MARITTA UKKONEN

Primary Progressive Multiple Sclerosis

Clinical, immunological and radiological study

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UNIVERSITY OF TAMPERE

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Mikko Reinikka

CONTENTS

LIST OF ORIGINAL COMMUNICATIONS

This thesis is based on the following original communications that are referred to in the text by the Roman numerals I-IV.

I Ukkonen M, Dastidar P, Heinonen T, Laasonen E, Elovaara I. Volumetric quantitation by MRI in primary progressive multiple sclerosis: volumes of plaques and atrophy correlated with neurological disability. Eur J Neurol. 2003;10:663-9.

II Ukkonen M, Elovaara I, Dastidar P, Tammela TLJ. Urodynamic findings in primary progressive multiple sclerosis are associated with increased volumes of plaques and atrophy in the central nervous system. Acta Neurol Scand. 2004;109:100-5.

III Ukkonen M, Wu X, Reipert B, Dastidar P, Elovaara I. Cell surface adhesion molecules and cytokine profiles in primary progressive multiple sclerosis. Mult Scler 2007;13:701-7.

IV Ukkonen M, Vahvelainen T, Hämäläinen P, Dastidar P, Elovaara I. Cognitive dysfunction in primary progressive multiple sclerosis: a neuropsychological and MRI study. Mult Scler 2009;15:1055-61.

ABBREVIATIONS

AM	adhesion molecule
BBB	blood brain barrier
BDI	Beck Depression Inventory
BNT	Boston naming test
c	cell surface
CIS	clinically isolated syndrome
CNS	central nervous system
CSF	cerebrospinal fluid
CYC	cyclophosphamide
DAWM	diffusely abnormal white matter
DIR	double inversion recovery
DIS	dissemination in space
DIT	dissemination in time
DSD	detrusor sphincter dyssynergia
DTI	diffusion-tensor imaging
EAE	experimental autoimmune encephalomyelitis
EBV	Ebstein-Barr virus
EDSS	expanded disability status scale
fMRI	functional magnetic resonance imaging
Gd	gadolinium
GM	grey matter
HLA	human leukocyte antigen
ICAM-1	intercellular adhesion molecule 1
IgG	immunoglobulin G
IL	interleukin
IFN	interferon
IM	immunomodulatory
LFA-1	lymphocyte function associated antigen 1
MAB	monoclonal antibody
MCP-1	monocyte chemoattractant protein-1
MIP	macrophage inflammatory peptide
MRI	magnetic resonance imaging
MRS	magnetic resonance spectroscopy
MS	multiple sclerosis
MSFC	multiple sclerosis functional composite
MTI	magnetisation transfer imaging
MTR	magnetisation transfer ratio
NAWM	normal appearing white matter
NAGM	normal appearing grey matter
OCBs	oligoclonal bands

PASAT	paced auditory serial addition test
PPMS	primary progressive multiple sclerosis
PRMS	progressive relapsing multiple sclerosis
RRMS	relapsing remitting multiple sclerosis
RFSS	regional functional scoring system
s	soluble
SD	standard deviation
SDMT	symbol digit modalities test
SE	spin echo
SPMS	secondary progressive multiple sclerosis
SRT	spatial recall test
T	tesla
Th	T helper cell
TNF	tumor necrosis factor
VCAM-1	vascular cell adhesion molecule 1
VEP	visual evoked potential
VLA-4	very late activation antigen 4
WAIS-R	Wechsler adult intelligence scale-revised
WM	white matter
WMS	Wechsler memory scale

ABSTRACT

Multiple sclerosis (MS), which is the most frequent demyelinating disease of the central nervous system (CNS) has very heterogeneous clinical presentations. The majority of patients have relapsing-remitting disease course characterised by episodes of acute exacerbation. Primary progressive MS (PPMS) is a rare subtype of MS that is characterized by a steady progression of irreversible disability from the onset of the disease.

The main goal of this thesis was to identify clinical and immunological features of PPMS and to correlate these with magnetic resonance imaging (MRI) findings. The specific aims were the following: 1) to characterise the neurological, cognitive and urological abnormalities in PPMS; 2) to identify whether there are specific immunological changes typical of progressive MS subtypes; 3) to characterise the volumes of MRI abnormalities in the brain and spinal cord in PPMS and 4) to evaluate whether the volumes of focal, diffuse and atrophic CNS abnormalities in MRI are associated with clinical and immunological features of PPMS.

The study included patients with PPMS (n=28), secondary progressive MS (SPMS, n = 28) and healthy control subjects (n = 20). All patients and controls underwent detailed neurological examination, MRI and neuropsychological examination. Expression of adhesion molecules (AMs) and levels of 17 different cytokines/chemokines were measured in blood and cerebrospinal fluid (CSF). Patients with PPMS and healthy controls underwent MRI. Furthermore, urodynamic investigations of patients with PPMS were performed.

The most common symptoms of PPMS at the onset of disease included motor, cerebellar and sensory disturbances. Disturbances in motor functions predominated, other neurological dysfunction involving cerebellar, brain stem, sensory, cerebral or visual problems. All patients with PPMS had at least one micturition complaint, with urgency and urge incontinence being the most common complaints. Urodynamic abnormalities were found in the majority of patients, the most common being detrusor sphinkter dyssynergia (DSD) and detrusor hyperreflexia. Neuropsychological examination showed that, patients with PPMS performed better than those with SPMS in a visual learning test, but other significant differences were not found. Immunological examinations showed that, the expression of most AMs on blood and CSF immune cells were higher in patients with PPMS than in patients with SPMS or healthy subjects, but no marked differences were detected in levels of cytokines and chemokines.

MRI showed that atrophic changes were more pronounced in patients with PPMS compared with healthy controls. Clinicoradiological correlations revealed weak but some significant correlations between MRI measures and disability measures, including ambulation index, arm index and higher cerebral disturbances were found. No MRI measures correlated with expanded disability status scale score. The number of diffuse brain lesions correlated with attention and information processing, concept formation, reasoning and executive functions, verbal production and learning, visuoperceptual and visuoconstructive function. All other cognitive domains except attention and information processing correlated with the volume of T2-weighted brain lesions. Atrophic changes in the CNS did not correlate with any cognitive measures. Considering urodynamics, detrusor hyperreflexia and DSD correlated with T2-weighted brain lesion load and hypotonic detrusor was associated with an increased number of thoracic plaques and decreased brain volume. The expression of AM very late activation antigen 4 on blood lymphocytes and CSF lymphocytes and monocytes correlated with the total volume of brain lesions and the number of diffuse brain lesions.

In summary, this thesis focusing on the identification of clinical, immunological and radiological characteristics of PPMS, revealed only weak correlations between clinical parameters and MRI measures. Patients with PPMS showed declines in several cognitive domains that did not differ markedly from those in patients with SPMS. Micturition problems were very common in PPMS and with typical urodynamic problems being DSD and detrusor hyperrefleksia. Upregulated expressions of AMs and some cytokines in the blood and CSF of MS patients compared with controls indicates the presence of persistent inflammation even in neurodegenerative subtypes of MS.

TIIVISTELMÄ

Multippeliskleroosi (MS) on yleisin demyelinoiva keskushermoston tulehduksellinen sairaus. MS-taudin kliininen kuva on hyvin monimuotoinen. Taudin yleisin ilmenemismuoto on aaltomainen MS-tauti (relapsoiva-remittoiva MS, RRMS), joka useimmilla muuttuu vuosien kuluessa toissijaisesti etenevään tautimuotoon (sekudaarisprogressiivinen MS, SPMS). Ensisijaisesti etenevä MS-tauti (primaarisprogressiivinen, PPMS) on harvinainen MS-taudin alatyyppi.

Tutkimukseen osallistuneille PPMS- ja SPMS-potilaille (n=56) sekä terveille verrokeille (n=20) tehtiin kliininen neurologinen tutkimus ja laaja neropsykologinen kartoitus. Selkäydinnestenäytteestä ja verestä tutkittiin adheesiomolekyylejä, sytokiinejä ja kemokiinejä. PPMS-potilaille ja terveille kontrolleille tehtiin koko keskushermoston magneettitutkimus (MRI) ja PPMS-potilaille tehtiin lisäksi urodynaaminen tutkimus.

PPMS-potilailla yleisimmät taudin alkuoireet olivat motoriset-, pikkuaivo- ja tuntohäiriöt. Motorisen toiminnan häiriöt olivat yleisimmät löydökset kliinisessä neurologisessa tutkimuksessa. Kaikilla PPMS-potilailla oli virtsaustoiminnan häiriöitä, joista tihentynyt virtsaamistarve ja siihen liittyvä virtsan karkailu olivat yleisimmät oireet. Urodynaamisen tutkimuksen yleisimmät löydökset olivat detrusor sphinkterin dyssynergia (DSD) and detrusorin hyperreflexia. Neuropsykologisessa kartoituksessa todettiin PPMS-potilailla heikentymistä useilla eri osa-alueilla verrattuna terveisiin verrokkeihin, mutta tilastollisesti merkitseviä eroja PPMS- ja SPMS-potilaiden välillä ei ollut. Vertailussa kolmen eri tutkimusryhmämme välillä todettiin, että adheesiomolekyylien ilmentymistä immuunisolujen pinnalla esiintyi eniten PPMS-potilailla, mutta merkitseviä eroja sytokiinien tai kemokiinien esiintymisessä ei ollut.

PPMS-potilailla esiintyi enemmän keskushermoston atrofialöydöksiä kuin terveillä verrokeilla. MRI-löydökset eivät kuitenkaan korreloineet MS-potilaan kokonaistoimintakykyä mittaavaan expanded disability status scale-pistetykseen. Aivojen T2-painotteisten leesioiden tilavuus ja diffuusien leesioiden määrä korreloivat tilastollisesti merkitsevästi PPMS-potilaiden neuropsykologisiin muutoksiin. Urodynaamisista löydöksistä detrusor hyperrefleksia ja DSD korreloivat aivojen T2-painotteisten leesioiden tilavuuteen ja hypotonisella detrusor-löydöksellä oli yhteys thorakaalisten plakkien määrään. Adheesiomolekyyleista ainoastaan VLA-4:n ilmentyminen veressä ja selkäydinnestenäytteessä korreloi aivoleesioiden kokonaistilavuuteen ja diffuusien leesioiden määrään. Sytokiinein tai kemokiinien osalta ei todettu merkitseviä korrelaatioita MRI-muutoksiin.

Yhteenvetona voidaan todeta, että PPMS-potilaiden MRI-löydökset korreloivat vain heikosti kliinisiin löydöksiin. PPMS-potilailla todettiin heikentymistä useilla eri osa-alueilla kognitiivisissa toiminnoissa, mutta merkitseviä eroja verrattuna SPMS-potilaihin ei löydetty. Virtsaustoiminnan häiriöt ovat erittäin yleisiä PPMS-potilailla. Adheesiomolekyylien ja joidenkin sytokiinien ilmentymisen lisääntyminen viittaa siihen, että tulehduksellista aktiviteettia esiintyy pidemmälle edenneessä PPMS-taudissakin.

INTRODUCTION

Multiple sclerosis (MS) is the most frequent chronic inflammatory demyelinating disease of the central nervous system (CNS) and the leading cause of non-traumatic neurological disability in young adults. The aetiology of MS is still unknown, but according to present knowledge both genetics and environmental factors contribute to its development (Ramagopalan et al 2010b). The pathogenesis of MS is also not fully understood. The concept that MS is a T cell-dependent autoimmune disease has been widely accepted, but new evidence indicates that B cells and antibodies are also involved in the pathology of MS (McFarland and Martin, 2007, Krumbholz et al 2012). The access of immune cells into the CNS is one of the key elements of MS pathogenesis. Neuropathological and imaging studies, together with studies of experimental autoimmune encephalomyelitis (EAE), an animal model of MS, indicate that adhesion molecules (AMs), integrins and chemokines are important for cell migration.

MS is classified into different subtypes based on different clinical features. The majority of patients have relapsing-remitting MS (RRMS) disease course characterised by episodes of acute exacerbation followed by complete or partial recovery. More than half of patients with RRMS progress to secondary progressive MS (SPMS) (Raine et al 2008). Primary progressive MS (PPMS) is a rare subtype of MS which is characterised by a steady progression of irreversible disability from the onset of the disease (Antel et al 2012). Although a diagnosis of MS can be made on clinical grounds, currently the evidence of dissemination in space (DIS) and dissemination in time (DIT) is based on magnetic resonance imaging (MRI) and lumbar puncture are taken into account (Polman et al 2011). Typical MRI findings include focal white matter (WM) lesions and gradually increasing central and cortical atrophy. Newer MRI techniques, such as magnetization transfer imaging (MTI), diffusion-tensor imaging (DTI), proton MR spectroscopy (MRS) and functional MRI (fMRI) show that these focal changes are found in grey matter (GM) as well (Horakova et al 2012). Also, changes in normal appearing WM (NAWM) and normal appearing GM (NAGM) are found by these new MRI techniques.

Typical symptoms of MS include optic neuritis, sensory and motor symptoms and fatique (Compston and Coles 2008). Cognitive dysfunction is also a common manifestation of MS. The prevalence of cognitive impairment in MS ranges from 50 to 70% of patients (Chiaravalloti and DeLuca 2008). Deficits typically involve in recent memory, attention, information processing speed and executive functions (Julian 2011). Also, more than half of patients have micturition difficulties, such as urgency, frequency and urge incontinence.

These problems often cause a marked reduction in the quality of life (Nortvedt et al 2001). The most common urodynamic findings are detrusor hyperreflexia and detrusor sphincter dyssynergia (DSD).

During the last 20 years, the treatment of MS has advanced substantially, and promising new therapies have become available (Fox and Rhoades 2012). Unfortunately, efficacious immunomodulatory (IM) treatment for PPMS is not yet available, despite many attempts in clinical trials, although it is likely that such treatments will be available in the near future. The aim of this study was to characterise the clinical, immunological and radiological characteristics of PPMS.

REVIEW OF THE LITERATURE

MS usually manifests as a RRMS disease course, during which the symptoms occur in episodes (i.e., relapses) lasting at least 24 hours to several weeks. In most cases, RRMS is followed by SPMS within 10 to 15 years (Weinshenker et al 1989). SPMS is defined as an initial relapsing-remitting disease course followed by progression with or without occasional relapses, minor remissions and plateaus (Lublin and Reingold 1996). PPMS is the subtype of MS with a progressive disease course from onset without relapses or remissions. In the rare subtype of progressive-relapsing MS (PRMS), a progressive disease course from onset is associated with clear acute relapses with or without full recovery and continuing progression during periods between relapses (Miller and Leary 2007).

1. Epidemiology

In Europe, the total estimated prevalence rate of MS is 83/100 000, with higher rates in northern countries (100-150/100 000) (Pugliatti et al 2006). In Finland, the prevalence is 108-202/100 000, and incidence per year in Finland varies from in 8.7/100 000 in western Finland to 5.1 / 100 000 in southern Finland (Sumelahti et al 2001, 2003, Krökki et al 2011). Recent data shows even higher incidence (14.7 / 100 000) in western Finland (Sumelahti, personal communication).

In recent years, an increase in MS incidence and prevalence has been reported (Sumelahti et al 2001, Hirst et al 2009), but the reason is still unclear. Genetic predisposition alone cannot explain this sudden increase in incidence. Rather, it is most likely that improved patient care, changes in the mortality and survival of MS (Ragonese et al 2008), and improved diagnostic methods partly explain this phenomenon. Several studies report an overall increase in incidence primarily due to increased incidence among females (Orton et al 2006, Hirst et al 2009, Krökki et al 2011). Obesity has been considered a factor that explains the increase in MS among women (Langer-Gould et al 2013).

1.1 Epidemiology of PPMS

Globally, approximately 20 % of MS patients have PPMS, but the European distribution of MS by disease subtype varies from 24- 88 % for RRMS, 4 -50 % for PRMS and/or SPMS, and 4 -35% for PPMS (Weinshenker et al 1989, Lublin and Reingold 1996, Thompson et al 1997, McDonnell and Hawkins 1998, Dujmovic et al 2004, Pugliatti et al 2006). In Finland, 22% of patients

with MS are reported to have PPMS (Sumelahti et al 2000). The incidence of PPMS is remarkably high in Seinäjoki (3.7/100 000) compared with Uusimaa (1.0/100 000) or Vaasa (0.2/100 000) (Sumelahti et al 2001). Conflicting estimates of PPMS incidence in Finland have also been reported, with decreased incidence in Vaasa and increased incidence in Seinäjoki (Sumelahti et al 2003). These different trends in incidence suggest that environmental factors have changed. Moreover, the same trends in incidence were also found for RRMS, suggesting that there are similar environmental factors influencing RRMS and PPMS.

2. Aetiology

2.1 Genes

The exact aetiology of MS is still unknown. According to current understanding, both genes and environmental factors are involved in the aetiology of MS (Figure 1). Twin studies show that the concordance rate of MS is approximately 30% for monozygotic twins, and 2-5% for dizygotic twins (Hansen et al 2005, Kuusisto et al 2008). In Finland, a high familial occurrence of MS has been reported in the Seinäjoki district, suggesting that the frequency of susceptibility genes is higher in that area (Sumelahti et al 2001). Regardless of these findings, a congruent genetic profile has not been identified, and it is thought that several genes contribute to susceptibility to MS (McElroy and Oksenberg 2011). The human leukocyte antigen (HLA) has been unambiguously associated with MS susceptibility, and the strongest association is found between HLA DRB1*1501 antigen and MS (Olerup and Hillert 1991, Barcellos et al 2006). Moreover, interleukin (IL) 2 receptor and IL7 receptor genes are found to associate to MS (Sawcer 2008). A recent genome-wide association study of 15 different countries identified 29 novel susceptibility loci (Sawcer et al 2011). In Finnish MS patients, the protein kinase C alpha gene and C7 on chromosome 5p are associated with MS (Saarela et al 2006, Kallio et al 2009).

Figure1. Aetiology of MS

GENES
-HLA
-IL2 and IL7 receptor

ENVIRONMENTAL FACTORS
- viruses (EBV, HHV6)
- vitamin D
- smoking

MS

KNOWN OTHER FACTORS
- latitude
- sex
- place of birth
- month of birth

UNKNOWN OTHER FACTORS
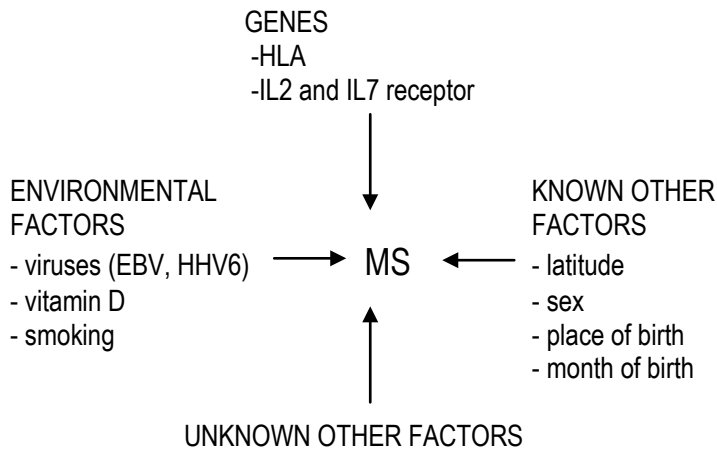
HLA = human leu= human leukocyte antigen, IL = interleukin, EBV = Ebstein-Barr virus, HHV = human herpes virus

2.1.1 Genes in PPMS

Genetic factors also affect the phenotypic expression of MS (Ramagopalan et al 2008), but according to present knowledge, the clinical phenotype of MS correlates only modestly with the HLA allele (Gourraud et al 2012). A few studies have found an association between PPMS and cytotoxic T lymphocyte antigen-4 G49, IL-4 E1(33)TT genotype, apolipoprotein APOE ε4 and chemokine receptor 5 but these findings could not be confirmed by other studies (Ramagopalan et al 2008). An association between PPMS and the DR2 and DRB1*1501 and DQB1*0602 alleles have been reported, and severe morbidity was found among DRB1*1501-positive PPMS patients (Vasconcelos et al 2009). Furtermore, cysteine protease caspase-8 polymorphism was suggested as a genetic modifier of PPMS risk and progression (Camina-Tato et al 2010).

2.2 Environmental factors

2.2.1 Viruses

Many infectious agents such as human herpes virus-6, Epstein-Barr virus (EBV) and Chlmydia pneumoniae are suggested to be associated with MS

(Pugliatti et al 2008). Different viruses can cause autoimmunization of T lymphocytes against myelin basic protein (Bagert 2009). Among the infectious agents proposed to be associated with MS, only EBV stands out as an important risk factor (Ascherio and Munger 2007a, Levin et al 2010).

2.2.2 Vitamin D

The association between increasing latitude and increasing risk of MS was first observed many decades ago (Myhr 2009). MS is more common in the Northern hemisphere, where people have less lifetime ultraviolet light exposure, than in southern parts of the world (Kurtzke 1977). Consistently, a prospective study indicates that vitamin D supplementation is associated with a 40% reduction in the risk of developing MS (Munger et al 2006). The relative risk of developing MS also seems higher for people born in May compared with people born in November (Willer et al 2005). According to present knowledge, an active metabolite of vitamin D (1,25 dihydroxyvitamin D) has several immunomodulatory effects (Correale et al 2009). Recent studies suggest that vitamin D supplementation has disease-modifying effects and decrease exacerbation of MS symptoms (Myhr 2009, Correale et al 2009, Simpson et al 2010). Compared with healthy controls, RRMS patients have significantly lower vitamin D levels, but a similar observation has not been found for PPMS patients (Correale et al 2009). Furthermore, vitamin D3 as on add-on treatment with interferon (IFN) β reduces MRI disease activity in MS (Soilu-Hänninen et al 2012).

2.2.3 Smoking

According to several studies, smoking is associated with an increased risk of MS (Hernan et al 2001, Hernàn et al 2005, Ascherio and Munger 2007b), and even passive exposure to smoking may increase risk (Sundström et al 2008). This increased risk is linked to exposure of axons to nitric oxide, which may cause axonal degeneration or block axonal conduction, especially in axons that are physiologically active or demyelinated (Smith et al 2001, Kapoor et al 2003, Redford et al 1997). Smoking increases the risk of development of MS and may be associated with a progressive clinical course (Deisenhammer et al 2008, Di Pauli et al 2008, Hernàn et al 2005, Healy et al 2009). Smoking is also associated with higher lesion volume and greater atrophy in MS (Zivadinov et al 2009). According to a recent study, smoking and two HLA genes interact to increase the risk of MS (Hedström et al 2011).

2.2.4 Sex hormones

It is well known that there is a female majority in MS and sex steroids may act directly on the immune system to modulate antigen presentation, lymphocyte activation, cytokine gene expression and homing of immune cells (Whitacre 2001). Increased levels of estrogens can inhibit expression of proinflammatory cytokines and stimulate the production of regulatory cytokines (Gilmore et al 2004, Airas et al 2008, Tomassini and Pozzilli 2009). This effect may explain the decrease in number of MS relapses during pregnancy, when levels of estrogens are high (Confavreux et al 1998, Saraste et al 2007, Rinta et al 2010). This benefit of estrogens appears to be transient, as oral contraceptive use, number of pregnancies or age at first birth are not associated with long-term MS risk, although oral contraceptives might delay the onset of the disease (Hernàn et al 2000, Alonso et al 2005, Alonso and Clark 2009).

3. Pathology and pathogenesis

The central pathological findings of MS are inflammation, demyelination, remyelination, neurodegeneration and glial scar formation occurring either focally or diffusely in WM and GM of the CNS (Lassmann et al 2007). Focal plaques of demyelination are an essential part of the diagnosis of MS. These plaques are present in both the WM and GM of the brain. Focal plaques of demyelination can be highly inflammatory classic active plaques, slowly expanding lesions (accounting for about half of lesions in progressive MS), inactive lesions (the most frequent lesion type in MS) or remyelinated shadow plaques (Lassmann et al 2012).

The pathogenesis of MS is not fully understood. Early research in EAE, indicated that CD4+ effector T cells have a central role in the pathogenesis of MS. During the last years it has been shown that tissue damage in the lesions of MS in CNS may be induced by numerous mechanisms, such as cell-, cytokine, antibody- and radicals-mediated mechanism (Lassman 2007).

The interaction between the antigen-presenting cells and T cell is an essential step in immune cell activation (Kasper and Shoemaker, 2010) (Figure 2). Activated naive T cells differentiate to T helper (Th) 1, Th2, Th3 or Th17 cells, depending on the cytokine milieu. Activated T cells can transmigrate into the CNS via the blood-brain barrier (BBB), because they express AMs, chemokine receptors and integrins (Engelhardt 2008). In the CNS, T cells are reactivated and then mediate activation of macrophage/microglia, B cells and cytotoxic T cells. Reactivated T cells start to produce cytokines, like tumor necrosis factor (TNF) α and IFNγ, which may damage oligodendrocytes and neurons. Th2

cells secrete cytokines, which provide help to B cell-mediated tissue damage. According to current understanding, tissue damage in MS lesions may be induced by different cells, such as interleukin-17-producing Th 17 cells, B cells, CD8+ T cells, and CD4+ and CD8+ T-regulatory cells (Kasper and Shoemaker 2010).

