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A PET imaging study

Sini Laaksonen

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To my Lauri and our lovely children

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ABSTRACT

Multiple sclerosis (MS) is a chronic inflammatory and neurodegenerative autoimmune disease of the central nervous system (CNS). In the majority of patients, MS manifests as a relapsing-remitting (RR) disease, which may evolve over time into secondary progressive (SP) disease, leading to irreversible disability. The exact pathogenic mechanisms underlying disease progression are unknown. However, compartmentalized chronic inflammation, including activation of microglial cells, likely plays a central role. Neuropathological studies have demonstrated that activated microglia are present at the rims of chronic active lesions as well as diffusively in the normal-appearing white matter (NAWM). Microglial activation has been shown to correlate with disease severity and predicts disease progression. Positron emission tomography (PET) imaging using radioligands binding to translocator protein (TSPO) enables *in vivo* quantification of microglial activation in the brain, and TSPO-PET is considered a marker of inflammatory activity in MS.

This thesis consists of three studies. Study I assessed, which demographic, clinical, and paraclinical MS disease-related parameters are associated with later TSPO-PET measurable microglial activation. Study II evaluated the effect of sex on [^{11}C]-PK11195 binding in people with MS (pwMS) and in healthy individuals. Study III explored the usability of various imaging and serum biomarkers in predicting conversion to SPMS.

In these studies, a higher number of WM lesions on diagnostic brain MRI, a higher immunoglobulin (IgG) index, and an early increase in the Expanded Disability Status Scale (EDSS) score were associated with increased microglial activation an average of 12 years later. Compared to women, [^{11}C]-PK11195 binding was more prominent in both healthy men and male pwMS. Increased microglial activation, as quantified by TSPO-PET, elevated serum glial acidic fibrillary protein (GFAP) concentration, and thalamic atrophy predicted SPMS conversion.

This thesis demonstrates an association between early clinical factors and later microglial activation that is detrimental to disease progression, and suggests that TSPO-PET, along with serum GFAP concentration and thalamic volume measurement, may be useful in assessing disease prognosis in pwMS.

KEYWORDS: progressive multiple sclerosis, PET imaging, TSPO, microglia, biomarker, prognosis, gender

TURUN YLIOPISTO
Lääketieteellinen tiedekunta
Kliinisten neurotieteiden oppiaine
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TIIVISTELMÄ

Multippeliskleroosi eli MS-tauti on keskushermoston tulehduksellinen ja hermostoa rappeuttava autoimmuunisairaus. MS-tauti alkaa usein aaltomaisena tautimuotona ja voi edetä myöhemmin toissijaisesti eteneväksi MS-taudiksi (SPMS). Mikrogliasolujen aktivoitumisen on osoitettu liittyvän keskeisesti SPMS-taudissa nähtävään hermosolujen vaurioitumiseen. Neuropatologisissa tutkimuksissa aktivoituneita mikrogliasoluja on löydetty tulehduspesäkkeiden ympäriltä ja magneettikuvauksessa (MK) normaalilta näyttävästä valkeasta aivoaineesta. Mikrogliasolujen aktivoitumisen asteen on osoitettu korreloivan taudin vaikeusasteen ja ennustavan taudin etenemistä. Positroniemissiotomografialla (PET) translokaatioproteiiniin (TSPO) sitoutuvia radioaktiivisesti leimattuja merkkiaineita käyttäen voidaan tutkia aktivoituneita mikrogliasoluja aivoissa *in vivo*. Oletuksena on, että TSPO-PET-kuvauksella voidaan arvioida aivojen tulehdusreaktion voimakkuutta MS-taudissa.

Väitöskirja koostuu kolmesta tutkimuksesta. Ensimmäisessä tutkimuksessa arvioitiin, mitkä tekijät MS-taudin diagnoosivaiheessa vaikuttavat myöhempään TSPO-PET-kuvauksella mitattavaan mikrogliasolujen aktiivisuuteen. Toisessa tutkimuksessa arvioitiin sukupuolen vaikutusta TSPO-molekyyliin sitoutuvan [^{11}C]-PK11195 merkkiaineen sitoutumiseen terveillä ja MS-tautia sairastavilla. Kolmannessa tutkimuksessa selvitettiin, voidaanko erilaisia kuvantamis- ja verestä mitattavia biomarkkereita hyödyntää arvioitaessa SPMS-taudin riskiä.

Tutkimuksissa suurempi valkean aineen muutosten määrä diagnostisessa MK:ssa, korkeampi selkäydinnesteen immunoglobuliini G (IgG) indeksi ja varhainen MS-taudin aiheuttama haitta liittyivät lisääntyneeseen mikrogliasolujen aktiivisuuteen keskimäärin 12 vuotta myöhemmin. Verrattuna naisiin, [^{11}C]-PK11195 merkkiaineen sitoutuminen oli voimakkaampaa sekä terveillä että MS-tautia sairastavilla miehillä. Lisääntynyt TSPO-PET-kuvauksella todettu mikrogliasolujen aktiivisuus, korkeampi veren GFAP (glial fibrillary acidic protein) pitoisuus ja talamuksen pienempi koko ennustivat SPMS-taudin kehittymistä.

Väitöskirja osoittaa varhaisten kliinisten tekijöiden yhteyden myöhempään mikrogliasolujen aktiivisuuteen. TSPO-PET kuvausta, GFAP pitoisuutta ja talamuksen tilavuutta voidaan käyttää arvioitaessa MS-taudin ennustetta.

AVAINSANAT: etenevä MS-tauti, PET-kuvantaminen, TSPO, mikrogliasolut, biomarkkeri, ennuste, sukupuoli

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Abbreviations

AHSCT	Autologous hematopoietic stem cell transplantation
AI	Artificial intelligence
ANCOVA	Analysis of covariance
APC	Antigen presenting cell
ARR	Annual relapse rate
BBB	Blood-brain barrier
BTK	Bruton tyrosine kinase
BMI	Body mass index
BP	Binding potential
BRL	Broad rim lesion
CHI3L1	Chitinase-3-like protein 1
CIS	Clinically isolated syndrome
CL	Cortical lesion
CNS	Central nervous system
CSF	Cerebrospinal fluid
CVS	Central vein sign
DAM	Disease-associated microglia
DIS	Dissemination in space
DIT	Dissemination in time
DMF	Dimethyl fumarate
DMT	Disease modifying therapy
DVR	Distribution volume ratio
EAE	Experimental autoimmune encephalomyelitis
EBV	Epstein-Barr virus
EDSS	Expanded Disability Status Scale
EMA	European Medicines Agency
ER	Estrogen receptor
FDR	False discovery rate
FLAIR	Fluid-attenuated inversion recovery
FS	Functional system
GAMLSS	Generalized Additive Model for Location, Scale and Shape

GFAP	Glial fibrillary acidic protein
GM	Gray matter
GRE	Gradient echo
HC	Healthy control
HLA	Human leukocyte antigen
IFN	Interferon
IgG	Immunoglobulin G
IL	Interleukin
IQR	Interquartile range
JC	John Cunningham
LOR	Line of response
LPS	Lipopolysaccharide
LST	Lesion segmentation tool
MAGNIMS	Magnetic Resonance Imaging in MS
MOG	Myelin oligodendrocyte glycoprotein
MRI	Magnetic resonance imaging
MS	Multiple sclerosis
NAGM	Normal-appearing gray matter
NAWM	Normal-appearing white matter
NfL	Neurofilament light chain
OCB	Oligoclonal band
OCT	Optical coherence tomography
PET	Positron emission tomography
PF	Parenchymal fraction
PIRA	Progression independent of relapses
PMS	Progressive MS
PPMS	Primary progressive multiple sclerosis
PRL	Paramagnetic rim lesion
PVE	Partial volume effect
pwMS	Person/people with MS
QSM	Quantitative susceptibility mapping
RAL	TSPO-PET rim-active lesion
RIS	Radiologically isolated syndrome
ROI	Region of interest
RRMS	Relapsing-remitting multiple sclerosis
SAW	Smoldering associated worsening
scRNA-seq	Single-cell RNA sequencing
SD	Standard deviation
SEL	Slowly expanding lesion
SPM	Statistical parametric mapping

SPMS	Secondary progressive multiple sclerosis
SWI	Susceptibility-weighted imaging
SVCA	Supervised cluster analysis
S1P	Sphingosine-1-phosphate
T	Tesla
TAC	Time-activity curve
TMEM	Transmembrane protein
TNF-α	Tumor necrosis factor alfa
TPC	Turku PET Centre
TSPO	Translocator protein
T25FW	Timed 25-Foot Walking
V_T	Distribution volume
WM	White matter
WMIC	Wolfson Molecular Imaging Centre in Manchester UK
κ-FLC	Kappa (κ) free light chain
3D	3-dimensional
9HPT	9-Hole-Peg Test

List of Original Publications

This dissertation is based on the following original publications, which are referred to in the text by their Roman numerals:

I Laaksonen S, Saraste M, Sucksdorff M, Nylund M, Vuorimaa A, Matilainen M, Heikkinen J, Airas L. Early prognosticators of later TSPO-PET-measurable microglial activation in multiple sclerosis. *Multiple Sclerosis and Related Disorders.* 2023;75: 104755

II Laaksonen S, Saraste M, Nylund, M, Hinz R, Snellman A, Rinne J, Matilainen M, Airas L. Sex-driven variability in TSPO-expressing microglia in MS patients and healthy individuals. *Frontiers in Neurology.* 2024;15: 1352116

III Laaksonen S, Sucksdorff M, Vuorimaa A, Kuhle J, Nylund M, Rajander J, Wahlroos S, Matilainen M, Saraste M, Airas L. Predictors of risk of secondary progression in multiple sclerosis. *Therapeutic Advances in Neurological Disorders*. 2025;18: 17562864251357276

1 Introduction

Multiple sclerosis (MS) is a chronic inflammatory and neurodegenerative disease affecting the central nervous system (CNS). Traditionally, it has been classified into distinct phenotypes based on the clinical disease course. In most patients, MS presents as relapsing-remitting disease (RRMS), which is characterized by periods of new or worsening neurological symptoms, referred to as relapses, followed by periods of remission where symptoms either partially or completely resolve or stay stable. In the majority of untreated patients, RRMS evolves with time into secondary progressive MS (SPMS). SPMS is characterized by the gradual progression of symptoms and accumulating neurological disability (Lublin et al., 2014). This traditional classification into distinct clinical phenotypes has been lately challenged. Instead, MS is considered a single disease continuum from relapsing to progressive stages, and the underlying pathophysiological mechanisms vary in their contribution in different disease phenotypes but are all present already from early disease stages (Kuhlmann et al., 2023; Lassmann, 2018). An evolving understanding of the pathological mechanisms underlying disease progression has emphasized the role of chronic, compartmentalized, low-grade inflammation within the CNS, commonly referred to as smoldering inflammation. This smoldering disease component is characterized by chronic active lesions, with a rim of activated microglia/macrophages, and by diffuse damage in normal-appearing brain regions (Giovannoni et al., 2022). These processes are not visible on conventional magnetic resonance imaging (MRI), but can be assessed with positron emission tomography (PET) imaging using radioligands binding to translocator protein (TSPO), as well as with advanced MRI techniques (Banati et al., 2000; Bodini et al., 2021; Dal-Bianco et al., 2024).

Current disease modifying treatments (DMTs) effectively suppress inflammatory activity prominent in early disease stages, but their capacity to hinder the neurodegenerative disease mechanisms leading to progressive disease is limited (Amin and Hersh, 2023). Thus, the lack of effective treatment options for progressive MS (PMS) remains a great unmet need in the MS field, and MS is still one of the leading causes of neurological disability among young adults worldwide (Walton et

al., 2020). Deeper understanding of the mechanisms driving disease progression could provide new targets for future treatments.

The course of MS is heterogeneous and unpredictable. Large natural history studies have shown an association between several demographic and clinical factors, such as longer disease duration, age and male gender, with disease progression and a poor prognosis (Koch et al., 2010; Leray et al., 2010; Tremlett et al., 2008; Tutuncu et al., 2013). Additionally, early imaging parameters like T2 lesion load on diagnostic MRI may help predict the disease course (Fisniku et al., 2008; Uher et al., 2017). In recent years, numerous potential biomarkers for disease progression have been studied. Examples include serum biomarkers like glial fibrillary acidic protein (GFAP) and neurofilament light chain (NfL) (Abdelhak et al., 2025; Meier et al., 2023), new MRI techniques such as paramagnetic rim lesions (PRL) (Absinta et al., 2019; Altokhis et al., 2022; Reeves et al., 2024b) and different brain volume measures (Cagol et al., 2022; Cree et al., 2019; Eshaghi et al., 2018), and TSPO-PET imaging (Bodini et al., 2021). Despite the improvements in the biomarker field, there is currently no reliable biomarker to predict disease progression and treatment responses, which creates challenges for the MS clinician.

This study evaluated the usability of TSPO-PET imaging and other recent biomarkers in predicting evolution to SPMS. Besides, the association of recognized risk factors of a poor disease prognosis with later TSPO-PET measurable microglial activation, and the effect of sex on TSPO binding were explored. Broadly, this work aimed to provide knowledge of the pathological mechanisms underlying progressive MS to ultimately improve the care and long-term prognosis of this patient group.

2 Review of the Literature

2.1 Multiple Sclerosis

2.1.1 Epidemiology

Based on the latest Multiple Sclerosis Atlas, in total 2.8 million people are estimated worldwide to have MS, with a global prevalence rate of 35.9 cases per 100 000 people (Walton et al., 2020). The prevalence varies across different geographical areas and ethnic populations (Howard et al., 2016; Wallin et al., 2019). Most studies indicate that the incidence and prevalence of MS have increased from 1950s until around year 2000, and thereafter incidence and prevalence have remained stable (Howard et al., 2016; Koch-Henriksen and Magyari, 2021; Walton et al., 2020). Similar trends in incidence and prevalence from 1974 to 2021 were observed in a recent Finnish nationwide population-based registry study (Maunula et al., 2025). Several factors may explain the increased prevalence of MS, such as changing exposure to risk factors, improved MS diagnosis, better access to neurological services, introduction of effective MS treatments, global population growth, aging, and greater life expectancy (Goldman et al., 2022; Koch-Henriksen and Magyari, 2021). MS is more common in women than in men, and the incidence among women has increased over time. Globally, the female to male ratio is 2:1, but varies between regions up to 4:1 (Koch-Henriksen and Magyari, 2021; Wallin et al., 2019; Walton et al., 2020).

