the importance of JCV antibody screening index measurements in patients with MS. Higher anti-JCV antibody levels in the serum/plasma of MS patients with no prior immunosuppressant use have been associated with an increased PML risk compared with patients with lower screening index values (27).

Our results indicating the link between the number of relapses and JCV seroprevalence may not be explained simply by the influence of the treatment, as the number of patients undergoing any prior and current MS therapy was similar in the anti-JCV antibody positive and negative groups. It has been shown that the cytokines TNF-α, IL-1β, and IL-6 can stimulate the transcription factors involved in JCV replication (28). Moreover, the changes in the levels of these proinflammatory cytokines are associated with disease activity in patients with MS (29–32). Therefore, it may be that peripheral immune activation in patients with active MS could potentially affect the JCV replication and the production of anti-JCV antibodies. One way to study this further would be to relate the anti-JCV antibody levels to the levels of total IgG in the same sample.

The temporal instability of the JCV seroprevalence during the follow-up period was detected in this study. Marked and consistent increases in the screening indices in the patients who converted to JCV seropositivity may suggest the activation of a humoral immune response to a new JCV infection, or alternatively, to a new site of infection or change in the viral load due to a re-exposure to JCV (22). Earlier studies using the first- and second-generation two-step ELISA reported seroconversion rates ranging from 2% to 36% and seroreversion rates between 1.5 and 4.7%, respectively (18, 20, 33, 34). As the second-generation assay is in good agreement with the original test showing similar seroprevalence (12), the differences in the results may not be explained only by the different methodologies, as suggested by others (34).

It is noteworthy that the screening indices of six patients fluctuated around the cut-off value of the assay. This result appears to reflect the natural fluctuation of antibodies or the analytical variability of the assay (18). Additionally, the potential cross-reactivity of the antibodies with other polyomaviruses, innate differences in individuals' production of antibodies, and non-specific changes in the levels of antibodies relating to disease activity may also influence the results of the assay. Because the risk of natalizumab-associated PML depends on the presence of anti-JCV antibodies (8), the interpretation of the test results may lead to uncertainty in patients with screening indices near the cut-off points. Moreover, the overestimation of PML risk may occur, as the high or increasing anti-JCV antibody levels have been associated with the onset of PML (18, 22, 27, 35). Additionally, the false-negative rate of JCV serology, ranging from 2.5% to 37% (20, 36), combined with the increase in JCV seroprevalence with age suggests the need for repeated testing of JCV antibody-negative patients treated with natalizumab (20, 36).

The definitive diagnosis of PML is based on the detection of virus DNA in the cerebrospinal fluid or on a brain biopsy to demonstrate the histopathological changes caused by the replicating JCV (37). The assessment of the L-selectin-expressing CD4 T cells (38) and the cerebrospinal fluid JCV antibody index (39) has been proposed recently for PML risk stratification. Testing for JCV DNA in the blood or urine was found to be non-predictive (reviewed by Rudick et al. (40)).

In summary, this study confirmed the high prevalence of anti-JCV antibodies among patients with MS. Although MS treatment does not seem to have an influence on JCV seropositivity, the patients using natalizumab had lower levels of anti-JCV antibodies, suggesting an immunosuppressive effect of natalizumab. Due to fluctuations in JCV serostatus, further studies focusing on the longitudinal changes in the anti-JCV antibody levels as well as the identification of new PML markers are needed.

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Conflict of interests

The authors have no conflict of interests.

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