Figure 2. Pathogenesis of MS
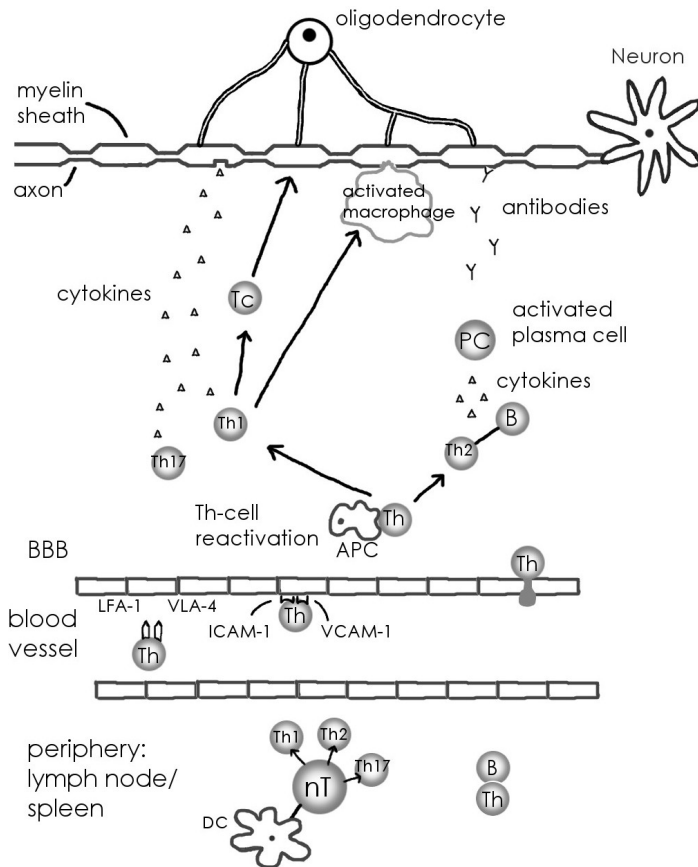
DC = dendritic cell, BBB = blood-brain barrier , APC = antigen presenting cell, nT = naïve T cell, Th = T helper cell, Tc = cytotoxic T-cell, LFA = lymphocyte function associated antigen, VLA = very late antigen, ICAM = intracellular adhesion molecule, VCAM = vascular cell adhesion molecule.

3.1. Pathology and pathogenesis of PPMS

Pathological studies have found fewer inflammatory cells and less acute axonal injury in PPMS lesions than in SPMS lesions (Revesz et al 1994, Bitch et al 2000). A later pathological study demonstrates extensive cortical demyelination and diffuse damage with microglia activation and axonal injury in the NAWM of patients with PPMS or SPMS (Kutzelnigg et al 2005). The investigators proposed that progressive forms of MS are characterised by a diffuse inflammatory process with widespread axonal injury in WM and cortical demyelination, whereas in RRMS, focal inflammatory lesions are the main feature. A recent study of progressive MS revealed evidence of leakage at the endothelial tight junctions of brain microvessels in lesions as well as in NAWM and NAGM (Leech et al 2007). In patient with progressive MS, most WM lesions are inactive demyelinated plaques or exhibit slow expansion at the lesion border (Bradl and Lassmann 2009). A recent study reports higher remyelination capacity in the brain of patients with PPMS than in those with SPMS, but this difference was not found in the spinal cord (Bramow et al 2010).

The progression of neurological impairment in PPMS could be due to a prolonged slow immune attack, and it has been proposed that this is more likely to occur when antibodies, rather than T cells, constitute the main mechanism of attack (Pender 2004). Antibodies to CNS antigens could contribute to progression of the disease by causing demyelination or axonal damage or by inhibiting remyelination. According to recent understanding, inflammation occurs at least partly behind the BBB in progressive stages of MS (Lassmann et al 2012). A recent pathological study found that generalised diffuse meningeal inflammation and associated inflammatory milieu in the subarachnoid compartment plays a role in the pathogenesis of cortical GM lesions and more severe clinical courses of PPMS (Choi et al 2012). Oxidative stress, resulting in mitochondrial injury, may be one of the factors initiating demyelination and neurodegeneration. Furthermore, age-dependent iron accumulation in the brain and mitochondrial gene deletions may amplify the effects of oxidative stress. In spite of these new findings the specific immunological pattern of PPMS is still unclear (Antel et al 2012).

4. Clinical presentation of MS

4.1 RRMS

The earliest clinical presentation of RRMS is clinically isolated syndrome (CIS), a first clinical episode with features suggestive of MS (Miller et al 2012). CIS

presents as an acute or subacute episode of neurological disturbance due to single or multifocal lesions. Typical clinical presentations include long-tract symptoms and signs, optic neuritis, brainstem syndrome or multifocal abnormalities (Confavreux et al 2000, Miller et al 2012).

In RRMS the age of onset is usually between 25-35 years of age and the female to male ratio is approximately 2 (Pugliatti et al 2006). The latest epidemiological studies show an increasing female to male ratio for RRMS (Celius and Smestad 2009, Ramagopalan et al 2010a). In MS, relapses typically present subacutely, with symptoms developing over hours to several days, persisting for days to several weeks, and then gradually dissipating. In RRMS, there is no progression of symptoms between relapses. In the beginning of RRMS, most symptoms fully recover after a few days or weeks. On average, the yearly relapse rate is approximately 1 in 1-2 years, but the frequency decreases over time (Confavreux and Vukusic 2008). Several follow-up studies show that IM treatments reduce relapse rate by 30-60 % compared with placebo (IFNβ Multiple Sclerosis Study Group 1993, Jacobs et al 1996, Johnson et al 1995, Kappos et al 2010, PRISMS study group 1998 and 2001, Polman et al 2006). Other symptoms of MS are motor deficits, bladder dysfunction, coordination difficulties, cognitive problems, fatigue and pain (Compston and Coles, 2008). Furthermore, behavioural impairments and particularly subjective behavioural symptoms seem to be relatively frequent among MS patients (Rosti-Otajärvi and Hämäläinen 2012). Sensory symptoms and optic neuritis are more common in RRMS and whereas motor symptoms predominate in SPMS (Palace 2003). Over time, recovery from each episode is incomplete and persistent symptoms accumulate. Around 60% of RRMS patients enter SPMS after having MS for approximately 20 years (Tremlett et al 2008). In SPMS, symptoms increase gradually, but relapses may also occur. Male sex and motor symptoms at onset are associated with both a shorter time to and younger age at onset of SPMS (Koch M et al 2010a). Development of SPMS is associated with a higher risk of long-term accumulation of disability (Scalfari et al 2011).

4.2 PPMS

Patients with PPMS are older at onset, and there is less female predominance than that for RRMS patients (Table 1) (Minderhoud et al 1988, McDonnell and Hawkins 1998, Andersson et al 1999, Dujmovic et al 2004, Nilsson et al 2007). The age at onset is approximately 10 years older than that for RRMS but similar to that for SPMS (Confavreux and Vukusic 2006, Scalfari et al 2011). Familial PPMS has been reported to begin about five years earlier than sporadic PPMS, but sex has no significant effect on age at disease onset (Koch et al 2010b). In PPMS, symptoms develop slowly and increase steadily over months to years.

The most common presentation is progressive spastic paraparesis (80% of patients) characterised by weakness, increased tonus, sensory deficits and micturition problems. Motor onset is the most common mode of onset in patients with PPMS (Dujmovic et al 2004, Maghzi et al 2007). About 15% of patients show a progressive ataxic syndrome with prominent cerebellar features (Ingle et al 2003, Tremlett et al 2005). Other clinical phenotypes include hemiplegia, brainstem syndromes and cognitive decline (Stevenson et al 1999). Progressive optic neuropathy is a rare presentation of PPMS (Ormerod and McDonald 1984).

According to natural history data, the rate of neurological deterioration from disease onset is substantially more rapid for PPMS than for RRMS (Cottrell et al 1999, Confavreux and Vukusic 2006). However, the rate of progression is faster or similar for SPMS than for PPMS (Runmarker and Anderson 1993, Minderhoud et al 1988, Vukusic and Confavreux 2003). Slower speeds in timed walk test, male sex and deterioration in expanded disability status scale (EDSS) score (Kurtzke 1983), and reduction in brain volume over two years are reported to be independent variables that predict disease progression in PPMS (Khaleeli et al 2008). The latter study, however, could not confirm sex as a predictor of disease progression, but found that sensory onset symptoms are associated with both a longer time to and an older age at reaching an EDSS score 6.0 (Koch et al 2009).

Table1. Clinical features of patients with RRMS, SPMS and PPMS.

	RRMS	SPMS	PPMS
Mean age at onset (y)	30	40	40
Female:male ratio	2-3:1	2-3:1	1:1
Presenting syndrome	optic nerve, spinal cord (sensory>motor), brainstem	optic nerve, spinal cord (sensory>motor)	spinal cord (motor>sensory), brainstem, cerebellum
Brain lesions in coventional MRI	several	several	few
Gd-enhancing lesions	frequently	rarely	rarely
Spinal cord lesions	occasionally	occasionally	frequently
CSF oligoclonal bands	common	common	common
Spinal cord atrophy	rarely	occasionally	frequently

Abbreviations: RRMS = relapsing remittig MS, SPMS = secondary progressive MS, PPMS = primary progressive MS, MRI = magnetic resonance imaging, Gd = gadolinium, CSF = cerebrospinal fluid
(Raine et al 2008)

5. Diagnosis of MS

Diagnosis of MS is based on the objective clinical evidence of dissemination of inflammatory lesions in the CNS in time and space. Although a diagnosis is possible to make without any paraclinical evidence, MRI is highly recommended by current guidelines (Giesser 2011).

The oldest and most well known diagnostic criteria for MS are Schumacher's criterias, which are based on clinical features and require two or more episodes of neurological dysfunction or slow progression for more than six months in addition to objective signs of neurological dysfunction on examination (Schumacher et al 1965). Later, the Poser's criteria allowed the replacement of clinical evidence by laboratory findings (Poser et al 1983). Paraclinical evidence includes the hot bath test, evoked response studies, tissue imaging procedures such as computer assisted tomography and nuclear magnetic resonance and reliable expert urological assessment. Supporting laboratory findings include the examination of cerebrospinal fluid (CSF) for oligoclonal bands and increased production of immunoglobulin G (IgG).

An international panel developed new diagnostic criteria that simplified MS diagnostic classification and description and integrated MRI criteria into the overall diagnostic scheme (McDonald et al 2001). These criteria made possible to diagnose MS after CIS, because DIS and time DIT is possible to confirm by MRI findings. These McDonald criteria were revised to clarify the use of spinal cord lesion and to demonstrate the dissemination of lesion in time (Polman et al 2005). The next proposal for new MRI criteria for MS was presented by Swanton and collagues (Swanton et al 2007). According to these criteria, a new tesla (T)2-weighted lesion in a follow-up scan is considered as DIT irrespective of the timing of the baseline scan. According to McDonald criteria, baseline MRI had to be performed at least 30 days after the initial symptoms.

The most recent proposed criteria, called the 2010 McDonald criteria, simplify the requirements for DIS and DIT (Polmann et al 2011). According to these criteria, DIS can be demonstrated by one or more T2 lesions in at least two of four areas of the CNS (periventricular, juxtacortical, infratentorial or spinal cord). DIT can be demonstrated by any new T2-weighted or gadolinium (Gd)-enhanced lesion in a follow-up MRI, irrespective of the time between baseline and follow-up MRI examinations.

5.1 Diagnosis of PPMS

Neither Schumacher's criteria nor Poser's criteria are appropriate for PPMS because the basic requirement of two discrete episodes may not be fulfilled. Therefore experts have presented new diagnostic criteria for PPMS (Thompson et al 2000). A definite PPMS diagnosis (Table 2) is based on evidence of clinical progression for at least one year, positive CSF and MRI findings or equivocal MRI findings and a delayed visual evoked potential (VEP) (Thompson et al 2000) . Positive MRI finding requires nine brain lesions or two spinal cord lesions or 4-8 brain lesions and one spinal cord lesion (Barkhof et al 1997). The McDonald criteria for PPMS require positive CSF findings and evidence of DIS and DIT by MRI (McDonald et al 2001). A positive CSF finding is based on the presence of oligoclonal bands or an increased IgG index. Revised McDonald criteria allow the diagnosis of PPMS without abnormal CSF (Polman et al 2005). The Polman criteria are recommended for use in neurological practice to facilitate the diagnosis of PPMS (Vasconcelos et al 2008). According to the revised McDonald criteria (2010), the diagnosis for PPMS includes one year disease progression and two of the three criteria: in the brain at least one T2-weighted lesion in the periventricular, juxtacortical or infratentorial regions; in the spinal cord at least two lesions, and positive CSF (Polman et al 2011).

Table 2. Diagnostic criteria of PPMS

Thompson's criteria (Thompson et al 2000)	Original McDonald criteria (McDonald et al 2001)	Revised McDonald criteria (Polman et al 2005)	Revised McDonald 2010 criteria (Polman et al 2011)
Definite PPMS	1. Positive CSF and	1. One year disease progression	1. one year of disease progression
1. Clinical progression for at least 1 year and	2. Dissemination in space by MRI	2. Plus two of the following	2. Plus 2 of the 3 following criteria:
2. Positive CSF evidence and	evidence of nine or more T2 brain	a. Positive brain MRI (nine T2 lesions	a. Evidence for DIS in the brain
3. Positive MRI evidence or equivocal	lesion or	or four or more T2 lesions with	based one ≥1 T2 lesions in at least 1 area
MRI and delayed VEP	Two or more cord lesion or	positive VEP)	characteristic for MS (periventricular,
Probable PPMS: Either	Four to eight brain lesions and	b.Positive spinal cord MRI	juxtacortical or infratentorial)
1. Clinical progression for at least 1 year and	one cord lesion or	(two focal T2 lesion)	b. Evidence for DIS in the spinal cord
2. Positive CSF evidence and	Positive VEP with four to eight	c. Positive CSF (IEF evodence	based on ≥2 T2 lesions in the cord
equivocal MRI or delayed VEP	MRI lesions or	of oligoclonal IgG bands or	c. Positive CSF (IEF evidence of
Or:	Positive VEP with less than four	increased IgG index, or both)	oligoclonal bands and/or
1. Clinical progression for at least 1 year and	brain lesions plus		elevated IgG index
2. Positive MRI evidence or equivocal	one cord lesion and		
MRI evidence and delayed VEP	3. Dissemination in time by MRI or		
(CSF evidence either unavailable or negative)	continued progression for one year		
Possible PPMS			
1. Clinical progression for at least 1 year and			
2. Equivocal MRI evidence er delayed VEP			

Abbreviations: PPMS = primary progressive MS, MRI = magnetic resonance imaging, CSF = cerebrospinal fluid, VEP = visual evoked potential, DIS = dissemination in space, IgG = immunoglobulin, IEF = isoelectric focusing

5.2 Differential diagnosis of MS

Many systemic disorders have similar symptoms as MS. If the first symptoms occur before 15 years or after 59 years of age and progress slowly or if the disease has an acute or fulminate onset, differential diagnoses have to be considered carefully. Furthermore, if clinical or laboratory findings are atypical, differential diagnoses have to be taken in consideration (Rolak and Fleming 2007). A number of illnesses can mimic MS, such as neurosarcoidosis, neuroborreliosis, vascular occlusive disease including vasculitis, neuromyelitis optica, systemic lupus erythematosus, Sjögren syndrome, neurosyphilis, acute disseminated encephalomyelitis, idiopathic transverse myelitis, HIV-related disease and tropical spastic paraparesis (Fadil et al 2007). Many genetic or degenerative diseases such as hereditary cerebellar ataxias and Fabry's disease may also mimic MS. Over-interpretation of false-positive MRI is the most common mistake in diagnosing MS (Rolak and Fleming 2007). Signal abnormalities in MRI that mimic MS can originate, for example, from age-related changes, hypertension, migraine, cerebrovascular disease or primary CNS vasculitis (Carmosino et al 2005). Guidelines for MS differential diagnoses can help clinicians with diagnostic problems (Miller et al 2008).

5.2.1 Additional differential diagnosis of PPMS

For PPMS, the differential diagnoses depend on the clinical phenotype. In most cases, the differential diagnosis of progressive spastic paraplegia has to be taken into consideration (Table 3). In cases of progressive ataxic syndromes, differential diagnostic spectrums include hereditary spinocerebellar ataxias, structural abnormalities of the posterior fossa or foramen magnum and paraneoplastic disease. Diagnosing PPMS can be difficult because symptoms proceed slowly, and MRI abnormalities may be minimal. Furthermore, separating PPMS from RRMS can be challenging because the symptoms of PPMS can sometimes worsen faster and thus resemble a relapse.

Table 3. Differential diagnosis of PPMS

Spinal cord compression
    -cervical spondylosis
    -tumour
    -vascular anomalies

Hereditary
    -hereditary spastic paraplegia
    -Friedreich´s ataxia
    -leucodystrophies
    (adrenomyeloneuropathy, Krabbe´s disease)
    - CADASIL

Metabolic
    -B12 deficiency
    -phenylketonuria
    -copper deficiency

Inflammatory
    -neuromyelitis optica
    -neurosarcoidosis
    -CNS vasculitis

Infection
    -borreliosis
    -human T-lymphotrophic virus 1 (HTLV-1)
    -schistosomiasis
    -syphilis
    -HIV
    -brucellosis

Degenerative
    -motoneuron disease

Toxic
    -lathyrism
    -nitrous oxide

Vascular
    -dural arteriovenous malformation

Paraneoplastic

_____

(Miller and Leary 2007, Antel et al 2012)

6. Imaging

6.1. White matter lesions

MRI has a central role in diagnosing MS. Typically, MRI findings include multiple lesions in the WM and gradually increasing brain atrophy. Some lesions may eventually disappear, but most lesions are permanent. MS lesions are usually situated along the vessels periventriculary, infratentorially, subcortically and in the corpus callosum (Nijeholt et al 1998, Charil et al 2003).

The abnormalities of MS are seen as hyperintense lesions on T2-weighted images (Figure 3) or as hypointense lesions in T1-weighted images (Figure 4, Table 4). Enhanced MRI lesions after application of Gd are consistent with local breakdown of the BBB and are associated with transmigration of inflammatory cells through cerebral vessels walls. This enhancement is transient, with most lesions disappearing within 4 weeks (range: 1-16 weeks) (Cotton et al 2003). Approximately 75 - 90% of MS patients have spinal cord lesions visible in T2-weighted images (Figure 5). The cervical cord is most frequently affected (Bot et al 2004). Spinal cord lesions tend to involve posterior and lateral regions, are asymmetric, occupy less than half the area of the cord on axial images, and are limited to two or less vertebral segments in length (Lycklama et al 2003). Detection of cord lesions, is difficult, although new techniques have improved detection sensitivity (Ceccarelli et al 2012). Spinal cord lesions have been described even in patients with CIS, and their presence increases risk of developing MS (Okuda et al 2011).

In addition to focal lesions, diffuse brain abnormalities are also found in MS. Diffusely abnormal WM (DAWM) is defined as a region with ill-defined borders of intermediate signal intensity between that of NAWM and that of plaque in T2-weighted and proton density images (Moore et al 2008). These lesions are most commonly found around the ventricles and may develop independently from focal WM lesions (Traboulsee and Li 2008)Rovaris et al 2001, Pelletier et al 2003, Rocca et al 2003, Kutzelnigg et al 2005). Diffuse lesions are also found in the spinal cord (Bot et al 2004).

Table 4. Conventional MRI-findings in MS.

MRI finding	MS subtypes	patology	clinical correlation
Hyperintense lesions in T2-weighted images	typical in RRMS, common in SPMS rare in PPMS	inflammation, oedema, demyelination, axonal loss, gliosis	weak
Hypointense lesions in T1-weighted images			
- acute black holes	in relapse in RRMS and SPMS	oedema, active inflammation, demyelination	weak, enhancing correlate with disease activity
-chronic black holes	typical in SPMS and PPMS, also found in RRMS, especially after some years	irreversible axonal loss, gliosis	weak
Atrophy			
- white matter	typical in SPMS and PPMS less common in RRMS	axonal loss	some correlation with disability
-grey matter	typical in SPMS and PPMS, Less common in RRMS	neuronal and glial loss	greater correlation with disability
Diffuse lesions	typical in PPMS, found also in RRMS, and SPMS	demeylination, axonal loss	weak

Abbreviations: RRMS= relapsing remittig MS, SPMS= secondary progressive MS, PPMS= primary progressive MS
Recreated from Filippi and Rocca 2011 and Filippi et al 2012b.

6.2 Grey matter lesions

In addition to WM lesions, lesions in cortical and deep GM are also known to be involved in MS (Pirko et al 2007, Horakova et al 2012). GM damage can occur from the earliest stages of MS and may even precede development of WM damage (Chard and Miller 2009, Giorgio et al 2011, Pirko et al 2007). Changes in GM are also found in the cerebellum (Calabrese et al 2010b, Lucchinetti et al 2011). Recently, it was recommended that special attention be paid to changes in the thalamus, as this region is involved in a wide range of neurological functions including motor, sensory, ocular motility, fatique, attention and memory (Minagar et al 2013).

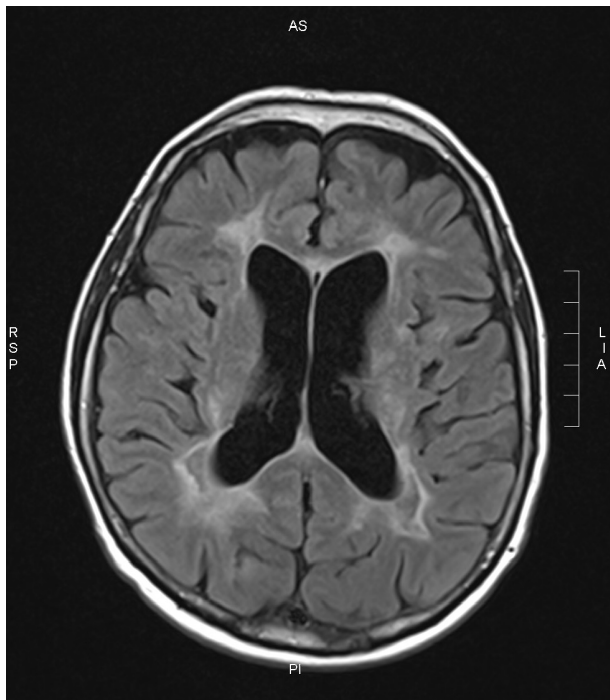Figure 3. Hyperintense lesions in T2-weighted image.

Figure 4. Hypointense lesions. in T1 weighted image.
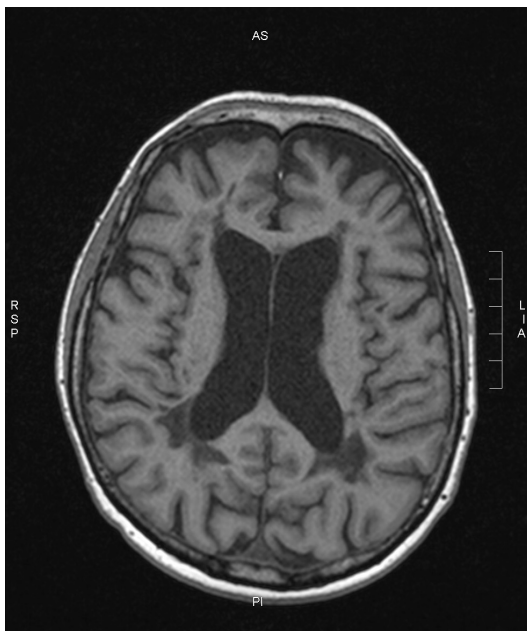
Figure 5. Spinal cord lesion in T2 weighted image.

6.3 Atrophic changes

Brain volume loss occurs at a rate of around 0.5%-1% per year in MS patients, which is higher than the rate of around 0.1%-0.3% per year in healthy subjects (Coffey et al 1992, Pfefferbaum et al 1994, Bermel and Bakshi 2006, Anderson et al 2006). In MS brain, atrophy is found as early as clinical disease onset and is more marked in SPMS than in RRMS (Brex et al 2000, Miller et al 2002, Dalton et al 2006). The rate of atrophy seems to be heterogenous at each stage of MS and is characterised by intra- and inter-individual variation (Zivadinov and Bakshi 2004). Both local and general atrophy is also found in the spinal cord (Tartaglino et al 1995). Cord atrophy is found in all MS subtypes and is correlated better with disability (Bonati et al 2011, Rocca et al 2011, Cohen et al 2012).

Atrophy is also found in GM and can be detected at the earliest stages of MS (Fisher et al 2008, Horakova et al 2009, Zivadinov and Minagar 2009). Atrophy of WM seems to be greater than that of GM during early stages of MS but evolves more slowly than GM atrophy (Chard et al 2002, Chard et al 2004, Tiberio et al 2005, Pirko et al 2007). GM atrophy is more pronounced in SPMS than in RRMS (Roosendaal et al 2011). In all MS subtypes, regional GM loss correlates with the volume and location of WM lesions in T2-weighted images (Ceccarelli et al 2008).