2.1.2 Risk Factors

2.1.2.1 Genetic Risk Factors

An apparent familial aggregation of MS has been observed in several studies. The concordance rate of MS in monozygotic twins is 25–30% whereas the recurrence risk in dizygotic twins and other siblings is remarkably lower, varying between 2–5% (Ebers et al., 1986; Willer et al., 2003). First-degree relatives of people with MS (pwMS) have an excess familial lifetime risk of approximately 2.5% of developing MS (Nielsen et al., 2005).

Genome-wide association studies have identified more than 200 genetic risk variants associated with MS susceptibility. Each single variant explains only a small proportion of increased risk, but increases individuals' total risk in an additive manner. Most of these identified genetic risk variants seem to be associated with subtle changes in gene regulation both in innate and adaptive immune system (Goris et al., 2022; Patsopoulos et al., 2019). Human leukocyte antigen (HLA) alleles are the strongest identified genetic risk factors for MS. HLA alleles can either increase or decrease the risk of developing MS. HLA-DRB1*15:01 is the most important risk allele and it increases the odds of developing MS by 3.92 times in heterozygous and 8.30 times in homozygous patients (Moutsianas et al., 2015).

Besides influencing the risk of disease onset, genetic factors may also affect the long-term prognosis. For example, a recent longitudinal study with a 15-year follow-up linked HLA-DRB1*1501 allele to a faster rate of disability accumulation, more pronounced brain atrophy, an increase in T1 and T2 lesion burden, and a higher number of gadolinium-enhancing lesions in all studied time points (Brownlee et al., 2023).

2.1.2.2 Environmental and Lifestyle Risk Factors

The association between Epstein-Barr virus (EBV) infection and the risk of developing MS has been proposed for years. Studies have shown that nearly all pwMS have EBV-specific antibodies, and the risk of MS in EBV-seronegative patients is extremely low (Hedström, 2023; Pakpoor et al., 2013). A recent large study following over 10 million young adults from United States military demonstrated a 32-fold increased risk for MS following EBV seroconversion, suggesting even a causal link between EBV infection and MS (Bjornevik et al., 2022). To date, the exact pathogenic mechanisms by which EBV increases the risk of MS and the role of EBV in disease progression remain unclear.

The prevalence of MS increases with latitude and the highest MS prevalence is observed in areas distant from the equator. These latitude-dependent changes are suggested to be related to exposure to ultraviolet radiation and vitamin D levels (Simpson et al., 2011) as low vitamin D levels and low sun exposure especially in childhood have been repeatedly associated with increased MS risk (Gallagher et al., 2019; Hewer et al., 2013; Munger et al., 2004; Sebastian et al., 2022; Tremlett et al., 2018). Additionally, people born at the end of spring show an increased risk and people born in late autumn show a reduced risk of MS, which might be explained by differences in maternal vitamin D levels and sun exposure during pregnancy (Dobson et al., 2013a). Low vitamin D levels have also been linked to clinical and radiological disease activity (Fitzgerald et al., 2015; Giordano et al., 2025; Mowry

et al., 2012; Smolders et al., 2019) and even to earlier conversion to SPMS (Muris et al., 2016).

Smoking is associated with increased risk of MS and the risk correlates with the duration and intensity of smoking (Degelman and Herman, 2017; Hedström et al., 2013). Smoking associates also with disability progression (Manouchehrinia et al., 2013) and the risk of conversion to SPMS (Degelman and Herman, 2017; Ramanujam et al., 2015). Obesity in early life seems to increase the risk of both pediatric and adult-onset MS, particularly among women (Munger et al., 2013; Schreiner and Genes, 2021). Abdominal obesity and high body mass index (BMI) have also been associated with more severe clinical disability (Fitzgerald et al., 2020; Stampanoni Bassi et al., 2020; Van Hijfte et al., 2022). Other environmental and lifestyle factors associated with increased MS risk include changes in gut microbiota composition (Rouzitalab et al., 2023; Stoiloudis et al., 2022), shift work (Hedström et al., 2015), poor sleep in adolescence (Åkerstedt et al., 2023), lack of physical exercise (Yuan et al., 2021), and air pollution (Rouzitalab et al., 2023). Besides EBV, other viruses such as human herpesvirus 6A and SARS-CoV-S (Bakhshi et al., 2023; Biström et al., 2021; Engdahl et al., 2019) have also been linked to an increased risk of MS. The impact of environmental and lifestyle risk factors is probably most prominent during childhood as migration studies indicate that individuals moving from a low-risk to a high-risk country before adolescence have a risk of MS comparable to the population of the high-risk country (Ahlgren et al., 2010; Berg-Hansen et al., 2015).

Despite several different identified risk factors, the definite cause of MS remains unknown. Presumably complex interactions between environmental and lifestyle risk factors and genetic susceptibility contribute to MS development, which is potentially triggered by a viral infection such as EBV infection (Goldman et al., 2022). Importantly, as many lifestyle and environmental risk factors are modifiable, there is an opportunity to influence an individual's MS risk especially among those with high genetic MS susceptibility (Alfredsson and Olsson, 2019). In the future, prophylactic vaccination against EBV may reduce the incidence of MS, and antiviral therapies targeting EBV may play a role in prevention and/or treatment of MS (Aloisi et al., 2023).

2.1.3 Pathogenesis

The exact pathogenetic mechanisms of MS are also still largely unknown. The current consensus suggests an interplay between genetic, environmental, and immunological factors triggering an autoimmune-mediated inflammatory process, which is accompanied by progressive neurodegeneration already from the onset of the disease (Kuhlmann et al., 2023; Kutzelnigg and Lassmann, 2014). The most

proposed hypothesis is that autoreactive T cells are primed in the periphery following complex interactions between genetic and environmental factors. To date, the specific target antigen or antigens and exact mechanisms by which autoreactive T cells become activated remain unclear. Once activated, autoreactive T cells migrate into the CNS across the blood-brain barrier (BBB). Inside the CNS, local antigen presenting cells (APCs) can reactivate these T cells, which results in the release of cytokines and chemokines, recruitment of additional immune cells (T cells, monocytes, B cells), and persistent activation of microglia and macrophages within the CNS tissue. This whole cascade leads to an inflammatory reaction, destruction of myelin and oligodendrocytes, and damage of neurons and axons. Myelin proteins, such as myelin basic protein and myelin oligodendrocyte glycoprotein (MOG), are proposed targets of autoreactivity in the CNS as demyelination is a hallmark of MS pathology (Bar-Or and Li, 2021; Dendrou et al., 2015).

2.1.3.1 Early and Relapsing MS

The main pathological feature of MS is the presence of demyelinated lesions in the white matter (WM) and gray matter (GM) of the brain and spinal cord. The formation of these focal MS lesions is considered a manifestation of acute inflammatory damage, which is most prominent in early stages of the disease (Frischer et al., 2009). The inflammatory reaction usually initiates around small postcapillary venules and veins and is associated with severe BBB disruption. Demyelinated lesions are typically hypercellular, consisting mainly of phagocytotic cells (blood-derived monocytes and microglia) located throughout the lesion, along with a smaller number of T cells (predominantly CD8+, fewer CD4+), B cells, and plasma cells (Frischer et al., 2015; Kuhlmann et al., 2017; Luchetti et al., 2018). Inflammation leads to loss of myelin sheaths and oligodendrocytes, astrocytic activation, and reactive gliosis.

In early MS, axons and neurons are mainly preserved. However, as the disease progresses, gradual axonal and neuronal injury develops, although the exact underlying mechanisms are not completely understood (Bitsch et al., 2000; Kutzelnigg and Lassmann, 2014; Lassmann, 2018). Focal demyelinated lesions can be partly or completely repaired by remyelination, or lesions may evolve to chronic active or inactive lesions, which are the main lesion types in PMS (Frischer et al., 2015; Kuhlmann et al., 2017; Luchetti et al., 2018). Remyelination is more common in the early disease stages than in progressive disease and depends at least partly on lesion location; however, extensive remyelination occurs only in 20–30% of pwMS (Albert et al., 2007; Goldschmidt et al., 2009; Patrikios et al., 2006). Importantly, beyond focal lesion pathology, diffuse neurodegenerative damage is present in the normal-appearing WM (NAWM) and normal-appearing GM (NAGM) of the brain

and spinal cord from the very beginning of the disease (Kutzelnigg and Lassmann, 2014).

2.1.3.2 Progressive MS

Inflammation is likely the main pathological driver of tissue injury even in progressive disease, although the magnitude of inflammation declines with increasing age and disease duration (Frischer et al., 2009). In PMS, only minor or no BBB damage is present, and thus, the infiltration of immune cells from the peripheral blood into the CNS is reduced (Hochmeister et al., 2006; Kwon and Prineas, 1994; Lassmann et al., 2012). Consequently, inflammation becomes compartmentalized within the CNS (Lassmann et al., 2012, 2007). Inflammatory cell infiltrates are diffusively located around small veins and venules in the NAWM and NAGM (Lassmann et al., 2012). Large aggregates of inflammatory cells resembling lymphoid follicles can also be observed in connective tissue spaces of the brain, such as the meninges and Virchow-Robin spaces (Howell et al., 2011; Lucchinetti et al., 2011).

In PMS, new and active WM lesions become rare, but pre-existing lesions may slowly expand (Lassmann et al., 2012). Instead, chronic active (mixed active/inactive or smoldering) and chronic inactive lesions are the dominant lesion types (Frischer et al., 2015; Kuhlmann et al., 2017; Luchetti et al., 2018). Chronic active lesions are characterized by a hypocellular lesion core surrounded by a rim of activated phagocytes (microglia/macrophages). T cells are located in perivascular area and diffusively within the lesion core. Additionally, small numbers of B cells or plasma cells are present (Frischer et al., 2015; Kuhlmann et al., 2017; Luchetti et al., 2018). Chronic inactive lesions are hypocellular, sharply demarcated, and gliotic throughout the lesion, containing only few T cells and macrophages/microglia (Frischer et al., 2015; Kuhlmann et al., 2017; Luchetti et al., 2018). In neuropathological studies, chronic active lesions have been associated with disease severity and are mainly observed in pwPMS with longer disease duration (Frischer et al., 2015; Luchetti et al., 2018). Chronic inactive lesions are most frequently seen in pwPMS with a disease duration longer than 15 years and no relapses (Frischer et al., 2015).

Beyond focal lesions, diffuse changes in the NAWM are typical for PMS. These changes include small, perivascular inflammatory cell infiltrates predominantly consisting of CD8+ T cells (Frischer et al., 2009; Seewann et al., 2009), widespread microglial activation and the formation of microglial nodules (Howell et al., 2010; Kutzelnigg et al., 2005; van Horssen et al., 2012), axonal injury, mild or absent demyelination, and astrocytic scarring. Altogether, these processes result in global tissue loss and prominent brain atrophy (Kutzelnigg et al., 2005; Lassmann, 2018; Lassmann et al., 2012). Diffuse WM injury develops independently of focal WM

lesions but correlates moderately with cortical demyelination (De Stefano et al., 2002; Kutzelnigg et al., 2005), and its severity increases with disease duration (Kutzelnigg and Lassmann, 2014; Lassmann et al., 2012). In addition to diffuse damage of the NAWM, cortical demyelination is prominent in PMS. Cortical demyelination is dominated by subpial cortical lesions, associates with meningeal inflammatory cell infiltrates (Haider et al., 2016; Howell et al., 2011; Magliozzi et al., 2010), and is accompanied with widespread loss of axons, neurons and glial cells, finally leading to cortical atrophy (Kutzelnigg et al., 2005; Wegner et al., 2006).

The pathogenic mechanisms underlying the neurodegenerative process are still not completely understood. Oxidative stress, followed by mitochondrial injury, can lead to "virtual hypoxia", resulting in energy failure in oligodendrocytes, axons, and neurons. Age-related hypoxic tissue injury and iron accumulation in the CNS may amplify the extent of oxidative damage (Haider et al., 2011; Lassmann et al., 2012; Trapp and Stys, 2009). Moreover, microglia and macrophage persisting in an activated state may further augment the tissue injury (Fischer et al., 2012; Lassmann et al., 2012). Loss of trophic support and failure of remyelination probably also contribute to disease progression (Goldschmidt et al., 2009; Lassmann, 2018). When the accumulation of tissue damage exceeds the functional reserve capacity of the brain, even a minor additional axonal injury may accelerate the clinical deterioration of patients´ symptoms. (Lassmann et al., 2012; Trapp et al., 1998). A summary of the differences in pathogenetic mechanisms and pathological features in RRMS and progressive MS is presented in Table 1.

Table 1. A summary of pathogenetic mechanisms and pathological features in relapsing-remitting MS and progressive MS. Writers own drawing based on Kutzelnigg and Lassmann 2014, and Lassmann 2018.

RELAPSING-REMITTING MS	PROGRESSIVE MS
Formation of new WM and GM lesions	Enlarging pre-existing WM lesions, chronic active lesions and chronic inactive lesions
Infiltration of immune cells from peripheral blood to CNS through damaged BBB	Compartmentalized inflammation in CNS behind intact BBB
Diffuse damage of NAWM and NAGM already present	Prominent widespread damage of NAWM and NAGM
Axonal and neuronal loss	Prominent brain atrophy
Remyelination	Failure of remyelination
Cortical lesions already present	Cortical lesions typical, widespread cortical demyelination and atrophy

Abbreviations: BBB = blood-brain barrier; CNS = central nervous system; GM = gray matter; MS = multiple sclerosis; NAGM = normal-appearing gray matter; NAWM = normal-appearing white matter; RRMS = relapsing-remitting multiple sclerosis; WM = white matter

2.1.4 Clinical Features

2.1.4.1 Symptoms

The clinical presentation of MS is heterogeneous, as the signs and symptoms depend on the affected area of the CNS. Classic manifestations of MS include optic neuritis (unilateral painful loss or blurring of vision), partial myelitis (impaired sensation, weakness, and/or ataxia in the extremities and torso), focal sensory symptoms (e.g., paresthesia or numbness in the limbs), and brainstem syndromes (vertigo, hearing loss, diplopia, facial sensory disturbances). Other symptoms may also occur, and while the signs and symptoms are not MS specific, Lhermitte´s symptom (an electrical sensation radiating down the spine during neck flexion) and the Uhthoff phenomenon (worsening of signs and symptoms with increased body temperature) are quite characteristic of MS (Compston and Coles, 2008; McGinley et al., 2021). Typical symptoms of PMS include progressive loss of walking ability, bladder and bowel dysfunction, and increasing cognitive impairment.

In recent years, studies focusing on the period before the onset of MS, termed MS prodrome, have emerged (Makhani and Tremlett, 2021). These population-based studies have demonstrated increased health-care usage among individuals who develop later MS due to various symptoms such as sleep disorders, fatigue, anemia, headache and other types of pain, gastrointestinal and urinary symptoms, anxiety, and depression (Disanto et al., 2018; Wijnands et al., 2017; Yusuf et al., 2021). However, these prodromal symptoms and diseases are not specific to MS (Guinebretiere et al., 2023).

2.1.4.2 Clinical Course

Assessing the clinical phenotype of MS is essential at the time of diagnosis. The clinical subtypes of MS were defined for the first time in 1996 (Lublin and Reingold, 1996), and the current classification, based on re-evaluation in 2013, categorizes the disease into clinically isolated syndrome (CIS), radiologically isolated syndrome (RIS), RRMS, SPMS, and primary progressive MS (PPMS) (Lublin et al., 2014). A growing understanding of MS pathophysiological mechanisms has led to the emerging view that all MS phenotypes are part of a single disease continuum, with no clear boundaries between clinical stages (Kuhlmann et al., 2023; Lassmann, 2018). Although clinical phenotyping may lose significance in the future, classification into clinical phenotypes is still widely used in current clinical practice and clinical trials.

CIS refers to the first neurological episode suggestive of the onset of MS that does not yet fulfil the diagnostic criteria for MS. The symptoms are usually

monofocal and involve optic nerve, spinal cord, brainstem, or cerebellum (Miller et al., 2012). Earlier risk factors for the development of MS in individuals presenting with CIS included the presence of oligoclonal bands (OCBs) in cerebrospinal fluid (CSF) and number and location of T2 lesions in brain MRI (Filippi et al., 1994; Fisniku et al., 2008; Giorgio et al., 2013; Kuhle et al., 2015; Tintore et al., 2010). However, as changes in the diagnostic criteria for MS (see also Chapter 2.1.5) (Montalban et al., 2025; Thompson et al., 2018) now allow an earlier diagnosis of MS, a substantial proportion of individuals previously diagnosed with CIS (presenting above mentioned risk factors for developing MS) are now diagnosed with MS (Filippi et al., 2022; Habek et al., 2025; Vidal-Jordana et al., 2024).

RIS refers to the presence of typical MS lesions on brain MRI in an asymptomatic individual imaged for reasons other than suspected demyelinating disease (De Stefano et al., 2018; Moore, 2009). Previous studies have shown that up to half of patients (20–50%) presenting with RIS are diagnosed with MS within 5-10 years (Lebrun-Frenay et al., 2020; Okuda et al., 2014). Identified risk factors for developing MS include younger age (< 35–37 years), the presence of OCBs or an elevated immunoglobulin G (IgG) index in CSF, increased serum NfL (sNfL) levels, infratentorial and spinal cord lesions, gadolinium enhancement, and a high number of WM lesions in the index MRI. The risk of developing MS is especially high when a combination of these risk factors is present (Fissolo et al., 2025; Lebrun-Frénay et al., 2021; Lebrun-Frenay et al., 2020; Maunula et al., 2023; Okuda et al., 2014; Rival et al., 2023). Recently, RIS was included in the revised 2024 MS diagnostic criteria (Montalban et al., 2025) (see also Chapter 2.1.5), and in the future, a significant proportion of patients previously diagnosed with RIS will likely be diagnosed with MS.

In the majority (85–90%) of patients, MS manifests as RRMS, a disease course characterized by acute attacks (relapses) followed by partial or complete recovery and relatively stable neurological disability between relapses (Lublin et al., 2014). A relapse is defined as symptoms or signs of an acute or subacute focal or multifocal inflammatory demyelinating event in the CNS with a minimum duration of 24 hours, in the absence of fever or infection (McDonald et al., 2001).

Before the introduction of DMTs, most pwRRMS (55–60%) converted to SPMS, typically after 10–20 years of disease duration between the ages of 40 and 50 years (Koch et al., 2010; Tedeholm et al., 2015; Tremlett et al., 2008; Tutuncu et al., 2013; Weinshenker et al., 1989). In recent years, studies have demonstrated a decreased incidence of SPMS in the modern treatment era (Tedeholm et al., 2022; Zanghì et al., 2025). Moreover, the proportion of patients transitioning to SPMS has markedly decreased (to approximately 10–20% of pwRRMS after 8–17 years), and the time to SPMS conversion has significantly lengthened compared to pre-DMT era (Barzegar et al., 2021; Brown et al., 2019; Cree et al., 2016; Fambiatos et al., 2020; Iaffaldano

et al., 2021a; Tedeholm et al., 2022; Zanghì et al., 2025). For example, a longitudinal study following 517 pwMS (most RRMS or CIS) treated with various DMTs reported that 18% of pwMS converted to SPMS and 11% reached an EDSS score ≥ 6 after a median of 16.8 years from disease onset (Cree et al., 2016). A recent retrospective population-based registry study including 9958 pwMS demonstrated a higher rate of SPMS conversion among pwMS who were never exposed to DMTs compared with those who had used DMTs (9.9% vs. 5.4%) (Zanghì et al., 2025). However, after entering the progressive disease stage and reaching moderate level of disability, the trajectory of disease progression appears to be identical between individuals regardless the earlier disease course (Kremenchutzky et al., 2006; Leray et al., 2010).

No established diagnostic criteria for SPMS exist. SPMS is defined by gradual progression of symptoms, with or without occasional concomitant relapses, following an initial relapsing disease course. The diagnosis of SPMS is often retrospective, and determining the exact transition point from RRMS to SMPS is challenging (Lublin et al., 2014). A minority of patients (10–15%) experience a progressive disease course without relapses from the disease onset and are diagnosed with PPMS; diagnostic criteria for PPMS were revised 2024 (Montalban et al., 2025; Thompson et al., 2000).

2.1.4.3 Clinical Measures

Expanded Disability Status Scale (EDSS) is a 0-to-10 scale describing the level of disability caused by MS. EDSS is based on clinical neurological examination and assessment of disability in eight functional areas: pyramidal, cerebellar, brainstem, sensory, bowel and bladder, visual, cerebral, and other (Kurtzke, 1983). EDSS has several limitations such as rather high interrater variability, emphasis on ambulation and lower limb function (ambulatory function being the main factor determining the EDSS score for values > 4.0) and underestimation of certain functional areas (e.g., upper limb function and cognition). Despites its shortcomings, EDSS is widely used in clinical practice and as on endpoint in clinical trials to evaluate disease progression and describe disease severity (Meyer-Moock et al., 2014).

Other tests, such as 9-Hole-Peg Test (9HPT) assessing upper limb function and Timed 25-Foot Walking (T25FW) measuring leg function performance can be used alongside EDSS (Feys et al., 2017; Kalinowski et al., 2022). A recent post hoc analysis including data from six phase 3 clinical trials showed that 9HPT and T25FW events were associated with an increased risk of later EDSS events, and that T25FW events additionally predicted treatment effects on EDSS outcomes supporting the use of a combination of these performance tests in progressive MS trials (Kappos et al., 2025).

2.1.4.4 Progression Independent of Relapses and Smoldering Associated Worsening

Increased understanding of biological mechanisms behind MS has led to the introduction of the concepts of progression independent of relapses (PIRA) and smoldering associated worsening (SAW), which both reflect the diffuse clinical disease worsening that occurs before the transition from RRMS to PMS.

PIRA is defined as gradual worsening of neurological symptoms in the absence of relapses. The accumulation of disability is typically measured by repeated EDSS score assessments (BAC et al., 2019; Müller et al., 2025, 2023). As EDSS primarily focuses on ambulation at higher end of the scale, PIRA also emphasizes motor performance and fails to capture the broader aspects of clinical worsening experienced by patients (BAC et al., 2019; Müller et al., 2025, 2023). Data from clinical studies (BAC et al., 2019; Portaccio et al., 2022; Tur et al., 2023; Zárate et al., 2025) and clinical trials (Kappos et al., 2020, 2018b; Lublin et al., 2022) over the last decade have demonstrated that disability accumulation due to PIRA occurs already in early phases of MS. For example, in a large real-world observation cohort of over 5100 patients with CIS or early RRMS, 27.6% of patients demonstrated PIRA over an average follow-up of 11.5 years (Portaccio et al., 2022). Moreover, a pooled post hoc analysis of 1656 pwRRMS participating in ocrelizumab trials OPERA 1 and OPERA 2 demonstrated that the majority of disability accrual events (89%) over the 96-week follow-up period were associated with PIRA, highlighting the insufficient efficacy of current DMTs in preventing PIRA (Kappos et al., 2020).

SAW is a recent definition developed by an international panel of neurologists in 2024. It also describes the gradual accumulation of disability independent of inflammatory disease activity (relapses, new MRI lesions), but encompasses broader spectrum of symptoms than PIRA. Besides the decline in motor performance, manifestations of SAW include reduced endurance and exercise induced symptoms, cognitive slowing, worsening fatigue, bladder and bowel symptoms, sexual dysfunction, neuropathic pain, and general deterioration (“feeling worse”) (Scalfari et al., 2024).

Despite intensive research, the mechanisms underlying PIRA and SAW are still not fully understood. Presumably the main pathological substrates of this “smoldering” disease activity are chronic, low-grade compartmentalized inflammation and widespread progressive neurodegenerative injury coupled with failure of compensatory mechanisms (Calabrese et al., 2024; Giovannoni et al., 2022; Kuhlmann et al., 2023; Liddelow et al., 2017; Scalfari et al., 2024). Additionally, smoldering disease activity probably promotes premature biological aging of the CNS and, conversely, aging may reinforce smoldering disease mechanisms (Graves et al., 2023; Kuhlmann et al., 2023).

2.1.5 Diagnosis

The diagnostic criteria for MS combine clinical, imaging and laboratory findings. The first diagnostic criteria, introduced in 1965, were completely clinical (Schumacher et al., 1965). The 1985 Poser criteria additionally included paraclinical evidence of CNS lesions, such as CSF analysis, neuroimaging, evoked potential studies and other special tests (urological studies, induced hyperthermia) (Poser et al., 1983). The 2001 McDonald criteria included MRI findings for the first time, and since then, the McDonald criteria have been revised several times (2005, 2010, 2017 and 2024) (McDonald et al., 2001; Montalban et al., 2025; Polman et al., 2011, 2005; Thompson et al., 2018).

The 2024 McDonald criteria were introduced for the first time at the 40th congress of the European Committee for Treatment and Research in Multiple Sclerosis in autumn 2024 and were published in September 2025 (Montalban et al., 2025). The 2024 revision highlights the essential role of paraclinical tests to facilitate early diagnosis of MS, alongside global applicability. As the MS disease course is now considered a single disease continuum with shared pathological mechanisms across all disease stages (Kuhlmann et al., 2023), the 2024 criteria present a diagnostic framework, which can be used both in patients with relapsing-onset and progressive-onset clinical presentation instead of the earlier separate diagnostic criteria for RRMS and PPMS. Additionally, the same diagnostic criteria apply to pediatric and adult-onset MS, but specific consideration must be given to differential diagnoses.

The initial diagnostic workup includes evaluation of clinical presentation, brain MRI and consideration of possible alternative diagnoses. A typical clinical presentation of MS is defined as (1) an attack with typical symptoms and signs of MS or (2) progressive symptoms lasting at least 12 months. Brain MRI remains the cornerstone of MS diagnosis and demonstrates typical MS lesions in distinct anatomical locations indicating the multifocal CNS process (dissemination in space, DIS). Besides the four previous anatomical locations (juxtacortical or cortical, periventricular, infratentorial, spinal cord), the 2024 McDonald criteria include the optic nerve as a fifth anatomical location demonstrating DIS. Optic nerve lesions can be demonstrated by MRI, optical coherence tomography (OCT) or visual evoked potentials. In patients with a typical clinical presentation, DIS is fulfilled when typical lesions (symptomatic or asymptomatic) are present in at least two of the five regions. In patients presenting with progressive symptoms, two spinal cord lesions are enough to identify DIS. If typical lesions are present in at least four anatomical locations and patient has a typical MS presentation, MS can be diagnosed without further requirements. If lesions are present only in one anatomical location, diagnosis of MS is still possible if clinical presentation is typical to MS and additional requirements are fulfilled. In these cases, evaluation

of dissemination in time (DIT) and the use of highly specific tools (presented below) is recommended.

DIT refers to the development of new CNS lesions over time. DIT can be demonstrated with MRI (the appearance of new T2 lesions, or one or more gadolinium-enhancing lesion at any time point), with a new clinical attack, or immunopathology in CSF (CSF positivity). The 2017 McDonald criteria already included the presence of OCBs as a substitute for DIT, and in the 2024 McDonald criteria, also presence of kappa free light chains (κ-FLC) in CSF can demonstrate CSF positivity.

The 2024 McDonald revision included two highly MS-specific MRI features – the central vein sign (CVS) and paramagnetic rim lesions – which can be used in the diagnostic process, in selected cases, to fulfill the diagnostic criteria without traditional demonstration of DIT. CVS refers to a centrally located hypointense line or small dot running partially or entirely through a lesion on T2-weighted MRI and can differentiate MS from most diseases that mimic MS (Barkhof et al., 2025; Sati et al., 2016). CVS positivity is demonstrated if six or more WM lesions with CVS are present (the "select 6" method). If fewer than ten lesions are present, CVS positivity requires that the majority of lesions have CVS (Montalban et al., 2025). PRLs are lesions with a paramagnetic rim surrounding the lesion core, visible on different susceptibility-based MRI sequences that detect iron, and also helpful in differentiating MS from other conditions (Absinta et al., 2018; Bagnato et al., 2024; Barkhof et al., 2025). Demonstration of CVS positivity and/or PRLs is not required for a diagnosis of MS, but both can be used in the diagnostic process in specific situations and can increase the specificity of MS diagnosis (Cagol et al., 2024b; Calvi et al., 2020; Clarke et al., 2020; Toljan et al., 2024). Table 2 presents the 2024 McDonald diagnostic criteria of multiple sclerosis.