6.4 Correlations between MRI findings and disability

Measures of lesions using conventional MRI have not shown strong associations with clinical disability (Bakshi et al 2008). In patients with CIS, a higher number of T2-weighted lesions is associated with a more severe disease course and a higher likelihood of developing clinically definite MS (Tintore et al 2006). Also in RRMS, the T2-weighted lesion load has a slight predictive value for progression of disability and is associated with higher risk of progressing to SPMS, but this predictive value is not observed for progression of SPMS or PPMS (Mostert et al 2007, 2010). An assessment of the regional distribution of WM lesions has shown that this measure correlates better with long-term disability (Dalton et al 2012). Several studies show that changes in GM are associated with disability, and a recent five-year longitudinal study reports that cortical GM damage, more than WM damage, is associated with disability progression (Zivanidov and Minagar 2009, Horakova et al 2012, Calabrese et al 2012).

Earlier studies showed that brain atrophy is a fairly reliable predictor of subsequent neurological impairment and that atrophy results in irreversible

damage (Rudick et al 2000, Zivadinov et al 2001, Fisher et al 2002, Ingle et al 2003). Central atrophy rate seems to be even more strongly predictive of disease progression than whole brain atrophy rate (Lukas et al 2010). Recent studies report that GM atrophy correlates with disability better than WM atrophy, and thalamic atrophy predicts long-term progression of disability in MS (Roosendaal et al 2011, Rocca et al 2010a). Cervical spinal cord atrophy correlates most strongly with disability when both lesions and atrophy of the brain and spinal cord are taken into consideration (Cohen et al 2012).

6.5. Advanced MR techniques

Advanced MR techniques provide more detailed information about focal lesions and normal-appearing tissue. Quantitative MRI techniques, such as MTI and DTI, are able to characterise diffuse abnormalities in NAWM better than conventional MRI (Filippi et al 2013). Furthermore, cortical lesions are typically not seen in conventional MR images but use of double-inversion-recovery (DIR) MR sequences increases sensitivity by over 50% (Geurts et al 2005). On the other hand, MRS has the potential to characterise the chemical pathology of lesions (Filippi et al 2013).

In recent years, fMRI has provided interesting information about cortical reorganisation following MS tissue damage, showing altered recruitment of task-related regions and additional areas that are not typically activated in healthy people (Filippi and Rocca 2004, Rocca et al 2005). Increased recruitment of task-related brain regions has been found even in early phase in RRMS and also during the progressive phase of MS (Filippi and Rocca 2004). This cortical reorganisation may have a role in compensating for tissue damage in MS.

6.6 Imaging in PPMS

Conventional MRI shows smaller lesion loads and lower frequency of Gd-enhanced lesions in PPMS than in RRMS or SPMS (Thompson et al 1990, 1991, 1997, Wolinsky 2004, Bieniek et al 2006). Therefore, clinicoradiological correlations for PPMS have been limited (Lycklama a Nijeholt et al 1998, Stevenson et al 1999, Rovaris et al 2001). In follow-up studies, early enhancement, an increase in T2-weighted lesion load and the development of new lesions over two years predicted long-term clinical outcomes for PPMS (Stevenson et al 2004, Ingle et al 2003, 2005, Sastre-Garriga et al 2005b). Later studies of patients with PPMS confirmed that an increased number of enhanced lesions at baseline predict greater decline in walking over five years

(Khaleeli et al 2010), but T2- or T1-weighted lesion load did not predict clinical deterioration over 10-15 years (Khaleeli et al 2008, Mostert et al 2010).

Diffuse brain abnormalities are more frequent in PPMS than in RRMS or SPMS (Thompson et al 1990, 1991, Lycklama a Nijeholt et al 1998, 2001, van Walderween et al 1998, 2001), but no significant associations between clinical disability and diffuse lesions were detected with conventional MRI (Rovaris et al 2001). New imaging techniques show the presence of diffuse damage in the WM, GM and spinal cord (Rocca et al 2003, Rovaris et al 2001, 2002, 2005, 2008, Agosta and Filippi 2007, De Stefeno and Filippi 2007). These new MR techniques also show that cortical, diffuse brain and spinal cord lesions are associated with clinical disability and damage in NAGM may predict the progression of PPMS (Sastre-Garriga et al 2005b, Ramió-Torrentá et al 2006, Calabrese et al 2009b, Khaleeli et al 2007, Rocca et al 2012, Rovaris et al 2006). Furthermore, the combined assessment of thalamic T2-weighted lesion load and GM magnetisation transfer ratio (MTR) (Tur et al 2011a) and thalamic DTI can be useful for predicting progression of PPMS (Mesaros et al 2011). Studies usinging MRS data or DIR images found no significant differences between PPMS and other MS phenotypes, but a study using quantitative MRI (including MTR and DTI measures) suggests that DAWM differs between PPMS and SPMS (Calabrese et al 2010a, Rocca et al 2012, Vrenken et al 2010).

Atrophy of both WM and GM is seen early in PPMS (Sastre-Garriga et al 2004, 2005a). Regional analysis shows that atrophy is most pronounced in deep GM (Sepulchre et al 2006). Inflammation in MRI during early stages of PPMS predicts greater development of brain atrophy (Ingle et al 2005). PPMS is characterised by a slightly higher annual rate of ventricular enlargement than that for SPMS; however, this rate is lower than that in patients with RRMS (Zivadinov and Bakshi 2004). A recent study found higher GM volume, but similar low WM volume in PPMS compared with SPMS (Roosendaal et al 2011). A five-year follow-up study of patients with PPMS showed that progressive brain atrophy tends to be constant within individuals but varies considerably between individuals (Ingle et al 2003). In the same study, a worsening of MS functional composite (MSFC) score (Fisher et al 1999) was found to correlate with ventricular volume.

In the spinal cord, atrophy is present during in early PPMS, but not during early RRMS and it increases from 0.83% to 5.2% at one-year follow-up (Bieniek et al 2006, Agosta et al 2007, Laule et al 2010). In PPMS, spinal cord atrophy develops at a faster rate than brain atrophy and is correlated with EDSS score (Ingle et al 2002, 2003, Ingle et al 2003, Laule et al 2010). Changes in the spinal cord are though to play an important role in determining disability in PPMS,

but a recent study shows that the location of T2-weighted brain lesions in motor and associative tracts is also an important independent factor predicting the progression of disability (Rovaris et al 2001, Bieniek et al 2006, Bodini et al 2011). It is likely that several area of the CNS affect disability, as an fMRI study reports that PPMS patients show increased activation and abnormal functional connectivity measures in several areas of the sensorimotor network (Ceccarelli et al 2010).

7. Neuropsychological deficits

Cognitive impairment occurs in 30-70% of patients with MS (Amato et al 2006, Chiaravalloti and DeLuca 2008, Patti 2009). Unlike the decline of semantic memory, language and visuospatial function associated with cortical dementias such as Alzheimer´s disease, the cognitive decline associated with MS resembles subcortical dementias characterised by slowed information processing, frontal lobe deficits and memory retrieval problems (Calabrese 2006, Chiaravalloti and DeLuca 2008). Impairment typically involve recent memory, attention, information processing speed and executive functions (Zakzanis 2000, Winkelmann et al 2007, Julian 2011), which often have a large impact on work and social abilities and decrease quality of life (Benedict et al 2005, Chiaravalloti and DeLuca 2008).

Typically, neuropsychological impairment develops slowly across several years, but a clear correlation between disease duration and cognition has not been confirmed (Rao et al 1991, Lynch et al 2005). Cognitive deficits can occur from the early stages of MS and even in CIS, and initial cognitive deficits predict further cognitive decline (Deloire et al 2005, Schulz et al 2006, Amato et al 2010, Glanz et al 2012, Feuillet et al 2007, Potagas et al 2008, Bergendahl et al 2007). Semestad and colleagues report that younger age at onset is significantly associated with cognitive impairment (Smestad et al 2010). In contrast to previous studies, a later study reports an association between physical disability and cognitive impairment (Rao et al 1991, Gaudino et al 2001, Ruggieri et al 2003, Lynch et al 2005). A relationship between depression and cognitive impairment was not found in earlier studies, but recent work suggests that moderate or severe depression significantly correlates with information processing speed, working memory and executive functioning (Siegert and Abernethy 2005). A Finnish study reports that increase in core body temperature during heat stress has some effects on cognitive performance (Hämäläinen et al 2012). A recent review concludes that early age of onset, male sex, secondary progressive course, neurodegeneration (indicated by GM atrophy) and low average or inferior intelligence are noteworthy risk factors for cognitive dysfunction (Benedict and Zivadinov 2011).

The basic mechanisms underlying cognitive impairment in MS are not fully understood but it seems that the cholinergic system is unlikely to explain the majority of these problems. The few studies of acetylcholinesterase inhibitors have not proved the advantage of these medicines and thus the use of symptomatic agents for cognitive decline is not recommended (Krupp et al 2011, O'Carroll et al 2012, Langdon 2011, O'Carroll et al 2012). Trials of disease-modifying drugs and cognitive rehabilitation have reported some positive outcomes of cognitive measurements (Chiaravalloti et al 2005, Fischer et al 2000, Filippi et al 2012a, Iaffaldano et al 2012, Penner et al 2012).

7.1 Neuropsychological deficits in PPMS

Higher numbers of cognitive deficits have been reported in chronic progressive forms of MS than in RRMS (Amato et al 2001, Huijbregts et al 2004, Rao et al 1984, 1987, Heaton et al 1985, De Sonneville et al 2002, Lynch et al 2005, Potagas et al 2008, Smestad et al 2010). Patients with chronic progressive subtypes of MS are more likely than patients with RRMS to have attention deficits, in particular reduced speed of information processing, executive dysfunctions, and deficits in verbal intelligence and abstraction (Zakzanis 2000). In particular, attention, working memory, verbal memory, spatial memory, spatial reasoning and verbal fluency are impaired in PPMS (Camp et al 1999, Foong et al 2000, Gaudino et al 2001, De Sonneville et al 2002, Huibregts et al 2004). The frequency of cognitive impairment in PPMS varies from 7% to 29% (Comi et al 1995, Camp et al 1999).

In the few studies that distinguished between patients with PPMS and SPMS, greater cognitive dysfunction was seen in SPMS. Patients with SPMS have greater cognitive impairment in visuospatial working memory, visuospatial new learning, verbal memory and verbal fluency (Comi et al 1995, Foong et al 2000, Gaudino et al 2001, Huijbregts et al 2004). Slowed speed of information processing and especially for visual information processing is also more pronounced in SPMS than in PPMS (Denney et al 2005, Bergendahl et al 2007). Only one study has reported that patients with PPMS tend to be more frequently and severely affected than SPMS patients (Wachowius et al 2005).

Longitudinal studies of cognition in PPMS do not show a significant change in mean cognitive scores at two-year follow-up, but initial cognitive status is a good predictor of cognitive ability at this time point (Camp et al 2005, Huijbregts et al 2006). A later three-year longitudinal study of cognitive impairment in PPMS showed that declines in cognitive functioning can be indexed by changes in processing speed (Denney et al 2008).

8. Urinary disturbances

Micturition is complex system that involves an intact neural pathway between bladder, spinal cord, pons and higher centres (Table 5). Lower urinary tract dysfunction is a common problem in MS, causing a marked reduction in the quality of life even during early stages of the disease (Nortvedt et al 2001, 2007). As many as 50-100% of patients report voiding problems (Awad et al 1984, Goldstein et al 1982, Borello-France et al 2004). Overall only 2% of MS patients have voiding difficulties as the initial symptom, but about 10% of patients have micturition difficulties as one of their first symptoms (Blaivas et Barbalias 1984, Beck et al 1981, Miller et al 1965).

Symptoms include urgency, frequency and urge incontinence, and the most common urodynamic findings are involuntary contractions of the bladder (detrusor hyperreflexia) and the external urinary sphincter (DSD) (Betts et al 1993, Goldstein et al 1982, Ciancio et al 2001). In most cases voiding symptoms and urodynamic findings have no association, although hesitancy and areflexia, irritative urinary complaints and detrusor hyperreflexia are reported to relate (Goldstein et al 1982, Philp et al 1981, Awad et al 1984, Betts et al 1993, Bemelmans et al 1991). Heterogeneity in bladder function among patients makes treatment difficult but several treatment options are available (Tubaro et al 2012).

Most studies show no correlation between voiding symptoms and either the duration of MS or the age of the patient (Koldewijn et al 1995, Blaivas and Barbalias 1984, Goldstein et al 1982, Awad et al 1984, Araki et al 2002), but Araki and colleagues (2003) reported correlation between filling symptom score and EDSS and disease duration. Urodynamic abnormalities such as areflexia, hyperactivity and DSD are reported to relate to disease parameters such as duration and disability status (Koldewijn et al 1995, Betts et al 1993, Araki et al 2002). Upper urinary tract complications are rare, but duration of MS, an indwelling catheter, high-amplitude neurogenic detrusor contractions and permanent high detrusor pressure are risk factors for upper urinary tract damage (Krhut J et al 2008, De Sèze et al 2007).

8.1 Urinary disturbances in PPMS

Micturition problems are correlated with motor disturbances and because spastic paraparesis is the most common phenotype of PPMS, urologic

problems are typical in patients with PPMS (Awad et al 1984, Betts et al 1993, McDonnell et al 1998, Minderhoud et al 1988, Kirchhof and Fowler 2000). PPMS can even start only with micturition diffuculties. Urgency of micturition is common and can be accompanied by urge incontinence (Miller and Leary 2007). However, despite their frequency, urinary disturbances in PPMS have been studied very little.

Table 5. CNS areas and corresponding urodynamic findings.

Nervous system area	Urodynamic findings	Symptoms
Brain	detrusor hyperreflexia	pollakisuria, urgency, urge incontinence
Suprasacral spinal cord	detrusor hyperreflexia, sfinkter dyssynergia	pollakisuria, urgency, urge incontinence, retentio, hesitancy
Epiclonal spinal cord	detrusor hyperreflexia, sphinkter dyssynergia	pollakisuria, urgency, urge incontinence, hesitancy, retention
Conus medullaris, cauda equina	detrusor arefleksia, atonic sphinkter sressincontinence	hesitancy, retention overflowincontinence,
Periferal	detrusor areflexia	hesitancy, retention
Sensory track	bladder sensory loss, detrusor areflexia	hesitancy, retention

Abbreviations: CNS= central nervous system, (Blok 2002)

9. Immunological changes in blood and CSF

In the diagnosis of MS, the analysis of CSF has a central role. The most common abnormalities reflect the presence of intrathecal IgG synthesis, which can be expressed by a formula: presence of oligoclonal bands (OCBs) and elevated IgG index (= IgG in CSF/ IgG in serum : album in CSF/album in serum) (Giesser 2011). OCBs and elevated IgG index are present in 95% and 75% of patients, respectively. CSF abnormalities also include mildly elevated

CSF white blood cell count (Tintore et al 2008). In PPMS the presence of OCBs is less frequent (80-94%) (McDonnell and Hawkins 1998, Sumelahti et al 2003). Oligoclonal IgM bands have a prognostic role in RRMS and SPMS (Mandrioli et al 2008, Mero et al 2013) but not in PPMS (Sola et al 2011).

9.1. Adhesion molecules

AMs including selectins, integrins and members of immunoglobulin gene superfamily play an important role in the transmigration of monocytes and lymphocytes into the CNS. Slow-flowing leukocytes in the blood roll along the vessel wall and may weakly attach to the endothelium via the selectin family of AMs such as L-, P- and E-selectin (Engelhardt 2008). Chemokines, lipid mediators and other pro-inflammatory molecules activate integrins very late activation antigen 4 (VLA-4) and lymphocyte function associated antigen 1 (LFA-1) and allow cells to adhere firmly. Intercellular adhesion molecule 1 (ICAM-1) and the vascular cell adhesion molecule 1 (VCAM-1) strengthen the unstable interaction of leukocytes with endothelium. AMs are found on cell surface (c) and soluble (s) forms in blood and CSF.

An increased proportion of cVLA-4 and cLFA-1 in blood and CSF of patients with CIS, RRMS or SPMS compared with healthy controls was reported by early studies (Elovaara et al 2000, Correale et al 2003). Levels of sAMs in CSF may be normal or elevated in active MS (Dore-Duffy et al 1995, Droogan et al 1996, Correale et al 2003). A comparison between different subtypes of MS showed significantly elevated serum sVCAM-1 and sICAM-1 in PPMS compared with RRMS or SPMS in relapse or remission (McDonnell et al 1999). Increased sE-selectin in serum has been demonstrated in PPMS (Giovannoni et al 1996, McDonnell et al 1999). Reports of cAMs in PPMS and SPMS are conflicting (Duran et al 1999, Eikelenboom et al 2005).

The increasing number of disease-modifying therapies puts pressure on early diagnosis, and the identification of novel biomarkers that could predict and optimise of therapeutic responses is an important issue in this context. AMs are proposed to be one type of biomarker for response to natalizumab or cladribine treatment but not for response to INF-β treatment (Mitosek-Szewczyk et al 2010, Sega et al 2008, Wipfler et al 2011).

9.2 Cytokines

Cytokines are critical components of the immune inflammatory process and are implicated in oligodendrocyte cell death and axonal degeneration (Bjartmar et al 2003). Cytokines are predominantly produced by immune cells that are

characterised by their cytokine production profiles (Codarri et al 2010). Th1 cells produce pro-inflammatory cytokines IL-2, INF-γ and TNF-α. Th2-type cytokines such as IL-4, IL-5, IL-10 and IL-13 promote B cell differentiation and antibody production. These cytokines are regulatory and anti-inflammatory and involved in the termination of inflammatory response (Imitola et al 2005). Both Th1 and Th2 cytokines have been found in MS lesions (Cannella and Raine 1995). In recent years, many studies report that IL-17 has a central role in MS (Kawanokuchi et al 2008).

Increased levels of cytokines are reported during exacerbation in RRMS and SPMS (Beck et al 1988, Imitola et al 2005, Wang et al 2011). Only some studies have analysed cytokine profile in the three different subtypes of MS. Osteopontin, a cytokine that promotes Th1 response, is up-regulated in the plasma of RRMS patients, but levels are similar in PPMS and SPMS patients compared to healthy controls (Vogt et al 2003). In addition, the secretion of pro-inflammatory cytokines is increased in patients with RRMS and SPMS patients but not in patients with PPMS (Balashov et al 2000). A later study reports higher levels of TNF-α in the serum of PPMS patients, which is consistent with the inflammatory activity also observed for this subtype (Hagman et al 2011).

Cytokines are also proposed as possible biomarkers. A positive correlation has been reported between high serum levels of IL-17 in RRMS patients and good response to INF-β treatment but this finding was not confirmed by later studies (Lee et al 2011, Bushnell et al 2012, Bustamante et al 2013). A recently published study found more inflammatory (IL-2 and INF-γ) and anti-inflammatory (IL-4 and IL-10) cytokines in NMO than in MS and the researchers concluded that serum cytokine levels can differentiate NMO cases from MS (Wang et al 2013).

9.3 Chemokines

Chemokines are small molecules that direct the movement of leucocytes to sites of inflammation or injury (Charo and Ransohoff 2006). Approximately 50 human chemokines have been identified and divided into four groups (CXC, CC, CX_3C, C) on the basis of their structure and function. The largest group is CC chemokines, which attract mononuclear cells to site of chronic inflammation. Many of the chemokines and their receptors are highly expressed on infiltrating leukocytes and in MS lesions (Subileau et al 2009, Broux et al 2012, Réaux-Le Goazigo et al 2013).

The levels of some chemokines are linked to MS activity. For example, increased levels of CCL5, CXCL8 and CXCL13 in CSF are founf in patients suffering from clinical relapse (Bartosik-Psujek and Stelmasiak 2005, Sellebjerg et al 2009). Furthermore, high levels of CSF CXCL13 predict the conversion of CIS to MS and CXCL13 is associated with unfavourable prognosis (Brettschneider et al 2010, Khademi et al 2011). In PPMS, chemokines have not been studied extensively. Furlan and colleagues reported decreased mRNA levels of CCL5 and its receptor CCR3 in PPMS compared with other MS subtypes (Furlan et al 2005). A more recent study reports higher CCL2 levels in the serum of PPMS patients compared with that of healthy controls, but no significant differences in cytokine levels were found between PPMS and other subtypes of MS (Hagman et al 2011).

10. Disease-modifying therapies for MS

The standard treatments for MS consist of five disease-modifying drugs that are approved for patients with RRMS: IFN-β-1b, IFN-β-1a, glatiramer acetate, natalizumab and fingolimod (PRISMS Study Group 1998, IFNB Multiple Sclerosis Group 1993, Jacobs et al 1996, Polman et al 2006, Kappos et al 2010). These treatments reduce mean annual relapse rates and MRI activity, but their effect on the progression of disability is not as obvious (Nosewortthy et al 2000, Rudick et al 2005, Paty and Li 1993, Simon et al 1998, Li and Paty 1999). A recently published review of long-term follow-up of clinical trials indicates that currently available disease-modifying therapies improve outcomes by delaying the time to significant disease progression (Freedman 2011). On the other hand, a recently published cohort study based on prospectively collected observational data from British Columbia, Canada concluded that the administration of IFN-β is not associated with slower progression of disability (Shirani et al 2012).

Natalizumab is the first monoclonal antibody (MAB) therapy approved for MS and its therapeutic mechanism is based on the blocking the α4-integrin subunit of the VLA-4. Natalizumab currently appears to be the most effective treatment for RRMS (Polman et al 2006). However, a serious side effect (i.e., progressive multifocal leukoencephalopathy) restricts its use, and patients treated with natalizumab should be carefully monitored (Chataway and Miller 2013). Recently, another MAB alemtuzumab, which causes rapid and prolonged lymphocyte depletion, was approved for treatment of active MS (Brown and Coles 2013). Furthermore, studies with rituximab and ocrelizumab, which are MABs against CD20 B cells, have shown promise in treating RRMS (Hauser et al 2008, Cross et al 2012, Kappos et al 2011). These new treatments seem to be

effective, but they require careful monitoring because of possible serious side effects (Lulu and Waubant 2013).

Recently, another oral medication was accepted for RRMS treatment. Teriflunomide is a dihydro-oratate dehydrogenase inhibitor that blocks pyrimidine synthesis (O´Connor et al 2011, Jeffery 2013). A third possible oral treatment for MS, dimethylfumarate (BG-12), a fumaric acid ester, is a promising first-line agent in the treatment of RRMS (Jeffery 2013). Statins have also been studied as an add-on therapy, but their combination with interferon therapy does not significantly influence relapse risk, disease progression or EDSS score compared with interferon treatment alone (Bhardwaj et al 2012).

10.1 Treatment of PPMS

In patients with PPMS, no treatment to date has proven efficacious. The first randomised controlled trial of IFN-β for PPMS used IFN-β-1a (Leary et al 2003). Patients receiving weekly intramuscular treatment of IFN-β-1a (30 µg) had less accumulation of cerebral T2-weighted lesion load than patients receiving placebo but no treatment effect on sustained disability progression was shown. Another trial of IFN-β-1b 8 MIU shows no evidence that IFN-β has any beneficial effect on MRI findings or disease progression in PPMS (Bitsch et al 2004). A randomised, placebo-controlled study of IFN-β-1b 8 MIU given subcutaneously shows a beneficial effect on MSFC score, cerebral lesion load and active lesions at 24 months (Montalban 2009), but there was no significant effect on EDSS score or brain atrophy. When patients were re-examined after five years without treatment, IFN-β-1b was found to produce modest beneficial effects on clinical variables and brain atrophy (Tur et al 2011b). The largest trial (943 patients) of PPMS therapy to date, which involved treatment with glatiramer acetate (20 mg daily) was terminated prematurely, as interim analysis indicated that no treatment effect could be expected (Wolinsky et al 2007).

Many immunosuppressive drugs, such as methotrexate, azathioprine and cyclophosphamide (CYC) have been tested for PPMS. CYC stabilised EDSS score over one year, but no conclusions could be drawn regarding treatment efficacy (Zephir et al 2004). Trials with azathioprine (British and Dutch Multiple Sclerosis Azathioprine Trial Group1988) or methotrexate (Goodkin et al 1995) showed no significant treatment effects. A cladribine study failed to show any significant treatment effects in terms of changes in EDSS score but reported reductions in the presence, number and volume of Gd-enhanced brain lesions in T1-weighted images (Rice et al 2000). An intravenous immunoglobulin trial suggested that monthly infusion delays disease

progression in patients with PPMS (Pöhlau et al 2007). Considering the small number of PPMS patients evaluated, these results need confirmation by further trials. Rituximab treatment produces no benefit in terms of time to confirmed progression of PPMS, but effects to median change in T2-weighted lesion volume (Hawker et al 2009). A study of intrathecal methotrexate treatment reports stabilization of EDSS score at one-year follow-up (Sadiq et al 2010). To date, the results of these clinical trials are disappointing. Studies of fingolimod and ocrelizumab treatment for PPMS are ongoing (Hohlfeld et al 2011).

AIMS OF THE STUDY

The purpose of this cross-sectional study was to characterise the clinical, imaging and immunological features of PPMS in a Finnish patient cohort and to compare these features with those of SPMS. The specific aims were:

1. To characterize the neurological, cognitive and urological abnormalities in PPMS.
2. To identify whether there are specific immunological changes typical of progressive MS subtypes.
3. To characterize the volumes of MRI abnormalities in the brain and spinal cord of patients with PPMS.
4. To determine whether the volumes of focal, diffuse and atrophic CNS abnormalities detected by MRI are associated with clinical or immunological features of PPMS.