Importantly, when suspecting MS in people aged ≥ 50 years, with headache disorders, and/or with significant vascular risk factors or comorbidities, additional features in the diagnostic workup are strongly recommended to reduce the risk of misdiagnosis. These features supporting MS diagnosis include a spinal cord lesion, CSF positivity, and CVS positivity. In children, testing for MOG antibodies is highly recommended to prevent misdiagnoses.

Table 2. The 2024 McDonald criteria for diagnosis of multiple sclerosis. Modified from Montalban et al. 2025.

Typical presentation of MS, initial diagnostic evaluation supporting diagnosis of MS and better alternative explanations excluded

Lesions present in **≥ 2** CNS areas OR Progressive symptoms for ≥ 12 months and ≥ 2 spinal cord lesions	Lesions present in **one** CNS area (including also patients with progressive symptoms for ≥ 12 months)
MS can be diagnosed if at least one of the following is also demonstrated	
CSF+	CSF+ and CVS+
CVS+	CSF+ and PRL+
DIT	DIT and CVS+
Lesions present in 4 or 5 CNS areas	DIT and PRL+

CSF+ is demonstrated by presence of oligoclonal bands or kappa free-light chains. CVS+ is demonstrated by finding at least 6 lesions with CVS (in cases with less than 10 lesions, majority of lesions need to demonstrate CVS). PLR+ is demonstrated by finding at least one lesion with paramagnetic rim. DIT is demonstrated by a new clinical attack, one or more gadolinium-enhancing lesions on MRI at any time and/or one or more new T2 lesions in follow-up MRI. Abbreviations: CNS = central nervous system; CVS = central vein sign; DIT = dissemination in time; MRI = magnetic resonance imaging; MS = multiple sclerosis; PRL= paramagnetic rim lesion

An additional major update in the 2024 McDonald criteria is the inclusion of RIS in the diagnostic criteria. In patients with incidental imaging findings suggestive of demyelinating disease and lesions present in two or more CNS areas, MS can be diagnosed if one of the following is also demonstrated: (1) CSF positivity, (2) CVS positivity, or (3) DIT. This diagnostic workup can also be applied to patients with clinical symptoms or signs not specific to MS, such as paroxysmal symptoms, seizures, or other neurological symptoms that do not constitute a clear attack or progression of disability.

To date, no established diagnostic criteria for SPMS exists. According to the Lublin criteria from 2013, SPMS can be diagnosed in a patient with an initial relapsing disease course who experiences gradual worsening of neurological function independent of relapses over the previous 3-12 months (Lublin et al., 2014). The diagnosis of SPMS based on Lublin criteria is challenging, often retrospectively established, and delayed up to 3 years (Sand et al., 2014). Hence objective, mostly EDSS based definitions for SPMS have been proposed. The Lorscheider definition is a data-derived definition based on a large patient cohort (17 356 patients from the MSBase registry) which consists of following: (1) disability progression unrelated to relapses demonstrated by worsening in EDSS

score (1.0 step increase in patients with follow-up EDSS score ≤ 5.5 or 0.5 step increase in patients with follow-up EDSS score ≥ 6.0), (2) EDSS score ≥ 4.0, (3) pyramidal functional system (FS) score ≥ 2, and (4) EDSS score progression confirmed over at least for 3 months, including confirmation withing the leading FS (Lorscheider et al., 2016). Another suggested objective definition for SPMS is the Modified EXPAND criteria, which is based on the inclusion criteria of the siponimod in SPMS phase 3 study (EXPAND) (Kappos et al., 2018a). The Modified EXPAND criteria consists of (1) EDSS score 3.0-6.5 at the time of SPMS conversion, (2) EDSS score worsening in the absence of relapses over at least 6 months (1.0 step increase in patients with follow-up EDSS score ≤ 5.5 or 0.5 step increase in patients with follow-up EDSS score ≥ 6.0), and (3) confirmation of EDSS score progression over at least 6 months. Both these definitions rely on EDSS (likewise the definition of PIRA) and may thus fail to capture the whole spectrum of symptoms related to progressive MS.

2.1.6 Treatment

2.1.6.1 General Perspectives

No curative treatment for MS exists. After MS diagnosis, most patients start DMTs, which have a beneficial effect on long-term disease outcomes, particularly when highly effective treatment is initiated early (Buron et al., 2020; Hänninen et al., 2022; Harding et al., 2019; He et al., 2020; Iaffaldano et al., 2021b). Interferon-beta 1b was the first DMT accepted for treatment of MS in 1993, and since then, significant progress in the field of MS treatment has occurred, and the therapeutic landscape still evolves constantly. Most currently available DMTs are approved for treatment of RRMS, and treatment options for patients with progressive MS forms are sparse. Initial treatment selection includes consideration of several clinical factors including disease type (RRMS, progressive MS), disease activity (active or highly active), and different patient-related clinical, imaging and other factors. Additionally, potential side effects and long-term safety profile of the DMT, age, possible comorbidities, other medications, family planning, and patients´ own preferences are considered (Bose and Freedman, 2020). Multiple recommendations and guidelines for the use of DMTs have been developed worldwide. In Finland, the Current Care Guidelines for MS guide treatment selection (Multiple Sclerosis: Current Care Guidelines, 2024). DMTs approved for treatment of MS in Finland are listed in Table 3.

Table 3. Disease modifying therapies available in Finland based on Current Care Guidelines for MS (2024).

MODERATE-EFFICACY	HIGH-EFFICACY
Dimethyl fumarate	Alemtuzumab
Diroximel fumarate	Cladribine
Glatiramer acetate	Fingolimod
Interferon betas (beta-1a and beta-1b)	Natalizumab
Ofatumumab	Ocrelizumab
Teriflunomide	Ofatumumab
Ponesimod	Rituximab*

*Rituximab does not have official therapeutic indication for MS treatment but is still widely used in Nordic countries for MS treatment.

After DMT initiation, pwMS are followed in neurological clinics in Finland. The efficacy of treatment is evaluated at least once a year. The follow-up visit should include patients´ interview for possible relapses and a neurological examination for assessment of EDSS score (Kurtzke, 1983). Brain MRI is used routinely (Wattjes et al., 2021) as it is more sensitive than clinical relapses in detecting inflammatory disease activity (Kappos et al., 1999). The general target of treatment is remission, which refers to the absence of relapses, stable MRI, and no observed disability worsening. In cases with insufficient treatment response, a switch of DMT from a moderate- to high-efficacy DMT should be considered. In patients with very aggressive relapsing-remitting disease despite high-efficacy DMTs, even autologous hematopoietic stem cell transplantation (AHSCT) should be considered (Multiple Sclerosis: Current Care Guidelines, 2024).

In addition to DMTs, comprehensive treatment of MS includes acute treatment of relapses (usually corticosteroids), management of comorbidities (e.g., hypertension, hyperlipidemia and obesity), symptomatic treatment (spasticity, bladder and bowel dysfunction, sexual dysfunction, walking impairment), psychological support, rehabilitation, lifestyle counselling (e.g., smoking cessation) and mood medication (if needed) (McGinley et al., 2021).

2.1.6.2 Disease Modifying Therapies for RRMS

The primary mechanism of action for all DMTs is to reduce neuroinflammation. All currently available DMTs have been shown to decrease the relapse rate and the accumulation of new MRI lesions in clinical trials, they are capable to reduce long-term disability, but their potential to treat SPMS is at most modest. Therefore, most DMTs are approved mainly for treatment of pwRRMS. Currently used DMTs have

several treatment targets, and they modulate or suppress the immune system in different ways. Moreover, administration routes and adverse event profiles vary as well as degrees of efficacy (Amin and Hersh, 2023; Hauser and Cree, 2020; McGinley et al., 2021). The European Medicines Agency (EMA) has approved a total of 20 DMTs for the treatment of MS (until December 2025).

Injectable DMTs, interferons (IFN) and glatiramer acetate, were the first DMTs introduced for treatment of RRMS. They are immunomodulatory drugs whose exact mechanisms of action remain unclear. IFN and glatiramer acetate have been shown to reduce the annual relapse rate (ARR) by 29-34% in randomized placebo-controlled trials, are moderately tolerated, and have strong long-term safety data (Jacobs et al., 2000; Johnson et al., 1995; Kappos et al., 2015; The IFNB Multiple Sclerosis Study Group, 1993). In recent years, the use of IFNs and glatiramer acetate has decreased as newer drugs with higher efficacy and better tolerability have become available.

Oral DMTs available include teriflunomide, fumarates (dimethyl fumarate (DMF), diroximel fumarate, monomethyl fumarate), sphingosine-1-phosphate (S1P) receptor modulators (fingolimod, siponimod, ozanimod, ponesimod) and cladribine. Oral DMTs demonstrate comparable or superior efficacy in reducing relapses compared with injectable DMTs, but they are associated with increased safety concerns (Amin and Hersh, 2023; D'amico et al., n.d.; Hauser and Cree, 2020; McGinley et al., 2021). Studies have reported subtle efficacy differences between oral DMTs. In randomized clinical trials, DMF reduced ARR by 48-53% compared with placebo, and demonstrated superior efficacy compared with teriflunomide and injectable DMTs (Fox et al., 2012; Gold et al., 2012). In observational real-world patient cohorts, fingolimod has demonstrated superiority over teriflunomide and, in some studies, over DMF (Kalincik et al., 2019; Ontaneda et al., 2019; Vollmer et al., 2017). Among the oral DMTs, cladribine has demonstrated the highest efficacy reducing ARR by 55-57% in randomized placebo-controlled clinical trials (Giovannoni et al., 2010). In large real-world observational studies, cladribine has shown superior efficacy in reducing ARR compared with injectable DMTs and DMF, and similar efficacy to fingolimod (Kalincik et al., 2018; Signori et al., 2020). An especially important safety concern related to oral DMTs is a rare risk of progressive multifocal leukoencephalopathy (PML), an opportunistic brain infection caused by John Cunningham (JC) virus, which has been associated with DMF (Jordan et al., 2022) and fingolimod (Berger et al., 2018). Moreover, a small risk of malignancies is associated with fingolimod treatment (Alping et al., 2020). Additionally, withdrawal of fingolimod can result in rebound effect (severe disease activity that exceeds the disease activity prior to treatment initiation and exposes patient to severe relapses), which should be carefully considered when switching or stopping fingolimod treatment (Barry et al., 2019; Ferraro et al., 2022).

Monoclonal antibodies (natalizumab, anti-CD20 monoclonal antibodies, alemtuzumab) have superior efficacy compared with injectable and oral DMTs. Earlier, the use of these high-efficacy DMTs was restricted to patients with highly active disease and/or to patients not responding sufficiently to lower efficacy DMTs. However, improvements in safety profiles and risk mitigation strategies have increased the use of these drugs already as first treatment choice (Amin and Hersh, 2023).

Natalizumab was the first approved infusion therapy for RRMS. It is a humanized monoclonal antibody that inhibits α4β1 integrin and prevents the migration of leucocytes across BBB into the CNS (Yednock et al., 1992). It is administered as an intravenous infusion or subcutaneous injection every 4 to 6 weeks. In randomized clinical trials, natalizumab has been highly effective in reducing relapses and slowing disease progression compared to both placebo and IFNβ-1a (Polman et al., 2006; Rudick et al., 2006). Real-world observational studies have demonstrated similar results (Butzkueven et al., 2020; Hersh et al., 2021) and additionally superior efficacy in reducing ARR compared with fingolimod and cladribine (Boz et al., 2023; Kalincik et al., 2018). Moreover, the efficacy of natalizumab in preventing relapses is only marginally lower than that of AHSCT (Kalincik et al., 2023). A major concern limiting the use of natalizumab is the increased risk of PML, particularly in JCV antibody-positive patients (Ho et al., 2017; Plavina et al., 2014). Moreover, the risk of rebound disease activity is considerable after discontinuation of natalizumab and should be considered when switching or stopping treatment (Prosperini et al., 2019).

Anti-CD20 monoclonal antibodies used in the treatment of MS include ocrelizumab, rituximab, ublituximab, and ofatumumab. All these drugs target CD20 molecule (primarily expressed by B lymphocytes and, to a lesser extent, by T lymphocytes) and lead to rapid depletion of circulating B lymphocytes (Mancinelli et al., 2021). Ocrelizumab has demonstrated a 45% reduction in ARR compared with IFN-β1a in pwRRMS in randomized clinical trials (Hauser et al., 2020b, 2017) and has also shown efficacy in pwPPMS (Montalban et al., 2017). After initiation, ocrelizumab is administered as an intravenous infusion every 6 months and is usually well tolerated (Hauser et al., 2020b; Wolinsky et al., 2020). Rituximab does not have an official indication for treatment of MS and but is widely used off-label. Real-world observational studies and one phase 3 randomized controlled trial have demonstrated its high efficacy in pwRRMS. Rituximab is also administered as an intravenous infusion, usually every 6 to 12 months (Roos et al., 2023; Svenningsson et al., 2022). Ublituximab is a third intravenously administered anti-CD20 monoclonal antibody, which has shown superior efficacy compared with teriflunomide (Steinman et al., 2022). Ofatumumab is administered as a subcutaneous injection every four weeks and its efficacy has been demonstrated in

randomized clinical trials where it was shown to be superior to teriflunomide (Hauser et al., 2020a).

Alemtuzumab is a humanized anti-CD52 monoclonal antibody that induces rapid depletion of CD52+ circulating B and T lymphocytes. This is followed by slow repopulation of lymphocytes with quantitative and qualitative alterations in the lymphocyte repertoire (Ruck et al., 2015). Superior efficacy of alemtuzumab compared with IFN-β1a has been demonstrated in randomized clinical trials (Cohen et al., 2012; Coles et al., 2012; Ziemssen et al., 2020). However, long-term safety concerns, particularly secondary autoimmunity, limit its use (Guarnera et al., 2017).