SUBJECTS AND METHODS

1. Subjects

1.1 Patients with PPMS (Studies I-IV)

Patients with a progressive course of MS were selected out of 300 MS patients
followed at the Department of Neurology, Tampere University Hospital, which
provides specialised neurological care for 400,000 Finns. The study was
approved by the Ethics Committee of Tampere University Hospital, and all
subjects gave written informed consent prior to study entry. All patients with
progressive clinical courses according to patient records were interviewed, and
those with relapses were excluded. All patients who fulfilled the criteria of
progressive clinical course from onset participated in the study. These 28
patients fulfilled the criteria of Lublin and Reingold (1996). According to
Thompson's criteria for PPMS (Thompson et al 2000), 71% of patients had
definite MS and 29% had probable MS. No patients had received corticosteroid
or other immunosuppressive treatment (Table 6).

1.2 Patients with SPMS (Studies III-IV)

The SPMS patients were selected out of the patients followed at the
Department of Neurology. All 28 patients fulfilled the criteria for definite MS
according to McDonald's criteria (McDonald et al 2001). Patients in the SPMS
group were matched to those in the PPMS group with respect to age, sex and
education (Table 6).

1.3 Healthy controls (Studies I-IV)

The control group consisted of Department of Neurology staff and their
relatives. This group was comprised of 20 subjects (10 male, 10 female) whose
age, sex and education were matched to patients. No controls had any organic
CNS disease, hypertension or head trauma.

Table 6. Clinical characteristics of MS patients and controls.

Characteristics	PPMS (Studies I-IV) n=28	SPMS (Studies III-IV) n=28	Control group (Studies I- IV) n=20
Age (mean±SD, y)	50.9±9.4	45.2±6.4	46.0±6.1
Gender (male/female)	14/14	12/15	10/10
Duration of MS (mean±SD, y)	12.3±9.2	15.0±5.8	NA
EDSS score [mean (range)]	4.9 (2.0-8.0)	4.8 (3.5-6.5)	
RFSS score [mean (range)]	35.5 (11-86)	13.9 (5.6-23.7)	NA
Arm index [mean (range)]	1.6 (0-4)	NA	NA
Ambulation index [mean (range)]	3.7 (1-9)	NA	NA
Education (mean±SD, y)	10.4± 3.5	10.4±3.3	11.9±2.3
BDI (mean±SD)	6.4±6.1	6.4±4.4	1.3±1.7

Abbreviations: PPMS = primary progressive MS, SPMS = secondary progressive MS, EDSS = expanded disability status scale, RFSS = regional functional scoring system, BDI = beck depression inventory, NA = not applicable

2. Neurological examination (Studies I-IV)

Patients and controls underwent neurological examination, including evaluation of disability as measured by EDSS score, regional functional scoring system (RFSS) score, arm index and ambulation index (Kurtzke 1983, Munsat 1989). The RFSS scoring system contains the same seven functional systems of Kurtzke that are the basis for EDSS, but each functional system is divided into several regions or items. The arm index assesses four functions of the upper limbs (dressing, washing hair, using knife and fork and handling coins). The ambulation index assesses walking.

3. MRI protocol (Study I-IV)

MRI was undertaken within two weeks of neurological examination. All PPMS patients and healthy controls were assessed using the same 1.5 T MRI unit (Signa Horizon LX Echospeed, Wisconsin, USA) to avoid inter-scanner variations. This MRI unit was in general use in the hospital at that time. Details of the MRI protocol are shown in Table 7. MRI of SPMS patients was previously performed using a different 0.5 T MRI unit (Vectra GE, Wisconsin, USA) (Dastidar et al 1999). Because of the differences between these two units, MRI findings for PPMS and SPMS patients were not compared.

3.1 Volumetric analysis of the brain

Segmentation and volumetric analyses were conducted using Anatomatic software operating in a PC/Windows95 environment (Heinonen et al 1998a, 1998b, Dastidar et al 1999). MS lesion volumes were estimated by multiplying the average cross-sectional area of plaques in two consecutive slices by the gap thickness. This method was applied in non-three-dimensional images (axial T1-weighted and sagittal T2-weighted images). Total intracranial volume was measured by summing the volumes of segmented GM and WM (i.e., total brain volume) and total intracranial CSF space. The total volume of intracranial CSF space consisted of the volumes of ventricular and peripheral CSF spaces. Relative brain atrophy was determined by a ratio of total brain volume to total intracranial volume.

3.2 Volumetric analysis of the spinal cord

In the spinal cord, plaques were located peripherally and adjacent to spinal CSF space, making their segmentation difficult. Therefore, we measured the number of plaques and their volumes manually. CSF and spinal cord volumes were determined by our segmentation technique using T2-weighted sagittal spin echo

(SE) images. The volumes of CSF between two adjacent sagittal slices were estimated separately by multiplying the average cross-sectional area of the CSF space and spinal cord in two consecutive slices by the gap thickness. Total spinal volume was obtained by summing the volumes of total spinal cord and total spinal CSF spaces. Relative spinal cord atrophy was determined by a ratio of total spinal cord volume to total spinal volume.

3.3 Determination of diffuse lesions

Typical MS lesions are oval-shaped with the long axis pointing toward the cortex (i.e., Dawson's fingers), but diffuse lesions have no specific size or shape and are assimilated by the surrounding brain or spinal cord parenchyma. Diffuse lesions were defined as poorly demarcated high-signal areas on both T2-weighted and/or fast fluid-attenuated inversion recovery images. Because they were small in volume, only the number of diffuse T2-weighted lesions was calculated from both cranial and spinal cord MR images.

Table 7. MRI protocol.

Sequence	TR (ms)	TE (ms)	NEX	Voxel size (mm^3)	turbo factor
Brain					
Sagittal T1 SE	400	8	2	5.88	
Axial T1 SE	540	12	2	4.21	
Axial T2 FSE	4300	106	1	0.74	48
Spinal cord					
Sagittal T1 SE	400	12	3	8.05	
Sagittal T2 FSE	8002	204	2	9.40	

Abbreviations: MRI = magnetic resonance imaging, TR = repetition time, TE = excitation time, NEX = number of excitations, SE = spin echo, FSE = fast spin echo

4. Neuropsychological evaluation (Study IV)

Within two weeks of the neurological examination and MRI scan, all subjects underwent an extensive battery of neuropsychological tests performed in a single session. Cognitive domains and the tests used for their evaluation are indicated in Table 8. These tests are widely used to assess cognitive impairment in MS and were divided into different domains according to Lezak's theoretical considerations (Lezak 1995). Depression was evaluated using the Beck Depression Inventory (BDI) test (Beck et al 1961).

5. Urological investigations (Study II)

The presence of urgency, urge incontinence, stress incontinence, voiding frequency and dysuria were evaluated from urological history. The urodynamic investigation was performed within three months of the neurological examination according to standards of the International Continence Society except when specifically noted (Abrams et al 1988). Medications that possibly influence bladder and urinary sphincter behaviour were discontinued two weeks before urodynamic tests. Urodynamic evaluation consisted of free-flow, static urethral pressure, cystometry and pressure-flow tests together with electromyographic registration of pelvic floor muscles. For cystometry, the bladder was filled with saline at room temperature (25°C) at a filling of 50 ml via a 10 F catheter with the patient in sitting position. Pressure-flow analysis was made on a flow chair after cystometry. Pressures were measured using external transducers connected to the patient by fluid-filled manometer lines and catheters using a 3.3 F catheter for intravesical pressure measurement and an 8 F catheter for abdominal pressure measurement.

Table 8. Neuropsychological evaluation.

Cognitive function	Test
Attention and information processing	-Wechsler Adult Intelligence Scale-Revised (WAIS-R), digit span backwards and total (Wechsler 1981) -Corsi Block-tapping Test (Corsi 1972) -Paced Auditory Serial Addition Test (PASAT), (Gronwall 1977) -Stroop test (Stroop 1935) -Symbol Digit Modalities Test (SDMT) (Smith 1982)
Concept formation, reasoning and executive functions	-WAIS-R, similarities and picture arrangement (Wechsler 1981) -Wisconsin Card Sorting test (WCST) (Heaton et al 1993)
Verbal production	-Boston Naming Test (BNT), finnish version (Laine et al 1993) -Verbal fluency, category and phonological (Lezak 1995)
Memory and learning	-Benton Visual Retention Test, BVRT (Benton 1963) -Wechsler Memory Scale, logical stories, immediate and delayed (WMS) (Wechsler 1987) -Spatial Recall Test 7/24, SRT, learning score and delayed recall (Barbizet and Cany 1970) -Shopping list, learning score and delayed recall (Buschke 1973)
Visuoperceptual and – constructive functions	-Hooper Visual Organization Test (Hooper 1983) -WAIS-R block design (Wechsler 1981)

6. Immunological analyses (Studies I, III)

Blood test was drawn from all MS patients and controls. CSF was obtained by lumbar puncture from 26 patients with PPMS, 18 patients with SPMS and 11 healthy controls. Leukocyte count, IgG index and OCBs were determined from CSF.

6.1 AMs

Mononuclear cells were separated from heparinised blood by Ficoll-Hypaque density gradient centrifugation, and cell smears were processed by cytocentrifugation. Cytologic specimens of CSF were prepared by cytocentrifugation and stained with May-Grünwald-Giemsa stain. The differentiation of lymphoid cells into lymphocytes and mononuclear phagocytes was done on cytocentrifuge smears.

AMs on the mononuclear cells were analysed by the three-layer indirect immunoperoxidase technique. Primary antibodies were LFA-1 (CD11a), VLA-4 (CDw49d), ICAM-1 (CD54), or VCAM-1 (CD106) (0157, 0764, 0544 and 1244 respectively; Immunotech, Marseille, France). The dilution was 1:100 for CSF specimens and 1:200 for blood specimens. Secondary antibody was peroxidase-conjugated rabbit anti-mouse antibody at a dilution of 1:10 (P0161; Dako, Glostrup, Denmark) and the third antibody was a peroxidase-conjugated goat anti-rabbit antibody at a dilution of 1:20 (L42007; Caltag, San Francisco, Calif). Specimens were analysed at $100 \times$ magnification. A total of 400 cells from blood and a mean of 330 ± 71 mononuclear cells from CSF were analysed for each cell surface marker. Results were expressed as the percentage of positively stained lymphocytes from the total number of lymphocytes counted and the percentage of positively stained monocytes from the total number of monocytes counted.

6.2 Cytokines and chemokines

In serum and CSF, we analysed IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IL-13, IL-17, IFN-γ, TNF-α, macrophage inflammatory peptide-1beta (MIP-1β), monocyte chemoattractant protein-1 (MCP-1), granulocyte-macrophage colony-stimulating factor, and granulocyte colony-stimulating factor. Multiple cytokine analysis kits were obtained from two sources, Linco Research Inc. (St. Charles, MO) and Upstate Cell Signaling Solutions (Lake Placid, NY). Assays were run in triplicate according to the manufacturers' protocol. Data were collected using the Luminex-100 system Version 1.7

(Luminex, Austin, TX). Data analysis was performed using the MasterPlex QT 1.0 system (MiraiBio, Alameda, CA). A five-parameter regression formula was used to calculate the sample concentrations from standard curves. All 96 samples were analysed with the LINCOplex kit (Linco Research Inc., Dupont et al 2005).

7. Statistical analyses

Statistical analyses were performed using SPSS 9.0 for Windows. Results were expressed as mean values±standard deviation (SD). Spearman's rank correlation coefficient was used to describe the relationship between two variables, and group comparisons between groups were conducted using Mann-Whitney U tests. P-values of < 0.01 (Study I) or < 0.05 (Studies II-IV) were considered statistically significant.

Results of immunological analyses were analysed using Kruskal-Wallis tests for group comparisons and were made by the Mann-Whitney U tests for multiple comparisons between two groups. A p-value of < 0.01 was considered statistically significant. Wilcoxon signed ranks test were used for paired serum and CSF cytokine comparisons.

In the neuropsychological study, comparisons of clinical characteristics between groups were conducted using Kruskal-Wallis tests with Bonferroni adjustment for multiple comparisons, and Mann-Whitney U tests were used for post-hoc analysis test. A p-value of < 0.05 with Bonferroni correction was considered statistically significant.

RESULTS

1. Neurological characteristics of patients and healthy controls (Studies I-IV)

Retrospectively, 22 of 28 PPMS patients fulfilled the criteria for definite PPMS, and 6 of 28 patients fulfilled the criteria for possible PPMS according to revised McDonald criteria (Polman et al 2005). Clinical characteristics of PPMS patients and controls are shown in Table 6. The most common symptoms at the onset of PPMS included motor, cerebellar and sensory disturbances (68, 39 and 25%, respectively). Disturbances in bowel and/or bladder function at onset occurred in 18% of patients, visual disturbances occurred in 14 % of patients and brainstem abnormalities occurred in 7% of patients. Sixty-eight % of patients had at least two disturbances at onset. EDSS subscores showed that motor (mean: 3.3), cerebellar (1.7), sensory (1.7) and bowel/bladder functions (1.7) were the most commonly disturbed.

2. MRI findings (Studies I-IV)

Cerebral lesions in T1- and/or T2-weighted images were detected in all patients but not in controls (Table 9). T1-weighted brain lesions were seen in 26 of 28 patients and T2-weighted lesions were seen in all patients. Diffuse changes in the brain and spinal cord were seen in 25 and 23 patients, respectively, but these changes were not detected in controls. Diffuse lesions were more frequent in the brain than in the spinal cord (means: 7.1 vs. 1.8). T2-weighted spinal lesions were observed in 23 of 28 patients (82%), but T1-weighted spinal lesions were not observed. In PPMS patients the number of T2-weighted lesions in the cervical region appeared to be greater than that in the thoracic cord (57 vs. 37). Relative atrophy in PPMS patients was significantly more pronounced in the brain and spinal cord compared to controls. No significant differences in MRI parameters were found between men and women.

Table 9. MRI findings for PPMS patients and controls.

MRI characteristics	PPMS mean±SD	Controls mean±SD	p-value*
Brain			
T1 lesion load, cm^3	0.9±0.7	0	-
T2 lesion load, cm^3	8.4±7.2	0	-
Total brain volume, cm^3	1047.8±128.7	1182.2±112.1	**0.001**
Total intracranial CSF space volume, cm^3	386.4±105.4	312.9±65.0	n.s.
Total intracranial volume, cm^3	1433.8±97.8	495.1±138.9	n.s.
Relative brain atrophy	0.73±0.07	0.79±0.04	**0.002**
Number of diffuse lesions	7.1±4.8	0	-
Spinal cord			
Number of T2-weighted lesions	3.4±2.0	0	-
Total spinal cord volume, cm^3	26.3±4.4	33.1±3.5	**0.000**
Total spinal CSF space volume, cm^3	46.1±2.7	44.8±3.0	n.s.
Total spinal volume, cm^3	72.5±4.2	77.9±4.1	**0.000**
Relative spinal cord atrophy	0.36±0.05	0.42±0.03	**0.000**
Number of diffuse lesions	1.8±1.2	0	-

Abbreviations: PPMS = primary progressive MS, CSF = cerebrospinal fluid, * Mann-Whitney U test, n.s. = not significant

2.1 Clinicoradiological correlations in PPMS (Study I)

No significant correlations were detected between MRI findings and total EDSS score, but total RFSS score significantly correlated with relative brain atrophy (p = 0.004) and the volume of total intracranial CSF space (p = 0.006). Volumes of cerebral T1- and T2-weighted lesions correlated with ambulation index (p = 0.005 and 0.004, respectively). Both total brain volume and relative brain size both correlated with arm index (p = 0.001 and 0.004, respectively) and higher cerebral disturbances as measured by EDSS subscore (p = 0.008 and 0.005, respectively). Atrophy in the spinal cord was not associated with disability as measured by EDSS score, but the number of T2-weighted plaques in the spinal cord correlated with sensory disturbance subscore (p = 0.001). Diffuse lesions in the brain and the spinal cord did not significantly correlate with disability.

Duration of disease did not correlate with EDSS or RFSS scores, arm index or ambulation index. Total brain volume correlated with the duration of PPMS (r = −0.55, p = 0.001), but other MRI parameters did not significantly correlate with disease duration.

3. Neuropsychological findings (Study IV)

No significant differences in age, gender or education were detected among PPMS, SPMS and control groups. Age, years of education, disease duration, depression score and EDSS score did not significantly correlate with the results of any neuropsychological test. Comparisons between men and women showed no differences for any groups. Patients with MS had higher depression scores than controls, but no significant differences in BDI scores were found between the two MS groups.

Patients with PPMS performed better than patients with SPMS in the visual learning test (7/24 spatial recall test, SRT) (Table 10), but no other significant differences were found. Patients with PPMS performed worse than the control group in attention and information processing, concept formation, reasoning and executive functions, verbal production and memory and learning. Patients with PPMS also scored worse than controls in visuoperceptual and visuoconstructive functions but these differences did not reach statistical significance.

Table 10. Neuropsychological performance of patients with PPMS, SPMS and controls.

Tests	(1) PPMS Mean± SD	(2) SPMS Mean±SD	(3) Controls Mean±SD	Kruskall Wallis test Bonferroni corrected p-value
ATTENTION AND INFORMATION PROCESSING				
WAIS-R/digit span, backwards	5.3±1.5	5.7±1.7	6.2±1.0	1.0
WAIS-R/digit span, total	11.4±2.6	12.0±3.1	13.1±1.7	1.0
Corsi block, total	11.7±1.9	10.6±2.7	12.6±2.8	0.836
PASAT 3 sec, correct	39.0±12.1	41.1±15.7	46.7±10.1	1.0
PASAT 2 sec, correct	27.6±7.7	32.5±12.3	36.3±6.9	0.308
STROOP interference time	60.2±28.5	49.7±53.6	31.8±10.9	**0.009**[1,2<3]
SDMT	38.0±12.3	38.2±12.1	52.9±9.5	**0.002**[1,2<3]
CONCEPT FORMATION, REASONING AND EXECUTIVE FUNCTIONS				
WAIS-R similarities	22.9±7.4	24.3±5.4	27.8±2.3	0.616
WAIS-R picture arrangement	9.3±5.1	10.6±5.9	13.0±3.0	1.0
WCST, correct	72.3±13.4	79.7±22.8	97.1±22.5	**0.022**[1,2<3]
VERBAL PRODUCTION				
BNT, correct	25.5±3.3	26.2±3.4	28.1±1.6	0.176
Verbal fluency, category	20.5±5.6	18.1±6.4	24.8±5.1	**0.022**[1,2<3]
Verbal fluency, phonological	11.4±5.1	10.4±5.5	16.7±3.6	**0.004**[1,2<3]
MEMORY AND LEARNING				
BVRT, correct	6.7±1.5	6.8±1.5	8.0±1.2	0.308
WMS, logical stories, immediate	10.6±3.8	10.2±3.7	13.8±2.8	0.132
WMS, logical stories, delayed	7.8±4.4	8.3±3.8	13.0±2.8	**0.002**[1,2<3]
7/24 SRT, learning score	28.3±6.4	26.1±6.2	31.8±3.6	0.110
7/24 SRT, delayed recall	5.4±1.9	4.4±2.2	6.2±1.5	0.374
Shopping list, learning score	36.0±8.4	39.2±6.7	42.0±4.3	0.506
Shopping list, delayed recall	7.3±2.3	7.9±1.8	8.9±1.2	0.594
VISUOPERCEPTUAL AND –CONSTRUCTIVE FUNCTIONS				
Hooper, correct	11.2±1.7	11.0±2.5	12.5±1.3	0.616
WAIS-R Block Design	26.4±9.1	25.0±11.2	33.0±8.5	0.418

Abbreviations: PPMS= primary progressive MS, SPMS=secondary progressive MS, WAIS-R= Wechsler Adult Intelligence Scale-Revised, PASAT= Paced Auditory Serial Addition Test, SDMT= Symbol Digit Modalities Test, WCST= Wisconsin Card Sorting Test, BNT= Boston Naming Test, Finnish version, BVRT= Benton Visual Retention Test, MS=Wechsler Memory Scale-Logical Memory,SRT 7/24=Spatial Recall Test; Hooper=Visual Organization Test

3.1 Correlations with MRI findings

Correlation analyses showed that the number of diffuse brain lesions in MR images significantly correlated with performance in the Paced auditory serial addition test 3 (PASAT 3), Symbol Digit Modalities Test (SDMT), Wechsler Adult Intelligence Scale-Revised (WAIS-R) similarities test, Boston Naming Test (BNT), phonological fluency test, Wechsler Memory Scale (WMS) logical stories test, shopping list test, Hooper Visual Organisation test and WAIS-R block design test (Table 11). Volume of T2-weighted brain lesions correlated with performance in the SDMT, BNT, Hooper Visual Organisation test and WAIS-R block design test. Volume of T1-weighted brain lesions correlated with performance in the BNT and 7/24 SRT. Brain atrophic changes did not correlate with performance in any neuropsychological test.

4. Urinary symptoms and urodynamic findings in PPMS (Study II)

All 24 PPMS patients who participated in the urology study had at least one micturition complaint. Twenty-one% of patients (n= 5) had lower urinary tract symptoms as one of the initial symptoms of MS, but only one man had voiding problems as the only symptom at onset. The most common symptoms were urgency (83%) and urge incontinence (75%). Other symptoms were hesitancy (58%), frequency (54%), nocturia (38%) and stress incontinence (33%). No patients had dysuria. Urinary symptoms were not related to gender, age, disease duration or total EDSS score. Voiding complaints were not related to the severity of pyramidal or sensory disturbances.

Twenty-one PPMS patients showed abnormal urodynamics. The most common finding was DSD (71%). Fifty-eight % of patients had detrusor hyperreflexia, 17% had detrusor hypoflexia and 25% had normal detrusor function. Obstruction was found in 58 % of PPMS patients. Urodynamic findings were not related to disease duration or EDSS score. Eighty-three % of men and 33% of women had detrusor hyperreflexia, which was the only significant sex difference in urodynamic results.

4.1 Correlations with MRI findings

Although voiding symptoms were not associated with MRI measurements, some correlations between urodynamic findings and MRI measurements were detected. Detrusor hyperreflexia and DSD correlated with T2–weighted lesion load in the brain (p = 0.01 and p = 0.02, respectively), and hypotonic detrusor was associated with an increased number of thoracic plaques (p = 0.02) and decreased brain volume (p= 0.02).

Table 11. The significant correlations (p< 0.05) between neuropsychological tests and MRI findings in patients with PPMS.

	T1 lesion load	T2 lesion load	Number of brain diffuse lesion	Brain atrophy
ATTENTION AND INFORMATION PROCESSING				
WAIS-R/digit span, backwards				
WAIS-R/digit span, total				
Corsi block, total				
PASAT 3 sec, correct			-0.539	
PASAT 2 sec, correct				
STROOP interference time				
SDMT				
CONCEPT FORMATION, REASONING AND EXECUTIVE FUNCTIONS				
WAIS-R similarities			-0.422	
WAIS-R picture arrangement				
WCST, correct				
VERBAL PRODUCTION				
BNT, correct	-0.446	-0.385	-0.481	
Verbal fluency, category				
Verbal fluency, phonological			-0.427	
MEMORY AND LEARNING				
BVRT, correct				
WMS, logical stories, immediate			-0.425	
WMS, logical stories, delayed			-0.378	
7/24 SRT learning score	-0.461			
7/24 SRT delayed recall	-0.511			
Shopping list, learning score			-0.434	
Shopping list, delayed recall				
VISUOPERCEPTUAL AND –CONSTRUCTIVE FUNCTIONS				
Hooper, correct		-0.401	-0.457	
WAIS-R Block Design		-0.442	-0.556	

Abbreviations: PPMS= primary progressive multiple sclerosis, WAIS-R= Wechsler Adult Intelligence Scale-Revised, PASAT= Paced Auditory Serial Addition Test, SDMT= Symbol Digit Modalities Test, WCST= Wisconsin Card Sorting Test, BNT= Boston Naming Test, Finnish version, BVRT= Benton Visual Retention Test, WMS= Wechsler Memory Scale-Logical Memory, SRT 7/24= Spatial Recall Test, Hooper= Visual Organization Test

5. Immunological findings (Studies I, III)

5.1 Basic inflammation indices

CSF leukocyte count was $0.004\pm0.001\text{x}10^9$/l and $0.003\pm0.0002\text{x}10^9$/l (reference: $\leq0.005\text{x}10^9$/l) for PPMS and SPMS, respectively. IgG index was 1.0 ± 0.1 (reference: ≤0.70) for PPMS and 0.9 ± 0.1 for SPMS. Eighty-three % of PPMS patients had OCBs in their CSF. Twenty-one of the 26 CSF samples were abnormal.

5.2 Expression of AMs on immune cells

The significant proportions of AM expressions on blood and CSF immune cells (i.e., lymphocytes and monocytes) are presented in Table 12. In patients with PPMS, the expressions of AMs were not related to gender, age, EDSS score or disease duration. Comparison between PPMS and SPMS showed that most of the expressions of AMs were higher in PPMS that in SPMS. Only the expressions of VLA-4, LFA-1 and ICAM-1 on blood lymphocytes were higher in SPMS than in PPMS. These differences were significant for LFA-1 and VCAM-1 on blood lymphocytes, ICAM-1 on blood monocytes and LFA-1 and ICAM-1 on CSF monocytes. The expressions of AMs on blood and CSF immune cells were higher in PPMS patients than in controls. Only VCAM-1 expression on blood monocytes was higher in controls than in patients with PPMS, but this difference was not significant. In blood, significant differences were detected between expression of VLA-4 on lymphocytes and expression of LFA-1 and ICAM-1 on monocytes. In CSF, significant differences were found between expression of VLA-4 and LFA-1 on both lymphocytes and monocytes. Furthermore, the expression of ICAM-1 on CSF monocytes was significantly higher in PPMS patients than in controls.