Autologous hematopoietic stem cell transplantation is an off-label treatment for patients with highly aggressive disease and previous DMT failures (Paolo A. Muraro et al., 2025). AHSCT has demonstrated high efficacy in reducing clinical and MRI activity in pwRRMS (Burt et al., 2019; Mancardi et al., 2015; Nash et al., 2017), but its potential to reduce the risk of disability progression remains unclear (Burt et al., 2019; Paolo Antonio Muraro et al., 2025). Larger studies with longer follow-up time are needed to establish the efficacy, safety, and long-term impact of AHSCT.

In clinical practice, DMTs are often divided into moderate- and high-efficacy therapies according to their potential to reduce inflammatory activity. IFNs, glatiramer acetate, fumarates, and teriflunomide are considered moderate-efficacy drugs, whereas monoclonal antibodies, cladribine, and the rarely used mitoxantrone are considered high-efficacy therapies. S1P-receptor modulators are thought to have intermediate efficacy (Samjoo et al., 2021; Scolding et al., 2015). Based on this division, two common treatment strategies are used in treatment of RRMS: escalation approach (initiation of treatment with a DMT considered safe but of moderate efficacy, followed by escalation to a more potent DMT if there is evidence of disease activity) and induction approach (initiation of treatment with a high-efficacy DMT that has a less favorable safety profile) (Rjeily and Mowry, 2022). As presented earlier, results from several observational studies support the early use of high-efficacy DMTs (Brown et al., 2019; Buron et al., 2020; Harding et al., 2019; He et al., 2020; Iaffaldano et al., 2021a). Two large multicenter clinical randomized trials (DELIVER-MS [NCT03535298], TREAT-MS [NCT03500328]) comparing these two treatment strategies are ongoing and are expected to provide further information supporting treatment selections already in the near future.

2.1.6.3 Disease Modifying Therapies for Progressive MS

Treatment options for progressive MS are limited. Currently available DMTs effectively reduce peripheral immune cell activity or prevent the migration of activated immune cells from the periphery to the CNS, but they fail to target the neurodegenerative mechanisms that are crucial in progressive MS. To date

(December 2025), the EMA has approved only two DMTs for treatment of progressive forms of MS: ocrelizumab for active PPMS and siponimod for active SPMS.

The anti-CD20 monoclonal antibody ocrelizumab was approved for the treatment of PPMS in 2017 after a randomized phase 3 clinical trial demonstrated that the percentage of patients experiencing confirmed disability progression at 24 weeks was significantly lower in those treated with ocrelizumab compared with placebo (29.6% vs. 35.7%) (Montalban et al., 2017). Notably, the study included only patients younger than 55 years, and a substantial proportion of patients had evidence of inflammatory disease activity. These factors should be considered when initiating ocrelizumab.

Siponimod (an S1P modulator) was approved for the treatment of active SPMS in 2020 but is not currently available in Finland. The efficacy of siponimod was evaluated in a placebo-controlled phase 3 clinical trial in which a significant reduction in confirmed disability progression after 3 and 6 months was observed in patients treated with siponimod (risk reduction of 21% and 26% vs. placebo, respectively) (Kappos et al., 2018a). Noteworthy, a substantial (at least 20%) proportion of patients were still in RRMS phase or transitioning to progressive phase, as 21% had gadolinium-enhancing lesions on baseline MRI and 36% experienced a relapse within 2 years preceding study enrollment.

Increased understanding of the pathophysiological mechanisms involved in PMS has led to the development of new treatment approaches. In particular, therapies focusing on compartmentalized immune activation within the CNS have been an area of interest. High expectations have been addressed on Bruton tyrosine kinase (BTK) inhibitors, a new class of drugs that target microglia, macrophages, and B lymphocytes in both the peripheral and central nervous systems. BTK inhibitors are capable to cross the BBB and may therefore modulate microglial activity considered essential for disease progression (Brunner et al., 2005; Torke et al., 2020; Touil et al., 2023).

BTK inhibitors have been evaluated both in pwRRMS and pwPMS. In phase 2 clinical trials, BTK inhibitors evobrutinib, fenebrutinib, and tolebrutinib were effective in reducing acute inflammation in pwRRMS (Bar-Or et al., 2025, 2024; Montalban et al., 2019; Reich et al., 2021). However, in phase 3 studies, evobrutinib and tolebrutinib failed to demonstrate superiority over teriflunomide in reducing ARR. Additionally, serious adverse events (liver enzyme elevations) were observed among patients receiving evobrutinib (Montalban et al., 2024; Oh et al., 2025). Tolebrutinib has also been studied in non-relapsing SPMS. In the placebo-controlled phase 3 trial including 754 patients with non-relapsing SPMS, treatment with tolebrutinib was associated with a lower risk of disability progression compared with placebo (30.7% vs. 22.6%). Thus, tolebrutinib is the first drug shown to delay

disability progression in patients with non-relapsing SPMS (Robert J. Fox et al., 2025), and it is currently under review by the United States Food and Drug Administration and the EMA. Phase 3 clinical trials evaluating the efficacy and safety of other BTK inhibitors with distinct pharmacological properties in RRMS and PMS are ongoing.

Besides therapeutic agents targeting neurodegenerative pathology, neuroprotective and remyelinating agents have been of interest. Treatments aiming to promote remyelination or to prevent axonal degeneration could potentially repair damaged nerves and restore lost function. Several strategies have been investigated with initial promising early signals, but to date, no drug has shown a clinically meaningful effect on myelin repair in phase 3 trials (Amin and Hersh, 2023; Cree and Hartung, 2025). Additionally, immunomodulators, novel B cell targeting therapies, microbiome-modulating agents, and cell-directed therapies are under investigation (Cree and Hartung, 2025).

2.2 Role of Microglia in Multiple Sclerosis

2.2.1 Microglia in Healthy CNS

Microglia are myeloid cells that comprise 5% to 10% of the total cell population in the brain, depending on the region examined (Lawson et al., 1990). They are derived from the common myeloid precursor cells of the embryonic yolk sac early in ontogeny. Microglia migrate to the developing CNS and thus differ from monocyte derived macrophages (Alliot et al., 1999; Chan et al., 2007). Microglia are long-lived cells that renew slowly throughout life (Réu et al., 2017). Microglia constantly survey their microenvironment for potential signals requiring action and express numerous surface molecules and receptors that can interact with cytokines, chemokines, purines, hormones, and neurotransmitters (Arcuri et al., 2017). In the brain, microglia can interact with almost all cell types (including neurons, astrocytes, and oligodendrocytes) while maintaining brain homeostasis and participating in disease processes (Prinz et al., 2021).

Microglia have several important homeostatic functions in the healthy human brain. During brain development, microglia participate in shaping of neuronal networks (e.g., synaptic pruning and elimination), and later in life, microglia participate in regulation of synaptic density and connectivity by eliminating redundant neurons and dendritic spines. Microglia clear toxic factors such as protein aggregates and dying cells, clean cellular debris, and defend the brain against foreign intruders through phagocytosis and elimination of microbes. Microglia can also function as APCs to other immune cells and participate in tissue repair (Helmut et al., 2011; Tremblay et al., 2011).

In the context of disrupted brain homeostasis, microglia respond to injury or other potentially harmful stimuli with a complex reaction, commonly referred to as "microglial activation". Microglial activation encompasses morphological, molecular, and/or functional changes occurring in microglia from a naïve state (Arcuri et al., 2017). Reactive/activated microglia can release both pro- and anti-inflammatory mediators and hence have inflammatory and neuroprotective effects on the surrounding CNS tissue (Arcuri et al., 2017; Voet et al., 2019).

Previously activated microglia were categorized similar to macrophages in two types: pro-inflammatory and neurotoxic M1 type microglia, or anti-inflammatory and neuroprotective M2 type microglia (Arcuri et al., 2017). However, this dichotomic categorization has proven to be an oversimplification as studies have shown that microglia exhibit a spectrum of different activation states and intermediate phenotypes. For example, recent studies using single-cell RNA sequencing (scRNA-seq, a technology that allows analyzation of expression of hundreds to thousands genes concurrently in cell populations, and identification of subpopulations of cells with distinct molecular and functional properties) have revealed that microglia have multiple different activation states related to specific developmental stages (Hammond et al., 2019; Matcovitch-Natan et al., 2016), aging (Galatro et al., 2017; Hammond et al., 2019; Pettas et al., 2022), and disease processes (disease-associated microglia, DAM) (Guvenek et al., 2024; Krasemann et al., 2017).

2.2.2 Microglia and Gender

Microglia have an important role in brain masculinization during development and early postnatal life. Studies in mice have revealed sex differences in the number and morphology of microglia during the brain development and early adulthood (Lenz and McCarthy, 2015; Nissen, 2017). A higher number of microglia have been observed in the cortex, hippocampus, and amygdala of male mice during early postnatal period. In adult mice, microglia from female mice show more ramified and longer processes in similar regions (Guneykaya et al., 2018; Schwarz et al., 2012). Additionally, microglial density in the hippocampus is higher in male mice than in females (Guneykaya et al., 2018).

Cell culture studies using rodent microglia suggest several sex differences in functional properties and reactivity of microglial cells, especially during development and early life. During early postnatal period, microglia from female mice express higher levels of inflammatory cytokines such as tumor necrosis factor alfa (TNF-α), interleukin-1 beta (IL-1β), and IL-6 compared with males, whereas microglia from older mice do not show such sex differences (Crain et al., 2013). Neonatal male microglia have shown higher *in vitro* migration capacity both in basal

conditions and after pro-inflammatory stimulus, while microglia from neonatal female mice have shown greater phagocytic activity than male microglia both in basal and pro-inflammatory conditions (Yanguas-Casás et al., 2018). Later during the postnatal period, earlier increase and fall in microglial phagocytic capacity has been observed in female mice compared to males (Guneykaya et al., 2018; Weinhard et al., 2018). In adulthood, no sex difference in phagocytic activity is observed anymore, but microglia from adult male mice have shown higher antigen-presenting properties compared with female microglia (Guneykaya et al., 2018). When phagocytotic activity of aged microglia was studied in a cell culture study, a higher increase in phagocytotic activity was observed in females than in males. After pro-inflammatory stimulation, decrease in phagocytotic activity was observed in aged female microglia (Yanguas-Casás et al., 2020).

Moreover, gene studies have reported sex differences in gene expression of microglia. A RNA-seq study investigating differences in the transcriptome of adult male and female microglia isolated from healthy mice demonstrated that male microglia expressed more genes associated with inflammatory processes (such as regulation of cell migration and cytokine production), whereas genes preferentially expressed by female microglia were related to morphogenesis, development or cytoskeleton organization, inhibition of inflammatory response, and promotion of tissue repair mechanisms (Villa et al., 2018). In another scRNA-seq study, microglia isolated from female mice expressed more genes related to response to selenium, which is related to the ability of microglia to migrate and phagocyte (Barko et al., 2022). Proteomics analyses have also shown differences in proteins expressed by male and female microglia from mice. Proteins highly expressed by male microglia seem to involve cell motility and trafficking as well as proteins related to Toll-like receptor pathways, suggesting more pronounced responsiveness to immunological stimuli. In contrast, IFN regulatory factor 3, involved in regulation of IFN mediated inflammatory responses, was overexpressed by female microglia (Guneykaya et al., 2018). In line with this finding, female microglia have shown to be more responsive to IFN (Thion et al., 2018).

Microglia express receptors for sex hormones, which can influence microglial responses in several ways. Microglia from adult mouse brain express one or both estrogen receptor (ER) subtypes (ERα, ERβ) (Baker et al., 2004; Sierra et al., 2008), and several studies have suggested an anti-inflammatory effect of estrogen on microglia (Baker et al., 2004; Bruce-Keller et al., 2000; Ghisletti et al., 2005; Vegeto et al., 2003; Villa et al., 2016). In the experimental autoimmune encephalomyelitis (EAE), the most used animal model of brain inflammation and MS, estrogen has been shown to alleviate EAE severity in both males and females (Spence and Voskuhl, 2012). Estrogen treatment of rat microglial cells reduces lipopolysaccharide (LPS) -induced production of pro-inflammatory molecules such

as inducible nitric oxide, reactive oxygen species, and prostaglandin E2 (Vegeto et al., 2001). Moreover, changes in morphology and accumulation of mRNA encoding inflammatory mediators have been observed in ovariectomized mice (Benedusi et al., 2012). Microglia also express progesterone receptors. In a rodent study using cuprizone-induced demyelination mouse model, progesterone therapy suppressed inflammation and demyelination (Aryanpour et al., 2017). Additionally, testosterone administration has been shown to have anti-inflammatory effects following brain injury or LPS-induced neuroinflammation (Barreto et al., 2007; Yang et al., 2020).

In conclusion, animal and cell culture studies have reported notable sex differences in the number, morphology, and functional properties of microglia throughout life. Overall, studies suggest increased reactiveness of female microglia to inflammatory stimuli compared to males, which might be related to the predisposition of females for autoimmune diseases (Gleicher and Barad, 2007). Furthermore, sex differences in the state of inflammation during normal CNS development could possibly contribute, at least partially, to differences in the incidence and disease outcomes of several neurodevelopmental and neurodegenerative diseases, including MS (Hanamsagar and Bilbo, 2016).

2.2.3 Microglia in MS

Microglia have an important role in the pathogenesis of MS and are crucial in the maintenance of CNS compartmentalized chronic inflammation (O'Loughlin et al., 2018). In neuropathological studies, activated microglia are mainly observed at the rims of chronic active lesions, which are at least 1 mm wide (Frischer et al., 2015; Klotz et al., 2025; Kuhlmann et al., 2017; Luchetti et al., 2018), in the WM surrounding chronic active lesions (Hendrickx et al., 2017; Kessler et al., 2023), and diffusively in the NAWM at all disease stages (Allen et al., 2001; Kutzelnigg et al., 2005). Active demyelination around chronic active lesions associates with activated microglia (Lassmann et al., 2007), and the extent of axonal injury in acute WM lesions correlates with the number of activated microglia and T lymphocytes (Bitsch et al., 2000; Kessler et al., 2023; Trapp et al., 1998). Additionally, clusters of microglia have been observed in NAWM preceding lesion formation (Singh et al., 2013). Studies have also demonstrated that GM demyelination and neurodegeneration associate with microglial activation (Bø et al., 2003; Magliozzi et al., 2010), and increased densities of activated microglia have been demonstrated in NAGM, choroid plexus, and the meninges (Herranz et al., 2024; Kutzelnigg and Lassmann, 2014; Magliozzi et al., 2025; Okutan et al., 2025).

Microglia can induce tissue injury in MS by several mechanisms. Production of reactive oxygen species, proteases, cytokines, and chemokines appears to be particularly important in the induction of demyelination, axonal injury, and

neurodegeneration (Friese et al., 2014; Haider et al., 2011; Lassmann et al., 2007; van Horssen et al., 2012; Yong, 2022). Cytotoxic compounds released by microglia include e.g., semaphorin 4A, TNF-α, IL-1β, glutamate, and matric metalloproteinases (Kauppinen and Swanson, 2005; Yong, 2022). Microglia participate in the control of iron homeostasis in the brain. Previous studies have demonstrated that inflammation alters iron metabolism (Urrutia et al., 2013), and iron released from damaged oligodendrocytes and neurons accumulates in the extracellular space (Hametner et al., 2013), where it may be phagocyted by microglia. This induces microglial activation and/or degeneration leading to additional release of iron and amplification of the oxidative damage related to iron deposition (Hametner et al., 2013; Kenkhuis et al., 2022). Interactions between microglia and other cell types may also promote destructive effects. Interaction between microglia and infiltrating T cells may exacerbate inflammation (Absinta et al., 2021; Dong and Yong, 2019). Activated microglia can also activate astrocytes, which amplify neurodegenerative tissue injury through the release of pro-inflammatory mediators (Absinta et al., 2021; Liddelow et al., 2017). One more suggested neurotoxic mechanism associated with microglia in MS is elimination of synapses in MS lesions via complement C3 mediated pathway (Werneburg et al., 2020).

In recent years scRNA-studies examining transcriptional profiles of human microglia isolated from MS brain have emerged. Total tissue transcriptomic analysis of microglia from NAWM has demonstrated increased expression of genes associated with inflammation and cellular stress (Miedema et al., 2022). Also, differences have been observed in gene expression profile of microglia from GM and WM. Increased expression of transcripts associated with immune activation and iron homeostasis has been reported in WM microglia, whereas GM microglia exhibit higher expression of type I IFN related genes and increased transcription of genes associated with the canonical pathway, the role of which role in MS remains to date unclear (Chomyk et al., 2024; van der Poel et al., 2019). RNA sequencing of microglia from nodules in NAWM has demonstrated increased expression of genes previously found to be upregulated in MS lesions, as well as upregulation of genes related to immunoglobulin and cytokine signaling, complement cascade activation, lipid metabolism, and metabolic stress (van den Bosch et al., 2024). Subpopulations of microglia exhibiting an activated/phagocytic transcriptional signature have been identified in demyelinating WM lesions (Miedema et al., 2022), and distinct gene signatures have been observed across different lesion types and at the rims of active WM lesions (Alsema et al., 2024). Finally, transcriptional profiles described in MS are partly similar to those observed in other neurodegenerative diseases, suggesting shared biological mechanisms underlying neurodegeneration (Absinta et al., 2021).

Microglia may also have beneficial functions in MS. It has been suggested that the initial microglial response might be protective, whereas the prolonged microglial activation drives the neuropathological processes (Yong, 2022). In animal models and tissue culture studies, microglia are observed to secrete neurotrophic factors promoting repair of tissue damage (Lloyd and Miron, 2019; Sherafat et al., 2021). After demyelination, microglia phagocyte myelin debris, which may otherwise inhibit remyelination (Kotter et al., 2006), and microglia may also promote remyelination by phagocytosing damaged cells and secreting regenerative factors (Cignarella et al., 2020; Voet et al., 2019). Microglia can also destroy harmful T cells (Wasser et al., 2020) and may additionally secrete cytokines, chemokines, and factors that promote recovery and regeneration (Lloyd and Miron, 2019; Yong, 2022). The precise mechanisms by which microglia lose their homeostatic and protective functions and adopt a disease promoting (DAM phenotype) in MS remain unknown. Possible candidates driving this change include iron accumulation in the brain, aging, persistence of immune complexes, meningeal inflammation, and fibrin derived from blood fibrinogen (Kamma et al., 2022; O'Loughlin et al., 2018; Yong, 2022).

2.3 Magnetic Resonance Imaging

2.3.1 Conventional MRI

MRI is a non-invasive imaging method that produces detailed anatomical images. The MRI scanner consists of a powerful magnet, which produces a strong static magnetic field, gradient coils enabling spatial encoding by generating varying magnetic fields, and radiofrequency coils, which receive and send signals. The magnetic field strength of MRI scanner is measured in tesla (T). Most MRI scanners in clinical practice have a field strength of 1.5 T or 3 T whereas ultra-high field 7 T (or stronger) MRI scanners are used predominantly in research purposes (Grover et al., 2015; Minhas and Oliver, 2022).

The physical phenomenon behind signal generation in MRI is nuclear magnetic resonance. All atomic nuclei consist of protons and neutrons and odd nuclei have a positive net charge. Certain atomic nuclei, such as hydrogen (^{1}H), abundant in human body in water and fat, have an angular momentum called spin. Spinning nuclei create a local magnetic field known as magnetic moment. In the absence of external magnetic field, the spins are randomly oriented with no net magnetization. An external magnetic field aligns spinning nuclei parallel or anti-parallel with the external field leading to non-zero net magnetization. A radiofrequency magnetic field, typically applied in short pulses with particular frequency and perpendicular to the strong external magnetic field, excites the nuclei (nuclei move from a low-

energy stage to a high-energy excited stage). When the radiofrequency pulse is discontinued, the excited nuclei return to their normal state and release the energy received from radiofrequency pulse (a process known as relaxation). This signal is detected by receiver coils, and the signal is then processed mathematically to reconstruct the MR image (Minhas and Oliver, 2022).

Different MRI sequences are used when imaging pwMS. T1-weighted sequences provide good discrimination between fat and water, as fat appears bright (hyperintense) and fluid dark (hypointense). These sequences offer high structural resolution and clear differentiation between WM and GM. Therefore, T1-weighted sequences are commonly used to assess brain volume and tissue damage (atrophy and so-called “black holes”). Additionally, T1-weighted sequences allow detection of active lesions following an injection of a contrast agent, which are most commonly gadolinium-based. T2-weighted sequences are sensitive for detecting WM abnormalities in MS. On T2-weigted sequences, fat appears intermediate hyperintense and fluid hyperintense. Fluid-attenuated inversion recovery (FLAIR) sequences are based on T2-weighted imaging, but the signal of CSF is suppressed (black). This allows improved detection of WM abnormalities, particularly in the periventricular regions (Bakshi et al., 2001; Mitchell, 1999).

2.3.2 Susceptibility-weighted Imaging

Susceptibility-weighted imaging (SWI) is an advanced MR imaging method based on the magnetic susceptibility of tissue. Magnetic susceptibility refers to the degree to which the tissue/material becomes magnetized when exposed to an external magnetic field. Magnetic susceptibility varies between different biological tissues and differences in magnetic susceptibility form the physical basis of SWI. Substances with paramagnetic, diamagnetic, or ferromagnetic properties (such as iron or deoxidated blood) locally distort the magnetic field and induce phase shifts in the MR signal. These susceptibility-related phase differences result in signal changes that can be detected using T2-weighted gradient-echo (GRE) sequences. The raw phase data is processed using techniques such as high-pass filtering and local phase correction algorithms to create a filtered phase image. The filtered phase image is then processed to create a phase mask, which is combined with the corresponding magnitude image (signal intensity data acquired simultaneously with the phase data) to produce the final SW image (Haacke et al., 2004). Quantitative susceptibility mapping (QSM) and susceptibility tensor imaging (STI) allow additionally quantification of magnetic susceptibility (Li et al., 2017; Reichenbach et al., 2015).

2.3.3 Imaging Microglial Activation with MRI

2.3.3.1 Paramagnetic Rim Lesions

A subset of chronic active lesions has a paramagnetic rim, which can be visualized with different susceptibility-based MRI sequences detecting iron (Absinta et al., 2018; Bagnato et al., 2024). Combined MRI-neuropathological studies have repeatedly demonstrated that magnetic susceptibility contrast at the lesion rim reflects iron accumulation within activated microglia/macrophages at the lesion rim and, to a lesser degree, active demyelination (Absinta et al., 2021, 2019; Bagnato et al., 2011; Dal-Bianco et al., 2017; Gillen et al., 2021). Thus, PRLs show convincing histopathological correlation to chronic active lesions.

PRLs are highly specific to MS (Maggi et al., 2020; Martire et al., 2022) and predict development of MS in pwCIS (Renner et al., 2025). Therefore, PRLs were recently incorporated into the 2024 McDonald diagnostic criteria as presented earlier (see also Chapter 2.1.5) (Montalban et al., 2025). PRLs develop already during early disease stages and can persist for up to 7-10 years (Altokhis et al., 2022; Assunta Dal-Bianco et al., 2021; Dal-Bianco et al., 2017). A pooled data analysis including 31 MRI studies published between 2008 and 2022 estimated that the prevalence of PRLs is approximately 50% in pwCIS and pwRRMS, and approximately 60% in pwSPMS/pwPPMS (Martire et al., 2022). In a meta-analysis including 58 MRI studies published up to February 2024, the pooled prevalence of PRLs was 0.52 at the patient level and 0.12 at the lesion level, with no significant differences between MS phenotypes. Notably, substantial heterogeneity was observed across studies in the reported prevalence of PRLs, and the incidence of new PRLs over time was low (0.11 over a mean follow-up period of 4.7 years) (Tranfa et al., 2025). PRLs have also been detected in pwRIS (Suthiphosuwan et al., 2020).

Results from numerous studies suggest that PRLs are a potential prognostic biomarker in MS. The association of PRLs with a more aggressive disease course was demonstrated for the first time by Absinta et. al in 2019: in a cohort of 192 pwMS (CIS, RRMS or PMS), pwMS with four or more PRLs reached motor and cognitive disability at a younger age (Absinta et al., 2019). Since then, several cross-sectional and prospective studies have demonstrated association of PRLs with higher and worsening physical disability (Altokhis et al., 2022; Marcille et al., 2022; Preziosa et al., 2024; Reeves et al., 2024b; Treaba et al., 2021), and with cognitive impairment (Preziosa et al., 2024). Recently, an association of PRLs with PIRA was observed in a cohort of 445 pwMS over a 4-year follow-up both in subgroups of RRMS and PMS (Cagol et al., 2024a). In addition, a disappearing iron rim (interpreted as a lesion transitioning to an inactive state) was recently reported to

associate with a reduced risk of clinical disability progression and PIRA (Reeves et al., 2024a). Several studies have also reported an association of PRLs with worse radiological outcomes. PRLs associate with higher T1 and T2 lesion loads (Cagol et al., 2024a; A. Dal-Bianco et al., 2021; Reeves et al., 2024b; Weber et al., 2022), different brain atrophy measurements (A. Dal-Bianco et al., 2021; Treaba et al., 2021; Weber et al., 2022), and spinal cord atrophy (Weber et al., 2022). Additionally, advanced imaging techniques have demonstrated more destructive tissue pathology related to PRLs compared with lesions without iron rim (Absinta et al., 2019; Assunta Dal-Bianco et al., 2021; Klistorner et al., 2022; Krajnc et al., 2023). Finally, PRLs have been associated with more frequent relapses (Reeves et al., 2024b) and sNfL levels (A. Dal-Bianco et al., 2021; Maggi et al., 2021), but not with sGFAP levels (Sjöros et al., 2025).

Studies evaluating the impact of currently available DMTs on PRLs are few and have shown only modest effects. A study assessing PRL changes over a 2-year follow up in 46 pwMS treated with anti-CD20 antibodies and 26 untreated pwMS did not observe resolution of PRLs nor differences in PRL lesion volume or magnetic susceptibility between the two groups (Maggi et al., 2023). In a cohort of 76 Chinese pwMS, treatment with teriflunomide for 2 years did not reduce the number of PRLs (Tan et al., 2024). In another recent study, no resolution of PRLs was observed in patients treated with teriflunomide nor in patients treated with ocrelizumab after a follow-up of 1.5-2 years (Cagol et al., 2025). Positive effects of DMTs on PRLs have also been reported. In a study following 152 pwMS for an average of 8.2 years, DMTs reduced the odds of developing a new PRL. However, DMTs did not associate with disappearance of PRLs nor reduction of the size of PRLs (Reeves et al., 2024a). Additionally, DMTs may have positive influence on tissue damage related to PRLs. A study comparing patients initiating DMTs (DMF, fingolimod, or ocrelizumab) with patients staying without treatment (based on their own choice) demonstrated less pronounced tissue damage (measured as T1/T2 ratio) around PRLs in patients initiating DMTs after a 2-year follow-up (Eisele et al., 2022). In another study, greater reduction in the susceptibility of iron rims was observed in patients treated with DMF compared with those treated with glatiramer acetate (Zinger et al., 2022). More information on the impact of DMTs on PRLs will emerge in the near future as PRLs are increasingly integrated as an outcome measure into clinal phase 2 and 3 trials (Gaitán et al., 2024). Figure 1 demonstrates a PRL in a QSM MR image.