Table 12. The significant proportions (mean ± SD) of AM expression in the blood and CSF.

Lymphocytes	PPMS	SPMS	Controls	p^1	p^2
Blood					
VLA-4	29.1 ± 17.4	34.9 ± 11.4	13.1 ± 9.3	n.s	**0.003**
VCAM-1	0.7 ± 2.4	0.0 ± 0.0	0.0 ± 0.0	**0.006**	n.s.
LFA-1	24.9 ± 15.3	36.3 ± 12.1	13.0 ± 7.8	**0.005**	n.s.
CSF					
VLA-4	38.5 ± 22.6	27.0 ± 16.1	9.1 ± 8.3	n.s.	**0.004**
LFA-1	62.5 ± 25.9	42.4 ± 24.0	12.0 ± 5.6	n.s.	**0.003**
Monocytes					
Blood					
LFA-1	55.6 ± 32.2	34.6 ± 22.3	24.4 ± 12.9	n.s.	**0.005**
ICAM-1	55.5 ± 25.1	13.7 ± 17.5	20.0 ± 14.1	**<0.0005**	**<0.0005**
CSF					
VLA-4	42.3 ± 32.5	17.5 ± 24.4	0.0 ± 0.4	n.s.	**0.009**
LFA-1	75.8 ± 25.7	19.9 ± 17.6	0.0 ± 0.4	**0.006**	**0.003**
ICAM-1	61.0 ± 41.2	2.2 ± 6.6	0.0 ± 0.4	**<0.0005**	**0.009**

Abbreviations: SD = standard deviation, AM = adhesion molecule, PPMS = primary progressive MS, SPMS = secondary Progressive MS, CSF = cerebrospinal fluid, VLA-4 indicates very late activation antigen 4, LFA-1 lymphocyte-function associated antigen 1, ICAM-1 = intercellular AM 1, VCAM-1 = vascular cell AM 1.

p^1 = PPMS vs SPMS, p^2 = PPMS vs controls, n.s.= not significant

5.3 Levels of cytokines and chemokines

Levels of cytokines and chemokines in PPMS and SPMS were of similar magnitude. Serum MCP-1 levels appeared to be lower in PPMS patients than in healthy controls (p<0.05) but otherwise there were no significant differences among groups in serum or CSF. In PPMS, the levels of IL-8 and MCP-1 in CSF were higher than those in serum (p=0.000 and p=0.014, respectively). The levels of several proteins were below detection level for all groups. Cytokine/chemokine levels were not related to gender, age, EDSS score or disease duration in patients with PPMS. A slight correlation was detected only between serum MIP-1β and EDSS score in patients with PPMS (r=0.05, p=0.02).

5.4 Correlations with MRI findings

For blood immune cells, the only significant correlation was between VLA-4 expression on monocytes and the number of diffuse brain lesions (r = 0.561, p = 0.004). In CSF, the expression of VLA-4 on lymphocytes correlated with the total volume of brain plaques and the number of diffuse brain lesions(r =.0.528, p = 0.007 and r = 0.536, p= 0.006, respectively). A significant correlation was also found between the level of VLA-4 on CSF monocytes and the volume of brain T2-weighted lesions as well as the total volume of brain lesions (r = 0.550, p = 0.004 and r = 0.567, p = 0.003, respectively). Furthermore, the expression of LFA-1 on CSF lymphocytes correlated with the number of spinal plaques in T2-weighted images (r = 0.660, p = 0.000). No associations were found between the expressions of AMs in blood or CSF and atrophic changes in the brain or spinal cord.

In patients with PPMS the level of serum MIP-1β correlated with T2-weighted brain lesion volume (r = 0.54, p = 0.01). Other significant (p < 0.01) correlations between cytokines and MRI results were not detected.

DISCUSSION

During the last two decades the understanding of MS has increased dramatically. The development of MRI techniques has been a significant factor in the refinement of diagnostics and evaluation of treatment responses. New clinical diagnostic criteria have standardised diagnoses. EAE and other animal models together with neuropathological and imaging studies have provided information on the pathophysiology of inflammation and demyelination. A large portion of this new information applies to RRMS, and there are still many open questions regarding PPMS. Because PPMS is a rare subtype of MS, it has been studied less than other subtypes. The purpose of this thesis was to study clinical, immunological and radiological characteristics of PPMS in more detail and to compare these characteristics to those of SPMS. Currently, there is no effective treatment for PPMS, therefore more information about this rare subtype of MS is highly needed.

Clinicoradiological findings

This study confirmed previous observations of clinical characteristics of PPMS, including older age at disease onset, the predominance of motor dysfunctions over other neurological manifestations, and the high frequency of micturition problems (McDonnell and Hawkins 1998, Miller and Leary 2007). As expected, plaques in T1- and/or T2-weighted images were seen in the CNS of all MS patients. MRI images could not be compared between PPMS and SPMS because patients with SPMS were studied with a different 0.5 T MRI unit. Atrophy in both the brain and spinal cord was more pronounced in PPMS patients than in healthy subjects. This is in line with existing evidence indicating that loss of brain volume occurs at a rate of 0.5-1% per year in MS as compared to rate of 0.1% per year in healthy individuals (Pfefferbaum et al 1994, Anderson et al 2006).

Correlation analysis showed that ambulation index, arm index and higher cerebral disturbances were only weak correlated with MRI measures, including T1- and T2-weighted lesion load, number of diffuse lesions and brain atrophy. These weak correlations between MRI measurements and clinical disability were not surprising, as previously reported, correlations between clinical manifestations of PPMS and conventional MRI findings are also weak (Rocca et al 2012). One explanation for these poor correlations may be the insufficient sensitivity of MRI to detect changes typical of PPMS such as diffuse lesions. Newer MR techniques show GM abnormalities also in PPMS, but no significant differences in cortical pathology between RRMS and chronic progressive MS have been found (Antel 2012, Calabrese et al 2012). In the

present study, cortical abnormalities were not taken into account, which may be one reason for the weak correlations between MRI findings and clinical measurements.

Correlations between clinical findings and spinal cord atrophy could also not be detected. According to the latest studies, spinal cord atrophy is already present in early PPMS and it increases at follow-ups and correlates with clinical findings (Bieniek et al 2006, Agosta et al 2007, Laule et al 2010, Rocca et al 2011). Many previous studies used a cross-sectional area of the cervical cord to measure atrophy (Bieniek et al 2006, Rocca et al 2011). The weakness of this method is the variation in cross section at different levels. Although we used the whole spinal cord to determine spinal atrophy, clinicoradiological correlations were still weak. One reason might be that MRI of the spinal cord is challenging because of its long and narrow shape and the presence of motion artefacts due to CSF, cardiac pulsation and respiration (Agosta and Filippi 2007). Thus conventional MRI may not be sensitive enough to detect atrophy in the spinal cord.

Although newer MR techniques are more accurate, a specific pattern for PPMS has not been found (Rocca et al 2012). Conventional MRI remains sufficient for differential diagnostic purposes. In addition to imaging, the diagnosis of PPMS is based on symptoms and clinical findings. To diagnose PPMS, CSF analysis is also required (Nilsson et al 2007).

Cognition and its correlations with MRI findings

Over the past two decades, cognitive impairment in MS has been the focus of attention. It is reported that cognitive impairment is more severe in patients with a chronic progressive disease than in those with RRMS (Smestad et al 2010). The present study showed that PPMS patients exhibit decline in several cognitive domains, and no marked differences were found between the cognitive performances of PPMS and SPMS patients. Previously, only a few studies have separately examined cognitive impairment in these two chronic forms of MS (Foong et al 2000, Gaudino et al 2001, Huijbregts et al 2004, Denney et al 2005). Most of the earlier studies have showed that cognitive difficulties are more significant in SPMS patients compared with PPMS patients (Huijbregts et al 2004, Denney et al 2005, Bergendahl et al 2007). Comparisons among these studies are difficult, however, due to variability in neuropsychological measures as well as disease duration and severity. On the basis of the present study, it can be concluded that cognitive problems are also common in patients with PPMS and should be taken into consideration when treating these patients. It is also important to evaluate cognitive status even

during early phases of MS, because cognitive impairment at MS diagnosis increases the risk of changing vocational status at later time points (Ruet et al 2013). If cognitive impairments are found, the possibility of neuropsychological rehabilitation can be assessed during early stages. Therefore, if cognitive decline is suspected, a brief cognitive assessment for MS is recommended (Langdon et al 2012). When more specific information is needed, extensive neuropsychological evaluation, such as that performed in the present study, is needed.

Many previous studies report significant correlations between MRI measurements and cognitive impairments in patients with MS (Rao et al 1989, Roosendaal et al 2009, Julian 2011). In particular, both central and cortical brain atrophy seem to correlate well with cognitive problems (Benedict et al 2006, Bermel ja Bakshi 2006, Sanfilipo et al 2006, Tekok-Kilic et al 2007, Roosendaal et al 2011, Achiron et al 2012). Previously, a few studies analysing the relationship between MRI findings and cognitive deficits in PPMS reported only minor to moderate correlations (Foong et al 2000, Camp 1999, 2005). In a two year follow–up study by Camp and colleagues (2005), cognitive decline was associated with increased T1-weighted lesion load and atrophy, but a recent study reports T2-weighted and not T1-weighted lesion volume as being the best MRI predictor of cognition, in PPMS (Penny et al 2010). In the present study, verbal production and memory and learning domains correlated with T1-weighted lesion load, and verbal production and visuoperceptual and visuoconstructive functions correlated with T2-weighted lesion load. No correlations between atrophy changes in the brain and neuropsychological deficits were found.

Cortical lesions also seem to have an important role in cognition, and GM atrophy is found to correlate with cognitive impairment in RRMS (Benedict et al 2002, Amato et al 2007, Calabrese et al 2009a, Roosendaal et al 2009, 2011, Achiron et al 2012). A recent study using conventional MRI and MTI reports that GM MTR, NAWM volume and T2-weighted lesion load are strongly associated with cognitive impairments also in PPMS (Tur et al 2011c). In the present study, GM changes were not examined, which may be one reason for the weak correlations between MRI and cognitive measurements.

New MRI techniques have provided much important information about the correlation between cognitive problems and brain changes. Abnormalities in normal-appearing brain tissue detected by DTI, MTR and MRS, thalamic atrophy and corpus callosum damage are also reported to correlate with cognitive performance (Deloire et al 2005, Mathiesen et al 2006, Akbar et al 2010, Yu et al 2012, Houtchens et al 2007, Mesaros et al 2009). A study using

DTI tractography showed that lesions in spesific brain WM tracts contribute to cognitive impairment (Mesaros et al 2012). From a statistical point of view, the small size of our study population prevented the correlation of specific CNS areas with particular cognitive functions.

In the past few years, functional neuroimaging has also increased our knowledge about cognitive function. According to present knowledge, patients with MS show an increased magnitude of activation with more activated regions compared to controls (Forn et al 2006, 2007, 2012). fMRI also shows that with the progression of MS there are adaptive changes in neuronal activation and compensatory mechanisms in cognitive networks (Loitfelder et al 2011). On the other hand, a recent small study shows a change in the recruitment of different brain regions after cognitive rehabilitation (Filippi et al 2012a). A functional MRI study of patients with PPMS shows differences in brain regions engaged during the performance of a cognitive task between patients with preserved cognition and those with cognitive impairment (Rocca et al 2010b).

Urological findings and their correlations with MRI findings

Among micturition disturbances, urgency and urge incontinence were the most common abnormalities, consistent with previous findings (De Sèze et al 2007). The relatively weak correlation between urinary symptoms and urodynamic findings indicates the importance of urodynamic investigations in understanding the mechanisms of presenting symptoms. It was previously reported that the subjective assessment of bladder dysfunction correlates poorly with objective measurements (Kragt et al 2004). In the present study, the urinary symptoms or urodynamic findings were not related to disease duration or EDSS score. Therefore, ultrasound scanning of residual volume is recommended for every PPMS patient and urodynamic investigation is recommended if there are any micturition complaints. Knowing of the exact bladder dysfunction helps in the making of the right and individual therapeutic decision.

Only a few studies have investigated the association between urinary problems and MRI findings. Correlations are reported between urological complaints and cross-sectional area of spinal cord or presence of spinal cord lesions (Lycklama á Nijeholt et al 1998, Araki et al 2002). A study by Araki and colleagues reports correlations between pontine lesions and detrusor hyporeflexia and between cervical cord lesions and DSD (Araki et al 2003). Although some atrophic and focal changes detected by MRI correlated with urodynamic findings in the present study, MRI findings are not specific enough to define the type or severity of lower urinary tract abnormalities. Understanding the underlying

mechanisms of lower urinary tract dysfunction is crucial for effective disease management.

Immunology

Due to the central role of immunological responses in MS pathogenesis, we analysed whether the expressions of AMs, cytokines and chemokines could be used to evaluate disease activity and progression and whether immune abnormalities are correlated with MRI findings. The presence of several immunological abnormalities in both the blood and CSF of PPMS patients was detected. These changes included increased expression of cAMs, indicating that these inflammatory changes are of key importance even in PPMS. The present study extends previous reports showing similar immunological abnormalities in PPMS and SPMS. The expression some of AMs was higher in PPMS than in SPMS, but there was no significant difference between PPMS and SPMS in levels of cytokines/chemokines in the serum or CSF. This similarity between chronic progressive subtypes may due to neurodegenerative processes that prevail over inflammation in both PPMS and SPMS. Thus immunological differences between these two progressive subtypes are so minimal that they cannot be used for MS subtype classification.

Some correlations between AMs and disease activation detected by MRI are reported for RRMS but not for PPMS (Giovannoni et al 1997, Hartung et al 1995, Khoury et al 1999, Rieckmann et al 1997, 1998, Acar et al 2005). In the present study, the level of VLA-4 in CSF correlated with the total volume of brain lesions and the number of diffuse brain lesions. This finding may be important because the mechanism of natalizumab, the most effective treatment for MS, is the blockade of the α4-integrin subunit of the VLA-4 (Polman et al 2006). The weakness of this study is a small sample size and therefore the results need to be interpreted with caution.

The only correlation involving cytokines and MRI parameters in patients with PPMS was between the level of serum MIP-1β and T2-weighted brain lesion volume. Previously, a correlation was reported between the level of IL-6 and lesion volume in patients with RRMS and SPMS, but not in patients with PPMS (Hagman et al 2011). In recent years, some correlations between the expression of CXCL12 or CXCL13 and increased inflammatory activity in MS lesions were reported, but previous studies using conventional MRI could not detect significant correlations between MRI parameters and serum levels of cytokines, cytokine receptors and chemokines for different subtypes of MS (Kraus et al 2002, Furlan et al 2005, Moll et al 2009, Festa et al 2009, Brettschneider et al 2010).

As the immune molecules analysed in this study did not correlate with disability, and their correlation with MRI parameters was modest, their use in clinical practice is of limited value. However, it is likely that these correlations may become more pronounced with the use of new MRI techniques.

SUMMARY AND CONCLUSIONS

In this thesis PPMS was examined from many sides. Patients were comprehensively examined: imaging includes the whole CNS, patients and controls underwent an extensive battery of neuropsychological tests, controls were well matched and also the immunological side was studied. It is obvious that we need more information on the pathophysiology of PPMS to develop effective treatments for this MS subtype. Nowadays we use a MRI unit with higher T and it is probable that clinicoradiological correlations would be better now. The diagnosis of PPMS may be challenging. In the future, the goal is to identify biomarkers that can be used to make earlier and more accurate diagnoses and to evaluate disease subtypes. Hopefully, we can then make individualised therapeutic decisions.

The following conclusions are drawn from this study:

1. Brain and spinal cord atrophy are present in PPMS and are only weakly correlated with clinical disability. Conventional MRI is not specific enough to identify all changes associated with PPMS, and therefore newer MR techniques are needed to obtain better clinicoradiological correlations.

2. PPMS patients show decline in several cognitive domains compared with healthy controls. No significant differences in cognitive performance were found between PPMS and SPMS patients.

3. Micturition problems are very common in PPMS. Urodynamic findings correlate with lesion load in the brain and spinal cord as well as with brain atrophy. Urodynamic investigation is recommended for all PPMS patients who have micturition complaints so that the individual therapeutic decisions can be made.

4. Up-regulated expression of AMs in the blood and CSF compared to controls and higher levels of MCP-1 and IL-8 in CSF compared with blood, suggesting their local synthesis in the CNS, indicate inflammation in advanced PPMS.

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ORIGINAL PUBLICATIONS

Volumetric quantitation by MRI in primary progressive multiple sclerosis: volumes of plaques and atrophy correlated with neurological disability

M. Ukkonen[a,d], P. Dastidar[b], T. Heinonen[c], E. Laasonen[b,e] and I. Elovaara[a,e]

[a]*Neuroimmunology Unit, Department of Neurology,* [b]*Department of Diagnostic Imaging, Tampere University Hospital, Tampere;* [c]*Ragnar Granit Institute, University of Technology, Tampere;* [d]*International Programme in Neuroscience, Tampere University Graduate School in Life Sciences, Tampere; and* [e]*Medical School, University of Tampere, Tampere, Finland*

In primary progressive multiple sclerosis (PPMS) abnormalities in brain magnetic resonance imaging (MRI) differ from abnormalities in other subtypes of multiple sclerosis (MS). It was investigated whether the extent of brain and spinal cord MRI abnormalities is reflected in the neurological disability in PPMS. Focal and diffuse changes and atrophy in central nervous system (CNS) in patients with PPMS ($n = 28$) and healthy controls ($n = 20$) were assessed by semi-automatic MRI segmentation and volumetric analysis. The measurements were related to neurological disability as expressed by the expanded disability status scale (EDSS), the regional functional scoring system (RFSS), the arm index and the ambulation index. Plaques in T1- and/or T2-weighted images were seen in all brains, while spinal plaques were detected in 23 of 28 patients (82%). The total volumes of brain and spinal cord were significantly smaller in patients than in controls ($P = 0.001$ and 0.000, respectively). The volumes of T1 or T2 lesions in the brain correlated to the ambulation index ($r = 0.51$, $P = 0.005$ and $r = 0.53$, $P = 0.004$, respectively). No correlations were detected between MRI measurements and total EDSS score, but relative brain atrophy correlated inversely with the total RFSS scores, poor arm index and higher cerebral disturbances ($r = -0.53$, $P = 0.004$; $r = -0.53$, $P = 0.004$; and $r = -0.52$, $P = 0.005$, respectively). Although the number of spinal T2 lesions correlated with sensory disturbances ($r = 0.60$, $P = 0.001$), no correlations were found between EDSS subscores and spinal cord atrophy. These findings show that marked atrophy of brain and spinal cord detected by volumetric quantitation correlates with neurological disability. This observation indicates the importance of neurodegenerative events in PPMS.

Introduction

Multiple sclerosis (MS) is an inflammatory disease of the central nervous system (CNS). In most patients, it is manifested as a relapsing–remitting MS (RRMS) course, which is followed after 10–15 years by transition to the secondary progressive multiple sclerosis (SPMS) phase. In 10–20% of patients, the disease is progressive from the onset, with occasional plateaus and temporary minor improvements; this form is termed primary progressive MS (PPMS) (Lublin and Reingold, 1996; Thompson *et al.*, 1997). It has been suggested that the genetic and immunologic background of PPMS differs from that of RRMS and SPMS (Olerup *et al.*, 1989; Hillert *et al.*, 1992; Giovannoni *et al.*, 1994, 1996; Revesz *et al.*, 1994; Dore-Duffy *et al.*, 1995; Weinshenker *et al.*, 1995; Jalonen *et al.*, 2002).

The spectrum of CNS lesions detected by magnetic resonance imaging (MRI) in PPMS differs from the brain abnormalities seen in RRMS or SPMS. In particular, focal T1- and T2-weighted lesions are fewer in number and show less enhancement with gadolinium, while diffuse brain abnormalities, defined as poorly demarcated high-signal areas on both T2 and/or fluid attenuated inversion recovery (FLAIR) images, are more frequent (Thompson *et al.*, 1990, 1991; Filippi *et al.*, 1995a; Sinnige *et al.*, 1995; Kidd *et al.*, 1996; Lycklama à Nijeholt *et al.*, 1998, 2001; van Walderween *et al.*, 1998, 2001). Better correlations between MRI changes in the brain and neurological disability have been reported in RRMS and SPMS than in PPMS (Mammi *et al.*, 1996; Lycklama à Nijeholt *et al.*, 1998), although, in general, these correlations have been relatively weak (Gass *et al.*, 1994; Filippi *et al.*, 1995b; van Walderween *et al.*, 1995). Recent studies have brought out a correlation between brain atrophy and neurological disability (Losseff *et al.*, 1996a; Filippi *et al.*, 1998).

Using MRI, spinal lesions with a predominance in the cervical cord have been found in about 75% of

Correspondence: Irina Elovaara, Neuroimmunology Unit, Tampere University Hospital, Teiskontie 35, 33520 Tampere, Finland (tel.: 358 3 2476692; fax: 358 3 2474351; e-mail: ielovaara@tays.fi).

patients with MS (Kidd *et al.*, 1993). It is noteworthy that such lesions have been reported to be more clearly associated with neurological disability than those in the brain (Lycklama à Nijeholt *et al.*, 1998). Also spinal cord atrophy is associated more clearly with neurological impairment than spinal plaques (Kidd *et al.*, 1993; Filippi *et al.*, 1996, 1997). The diffuse spinal cord abnormalities typical of PPMS are associated with a progressive clinical course and greater disability (Lycklama à Nijeholt *et al.*, 1997).

This prospective study was undertaken to establish whether the extent of focal and diffuse lesions and atrophy in the brain and spinal cord correlates with neurological disability in PPMS. For this purpose, the volumes of T1- and T2-weighted lesions in the brain and the estimates of brain and spinal atrophy were correlated with neurological disability as expressed by the expanded disability status scale (EDSS), the regional functional scoring system (RFSS), the arm index and the ambulation index (Kurtzke, 1983; Munsat, 1989). The RFSS scoring system contains the same seven functional systems of Kurtzke, that are the basis for the EDSS, but each functional system is divided into a number of regions or items. The arm index includes four functions of upper limbs (dressing, washing hair, using knife and fork and handling coins). Ambulation index measures the walking. The numbers of spinal T1- and T2-weighted lesions as well as the numbers of diffuse cerebral and spinal lesions were also related to neurological disability.

Patients and methods

Twenty-eight patients (14 men and 14 women) with a progressive course of MS were selected from among the 300 MS patients followed at the Department of Neurology, Tampere University Hospital, which provides specialized neurological care for 400 000 Finns. All patients with progressive clinical course according to patient records were interviewed and those with relapses were excluded. The remaining 28 patients had progressive disease from the onset, with occasional plateaus and temporary minor improvements fulfilling the criteria of Lublin and Reingold (1996). None of them had received corticosteroid or other immunosuppressive treatment. The control group comprised 20 subjects (10 male and 10 female) whose age, sex and education were matched to the patients. None of the controls had any organic CNS disease, hypertension or head trauma. Patients and controls underwent neurological examination, including evaluation of disability. Cerebrospinal fluid (CSF) was obtained by lumbar puncture from 26 patients. Altogether 65% of the patients had elevated IgG index (reference ≤0.70), 83% had oligoclonal bands in their CSF and 28% had

Table 1 Clinical characteristics of primary progressive multiple sclerosis patients and controls

Characteristics	PPMS	Controls
No. of patients	28	20
Age (mean ± SD, years)	51 ± 9	46 ± 6
Gender (male/female)	14/14	10/10
Duration of MS (mean ± SD, years)	12 ± 9	NA
EDSS [mean (range)]	4.9 (2.0–8.0)	NA
RFSS [mean (range)]	35.5 (11–86)	NA
ARM index [mean (range)]	1.6 (0–4)	NA
Ambulation index [mean (range)]	3.7 (1–9)	NA

PPMS, primary progressive multiple sclerosis; EDSS, expanded disability status scale; RFSS, regional functional scoring system; NA, not applicable.

pleocytosis (≤5 cell/mm^3). Patients were classified as having definite, probable or possible PPMS according to the criteria of Thompson *et al.* (2000). This study was approved by the Ethical Committee of Tampere University Hospital, and informed consent was obtained from each patient.

The clinical characteristics of the PPMS and control groups are shown in Table 1. The duration of MS and the scores in EDSS, RFSS, arm index and ambulation index were similar in men and women. The most common symptoms at the onset of disease included motor, cerebellar or sensory disturbances (68, 39 and 25%, respectively). Disturbances in bowel and/or bladder function at onset occurred in 18% of patients, visual disturbances in 14% and brain stem abnormalities in 7%. Nineteen patients (68%) had at least two disturbances at onset. The subscores of EDSS show that the most common signs involved motor (mean 3.3), cerebellar (1.7), sensory (1.7) and bowel and bladder functions (1.7).

MRI protocol

MR imaging was undertaken within 2 weeks from the neurological examination. All patients and controls were studied on the same 1.5 Tesla MRI unit (Signa Horizon LX Echospeed, Wisconsin, USA) to avoid the influence of interscanner variations. The brain MRI protocol included sagittal T1-weighted spin echo (SE), [repetition time (TR) = 400 ms; time of echo (TE) = 8 ms; number of excitations (NEX) = 2; voxel size = 5.88 mm^3], axial T1-weighted SE (TR = 540 ms; TE = 12 ms; NEX = 2; voxel size = 4,21 mm^3), and axial three-dimensional T2-weighted fast SE (FSE), (TR = 4300 ms; TE = 106 ms; NEX = 1; turbo factor = 48; voxel size = 0.74 mm^3) images. This sequence was performed as single slab sequences.