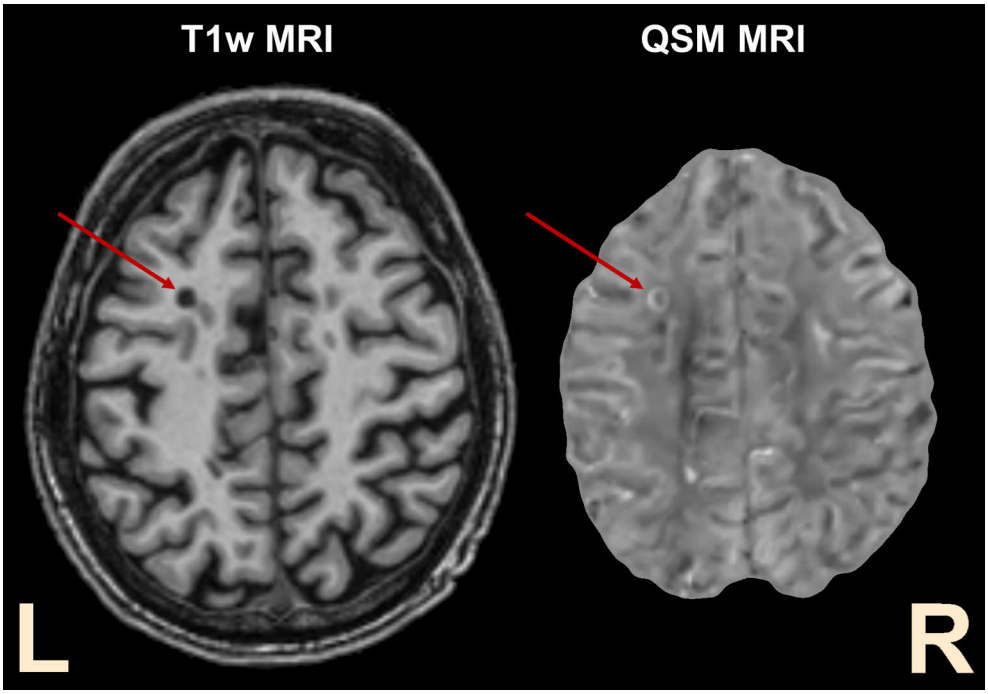

Figure 1. Visual demonstration of the axial T1 MR image (left) and respective QSM MR image (right). Red arrow points to a chronic T1 lesion showing bright paramagnetic rim in QSM MRI. Abbreviations: MRI = magnetic resonance imaging; QSM = quantitative susceptibility mapping.

2.3.3.2 Slowly Expanding Lesions

A subset of chronic WM lesions shows slow expansion over time and is referred to slowly expanding lesions (SEL). SELs can be detected from conventional T1-weighted and T2-FLAIR MR images by applying Jacobian determinant algorithms to at least three imaging time-points, ideally over a period of 1 to 2 years. SELs are considered an alternative MRI biomarker of chronic active lesions, even though no combined MRI-neuropathological studies demonstrating histopathological correlation between SELs and chronic active lesions exist (Bagnato et al., 2024; Elliott et al., 2019b).

SELs have been observed in all MS subtypes (Beynon et al., 2022; Calvi et al., 2024, 2022b; Elliott et al., 2019b). Studies have reported that SELs are present in 86% of pwRRMS (Calvi et al., 2022b) and in 99% of pwPMS (Calvi et al., 2022a). SELs are associated with clinical disability in both pwRRMS and pwPMS and predict disease worsening (Calvi et al., 2024, 2022b, 2022a; Elliott et al., 2019a; Preziosa et al., 2022). In a cohort of 52 pwRRMS, presence of four or more SELs and a higher proportion of lesions defined as SELs at baseline predicted EDSS score worsening after 9 years (Preziosa et al., 2022). Additionally, SELs are associated

with global and regional brain volume loss (Calvi et al., 2022b, 2022a; Yokote et al., 2024). Several studies have also reported more destructive tissue pathology and microstructural tissue abnormalities related to SELs (Calvi et al., 2022b, 2022a; Elliott et al., 2019b, 2019a; Vavasour et al., 2025). Longitudinal reduction in T1-weighted signal intensity (reflecting progressive tissue destruction) has been observed in SELs (Elliott et al., 2020) and is shown to predict later disability progression (Elliott et al., 2019a).

Some studies have assessed the effect of DMTs on SELs and have shown moderate positive effects (Arnold et al., 2024; Beynon et al., 2022; Calvi et al., 2022a; Elliott et al., 2019a; Preziosa et al., 2021). In a cohort of pwRRMS initiating fingolimod or natalizumab, only a limited effect of DMTs on occurrence of SELs was observed. However, a decrease in T1 signal intensity in SELs, suggesting positive effects on tissue pathology, was observed in both treatment groups (Preziosa et al., 2021). A post hoc analysis which evaluated the effect of BTK inhibitor evobrutinib on SEL volume demonstrated a reduction in SEL volume among pwRRMS receiving evobrutinib (Arnold et al., 2024). Moreover, a retrospective analysis of fingolimod trial in pwPPMS demonstrated reduction in the volume and number of SELs in patients treated with fingolimod (Calvi et al., 2024). Similar effects have been demonstrated in pwSPMS treated with natalizumab vs. placebo (Beynon et al., 2022). Like PRLs, SELs are increasingly incorporated as outcome measures in clinical phase 2 and 3 trials, and additional data on the effects of DMTs on SELs is expected to emerge in the coming years (Gaitán et al., 2024).

2.4 Positron Emission Tomography

2.4.1 Principles of PET Physics

Positron emission tomography is a molecular imaging method that utilizes radiolabeled compounds, referred to as radioligands. Radioligands bind to specific molecular, cellular, or metabolic targets, thereby enabling *in vivo* visualization and quantification of different biochemical and physiological processes in the target tissue. Following administration of radioligand to the patient, the radioactivity emitted by radioligands is detected by the PET scanner. The most commonly used radiolabels in PET imaging are positron-emitting radioisotopes such as carbon-11 (^{11}C), oxygen-15 (^{15}O) and fluorine-18 (^{18}F). Radioisotopes are produced in a cyclotron and then chemically incorporated into biologically active molecules that act as ligands for the process of interest. Radioligands are often produced on-site due to the short half-lives of many radioisotopes. For example, the half-life of ^{11}C – the most widely used radioisotope in MS studies – is only 20.3 minutes (Lameka and Farwell, 2016; Turkington, 2001).

The radioligand is usually injected intravenously and then delivered to the target region via blood flow. Radioisotopes undergo beta plus (β^+) decay (also known as positron emission decay), where a proton (p) is converted into a neutron (n) with the simultaneous emission of a positron (e^+) and an electron neutrino (ν_e).

$$p \rightarrow n + e^+ + \nu_e$$

The emitted neutrino is chargeless, very light, and interacts only very weakly with matter. The emitted positron is the positively charged antiparticle of the electron and usually travels approximately 1mm in matter, losing energy before colliding with an electron (e^-) in an event referred to as annihilation. Annihilation results in the production of a pair of two photons (γ), each with an energy of 511 keV, which are emitted simultaneously in almost opposite directions.

$$e^+ + e^- \rightarrow \gamma + \gamma$$

(Turkington, 2001)

2.4.2 The PET Scanner and Acquisition of Image Data

After annihilation, the two emitted photons (or gamma rays) travel to opposite directions at a speed of light. They hit the two opposing gamma-counter coincidence detectors, which are arranged in rings inside the PET scanner, approximately simultaneously (Figure 2).

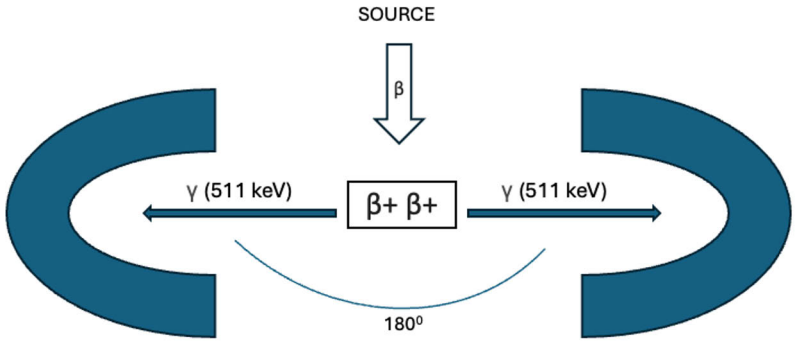

Figure 2. Illustration of positron emission in a PET scanner. After being emitted, positron (β^+) collides with an electron in an annihilation event. This results in production of two antiparallel photons (γ) which strike opposing detectors. Modified from Lameka and Farwell, 2016.

The path between the two detectors is called the line of response (LOR). Even though the exact location of annihilation is not known, it is possible to localize the annihilation to some point of LOR as the photons emit in almost opposite directions. The simultaneous detection of two photons is referred to as coincidence event or as a true count. The number of coincidence events occurring between the detectors indicates the amount of radioactivity on the LOR between detectors. Multiple detected coincidence events along all LORs allow spatial distribution of the radioligand to be reconstructed in a 3-dimensional (3D) image. Nevertheless, not all annihilation events lead to a detectable coincidence event. Detection of a single annihilated photon along LOR without a corresponding event in the opposing detector is referred to as a false count. A small proportion of annihilated photons originating from the imaged target are scattered and subsequently detected. Then the LOR indicates an incorrect location of the event, and this is referred to as a false LOR. The majority of single events (approximately 97%) can be eliminated using the window correction procedure (Lameka and Farwell, 2016; Zanzonico, 2004). Thereafter, the remaining data consisting of registered true counts is organized within a 2D coordinate system. Each LOR is identified and located based on its distance and angle and plotted as a discrete point on a graph. The plotted curve of the LORs is called a sinogram as it has a sinusoidal shape. Several physical factors degrade the quality of PET images, some of which can be corrected. Therefore, the sinogram data is further reconstructed with, for example, attenuation correction, correction for isotope decay, and filtering (smoothing). The final quantitative PET image represents the radioactivity of the used radioligand within the scanned object as a function of time (time-activity curve, TAC). Finally, the reconstructed PET images are overlaid with the anatomically corresponding MR images to relate the radioligand kinetics to anatomical structures (Lameka and Farwell, 2016; Turkington, 2001; Zanzonico, 2004).

Spatial resolution of PET images is relatively poor (for example, approximately 2.5 mm for the High-Resolution Research Tomograph (HRRT) PET scanner used in this thesis) due to PET scanner-related factors and physical factors, and this affects the quantification in PET (Zanzonico, 2004). The consequence of limited spatial resolution is, that a single voxel can represent a combination of different structures with different radioligand distribution, referred to as partial volume effect (PVE). Because of PVE, the intensity of a particular voxel reflects not only the radioligand concentration within the voxel, but also the radioligand concentration from the surrounding area. Thus, PVE can lead to substantial over- or underestimation of the radioactivity concentration. PVE becomes particularly important when the investigated target is less than two times the spatial resolution of the PET scanner (Erlandsson, Buvat, Pretorius, Thomas, & Hutton, 2012). Different methods for PVE correction have been developed to improve the quantitative accuracy of the PET images (Erlandsson et al., 2012; Thomas et al., 2016).

2.4.3 Modelling and Quantification of the Detected Radioligand Binding

Mathematical models describing radioligand kinetics are required to translate the PET-measured radioligand concentrations into quantitative estimates of the underlying biological processes. The modelling methods are applied in the dynamic PET data by fitting the model equation to the measured radioactivity over time in an individual voxel or regions of interest (ROI).

A simple way to quantify the uptake of the radioligand in the target tissue is the standardized uptake value (SUV). SUV is calculated by dividing the radioligand concentration by the injected dose normalized to body mass. It does not require dynamic imaging or blood sampling and is therefore easy to use. However, SUV does not allow pharmacokinetic modelling. Another semiquantitative approach to quantify the uptake of the radioligand is the tissue-to-reference tissue ratio method. This method allows calculation of binding potential (BP), which refers to the ratio of specifically bound radioligand in tissue compared with concentration of free radioligand in tissue at equilibrium (Bertoldo et al., 2014; Innis et al., 2007; Lammertsma, 2002).

More complex quantitative compartmental kinetic models with greater specificity are generally used in neurotransmitter PET studies, which evaluate specific receptor binding (Bertoldo et al., 2014; Lammertsma, 2002). The Logan plot is one of the most commonly used kinetic modelling methods for reversible binding radioligands, as it is easy to use, fast, and relatively simple to implement. The Logan plot enables estimation of the total distribution volume (V_T) and the distribution volume ratio (DVR), the latter used as the outcome measure in all the studies included in this thesis. V_T is the ratio of the radioligand concentration in the target tissue to that in plasma at equilibrium. When a reference region is used as the input (see the next paragraph), DVR can be calculated as the ratio of V_T in the receptor-containing target tissue region to V_T in a reference region assumed have negligible number of target receptors (Logan, 2000; Logan et al., 1996, 1990).

The tissue signal of radioligand depends on the delivery of the radioligand to the target issue (referred to as input function), which should be therefore considered in the modelling. Arterial cannulation for arterial plasma sampling is the gold standard for measurement of the input function. To avoid arterial sampling, other approaches such as reference tissue methods have been developed. Reference tissue can be used both in compartmental models and tissue-to-plasma ratio analysis. An optimal reference tissue is a region that is free of specific binding of the radioligand, has nonspecific binding similar to the target tissue, and is not affected by the disease process or treatment (Lammertsma, 2002). In neurodegenerative and neuroinflammatory diseases including MS, the disease

process virtually involves the whole brain making it challenging to find a reliably disease-free region. Therefore, supervised clustering methods that enable extraction of a pseudo-reference region are commonly applied. In a pseudo-reference region, the expression of the radioligand target is assumed not to change under pathological conditions. Supervised cluster analysis (SVCA) algorithms (such as SuperPK software used in the studies included to this thesis) are widely used in TSPO-PET studies. The SVCA algorithm segments PET voxels based on differences in their TACs, using four predefined kinetic classes representing radioligand binding in GM, WM, vasculature, and regions with specific radioligand binding. Reference tissue voxels are primarily selected from GM regions assumed to be free of specific binding. The reference region input function is calculated as a weighted average of all brain TACs, where the estimated GM coefficients from the linear combination are used as weighting factors (Turkheimer et al., 2007; Yaqub et al., 2012).

As presented above, the PET data acquisition, modelling, and quantification is a complex process, and several approaches are available for modelling and quantification of radioligand binding. Regardless of the selected method, PET analyses should produce quantitative values that describe specific radioligand binding within prespecified ROIs as reliably and accurately as possible.

2.4.4 Limitations of PET Imaging

PET scans are relatively expensive, and the availability of PET imaging is limited. Exposure to ionizing radiation limits the number of participants in PET studies and serial PET scans. Several technical challenges also need to be considered. Movement of the patient during image acquisition may produce motion artifacts that can degrade the image resolution. However, these artifacts can be mitigated using, for example, movement detection and correction techniques. Relatively poor spatial resolution hampers the assessment of small lesions, and the low signal-to-noise ratio affects the quality of imaging data. The specificity of the radioligand may be limited, and the ligand target may locate in several different cell types, which can complicate the interpretation of results. Furthermore, PET modelling and quantification processes are complex processes that require careful validation before clinical application. In addition, heterogenous methodologies are used across imaging centers, and harmonization of methodology is necessary to enable multicenter studies with larger patient cohorts. Despite these limitations, neuroinflammation can be quantified accurately with TSPO-PET when well-validated post-processing and image analysis methods are applied (Bodini et al., 2021; Kreisl et al., 2020; Rogasch et al., 2022).