The spinal cord MRI was performed using a phased array coil and the entire spinal cord from C1- to L2-level

was covered. The spinal cord MRI protocol included sagittal T1-weighted SE (TR = 400 ms; TE = 12 ms; NEX = 3; voxel size = 8,05 mm^3) and sagittal T2-weighted FSE (TR = 8002 ms; TE = 204 ms; NEX = 2; voxel size = 9,40 mm^3) images. The same sequences, 3 mm SE and 3D sequences were tried – FLAIR, Dual Echo (T2/PD) and PD sequences – but they proved to be suboptimal and artefactual, often because of time consuming (10–20 min) sequences.

From the brain MRI protocol, axial 3D T2-FSE and T1-SE data and from the spinal cord MRI protocol sagittal T1-SE and sagittal T2-FSE data were used for segmentation and volumetric analyses. These analyses were made using the Anatomatic software operating in a PC/Windows95 environment (Heinonen *et al.*, 1998a). The inter- and intra-rater reliability of the various MRI analysis methods have been reported in earlier studies (Heinonen *et al.*, 1998b; Dastidar *et al.*, 1999). The volumetric accuracy of this program has been previously validated using five fluid-filled syringes imaged on MRI. This test demonstrated a 1.5% relative error between the measured and real volume (Heinonen *et al.*, 1998b).

From the cranial MR images the volumes of T1- and T2-weighted lesions were defined. The MS lesion volumes in the gap between two slices on T1-weighted images were estimated by multiplying the average cross-sectional area of the plaques in two consecutive slices by the gap thickness. This method was applied to both the axial T1-weighted images in the cranial MRI and to the sagittal T2-weighted images in the spinal MRI because neither of these sequences was three-dimensional in nature. Total intracranial volume was measured by summing the volumes of segmented grey and white matter (=total brain volume) and total intracranial CSF spaces. The volume of the total intracranial CSF spaces consisted of the volumes of the ventricular and peripheral CSF spaces. Relative brain atrophy was obtained by the ratio of total brain volume to total intracranial volume.

From the spinal MR images the numbers of cervical and thoracic MS plaques were defined. Due to the peripheral location of the T2-weighted plaques in the spinal cord and their adjacency to the spinal CSF space, their segmentation proved difficult and therefore the number of plaques and volumes were measured manually.

The volumes of CSF and spinal cord were determined with the segmentation technique using T2-weighted sagittal SE images. As this sequence was not 3D in nature, the volumes of CSF in the gap between two adjacent sagittal slices were estimated separately. This was performed by multiplying the average cross-sectional area of the CSF space and spinal cord in two

consecutive slices by the gap thickness. The total spinal volume was obtained by summing the volumes of total spinal cord and total spinal CSF spaces. Relative spinal cord atrophy was defined by the ratio of total spinal cord volume to total spinal volume.

In addition, the number of diffuse T2-weighted lesions, was calculated from both cranial and spinal cord MR images. Diffuse lesions proved to be small in volume and were defined as poorly demarcated high-signal areas on both T2- and/or FLAIR images. In contrast to typical MS lesions that are oval and have the long axis pointing towards the cortex (Dawson's fingers), diffuse lesions have no specific size or shape but assimilated to the surrounding brain or spinal cord parenchyma. The diffuse cranial and spinal lesions were calculated numerically because of the extremely small volumes of these lesions.

Statistical analysis

Spearman's rank correlations were used to describe the dependence between two variables. Group data comparisons were made using Mann–Whitney test. A *P*-value of < 0.01 was considered statistically significant.

Results

According to Thompson's criteria for PPMS (Thompson *et al.*, 2000), 20 patients had definite and eight probable MS. Cerebral lesions in T1- and/or T2-weighted images were detected in all patients but not in controls (Table 2). T1-weighted brain lesions were seen in 26 of 28 patients and T2-weighted lesions in all of them. MRI and CSF results were abnormal in 20 of 28 patients (71%). Seven patients with probable PPMS had abnormal MRI, but in five of these the CSF was normal and two others refused a spinal tap. The remaining patient had MS-related CSF changes but MRI showed only seven brain lesions. Using the Anatomatic^{TM} segmentation technique (T. Heinonen) the mean total volumes for T1- and T2-weighted lesions in the brains of PPMS patients were estimated to be 0.9 cm^3 and 8.4 cm^3, respectively. Diffuse CNS changes were seen in the brains (*n* = 25 patients) and in the spinal cord (*n* = 23 patients) but were not detected in controls. These diffuse lesions were more frequent in the brain than in the spinal cord (7.1 vs. 1.8, mean values). T2-weighted spinal lesions were observed in 23 of 28 patients (82%), but no spinal lesions in T1-weighted images were seen. In the cervical region, the number of T2-weighted lesions appeared to be greater than in the thoracic cord (57 vs. 37).

In patients, the total brain volumes and the spinal cord volumes were smaller than in controls (13 and

Table 2 Magnetic resonance imaging results (mean ± SD) of primary progressive multiple sclerosis patients and controls

MRI measurements	PPMS	Controls	*P*-value*
Brain			
T1-weighted lesion volume (cm^3)	0.9 ± 0.7	0	–
T2-weighted lesion volume (cm^3)	8.4 ± 7.2	0	–
Total brain volume (cm^3)	1047.8 ± 128.7	1182.2 ± 112.1	**0.001**
Total intracranial CSF space volume (cm^3)	386.4 ± 105.4	312.9 ± 65.0	0.016
Total intracranial volume (cm^3)	1433.8 ± 97.8	495.1 ± 138.9	0.110
Relative brain atrophy	0.73 ± 0.07	0.79 ± 0.04	**0.002**
Number of diffuse lesions	7.1 ± 4.8	0	–
Spinal cord			
Number of T2-weighted plaques	3.4 ± 2.0	0	–
Total spinal cord volume (cm^3)	26.3 ± 4.4	33.1 ± 3.5	**0.000**
Total spinal CSF space volume (cm^3)	46.1 ± 2.7	44.8 ± 3.0	0.112
Total spinal volume (cm^3)	72.5 ± 4.2	77.9 ± 4.1	**0.000**
Relative spinal cord atrophy	0.36 ± 0.05	0.42 ± 0.03	**0.000**
Number of diffuse lesions	1.8 ± 1.2	0	–

CSF, cerebrospinal fluid.

*Mann–Whitney test, significant *P*-values are in bold.

26%, respectively). In patients, atrophy was more pronounced both in the brain and spinal cord compared with the controls. No significant differences in MRI parameters were found between men and women.

The significant correlations between MRI findings and neurological disability as assessed by EDSS, EDSS subscores, RFSS, arm index and ambulation index are shown in Table 3. No significant correlations were detected between the MRI findings and total EDSS scores, but total RFSS scores correlated significantly with relative brain atrophy ($P = 0.004$) and the volume of total intracranial CSF space ($P = 0.006$). The volumes of cerebral T1- and T2-weighted lesions correlated with the ambulation index ($P = 0.005$ and 0.004, respectively). The total brain volume and the relative brain size both correlated with the arm index ($P = 0.001$ and 0.004, respectively) and higher cerebral disturbances ($P = 0.008$ and 0.005, respectively). The atrophy in the spinal cord evinced no association with disability, but

the number of T2-weighted plaques in the spinal cord correlated with the subscore for sensory disturbances ($P = 0.001$). The diffuse lesions in brain or spinal cord had no significant correlations with disability.

Duration of disease did not correlate with EDSS, RFSS, arm index or ambulation index. The total brain volume correlated with the duration of PPMS ($r = -0.55$, $P = 0.001$), but the other MRI parameters showed no significant correlations with the disease duration.

Discussion

Few studies have reported the correlation between neurological disability and MRI findings in PPMS. In general, these correlations have been weak (Thompson *et al.*, 1990; Kidd *et al.*, 1993; Lycklama à Nijeholt *et al.*, 1998; Rovaris *et al.*, 2001). In order to establish whether the extent of changes in the CNS in PPMS is

Table 3 Correlations between clinical and magnetic resonance imaging parameters

MRI measurements	EDSS		RFSS		Arm index		Ambulation index		Sensory function		Higher cerebral function	
	*R**	*P*-level	*R**	*P*-level	*R**	*P*-level	*R**	*P*-level	*R**	*P*-level	*R**	*P*-level
T1-weighted plaque volume in brain (cm^3)	0.43	n.s.	0.43	n.s	0.16	n.s.	**0.51**	**0.005**	0.27	n.s.	0.31	n.s.
T2-weighted plaque volume in brain (cm^3)	0.36	n.s.	0.24	n.s.	0.05	n.s.	**0.53**	**0.004**	0.13	n.s.	0.21	n.s.
Total brain volume (cm^3)	−0.24	n.s.	−0.41	n.s.	**−0.60**	**0.001**	−0.33	n.s.	−0.48	n.s.	**−0.49**	**0.008**
Total intracranial CSF space volume (cm^3)	0.35	n.s.	**−0.51**	**0.006**	0.46	n.s.	0.37	n.s.	0.33	n.s.	**0.52**	**0.005**
Relative brain atrophy	−0.35	n.s.	**−0.53**	**0.004**	**−0.53**	**0.004**	−0.39	n.s.	−0.43	n.s.	**−0.52**	**0.005**
Number of T2-weighted spinal plaques	0.13	n.s.	0.19	n.s.	0.20	n.s.	0.18	n.s.	**0.60**	**0.001**	−0.13	n.s.
Total spinal cord volume	0.01	n.s.	−0.15	n.s.	0.39	n.s.	0.08	n.s.	0.24	n.s.	0.01	n.s.
Total spinal CSF volume	0.04	n.s.	0.30	n.s.	0.31	n.s.	0.17	n.s.	−0.03	n.s.	0.34	n.s.
Relative spinal cord atrophy	0.02	n.s.	−0.15	n.s.	0.27	n.s.	0.05	n.s.	0.29	n.s.	−0.06	n.s.

*R**, Spearman rank correlation coefficients, significant values are in bold; n.s., not significant; EDSS, expanded disability status scale; RFSS, regional functional scoring system; CSF, cerebrospinal fluid.

reflected in neurological disability, a quantitative segmentation and volumetric MRI analysis of focal and diffuse abnormalities and atrophy both in the brain and the whole spinal cord were performed.

Both brain atrophy and volumes of T1- and T2-weighted plaques in brain showed significant correlations with clinical disability. Relative brain atrophy correlated significantly with RFSS, arm index and higher cerebral disturbances. This is in line with the findings of a previous study, where ventricular dilatation in MRI correlated with cognitive impairment in RRMS, SPMS and PPMS patients (Comi *et al.*, 1993), but no studies of PPMS patients are available on the correlation between MRI and clinical disability as measured by RRMS and arm index. Similar to earlier reports no associations were found between EDSS and MRI findings (Thompson *et al.*, 1990; Kidd *et al.*, 1993; Lycklama à Nijeholt *et al.*, 1998; Rovaris *et al.*, 2001). In recent longitudinal studies, no correlations have emerged between progressive cerebral atrophy and worsening neurological disability in PPMS (Stevenson *et al.*, 2000), although in SPMS and RRMS (Losseff *et al.*, 1996a) such correlations have been reported. In SPMS patients, cerebral atrophy has even been proposed to be the most prominent among the MS subtypes (van Walderween *et al.*, 1998).

Although the PPMS patients had high EDSS subscores for pyramidal dysfunction, only sensory disturbances correlated with the number of T2-weighted spinal plaques. In addition, despite marked spinal atrophy in PPMS, no correlations were found between spinal MRI measurements and neurological disability. Earlier papers have reported a difference in cord cross-sectional area between PPMS and healthy controls, indicating spinal atrophy (Losseff *et al.*, 1996b; Stevenson *et al.*, 1998; Rovaris *et al.*, 2001), an increase in cervical cord atrophy over 1 year in PPMS, and correlations between spinal atrophy and EDSS (Kidd *et al.*, 1996; Losseff *et al.*, 1996b; Lycklama à Nijeholt *et al.*, 1998; Stevenson *et al.*, 1998). In these studies, the extent of spinal atrophy was evaluated by a cross-sectional area of the cervical cord used as a marker of cord atrophy. In this context, it is important to consider that marked variation in the cervical cord at different levels together with an effect of the patient's height and weight may influence interpretations of spinal atrophy. In the present study, the absence of correlation between spinal atrophy and EDSS may be related to suboptimal resolution on conventional MRI, as the frequency and severity of neurological findings in the patients were in accordance with those given in previous reports (Larsen *et al.*, 1985; Minderhoud *et al.*, 1988; McDonnell and Hawkins, 1998; Anderson *et al.*, 1999; Cottrell *et al.*, 1999).

Diffuse lesions were seen in the brain and spinal cord in most of our PPMS patients but no correlations between such lesions and neurological disability were detected. The conventional MRI technique may not in fact be optimal in revealing all lesions in the CNS. A recent study evaluated the cervical cord by fast short-inversion time recovery (fast-FLAIR) MR imaging, which has been shown to maximize MRI sensitivity in detecting lesions. This MRI sequence is a fat suppression technique where the synergistic effect of prolonged T1 and T2 relaxation times has been shown to be advantageous in detecting chronic MS lesions of the spinal cord (Rocca *et al.*, 1999). Despite using this methodology, no significant associations between clinical disability and diffuse lesions in PPMS have been detected (Rovaris *et al.*, 2001). In the future, new MR techniques such as, diffusion tensor imaging, magnetization transfer imaging and functional MRI will increase the understanding of changes in the CNS in PPMS (Dietemann *et al.*, 2000; Ciccarelli *et al.*, 2001; Mainero *et al.*, 2001; Filippi *et al.*, 2002; Rocca *et al.*, 2002).

As spinal cord is a long and thin structure, it is difficult to study. In the machine used in this study, the initial use of a sagittal PD- and FLAIR-weighted sequences gave poor results, i.e. they were artefactual and the quality of the image poor. The initial use of conventional axial T2 FSE images of the whole spinal cord proved to be very time consuming, i.e. 10–20 min. The trial use of 3D T1 and T2 sequences also led to suboptimal images. Because of these difficulties it was decided to use conventional T1 and T2 images, which proved to be optimal under the circumstances although they had their limitations. With the use of faster and modern 3D techniques available today, problems in spinal cord imaging could be overcome.

In conclusion, the present findings indicate that PPMS patients evince marked atrophy in brain and spinal cord, which correlate to clinical disability and this observation underlines the importance of neurodegenerative events in primary progressive MS.

Acknowledgements

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Urodynamic findings in primary progressive multiple sclerosis are associated with increased volumes of plaques and atrophy in the central nervous system

Ukkonen M, Elovaara I, Dastidar P, Tammela TLJ. Urodynamic findings in primary progressive multiple sclerosis are associated with increased volumes of plaques and atrophy in the central nervous system. Acta Neurol Scand 2004: 109: 100–105. © Blackwell Munksgaard 2003.

Objective – Voiding dysfunction is more frequent in primary progressive multiple sclerosis (PPMS) than in other subtypes of MS. We investigated whether lower urinary tract disorders are reflected in the extent of changes in brain and spinal cord detected by magnetic resonance imaging (MRI). *Methods* – Micturition symptoms and specific urodynamic findings in 24 patients with PPMS were related to MRI abnormalities as analysed by segmentation and volumetric analysis. *Results* – Urgency and urge incontinence were the most frequent urinary symptoms (83 and 75 %), while detrusor sphincter dyssynergia (DSD) (71%), detrusor hyperreflexia (58%) and obstruction (58%) were the most common micturition dysfunctions. Comparison between patients with detrusor hyperreflexia and those with normal bladder function revealed higher volumes of T2-weighted plaques in the brains of former ($P = 0.01$). In patients with hypotonic bladder the total brain volume was smaller ($P = 0.02$) and the number of thoracic plaques in T2-weighted images higher ($P = 0.02$) compared to patients with normal bladder function. Furthermore, DSD was associated with a higher volume of T2-weighted plaques in the brain ($P = 0.02$). *Conclusions* – Voiding dysfunction in PPMS is associated with increasing brain and spinal cord abnormalities. Urodynamic investigation is, however, needed for specific definition of micturition disturbances and should be made before therapeutic decisions.

M. Ukkonen[1,2], I. Elovaara[1,3], P. Dastidar[4], T. L. J. Tammela[3,5]

[1]Neuroimmunology Unit, Department of Neurology, Tampere University Hospital; [2]Tampere University Graduate School in Life Sciences, International Programme in Neuroscience; [3]Medical School, University of Tampere; [4]Department of Diagnostic Imaging, Tampere University Hospital; [5]Department of Urology, Tampere University Hospital, Tampere, Finland

Key words: multiple sclerosis; urinary symptoms; urodynamics; magnetic resonance imaging; atrophy

Maritta Ukkonen, Neuroimmunology Unit, Tampere University Hospital, Teiskontie 35, 33520 Tampere, Finland
Tel.: 358-3-2476692
Fax: 358-3-2474351
e-mail: irina.elovaara@uta.fi

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As multiple sclerosis (MS) can affect any level of the central nervous system (CNS), urinary problems are common among MS patients and can differ from patient to patient. The incidence of complaints of lower urinary tract dysfunction varies from 50 to 100% (1, 2). Approximately 10% of patients have voiding problems at the time of the initial clinical manifestation of MS, but only 2% complain of urinary problems as the only initial symptom (3–5). The commonest symptoms include urgency, frequency and urge incontinence, and the most frequent urodynamic findings include detrusor hyperreflexia and detrusor sphincter dyssynergia (DSD) (1–13). Some studies have found a correlation between the duration of MS and urological disturbances (4, 14–16), but the general conception is that urinary symptoms and lower urinary tract abnormalities can be found in every MS patient regardless of the state of the disease.

Correlations between neurological disability and magnetic resonance imaging (MRI) findings in patients with MS have been extensively studied, but there are few reports in which voiding dysfunction has been correlated to changes on MRI (17–20). The results of the studies in question have been contradictory, most likely due to the variability in the MS patient populations used. A further source of discrepancy is that most studies have analysed together patients with different subtypes of MS together.

The most common mode of presentation of primary progressive MS (PPMS) is progressive paraparesis, and voiding problems have been shown to correlate with motor disturbances (1, 6, 21, 22). However, no studies on the correlation between volumes of MS plaques or atrophy and voiding dysfunction in PPMS patients have hitherto been available (23).

Here we focused on PPMS in order to define more specifically the relationship between voiding dysfunction and clinical characteristics, including neurological disability as expressed by the Expanded Disability Status Scale (EDSS) (24). Our main goal was to investigate whether micturition dysfunctions detected by urodynamic investigation are reflected in the extent of MS-related changes detected by MRI of the brain and spinal cord.

Methods

Patients

In a prospective study 24 patients fulfilling the inclusion criteria were recruited in accordance with the Declaration of Helsinki. This study was approved by the Ethical Committee of Tampere University Hospital, and informed consent was obtained from each patient. According to the criteria of Thompson et al. (25), 16 patients had definite PPMS and eight probable PPMS. The clinical characteristics of the patients are shown in Table 1. They underwent a full neurological examination, including a detailed interview on the history of the clinical course of MS. Their neurological disability was moderate, with a mean EDSS of 5.1 ± 0.4 (SEM).

Urological investigations

Urological investigation consisted of micturition history and urodynamic examination. In the urological history urgency was considered as the sensation of an imminent desire to urinate and urge incontinence was defined as loss of urine associated specifically with this sensation. Stress incontinence was defined as loss of urine during

Table 1 Clinical characteristics of PPMS patients

Characteristics	
No. of patients (males/females)	24 (12/12)
Age (mean ± SEM, years)	51 ± 2
Duration of MS (mean ± SEM, years)	12 ± 2
EDSS score (mean ± SEM, range)	5.1 ± 0.4 (2–8)
CPR (mean ± SEM)	0.59 ± 0.07

CPR, clinical progression rate; EDSS, Expanded Disability Status Scale.

certain activities without a desire to void (26, 27). Frequency was defined as voiding more than eight times per day, nocturia as voiding more than once per night, dysuria as a sensation of pain accompanying micturition and hesitancy as difficulty to initiate voiding voluntarily.

All patients underwent a urodynamic investigation within 3 months from the neurological examination. It was performed according to the standards of the International Continence Society except when specially noted (26). Medication possibly influencing bladder and urinary sphincter behaviour was discontinued 2 weeks before the urodynamic tests. Urodynamic testing consisted of a free-flow study, static urethral pressure profile measurement, cystometry and pressure-flow study together with electromyographic registration of the pelvic floor muscles. In cystometry the bladder was filled with saline at room temperature (25°C) at a filling rate of 50 ml via a 10-F catheter with the patient in sitting position. Pressure-flow analysis was made on a flow chair after cystometry. Pressures were measured by external transducers connected to the patient by fluid-filled manometer lines and catheters using a 3.3-F catheter for intravesical pressure and an 8-F catheter for abdominal pressure measurement.

MRI examination

MRI was undertaken within 2 weeks from the neurological examination. All patients and controls were studied on the same 1.5 T MRI unit (Signa Horizon LX Echospeed, Milwaukee, WI, USA). The brain MRI protocol included sagittal T1-weighted spin echo (SE) [repetition time (TR) = 400 ms, excitation time (TE) = 8 ms, number of excitations (NEX) = 2, field of view (FOV) = 24 cm, matrix size = 256/192], axial T1-weighted SE (TR =540 ms, TE = 12 ms, NEX = 2, FOV = 22 cm, slice thickness 5 mm, slice gap 1 mm, matrix size = 256/224), and axial three-dimensional T2-weighted fast spin echo (FSE) (TR = 4300 ms, TE = 106 ms, NEX = 1, FOV = 22 cm, slice thickness, matrix size = 512/256) images.

The spinal cord MRI was performed using a phased array coil and the entire spinal cord from C1- to L2-level was covered. The spinal cord MRI protocol included sagittal T1-weighted SE (TR = 400 ms, TE = 12 ms, NEX = 3, FOV = 48 cm, slice thickness 4 mm, slice gap 1 mm, matrix size = 512/224) and sagittal T2-weighted FSE (TR = 8002 ms, TE = 204 ms, NEX = 2, FOV = 48 cm, slice thickness 4 mm, slice gap 1 mm, matrix size = 512/192) images.

In the brain MRI protocol axial 3-D T2 FSE and T1 SE data and in the case of spinal cord sagittal T1 SE and sagittal T2 SE data were used for segmentation and volumetric analyses. These analyses were made using the Anatomatic software operating in a PC/Windows95 environment (28). The inter-rater and intra-rater reliability of the various MRI analysis methods have been reported in earlier studies (29, 30). The volumetric accuracy of this programme has previously been validated using five fluid-filled syringes imaged on MRI. This test demonstrated 1.5% relative error between the measured and real volume (30).

From the cranial MR images we defined the volumes of T1- and T2-weighted lesions. The MS lesion volumes in the gap between two slices on T1-weighted images were estimated by multiplying the average cross-sectional area of the plaques in two consecutive slices by the gap thickness. This method was applied both in the axial T1-weighted images in the cranial MRI and in the sagittal T2-weighted images in the spinal MRI as neither of these sequences was not three-dimensional in nature. Total intracranial volume was measured by summing the volumes of segmented grey and white matter (total brain volume) and total intracranial cerebrospinal fluid (CSF) spaces. The volume of the total intracranial CSF spaces consisted of the volumes of the ventricular and peripheral CSF spaces. Relative brain atrophy was obtained by the ratio: total brain volume/total intracranial volume.

From spinal MR images we defined the numbers of cervical and thoracic T1- and T2-weighted plaques. Also the volumes of CSF and spinal cord were determined. Due to the peripheral location of the T2-weighted plaques in the spinal cord and their adjacency to the spinal CSF space, their segmentation proved to be difficult. Hence the number of plaques and the volumes were measured manually. The total spinal volume was obtained by summing the volumes of total spinal cord and total spinal CSF spaces. Relative spinal atrophy was defined by the ratio: total spinal cord volume/total spinal volume.

In addition, the number of diffuse T2-weighted lesions, defined as poorly demarcated high signal areas on both T2 and/or FLAIR images, was calculated from both cranial and spinal cord MR images. The cranial and spinal diffuse lesions were calculated numerically due to the extremely small volumes of these lesions.

The mean volumes $\pm$ SEM of T1- and T2-weighted plaques were 0.9 ± 0.1 and 8.5 ± 1.5 cm^3, respectively. The mean $\pm$ SEM of total intracranial volume and the volumes of total brain and intracranial CSF spaces were 1439.5 ± 20.3, 1053.0 ± 27.2 and 386.5 ± 20.9 cm^3, respectively. The mean relative brain atrophy was 0.73 ± 0.01. The mean $\pm$ SEM number of spinal plaques was 3.3 ± 0.4 and cervical plaques were more frequent than thoracic plaques: 2.0 vs 1.3 (mean values). The mean $\pm$ SEM of total spinal, total spinal cord and total spinal CSF space volumes were 72.4 ± 0.8, 26.3 ± 0.9 and 46.2 ± 0.5 cm^3, respectively. The mean $\pm$ SEM relative spinal atrophy was 0.36 ± 0.01. The mean numbers of diffuse lesions in brain and spinal cord were 7.0 ± 1.0 and 1.8 ± 0.3, respectively.

Statistical analysis

Statistical analysis was made on a microcomputer using the SPSS 9.0 for Windows. Spearman's rank correlation was used to describe the dependence between two variables and group data comparisons were made using Mann–Whitney test. A P-value < 0.05 was considered statistically significant.

Results

Urinary symptoms

All patients had at least one micturition complaint (Table 2). Five of 24 patients (four men and one woman) had lower urinary tract symptoms as one of the initial symptoms of MS and only one man had voiding symptoms as the only symptom at onset. Urgency and urge incontinence were the most common urinary symptoms and were present in most patients (20/24 and 18/24 patients, respectively). Urinary symptoms were not related to gender, age, disease duration or the total EDSS score.

Urodynamic investigation

The urodynamic findings are summarized in Table 3. Only three of 24 patients yielded normal urodynamic results. DSD, detrusor hyperreflexia

Table 2 Urinary symptoms in 24 PPMS patients

Symptom	Number of patients (%)
Urgency	20 (83)
Frequency	13 (54)
Nocturia	9 (38)
Dysuria	0
Urge incontinence	18 (75)
Stress incontinence	8 (33)
Hesitancy	14 (58)

Table 3 Urodynamic findings in PPMS patients

Urodynamic finding	Number of patients (%)			
	All patients (n = 24)	Men (n = 12)	Women (n = 12)	P*
Detrusor hyperreflexia	14 (58)	10 (83)	4 (33)	0.04
Detrusor hyporeflexia	4 (17)	1 (8)	3 (25)	n.s.
Normal detrusor function	6 (25)	1 (8)	5 (42)	n.s.
Detrusor sphincter dyssynergia	17 (71)	8 (67)	9 (75)	n.s.
Obstruction	14 (58)	9 (75)	5 (42)	n.s.

* Statistical difference between men and women was determined using Mann–Whitney test.

and obstruction were the most common micturition symptoms, found in 70 and 58% of the patients, respectively. Detrusor hyperreflexia was more frequent in male than in female patients ($P = 0.04$), but otherwise no significant differences between gender were detected. Urodynamic findings were not related to disease duration or EDSS. There was no relationship between urinary symptoms and urodynamic findings.

Relationship between urological symptoms and MRI abnormalities

Brain and spinal cord MRI measurements – Frequency, nocturia and urge or stress incontinence had no association with the brain and spinal cord MRI measurements.

Relationship between urodynamic and MRI findings

Brain MRI measurements – Comparison between patients with detrusor hyperreflexia and those with normal bladder function revealed higher volumes of T2-weighted plaques in the former ($P = 0.01$). DSD was also associated with increased volumes of T2-weighted plaques in the brain ($P = 0.02$) (Table 4). Patients with hypotonic bladder function showed smaller brain volume than those with normal bladder function ($P = 0.02$).

Spinal cord MRI measurements – Patients with hypotonic bladder function had more frequent T2-weighted thoracic plaques than those with normal bladder function ($P = 0.02$).

Discussion

Patients with PPMS have a higher frequency of urinary disturbances than patients with other subtypes of MS (1, 6, 21, 22), but it is not known whether voiding dysfunction is correlated to MS-induced CNS changes detected by MRI. To our knowledge this is the first study in which urological symptoms and urodynamic findings of patients with PPMS have been correlated to volumes of MS plaques and atrophy seen in MRI of brain and spinal cord. It emerged that specific urodynamic abnormalities were associated with brain or spinal MRI lesions, but urological symptoms showed no correlation with changes on MRI.

Urinary symptoms were noted in all patients. Urgency and urge incontinence were the most common complaints, which is in concordance with findings elsewhere (1, 6, 8, 12, 15). Although some investigators have reported a positive relationship between voiding complaints and the severity of pyramidal and sensory disturbances (1, 5, 6) or disease duration (1, 14, 16), we were not able to confirm these observations. Our results indicate that patients with a short duration of PPMS may equally suffer from voiding problems, as also concluded by Koldewijn et al. (15). These contrasting results may be related to differences in patient populations in the studies in question.

DSD was the most common urodynamic finding and detrusor hyperreflexia the principal bladder dysfunction, but no significant relationship between voiding symptoms and urodynamic findings was found. This is in accordance with the results of several studies (2, 12), although associations between hesitancy and areflexia (1), and irritative urinary complaints and detrusor hyperreflexia have been observed (6, 17). The reason for the poor correlation between symptoms and urodynamic

Table 4 Significant associations between MRI measurements and urodynamic findings

MRI measurements	Bladder storage function			Emptying of bladder	
	Normal cystometry	Hyperreflexia	Hypotonic detrusor	Normal voiding	Detrusor sphincter dyssynergia
T2-weighted plaque volume in brain (cm^3)	3.4 ± 1.1[a]	10.8 ± 1.9[a]	n.s.	2.7 ± 0.6[d]	10.9 ± 1.9[d]
Total brain volume (cm^3)	1071.8 ± 38.7[b]	n.s.	901.5 ± 32.3[b]	n.s.	n.s.
Number of T2-weighted thoracic plaques	0.33 ± 0.2[c]	n.s.	2.25 ± 0.6[c]	n.s.	n.s.

Data are given as mean ± SEM. Statistical differences determined using Mann–Whitney test were the following: a, detrusor hyperreflexia vs normal, $P = 0.01$; b and c, hypotonic detrusor vs normal, $P = 0.02$; d, detrusor sphincter dyssynergia vs normal, $P = 0.02$.

findings may be that patients with PPMS do not recognize their symptoms and are adapted to their voiding patterns because of the chronicity of the condition and the many other symptoms attending it. Importantly, even MS patients without micturition symptoms may yield pathologic urodynamic findings (17). Results on associations between urodynamic findings and EDSS or duration of MS also have been conflicting (1, 15, 16). In this study, no associations between urodynamic findings and disability or disease duration were detected.

Voiding symptoms in PPMS patients here were not associated with MRI measurements. One explanation for this may be the relatively small group size. One previous study has reported a correlation between urological complaints and spinal cord cross-sectional area used as a marker of spinal atrophy (19), but no significant association between cranial MRI parameters and urinary symptoms was found in another study (18).

Urodynamic findings were associated with brain and spinal cord abnormalities as seen on MRI. For example, both detrusor hyperreflexia and DSD were associated with increased total volumes of T2-weighted plaques in brain, while hypotonic detrusor was associated increased numbers of thoracic plaques and with decreased total brain volume. The only other study which has compared urodynamic findings to brain MRI parameters found no correlations between urodynamic results and atrophy or enhancing lesions, total number of lesions or size of largest lesion (18).

Micturition is a highly complex function. Normal voiding requires intact neural pathways between the bladder, pons and the higher centres. The conventional MRI used in this study may in fact have suboptimal resolution especially in the spinal cord. New MR techniques such as diffusion tensor imaging, magnetization transfer imaging and functional MRI may provide additional information on clinico-radiological correlations.

In conclusion, urodynamic findings in PPMS are associated with increased volumes of both atrophic and focal changes in brain and spinal cord. However, as the type or severity of lower urinary tract abnormality cannot be defined by MRI, urodynamic investigation is the only means of achieving a precise definition of micturition disturbances and should be undertaken prior to therapeutic decisions.

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Multiple Sclerosis

Cell surface adhesion molecules and cytokine profiles in primary progressive multiple sclerosis

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Cell surface adhesion molecules and cytokine profiles in primary progressive multiple sclerosis

Maritta Ukkonen[1,3–5], *Xingchen Wu*[1,3], *Birgit Reipert*[6], *Prasun Dastidar*[2,3] *and Irina Elovaara*[1,3]

Objective   We evaluated the utility of adhesion molecule (AM) and cytokine/chemokine expressions in blood and cerebrospinal fluid (CSF) as markers of disease activity in primary progressive multiple sclerosis (PPMS).

Methods   The expressions of AMs and the levels of 17 cytokines in patients with PPMS ($n = 25$) were compared with those in secondary progressive MS (SPMS) ($n = 18$) and controls ($n = 11$) and correlated with the volumes of focal and atrophic changes on MRI.

Results   The expressions of very late activation antigen 4 (VLA-4), lymphocyte function-associated antigen 1 (LFA-1) and intercellular adhesion molecule 1 (ICAM-1) in blood and CSF were higher in PPMS than in controls. Comparison between PPMS and SPMS showed higher levels of ICAM-1 in blood and CSF in PPMS, while the level of the vascular adhesion molecule (VCAM-1) was higher only in blood. There was no difference in the levels of cytokines in serum or CSF between PPMS and SPMS or controls, but evidence suggesting intrathecal synthesis of interleukin-8 (IL-8) and monocyte chemoattractant protein-1 (MCP-1) was found in PPMS. The expressions of CSF VLA-4 in PPMS correlated with the total volume of cerebral lesions and the number of diffuse brain lesions in MRI, while the amount of LFA-1 in CSF correlated with the number of spinal T2 lesions. The level of serum MIP-1β correlated with the T2 lesion load and EDSS score in PPMS.

Conclusions   The upregulated expressions of AMs in blood and CSF and evidence for intrathecal synthesis of MCP-1 and IL-8 in PPMS indicate the importance of inflammatory changes in the pathogenesis of PPMS. *Multiple Sclerosis* 2007; **13**: 701–707. http://msj.sagepub.com

Key words: adhesion molecules; blood; CSF; cytokines; magnetic resonance imaging, primary progressive multiple sclerosis; secondary progressive multiple sclerosis

Introduction

Primary progressive multiple sclerosis (PPMS) is a subtype of MS characterized by progressive course of disease and predominantly spastic paraparesis [1,2]. Identification of biomarkers for diagnostic use or monitoring of disease activity is currently one of the most important research areas in MS [3]. The most frequently reported immune abnormality in this MS subtype is increased intrathecal IgG synthesis and the presence of oligoclonal bands, although other factors such as osteopontin, matrix metalloproteinases-9 levels and autoantibodies have also been studied [4,5]. In general, however, the information on immunological findings in PPMS compared with other subtypes of MS is rather limited.

Adhesion molecules (AMs) and cytokines/chemokines participating in recruitment of immune cells into the CNS and regulating inflammation in brain tissue are potentially important diagnostic markers [6]. Increased levels of soluble AMs and

[1] Department of Neurology, Tampere University Hospital, Tampere, Finland
[2] Department of Diagnostic Imaging, Tampere University Hospital, Tampere, Finland
[3] Neuroimmunology Unit, Medical School, University of Tampere, Tampere, Finland
[4] Tampere University Graduate School in Life Sciences, International Programme in Neuroscience, Tampere, Finland
[5] National Graduate School of Clinical Investigation, Helsinki, Finland
[6] Baxter BioScience, Vienna, Austria

Author for correspondence: Maritta Ukkonen, Neuroimmunology Unit, Tampere University Hospital, Teiskontie 35, 33520 Tampere, Finland. E-mail: maritta.ukkonen@pshp.fi

cytokines have been reported during exacerbation in relapsing–remitting MS (RRMS) and secondary progressive MS (SPMS) [6–15], but for PPMS such information is very limited. The expressions of AMs on the surface of immune cells in MS have been analysed in only a few studies, which have reported partially conflicting results [11,16–18]. Duran and colleagues showed decreased expression of AMs in RRMS and SPMS compared to PPMS or healthy controls [16], while a group under Eikelenboom reported increased lymphocyte function-associated antigen 1 (LFA-1) and alpha4beta1-integrin expression in SPMS compared to RRMS or PPMS [18].

Upregulated levels of AMs and cytokines and their correlations with changes on brain MRI would indicate a use for these molecules in the evaluation of disease activity or in monitoring treatment efficacy. Previous studies have reported such correlations in RRMS [19–25], while in SPMS and PPMS the results have been conflicting [17,18,26,27]. In order to establish whether the levels of AM or cytokines could be used in the evaluation of disease activity in PPMS, we analysed the expressions of very late activation antigen 4 (VLA-4), LFA-1, intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1) on the surface of immune cells from blood and cerebrospinal fluid (CSF) in PPMS and compared their levels with those in controls and SPMS patients. The levels of 17 cytokines/chemokines, including interleukin (IL)-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IL-13, IL-17, interferon-γ (IFN-γ), tumour necrosis factor-α (TNF-α), macrophage inflammatory peptide-1beta (MIP-1β), monocyte chemoattractant protein-1 (MCP-1), granulocyte-macrophage colony-stimulating factor (GM-CSF) and granulocyte colony-stimulating factor (G-CSF) in serum and CSF were also measured and correlated with the volumes of focal or atrophic changes seen on brain and spinal MRI.

Material and methods

Patients and control subjects

We analysed the blood and CSF obtained from 54 subjects (25 PPMS, 18 SPMS and 11 controls). Twenty-five patients in the PPMS group were selected from among the 300 MS patients followed at the Department of Neurology, Tampere University Hospital, which provides specialized neurological care for 400 000 Finns. Patients with relapses and unwilling to undergo the lumbar puncture were excluded. All remaining patients had progressive disease from onset with occasional plateaus and temporary minor improvements fulfilling the criteria of Lublin and Reingold [28]. Altogether 19/25 PPMS patients fulfilled the criteria for definite PPMS and 6/25 fulfilled the criteria for possible PPMS according to McDonald and associates [29]. All SPMS patients fulfilled the criteria for definite MS according to McDonald *et al*. [29] The severity of the disease was scored using the Expanded Disability Status Scale (EDSS) [30] (Table 1). At the time of neurological examinations the clinical condition of the patients was stable. No patient had received corticosteroids or other immunosuppressive treatment in the eight weeks before entry into this study. Eleven controls were studied because of variable neurological symptoms, but they presented no evidence of organic CNS disease. All patients and controls underwent neurological examination. Blood and CSF samples were obtained by routine procedures after the neurological examination during the same day. The study was approved by the Ethical Committee of Tampere University Hospital, and informed consent was obtained from each patient.

MRI and volumetric quantitation

MR imaging was performed within two weeks from the neurological examination in all PPMS cases using the same 1.5-Tesla MRI unit (Signa Horizon LX Echospeed, Wisconsin, USA) in a blinded manner. The volumetric segmentation of plaques and atrophy in the brain and spinal cord was performed using semiautomatic software Anatomatic™ [31,32]. The details of MRI protocol [33] and volumetric analyses [34] have been described elsewhere.

From the cranial MR images the volumes of plaques on T1- and T2-weighted images and brain/CSF volumes were measured. From spinal MR images we defined the numbers of plaques on T1- and T2-weighted images. Relative brain and spinal cord atrophy was obtained by a ratio of total

Table 1 Clinical characteristics of primary and secondary progressive MS patients and controls

Characteristics	PPMS	SPMS	Controls
No. of patients	25	18	11
Age, mean $\pm$ SD, years	51 $\pm$ 10	46 $\pm$ 7	44 $\pm$ 8
Gender: male/female	12/13	9/9	1/10
Duration of MS, mean $\pm$ SD, years	13 $\pm$ 9	14 $\pm$ 4	–
EDSS, median (interquartile range)	5.0 (3.0–6.5)	4.8 (4.0–6.0)	–

brain or spinal cord volume to total intracranial or intraspinal volume. In addition, the number of diffuse lesions was calculated from both cranial and spinal cord MR images on T2 and/or FLAIR images according to Lycklama á Nijeholt and associates [35].

Determination of adhesion molecule expression

The methodology used for determination of AM expression has been described elsewhere [17,26]. In brief, for phenotyping of the mononuclear cells, the three-layer indirect immunoperoxidase technique was applied. The primary antibodies used were LFA-1 (CD11a), VLA-4 (CDw49d), ICAM-1 (CD54) or VCAM-1 (CD106) (0157, 0764, 0544 and 1244 respectively; Immunotech, Marseille, France). The secondary antibody was a peroxidase-conjugated rabbit anti-mouse antibody (P0161; Dako, Glostrup, Denmark) and the third was a peroxidase-conjugated goat anti-rabbit antibody (L42007; Caltag, San Francisco, CA). Results are expressed as the percentage of positively stained lymphocytes in the total number of lymphocytes counted and the percentage of positively stained monocytes in the total number of monocytes counted.

Multiple cytokine analysis using Luminex-100 system

Serum and CSF supernatants were collected and analysed simultaneously for 17 different cytokines and chemokines, including IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IL-13, IL-17, IFN-γ, TNF-α, MIP-1β, MCP-1, GM-CSF and G-CSF.

Multiple cytokine analysis kits were obtained from Linco Research Inc. (St. Charles, MO). Millipore multiscreen 96-well filter plates (Bedford, MA) were used for all multiple cytokine kits. Assays were

run in triplicate according to the manufacturers' protocols. Data were collected using the Luminex-100 system Version 1.7 (Luminex, Austin, TX). Data analysis was performed on MasterPlex QT 1.0 (MiraiBio, Alameda, CA). A five-parameter regression formula was used to calculate the sample concentrations from the standard curves. All 96 samples were analysed with the LINCOplex kit (Linco Research Inc.) [36]. The assay for each individual cytokine and chemokine was validated for intra-assay variation and interassay variation. The coefficient of intra-assay and interassay variations was found to be in the range of 5–20% and 10–50% (respectively), depending on the cytokine or chemokine.

Statistical methods

Statistical analyses were made on SPSS 9.0 for Windows. Correlations were explored by Spearman's rank correlation coefficient. The Kruskal–Wallis test was used in group comparisons and multiple comparisons between two groups were made by the Mann–Whitney test. The Wilcoxon signed ranks test was used in paired serum and CSF cytokine comparisons.

Results

Expression of adhesion molecules on immune cells from blood and CSF

The proportions of AM expressions on blood and CSF immune cells (lymphocytes and monocytes) are presented in Table 2.

Comparison between PPMS and controls showed upregulated expression of VLA-4 on blood lymphocytes in PPMS ($P = 0.003$), while increased VLA-4 was found on CSF lymphocytes and monocytes

Table 2 Proportions (mean ± SD) of adhesion molecule expression in blood and cerebrospinal fluid

	Lymphocytes					Monocytes				
	PPMS	Controls	SPMS	p^1	p^2	PPMS	Controls	SPMS	p^1	p^2
Blood										
VLA-4	29.1 ± 17.4	13.1 ± 9.3	34.9 ± 11.4	0.003	n.s.	36.9 ± 22.9	22.3 ± 11.8	26.7 ± 19.7	n.s.	n.s.
LFA-1	24.9 ± 15.3	13.0 ± 7.8	36.3 ± 12.1	n.s.	0.006	55.6 ± 32.3	24.4 ± 12.9	34.6 ± 22.3	0.005	n.s.
ICAM-1	9.4 ± 5.5	6.3 ± 6.0	12.3 ± 6.7	n.s.	n.s.	55.5 ± 25.1	20.0 ± 14.1	13.7 ± 17.5	<0.0005	<0.0005
VCAM-1	0.7 ± 2.4	0.0 ± 0.0	0.0 ± 0.0	n.s.	0.005	1.2 ± 3.1	2.5 ± 8.1	0.4 ± 1.5	n.s.	n.s.
CSF										
VLA-4	38.5 ± 22.6	9.1 ± 8.3	27.0 ± 16.1	0.004	n.s.	42.3 ± 32.5	0.0 ± 0.4	17.5 ± 24.4	0.009	n.s.
LFA-1	62.5 ± 25.9	12.0 ± 5.6	42.4 ± 24.0	0.003	n.s.	75.8 ± 25.7	0.0 ± 0.4	19.9 ± 17.6	0.003	0.006
ICAM-1	13.3 ± 12.8	1.3 ± 3.4	6.8 ± 8.2	n.s.	n.s.	61.0 ± 41.2	0.0 ± 0.4	2.2 ± 6.6	0.009	<0.0005
VCAM-1	1.0 ± 2.5	0.0 ± 0.0	0.0 ± 0.0	n.s.	n.s.	24.3 ± 33.8	0.0 ± 0.4	3.4 ± 8.0	n.s.	n.s.

p^1, PPMS versus controls; p^2, PPMS versus SPMS; n.s., not significant.

($P = 0.004$, $P = 0.009$ respectively). The expressions of LFA-1 and ICAM-1 also appeared to be higher on CSF immune cells ($P = 0.003$, $P = 0.009$, respectively) and blood monocytes in PPMS compared to their controls ($P = 0.005$, $P = 0.000$, respectively).

Comparison between PPMS and SPMS showed lower expression of LFA-1 on blood lymphocytes in PPMS ($P = 0.006$), while higher expression was found on CSF monocytes ($P = 0.006$). The level of ICAM-1 on blood and CSF monocytes was higher in PPMS than in SPMS ($P < 0.0005$ and $P < 0.0005$, respectively), while VCAM-1 was upregulated only on blood lymphocytes ($P = 0.005$).

Levels of cytokines/chemokines in serum and CSF

Serum MCP-1 levels appeared to be lower in both PPMS and SPMS when compared with controls ($P < 0.05$) (Table 3). However, in PPMS the levels of CSF IL-8 and MCP-1 were higher than those in corresponding sera ($P = 0.000$, $P = 0.014$, respectively). In SPMS, the levels of CSF IL-8 were likewise higher than in corresponding serum ($P = 0.023$) (Table 3). The levels of G-CSF, GM-CSF, IL-1β, IL-2, IL-4, IL-5, IL-7, IL-10, IL-12 and IL-17 in CSF were below detection level in both PPMS and SPMS, as were concentrations of IL-5, IL-12 and IL-17 in PPMS and of IL-4 in sera in SPMS (Table 3). There was no significant difference in the levels of 17 analysed cytokines/chemokines in serum or CSF between PPMS and SPMS.

Basic inflammation indices

The CSF leukocyte count was $4.3 \pm 1.0 \times 10^6$/L (means $\pm$ SEM, reference ≤ 5 cell/mm^3), IgG index 1.0 ± 0.1 (reference ≤ 0.7) and the CSF-serum albumin ratio $6.4 \pm 0.6 \times 10^3$ (reference ≤ 7.0). A total of 76% of the PPMS patients had oligoclonal bands (OCBs) in their CSF.

MRI results of PPMS patients

The MRI data on our PPMS patients are presented in Table 4.

Correlations between AM expressions or cytokine/chemokine levels and MRI measurements in PPMS

The expressions of AMs and the levels of cytokines/chemokines correlated with MRI measurements. Only correlations with a significance of $P < 0.01$ are shown. The expression of VLA-4 in blood monocytes correlated with the number of diffuse brain lesions ($r = 0.561$, $P = 0.004$), but no other correlations in blood were detected.

The expression of VLA-4 on CSF lymphocytes correlated with the total volume of brain plaques and the number of diffuse brain lesions ($r = 0.528$, $P = 0.007$ and $r = 0.536$, $P = 0.006$ respectively). A significant correlation also emerged between the level of VLA-4 on CSF monocytes and the volume of brain T2 lesions as well as the total volume of brain lesions ($r = 0.550$, $P = 0.004$ and $r = 0.567$,

Table 3 Concentrations of cytokines/chemokines in sera and CSF (pg/ml) of PPMS patients (mean $\pm$ SD)

	PPMS		SPMS		Controls	
	Sera	CSF	Sera	CSF	Sera	CSF
G-CSF	4.16 ± 13.00	–	67.67 ± 225.97	–	1.14 ± 3.22	–
GM-CSF	128.76 ± 444.36	–	21.51 ± 75.16	–	0.03 ± 0.07	–
IFN-γ	42.33 ± 80.23	8.36 ± 26.94	134.08 ± 325.61	5.95 ± 15.29	87.85 ± 127.40	–
TNF-α	17.09 ± 34.78	2.04 ± 6.28	50.06 ± 181.05	0.74 ± 2.77	50.03 ± 117.90	–
IL-1β	3.82 ± 10.93	–	17.18 ± 51.98	–	7.62 ± 18.59	–
IL-2	4.80 ± 19.83	–	5.30 ± 19.90	–	–	–
IL-4	0.81 ± 3.64	–	–	–	–	–
IL-5	–	–	5.70 ± 24.20	–	–	–
IL-6	28.35 ± 54.94	8.84 ± 16.02	43.22 ± 103.55	9.34 ± 12.50	49.83 ± 114.20	1.77 ± 3.07
IL-7	1.68 ± 5.60	–	2.70 ± 11.46	–	–	–
IL-8	1.83 ± 5.70	13.82 ± 5.97^b	2.39 ± 10.12	$12.48 \pm 7.39\#$	–	13.54 ± 3.77
IL-10	0.09 ± 0.43	–	2.01 ± 8.53	–	–	–
IL-12	–	–	4.73 ± 20.08	–	–	–
IL-13	28.48 ± 60.40	10.09 ± 35.01	85.62 ± 199.06	3.77 ± 10.57	75.97 ± 164.90	–
IL-17	–	–	4.03 ± 17.08	–	–	–
MCP-1	92.00 ± 200.78^c	226.31 ± 153.26^a	82.18 ± 245.68	203.67 ± 102.29	691.31 ± 1799.00	289.40 ± 132.7
MIP-1β	33.01 ± 41.78	17.25 ± 20.22	9.52 ± 19.53	18.79 ± 17.11	31.09 ± 30.66	8.76 ± 15.17

^{a}$P < 0.05$ compared between serum and CSF in the same group.
^{b}$P < 0.01$ compared between serum and CSF in the same group.
^{c}$P < 0.05$ compared with corresponding serum of control group.
–, below detection level.

Table 4 Brain and spinal cord MRI data on PPMS patients

Brain MRI parameters	Mean ± SD	Spinal MRI parameters	Mean ± SD
Total brain volume (cm^3)	1043.0 ± 129.8	Total spinal cord volume (cm^3)	26.2 ± 4.6
Total intracranial CSF volume (cm^3)	389.2 ± 109.7	Total intraspinal CSF volume (cm^3)	46.0 ± 2.8
Total intracranial volume (cm^3)	1433.1 ± 101.3	Total intraspinal volume (cm^3)	72.2 ± 4.3
Relative brain atrophy	0.73 ± 0.07	Relative spinal cord atrophy	0.36 ± 0.05
Number of diffuse lesions	7.2 ± 5.0	Number of T2-weighted plaques	3.2 ± 2.2
T1-weighted lesion volume (cm^3)	0.9 ± 0.8		
T2-weighted lesion volume (cm^3)	8.6 ± 7.6		

$P = 0.003$). Furthermore, the expression of LFA-1 on CSF lymphocytes correlated with the number of spinal plaques on T2-weighted images ($r = 0.660$, $P = 0.000$). No associations were found between the expressions of AMs in CSF and atrophic changes in brain or spinal cord.

In PPMS, the level of serum MIP-1β correlated with T2 lesion volume on the brain MRI ($r = 0.54$, $P = 0.01$).

Correlations between AM expressions or cytokine/chemokine levels and clinical data

In PPMS patients expressions of AM or cytokine/chemokine levels were not related to gender, age, EDSS or disease duration. A slight correlation was detected only between serum MIP-1β and EDSS score. The comparisons between patients with SPMS and controls have been previously described [17].

Discussion

The levels of soluble AMs in PPMS have been analysed in a few studies [8,16,17,20], but less information is available on the cell surface expression of these proteins. Here we analysed the expressions of AMs on the surface of immune cells and the levels of cytokines/chemokines in blood and CSF in PPMS in order to establish whether these measures could be used in the evaluation of disease activity or treatment efficacy. We detected increased cell surface expression of AMs, particularly of VLA-4, LFA-1 and ICAM-1, in blood and CSF in PPMS compared to controls. This indicates the potential of such activated cells expressing AMs to enter into the brain and participate in lesion formation. Lower expression of ICAM-1 in PPMS compared to healthy controls has been reported by Durán and associates [16]. This is most likely explained by differences in methodology as well as in patient characteristics. The lower expression of LFA-1 in PPMS compared to SPMS detected here is consistent with a lower level of brain inflammation in PPMS, which is in line with previous pathologi-

cal and MRI data showing fewer focal lesions and less gadolinium (Gd) enhancement in PPMS than in SPMS, despite similar levels of disability [37–39]. This phenomenon has been called a clinicoradiological paradox. The expressions of AMs were not related to EDSS, confirming earlier reports [20,27].

Correlation between the level of VLA-4 in CSF and the total volume of brain lesions or the number of diffuse brain lesions indicates the importance of inflammation in PPMS and suggests use of VLA-4 as a marker of inflammatory activity in this MS subtype. Previous correlation studies in PPMS report an inverse correlation between soluble ICAM-1 and the number of new Gd-enhanced lesions or increased total number of Gd-lesions [11]. This suggests that higher levels of soluble ICAM-1 may prevent adhesion of inflammatory cells to the vascular endothelium.

No correlations between the levels of AMs and the volumes of brain atrophy in PPMS were detected in this study. Likewise, earlier studies in RRMS or SPMS report no significant correlations between AMs and atrophic changes [17,26]. The lack of correlation between atrophy and AMs detected here is not surprising considering the fact that atrophy is related more to degeneration than to inflammatory changes [40]. As most of our PPMS patients had advanced disease, the lack of correlations between atrophic changes and AMs reflecting inflammation is not surprising. The relevance of inflammatory events in the early stages of PPMS has been shown in a study reporting correlations between cerebral atrophy and focal damage and inflammation in recent-onset PPMS [41]. This further confirms the conception that inflammatory events are more relevant in the early stages of PPMS than in the later course of disease.

The levels of MCP-1 and IL-8 in CSF from PPMS patients exceeded those in serum. Due to the intact BBB in our patients, as indicated by a normal range of CSF-serum albumin ratios, increased levels of these chemokines suggest their local synthesis within the CNS rather than diffusion from the blood. IL-8 is a chemoattractant for both monocytes and neutrophils and triggers their firm adhesion to the endothelium. It has been suggested that IL-8 may serve as a marker of monocyte

activity in MS and may play a role in monocyte recruitment to the CNS [42]. MCP-1 is considered a Th2 chemokine driven by IL-4. Variations in its level in MS are related to deviation in the Th1/Th2 balance. The level of serum MIP-1β in PPMS correlated with EDSS score or T2 lesion volume on brain MRI, suggesting a contribution of this protein to lesion development and disability progression. MIP-1β is a key player in the pathogenesis of MS and is produced and secreted by activated macrophages to attract other proinflammatory cells and macrophages themselves to sites of inflammation [43]. The detection of changes in chemokine/cytokine systems further stresses the role of inflammation even in relatively advanced PPMS.

In conclusion, upregulated expressions of AMs in blood and CSF in PPMS are consistent with transmigration of activated lymphocytes into the CNS and induction or maintenance of inflammation in the brain microenvironment. Elevated concentrations of MCP-1 and IL-8 in PPMS are in line with intrathecal synthesis of these chemokines, which further underlines the role of inflammation even in advanced PPMS. Taken together, our data indicate the importance of inflammatory changes in the pathogenesis of PPMS.

Acknowledgements

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Cognitive dysfunction in primary progressive multiple sclerosis: a neuropsychological and MRI study

M Ukkonen[1,2,3], T Vahvelainen[1], P Hämäläinen[4], P Dastidar[5] and I Elovaara[1,3]

Although cognitive dysfunction is known to occur in multiple sclerosis (MS), only few studies have reported cognitive performance in patients with primary progressive MS (PPMS). To find out the pattern of cognitive performance in PPMS, 28 PPMS patients underwent an extensive battery of neuropsychological tests. The results were compared to those of healthy controls ($n = 20$) and patients with secondary progressive MS (SPMS, $n = 28$). Furthermore, the results of neuropsychological tests in PPMS were correlated to magnetic resonance imaging findings. Our study showed that the PPMS patients have deficits in several cognitive domains when compared to age-matched and education-matched controls, but the cognitive impairment in the PPMS and SPMS patients appeared to be similar. Cognitive deficits in PPMS patients correlated with diffuse brain lesion, T1- and T2-lesion load, but no correlations were found with atrophy. *Multiple Sclerosis* 2009; **15**: 1055–1061. http://msj.sagepub.com

Introduction

Cognitive dysfunction is detected in approximately 50% of patients with multiple sclerosis (MS) [1–3] and occurs in all subtypes of MS [4]. Impairments are typically manifested in recent memory, attention, processing speed, and executive functions [5,6]. In studies, analysing cognitive performance in relapsing-remitting MS (RRMS), secondary progressive MS (SPMS), and primary progressive MS (PPMS), higher numbers of cognitive deficits have been reported in chronic progressive forms than in RRMS [2,4,7–12]. Few neuropsychological studies have shown some differences between PPMS and SPMS patients in visuospatial working memory, visuospatial new learning, verbal memory, and verbal fluency [4,13–16].

Studies correlating magnetic resonance imaging (MRI) abnormalities with severity of cognitive impairment have revealed relation between brain atrophy and cognitive decline [17–24]. In addition, some correlations have been reported between the severity of cognitive impairment and lesion load, especially T2-lesion load on MRI [23,25–28]. Previous studies have shown fewer focal T1- and T2-weighted lesions and lesser enhancement with gadolinium (Gd) in PPMS than in SPMS patients, despite similar levels of disability [29–31]. However, only minor-to-moderate correlations between MRI findings and cognitive deficits in PPMS have been reported [14,32].

The main aim of this study was to characterize the pattern of cognitive dysfunction in PPMS compared to matched patients with SPMS and controls, and to correlate cognitive findings in PPMS with MRI findings.

Methods

Subjects

All 28 PPMS patients (14 men, 14 women) involved in this study had progressive disease from onset,

[1]Department of Neurology and Rehabilitation, Tampere University Hospital, Tampere, Finland
[2]Medical School, University of Tampere, Tampere, Finland
[3]National Graduate School of Clinical Investigation, Finland
[4]Masku Neurological Rehabilitation Centre. Masku, Finland
[5]Department of Diagnostic Imaging, Tampere University Hospital, Tampere, Finland
Correspondence to: Maritta Ukkonen, MD, Neuroimmunology Unit, Tampere University Hospital, Teiskontie 35, 33520 Tampere, Finland. Email: maritta.ukkonen@pshp.fi
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with occasional plateaus and temporary minor improvements fulfilling the criteria of Lublin and Reingold [33]. A total of 22/28 PPMS patients fulfilled the criteria for definite PPMS and 6/28 of them for possible PPMS according to the McDonald revised criteria for PPMS [34]. All SPMS patients (12 men, 15 women) fulfilled the criteria for definite MS according to McDonald [35]. Severity of disease was scored using the Expanded Disability Status Scale (EDSS) [36], (Table 1). The evaluation of the possible influence of hand dysfunction on test result was made by specific questions. This arm index includes four functions of upper limbs: dressing, washing hair, using knife and fork, and handling coins. At the time of the neurological examinations the clinical condition of the patients was stable. None had received corticosteroids or other immunosuppressive treatment within the 8 weeks preceding the study. Twenty subjects (10 men, 10 women) without evidence of organic central nervous system disease were studied as healthy controls. The controls were matched with the patients with respect to age, sex, and education. None of the patients or controls had drug or alcohol abuse or psychiatric history. The patients had no nervous system disorder other than MS. The study was approved by the Ethics Committee of Tampere University Hospital and informed consent was obtained from each patient.

Neuropsychological evaluation

All subjects underwent an extensive battery of neuropsychological tests performed in a single session by an experienced neuropsychologist (TV). The assessment covered the following domains: attention and information processing; concept formation, reasoning and executive functions; verbal production; memory and learning; and visuoperceptual and visuoconstructive functions (Table 2). The tests were chosen according to clinical practice and were divided into different domains according to Lezak's theoretical considerations [37].

MRI and volumetric quantitation

MRI was performed within 2 weeks from the neurological and neuropsychological examination in all PPMS cases using the same 1.5 Tesla MRI unit (Signa Horizon LX Echospeed, Wisconsin, USA). Volumetric segmentation of plaques and atrophy in the brain was performed using semiautomatic software Anatomatic™ operating in a PC/Window 95 environment [38,39] and the images were analysed blindly. The details of the MRI protocol and volumetric analyses have been described elsewhere [40].

From the cranial MR images the volumes of plaques on T1- and T2-weighted images and brain/cerebrospinal fluid volumes were measured. Relative brain atrophy was obtained by a ratio of total brain volume to total intracranial volume. In addition, the number of diffuse lesions was calculated from T2 and/or fast fluid-attenuated inversion recovery (FLAIR) MR images according to Lycklama á Nijeholt and associates [41].

The data of MRI findings in SPMS are not shown because these patients underwent a different MRI procedure.

Statistical methods

Statistical analyses were carried out on a microcomputer using SPSS 9.0 for Windows. Correlations were calculated by Spearman's rank correlation coefficient test. Group data comparisons with clinical characteristics were made using Kruskal–Wallis test with Bonferroni adjustment for multiple comparisons and Mann–Whitney test was used as post-hoc analysis test. A *P*-value of less than 0.05 with Bonferroni correction was considered statistically significant.

Results

Clinical and neuropsychological findings

Table 1 shows the clinical characteristics of the PPMS and SPMS patients and controls. The two MS groups

Table 1   Clinical characteristics of multiple sclerosis (MS) patients and controls

Characteristics	(1) PPMS (N = 28)			(2) SPMS (N = 28)			(3) Controls (N = 20)			Kruskal–Wallis test
	Mean	Range	SD	Mean	Range	SD	Mean	Range	SD	*P*-value
Age (years)	50.9	36–76	9.4	45.2	33–57	6.4	46.0	36–58	6.1	0.072
Education (years)	10.4	6–22	3.5	10.4	7–19	3.3	11.9	7–15	2.3	0.052
BDI	6.4	0–23	6.1	6.4	0–17	4.4	1.3	0–7.0	1.7	0.001[1,2>3]
Duration of MS (years)	12.3	3–36	15.0	15.0	3–36	5.8	—	—	—	0.021
EDSS	4.9	2–8	1.9	4.8	3.5–6.5	1.0	—	—	—	0.760

PPMS, primary progressive multiple sclerosis; SPMS, secondary progressive multiple sclerosis; SD, standard deviation; BDI, Beck Depression Inventory (depression score); EDSS, Expanded Disability Status Scale.

Table 2 Neuropsychological performance of patients with PPMS, SPMS, and controls

Tests	(1) PPMS		(2) SPMS		(3) Controls		Kruskal–Wallis test
	Mean	SD	Mean	SD	Mean	SD	Bonferroni corrected *P*-value
Attention and information processing							
WAIS-R/digit span, backwards [51]	5.3	1.5	5.7	1.7	6.2	1.0	1.0
WAIS-R/digit span, total [51]	11.4	2.6	12.0	3.1	13.1	1.7	1.0
Corsi block, total [52]	11.7	1.9	10.6	2.7	12.6	2.8	0.836
PASAT 3 s, correct [53]	39.0	12.1	41.1	15.7	46.7	10.1	1.0
PASAT 2 s, correct [53]	27.6	7.7	32.5	12.3	36.3	6.9	0.308
Stroop interference time [54]	60.2	28.5	49.7	53.6	31.8	10.9	0.009[1,2<3]
SDMT [55]	38.0	12.3	38.2	12.1	52.9	9.5	0.002[1,2<3]
Concept formation, reasoning and executive functions							
WAIS-R similarities [51]	22.9	7.4	24.3	5.4	27.8	2.3	0.616
WAIS-R picture arrangement [51]	9.3	5.1	10.6	5.9	13.0	3.0	1.0
WCST, correct [56]	72.3	13.4	79.7	22.8	97.1	22.5	0.022[1,2<3]
Verbal production							
BNT, correct [57]	25.5	3.3	26.2	3.4	28.1	1.6	0.176
Verbal fluency, category [37]	20.5	5.6	18.1	6.4	24.8	5.1	0.022[1,2<3]
Verbal fluency, phonological [37]	11.4	5.1	10.4	5.5	16.7	3.6	0.004[1,2<3]
Memory and learning							
BVRT, correct [58]	6.7	1.5	6.8	1.5	8.0	1.2	0.308
WMS, logical stories, immediate [59]	10.6	3.8	10.2	3.7	13.8	2.8	0.132
WMS, logical stories, delayed [59]	7.8	4.4	8.3	3.8	13.0	2.8	0.002[1,2<3]
7/24 SRT, learning score [60]	28.3	6.4	26.1	6.2	31.8	3.6	0.110
7/24 SRT, delayed recall [60]	5.4	1.9	4.4	2.2	6.2	1.5	0.374
Shopping list, learning score [61]	36.0	8.4	39.2	6.7	42.0	4.3	0.506
Shopping list, delayed recall [61]	7.3	2.3	7.9	1.8	8.9	1.2	0.594
Visuoperceptual and visuoconstructive functions							
Hooper, correct [62]	11.2	1.7	11.0	2.5	12.5	1.3	0.616
WAIS-R Block Design [51]	26.4	9.1	25.0	11.2	33.0	8.5	0.418

PPMS, primary progressive multiple sclerosis; SPMS, secondary progressive multiple sclerosis; SD, standard deviation; WAIS-R, wechsler adult intelligence scale-revised; PASAT, paced auditory serial addition test; SDMT, symbol digit modalities test; WCST, Wisconsin card sorting test; BNT, Boston naming test, Finnish version; BVRT, Benton visual retention test; WMS, Wechsler memory scale-logical memory; SRT 7/24, spatial recall test.
Hooper, visual organization test.

did not differ significantly in gender, age, years of education, disease duration, or degree of physical disability (EDSS). Although the MS patients reported higher depression scores than the controls, no significant differences were found between the two MS groups in the Beck Depression Inventory (BDI) [42]. No significant difference in age, gender, or education was detected between the PPMS group and controls. However, the years of education was higher in the controls than in the PPMS group although the difference did not reach statistical significance. Age, years of education, disease duration, depression, EDSS, or arm index did not correlate significantly with the results of any of the neuropsychological tests. Comparison between men and women showed no differences in any of the groups.

Neuropsychological tests revealed significant differences between PPMS patients and controls in most of the tested cognitive domains including attention and information processing, concept formation, reasoning and executive functions, verbal production, and visual memory and learning (Table 2). In all of these areas, the controls performed better than the PPMS patients. Although the controls obtained higher scores than the PPMS patients in visuoperceptual and visuoconstructive functions, the differences did not reach statistical significance. Comparison between PPMS and SPMS patients showed that those patients with PPMS performed slightly better in the visual learning test (7/24 SRT learning score and 7/24 SRT delayed recall), but the difference did not reach statistical significance. No other differences were found between PPMS and SPMS patients on the other neuropsychological measures used.

MRI findings

The mean volumes (±SEM) of brain T1- and T2-weighted plaques were 0.9 (±0.1) cm^3 and 8.5 (±1.4) cm^3, respectively. The relative brain atrophy was 0.73 ± 0.01 cm^3. The mean number of diffuse lesions in the brain was 7.1 ± 0.9.

In the correlation analyses, the number of brain diffuse lesions in the MRI correlated significantly

with PASAT 3, WAIS-R similarities, BNT, verbal fluency, WMS logical stories, shopping list, Hooper and WAIS-R Block Design test (Table 3). The volume of brain T2 lesions correlated significantly with BNT, Hooper Visual Organisation Test and WAIS-R Block Design test. The volume of brain T1 lesions correlated with BNT and 7/24 SRT test. Brain atrophy did not correlate with any of these neuropsychological tests.

Discussion

In recent years, several studies have demonstrated a distinctive clinical course, pathology, and MRI findings in PPMS when compared to RRMS or SPMS [30,31,43,44]. In the view of these observations, PPMS should be evaluated as a discrete entity also in neuropsychological studies. The association between disease course and cognitive manifestations in MS has been difficult to explore because comparison between subgroups is often confounded by differences between groups in physical disability, duration of the disease, age, and education. In our study, the two MS groups were well

matched with respect to these disease characteristics. The results of neuropsychological tests can be confounded by several factors such as depression, fatigue, motivation, medications, and impairments of visual, sensory or motor deficits. In this study, the MS patients had higher depression scores than the controls and that may explain part of the differences in the cognitive performances observed between patients and the controls. However, none of our MS patients had severe depression and most of our patients manifested either normal or slightly lowered mood without depression. Although problems with mood may affect cognition, lowered mood alone cannot explain all the cognitive deficits observed in the patients.

The PPMS patients here performed significantly worse than the controls in almost all cognitive tests used. After Bonferroni corrections, significant differences were observed in measures of focused attention and interference (Stroop), information processing speed (symbol digit modalities test [SDMT]) and problem solving (Wisconsin card sorting test [WCST]), as well as in measures of verbal production (fluencies) and verbal memory (logical

Table 3 Correlation between neuropsychological tests and MRI findings in PPMS patients

	Volume of brain T1 lesions	Volume of brain T2 lesions	Number of brain diffuse lesion	Brain atrophy
Attention and information processing				
WAIS-R/digit span, backwards [51]	−0.204	−0.103	−0.297	−0.178
WAIS-R/digit span, total [51]	−0.104	−0.045	−0.253	−0.314
Corsi block, total [52]	0.046	0.050	−0.083	0.074
PASAT 3 s, correct [53]	−0.246	−0.443	−0.539*	−0.263
PASAT 2 s, correct [53]	0.080	−0.361	−0.188	−0.267
Stroop interference time [54]	−0.024	0.079	0.018	0.020
SDMT [55]	−0.183	−0.058	−0.061	0.207
Concept formation, reasoning and executive functions				
WAIS-R similarities [51]	−0.277	−0.304	−0.422*	−0.095
WAIS-R picture arrangement [51]	−0.335	−0.335	−0.181	−0.374
WCST, correct [56]	−0.238	−0.186	−0.370	0.135
Verbal production				
BNT, correct [57]	−0.446*	−0.385*	−0.481*	−0.291
Verbal fluency, category [37]	−0.116	−0.022	−0.123	−0.207
Verbal fluency, phonological [37]	−0.285	−0.203	−0.427*	−0.161
Memory and learning				
BVRT, correct [58]	−0.076	−0.149	−0.161	−0.145
WMS, logical stories, immediate [59]	−0.045	−0.079	−0.425*	−0.272
WMS, logical stories, delayed [59]	−0.054	−0.078	−0.378*	−0.065
7/24 SRT learning score [60]	−0.461*	−0.305	−0.284	0.226
7/24 SRT delayed recall [60]	−0.511*	−0.362	−0.084	0.175
Shopping list, learning score [61]	−0.347	−0.194	−0.434*	−0.206
Shopping list, delayed recall [61]	−0.009	−0.052	−0.172	−0.120
Visuoperceptual and visuoconstructive functions				
Hooper, correct [62]	−0.270	−0.401*	−0.457*	−0.225
WAIS-R Block Design [51]	−0.363	−0.442*	−0.556*	−0.280

PPMS, primary progressive multiple sclerosis; WAIS-R, Wechsler adult intelligence scale-revised; PASAT, paced auditory serial addition test; SDMT, symbol digit modalities test; WCST, Wisconsin card sorting test; BNT, Boston naming test, Finnish version; BVRT, Benton visual retention test; WMS, Wechsler memory scale-logical memory; SRT 7/24, spatial recall test.
P = Spearman's rank correlation coefficient test.
Hooper, visual organization test.
*$P < 0.05$.

stories). In accordance with previous studies on cognitive deficits in MS, especially information processing and attention together with executive functions, verbal production and verbal memory seemed to be the most vulnerable domains.

The cognitive performance of PPMS patients did not differ from that in SPMS. Recent comparative studies on neuropsychological test performance in PPMS and SPMS suggest only few and subtle differences to the disadvantage of SPMS patients in visuospatial working memory [14] and in visuospatial new learning [15]. Moreover, studies by groups under Comi [13] and Huijbregts [4] reported greater cognitive impairment in SPMS than in PPMS patients in the spatial recall and word list generation tests. However, in the study by Comi and associates, significantly longer disease duration in SPMS than in PPMS patients may have influenced the results obtained. In our study, the SPMS group performed slightly worse than the PPMS group only in the 7/24 Spatial recall test, a measure of visual memory, but the difference did not reach statistical significance. Our results support those of de Sonneville and colleagues [12], who reported similar cognitive deficits in PPMS and SPMS patients. Only Wachowius and associates [16] found more severe impairment in verbal memory and verbal fluency in PPMS than in SPMS. Comparison between these studies is, however, difficult in consequence of variability in the neuropsychological measures employed as well as in disease duration and severity.

A measure of information processing speed, the SDMT [45], has been suggested as one possible screening method to evaluate MS patients' cognitive abilities and a need for more comprehensive neuropsychological assessment. According to our results, SDMT differentiated MS patients well from the healthy controls. Although PPMS and SPMS patients performed at similar levels, controls performed better. Another measure of information processing speed and sustained attention, the PASAT, did not differentiate MS patients from the controls. Thus, the PASAT cannot be suggested as an appropriate screening method for patients with PPMS or SPMS, although it has been suggested as a reliable method in RRMS [46].

Several studies involving MS patients have reported a correlation between the severity of cognitive impairment and lesion load on conventional T2-weighted images [13,23,25–28] and brain atrophy [18,21–24]. One recent study concluded that in MS, the extent of brain atrophy predicts cognitive impairment better than the lesion burden, and central atrophy, in particular, was found to be strongly associated with neuropsychological morbidity [22].

In our study, the number of diffuse brain lesions correlated significantly with all the cognitive domains tested, including attention and information processing, concept formation, reasoning and executive functions, verbal production, verbal memory and learning, and visuoperceptual and visuoconstructive functions. Cerebral T1-lesion load has some correlation with verbal production and visual memory and learning, whereas T2-lesion load correlated significantly with the test of verbal production, and visuoperceptual and visuoconstructive functions. The earlier study by Foong and associates [14], did not find significant relationship between lesion load and neuropsychological test results in PPMS patients. The other study by Camp and colleagues [32] reported moderate correlations between the cognitive impairment index and T2-lesion load, T1-hypointensity load and cerebral volume. However, they did not correlate separate test measures for MRI findings.

It has been reported that in PPMS patients, diffuse brain damage remains undetected when using conventional MRI [30,31,41,47–49]. New techniques such as diffusion tensor MRI have shown that PPMS patients have significant microscopic damage of the normal-appearing brain tissue, which is independent of the extent of T2-visible abnormalities [50]. It is conceivable that this microscopic damage explains the lack of correlation between cognitive deficits and MRI findings in our study.

In conclusion, our findings show that PPMS patients have deficits in several cognitive domains when compared to age- and education-matched controls. Furthermore, our study showed similar cognitive impairment in patients with PPMS and SPMS. In PPMS patients, we found some correlations between cognitive deficits and diffuse brain lesions, T1- and T2-lesion load but no correlations were found with atrophy. Studies employing non-conventional MRI techniques are needed for better understanding of the mechanisms underlying cognitive impairment in MS.

Acknowledgements

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