2.4.5 Variability in the TSPO Uptake

Test-retest reliability describes the consistency of a measurement to produce similar results in the same individual when administered at different time points, and good test-retest reliability is essential in research and clinical practice. Only few studies with low number of participants have evaluated the test-retest reliability of [^{11}C]-PK11195 TSPO-PET in healthy individuals. These studies have demonstrated poor to moderate test-retest reliability with high variability in [^{11}C]-PK11195 binding (GM from 5% to 27%, WM 44%, whole brain 4%, and thalamus from 8% to 22%) (Jučaite et al., 2012; Kaunzner et al., 2017; Plavén-Sigray et al., 2018). To date, no test-retest [^{11}C]-PK11195 TSPO-PET studies in pwMS have been published. In patients with Alzheimer´s disease, good test-retest reliability, and approximately only 11% variability in [^{11}C]-PK11195 binding in GM has been reported (Turkheimer et al., 2007a). Additionally, one test-retest study using second-generation TSPO-radioligand [^{11}C]-PBR28 in pwMS has been published. In that study, good test-retest reliability with variability in [^{11}C]-PBR28 binding ranging from 7% to 9% across GM, NAWM and MS lesions was reported in four stable pwRRMS (Park et al., 2015). Of note, a higher variability (18%) in [^{11}C]-PBR28 binding in GM has been reported in healthy individuals (Collste et al., 2016).

In terms to second-generation TSPO radioligands, the TSPO genotype (rs6971 polymorphism) strongly affects the TSPO binding affinity. Individuals must be genotyped prior to TSPO-PET imaging to determine their binding affinity (high-, low-, or mixed-affinity binding phenotype), and low-affinity binders are typically excluded from studies (Owen et al., 2012). Hence, third-generation TSPO radioligands with specific binding comparable to that of second-generation radioligands, but with reduced sensitivity to the rs6971 polymorphism, are currently under investigation (Salerno et al., 2024). Other factors that may additionally influence TSPO binding include BMI, age, and sex (Ekblad et al., 2023; Kumar et al., 2012; Tong et al., 2019; Tuisku et al., 2019).

2.4.6 Imaging Microglial Activation with PET Imaging

2.4.6.1 Translocator Protein (TSPO) Expression as a Marker of Microglial Activation

Microglial activation can be assessed *in vivo* using PET imaging and radioligands binding to TSPO (Banati et al., 2000; Bodini et al., 2021). TSPO is an 18 kDa protein mainly located on the outer mitochondrial cell membrane (Papadopoulos, 1998). TSPO is expressed in different tissues and is proposed to participate in a wide range of cellular functions such as regulation of cellular proliferation, apoptosis,

cholesterol transport, steroid hormone synthesis, regulation of mitochondrial homeostasis, and heme synthesis (Y. Lee et al., 2020; Nutma et al., 2021a).

In the CNS, TSPO is upregulated by activated microglia after pro-inflammatory stimulus and it has therefore been widely used as a marker for neuroinflammation (Banati et al., 2000; Colasanti et al., 2014; Venneti et al., 2006). Of note, TSPO expression is not specific to microglia as also blood-derived macrophages, astrocytes, some neurons, and endothelial cells express TSPO but to a lesser extent (Guilarte et al., 2022; Lavisse et al., 2012; Nutma et al., 2021b, 2019; Wijesinghe et al., 2025). When cellular sources of TSPO expression were studied in postmortem MS brain samples, 40% of all TSPO-expressing cells were microglia/macrophages, 25% astrocytes and less than 5% vascular endothelial cells. Microglia/macrophages in active lesions and at the rims of chronic active lesions expressed TSPO. Additionally, approximately 25% of TSPO expression in active lesions and chronic active lesions was derived from astrocytes, which were also the main cell population expressing TSPO in the hypocellular centers of chronic active lesions and in inactive lesions (Nutma et al., 2019). Another neuropathological study by the same group, which used a wide range of microglia/macrophage markers, demonstrated that over 95% of TSPO-expressing cells in NAWM, active MS lesions and at the rims of chronic active lesions in postmortem MS brain samples were microglia/macrophages, suggesting that TSPO signal in these brain areas arises mainly from microglia/macrophages (Nutma et al., 2021b).

The presumption that TSPO expression increases upon microglial activation has been questioned in recent years, and the biological specificity of TSPO as a marker of microglial activation remains unclear. Previously, differentiation between blood-derived monocytes/macrophages and microglia has been challenging, as both cells share similar morphological features and express similar mononuclear phagocyte markers. Novel microglia-specific markers, such as transmembrane protein (TMEM) 119, allow improved identification and discrimination of these cells (Bennett et al., 2016; Böttcher et al., 2020; Jäckle et al., 2020; Zrzavy et al., 2017). In contrast to the findings from rodent studies, no increase in TSPO expression was observed following pro-inflammatory stimulus in cell cultures consisting of human microglia and macrophages (Owen et al., 2017). Moreover, studies using postmortem MS brain samples have demonstrated that TSPO expression is not restricted to pro-inflammatory or anti-inflammatory microglial phenotypes (Nutma et al., 2019). Although increased expression of microglial activation markers and loss of homeostatic markers was observed in microglia from active lesions and the rims of chronic active lesions (Nutma et al., 2021b), pixel analysis of TSPO expression per cell revealed no differences in TSPO expression between WM regions, active lesions, and the rims of chronic active lesions. (Nutma et al., 2021b). Similar findings according to TSPO expression per cell were reported in a recent study using brain

samples from patients with progressive supranuclear palsy (Wijesinghe et al., 2025). These findings suggest that TSPO expression at the protein level does not change despite alterations in microglial marker expression. It has therefore been suggested that increased TSPO levels reflect higher microglial density rather than an activated microglial phenotype or microglial reactivity (Nutma et al., 2021b; Owen et al., 2017; Wijesinghe et al., 2025).

Nevertheless, after the publication of studies by Nutma et al. a few years ago, a re-analysis of three previously published snRNA-seq studies (Absinta et al., 2021; Jäkel et al., 2019; Schirmer et al., 2019) demonstrated that TSPO is predominantly expressed by microglia and macrophages with an activated phenotype at the rims of chronic active lesions (Hamzaoui et al., 2023). In addition, a recent immunohistochemical analysis of postmortem MS brain meninges and cortical GM lesions showed that most TSPO-positive cells in these regions express MHC class II, a marker of activated microglia/macrophages. In cortical GM lesions, TSPO-positive cells expressed also TMEM119, a marker of activated resident microglia (Herranz et al., 2024). In conclusion, these observations suggest that the TSPO signal detected with PET imaging probably reflects both a phenotypic shift of microglia toward a pro-inflammatory state and an increased number of microglia. Regardless of the exact source of TSPO signal in PET imaging, studies in the context of MS have repeatedly demonstrated that increased TSPO binding in the MS brain is associated with important adverse clinical and MRI outcomes, treatment response, and disease progression, as reviewed in the next chapter (Chapter 2.4.6.2) (Banati et al., 2000; Debruyne et al., 2003; Giannetti et al., 2014; Herranz et al., 2016; Pitombeira et al., 2022; Rissanen et al., 2018, 2014; Sucksdorff et al., 2019).

2.4.6.2 PET Imaging Studies Detecting Microglial Activation in MS

Radioligands binding to the TSPO molecule have been widely used in MS research to evaluate microglial activation and neuroinflammation. The first PET imaging studies in pwMS were performed using the first-generation TSPO radioligand [^{11}C]-PK11195, which was synthesized in 1983 (Benavides et al., 1983). [^{11}C]-PK11195 is also the radioligand used in all the studies included in this thesis. There are several limitations related to [^{11}C]-PK11195, such as low brain permeability and a high non-specific binding, which result in a low signal-to-noise ratio that affects the quality of images. Therefore, novel TSPO radioligands with lower signal-to-noise ratio and higher affinity to TSPO have been developed. Examples of these second- and third-generation TSPO radioligands include [^{11}C]-PBR28, [^{18}F]-PBR111, [^{18}F]-PBR06, [^{11}C]-DPA713, [^{18}F]-DPA714, [^{18}F]-GE180, and [^{18}F]-FEDAA1106, which have been increasingly used over the last decade.

Additionally, as [^{11}C]-PK11195 has a relatively short half-life, fluorinated (^{18}F) radioligands with a longer half-life have been examined (Bodini et al., 2021; Guido et al., 2025; Salerno et al., 2024).

The first PET imaging study in pwMS using [^{11}C]-PK11195 radioligand was performed in 1997. Two pwMS were imaged and increased [^{11}C]-PK11195 binding was observed at the sites of acute MS lesions, but no increase in radioligand binding was observed in chronic lesions (Vowinckel et al., 1997). The next published [^{11}C]-PK11195 PET imaging study compared [^{11}C]-PK11195 binding in 12 pwMS and 8 healthy controls (HC). Higher [^{11}C]-PK11195 binding was observed in brainstem and thalami in pwMS compared to HCs, and only minimal [^{11}C]-PK11195 binding was observed in healthy brains. In addition, in pwMS [^{11}C]-PK11195 binding was higher in gadolinium-enhancing lesions compared with non-enhancing lesions (Banati et al., 2000).

Over the years numerous TSPO-PET studies have highlighted the harmful nature of increased TSPO binding for the disease course. Several cross-sectional studies have demonstrated higher TSPO binding in pwSPMS compared to pwRRMS and HCs (Banati et al., 2000; Bezukladova et al., 2020; Debruyne et al., 2003; Herranz et al., 2024, 2016; Nylund et al., 2025; Politis et al., 2012; Rissanen et al., 2014; Singhal et al., 2019). One study has also demonstrated increased widespread TSPO binding in a single pwPPMS compared to HCs (Singhal et al., 2021). In a cohort of 18 pwCIS, higher TSPO binding in NAWM was associated with increased risk of developing clinically definite MS during a 2-year follow-up, emphasizing the importance of microglial activation already in the very early stages of the disease (Giannetti et al., 2015). Moreover, studies have demonstrated an association between TSPO binding and disease duration (Debruyne et al., 2003; Oh et al., 2011).

The association between TSPO-PET measurable microglial activation and clinical disability was demonstrated for the first time already in 2000. In the study of Banati et al. the percentage of TSPO binding in T1-weighted MRI lesions correlated with increasing EDSS score in a cohort of 12 pwMS (Banati et al., 2000). Since then, numerous TSPO-PET studies have demonstrated an association between physical disability (EDSS score) and increased TSPO binding in various brain regions including NAWM (Giannetti et al., 2015; Pitombeira et al., 2022; Rissanen et al., 2018; Sucksdorff et al., 2019; Treaba et al., 2024), the thalamus (Herranz et al., 2016; Misin et al., 2022; Pitombeira et al., 2022; Singhal et al., 2019; Zeydan et al., 2025), and cortical GM (Herranz et al., 2024, 2016; Politis et al., 2012). In accordance with findings from neuropathological studies showing activated microglia/macrophages at the rims of chronic active lesions (Frischer et al., 2015; Kuhlmann et al., 2017; Luchetti et al., 2018), TSPO-PET imaging studies have repeatedly demonstrated increased TSPO binding in the perilesional

NAWM, which additionally correlates with disease progression and disability accumulation (Banati et al., 2000; Giannetti et al., 2014; Lehto et al., 2023b; Nylund et al., 2022; Rissanen et al., 2018; Sucksdorff et al., 2020). Finally, in a cohort of 69 pwMS, TSPO binding in NAWM and perilesional NAMW predicted disease progression independent of relapses during a 4-year follow-up (Sucksdorff et al., 2020).

Studies have also demonstrated an association between increased TSPO binding and cognitive impairment (Herranz et al., 2016; Pitombeira et al., 2022; Zeydan et al., 2025), brain atrophy measures (Datta et al., 2017; Nylund et al., 2025; Singhal et al., 2019; Versijpt et al., 2005), signs of brain microstructural damage (Bezukladova et al., 2020; Rissanen et al., 2018), sNfL levels (Saraste et al., 2023), and lately, chitinase-3-like protein 1 (CHI3L1) levels (Ahola et al., 2025). A recent study combining [^{11}C]-PK11195 PET imaging and neuroimaging software allowing assessment of WM integrity observed higher TSPO binding in WM tracts in patients with PRLs compared to patients without PRLs. Additionally, TSPO binding was higher in WM tracts disrupted by PRLs compared to tracts disrupted by non-PRLs. These results suggest that patients with PRLs have more widespread chronic inflammation than patients without PRLs (Tozlu et al., 2025). In addition, studies assessing TSPO binding in the choroid plexus and meninges and their clinical correlations are emerging (Herranz et al., 2024; Ricigliano et al., 2021).

Studies assessing longitudinal alterations in TSPO binding are scarce, but suggest a continuous increase in TSPO-PET measurable microglial activation in pwPMS. In a cohort of 14 pwPMS (SPMS and PPMS) with stable moderate-efficacy DMTs for at least 6 months prior to PET imaging and throughout the study period, an increase in TSPO binding in NAWM, putamen, thalamus, and cortical GM was observed over a 12-month follow-up period (Kang et al., 2021). Previously, in a small cohort consisting mainly of untreated pwSPMS, an increase in perilesional TSPO binding during a 1-year follow-up was reported (Sucksdorff et al., 2019). In a recent study, an increase in the proportion of active voxels in NAWM was observed in untreated pwSPMS, whereas no changes in TSPO binding were observed in pwRRMS during a 1-year follow-up (Nylund et al., 2025). In the latter study, BMI and change in the proportion of active voxels in NAWM over one year were the best predictors of later brain volume loss (Nylund et al., 2025).

TSPO-PET also enables classification of chronic WM lesions into active or inactive according to the lesional TSPO binding (Hamzaoui et al., 2023; Nylund et al., 2022). To date, two different methodologies with different categories have been described. First, chronic T1 lesions can be categorized with an automated method into TSPO-PET rim-active (RAL), overall active, or inactive based on [^{11}C]-PK1195 binding (proportion of active voxels) at the lesion rim versus lesion core. Using this

method, the number of RALs (reflecting chronic active lesions) and the proportion of RALs of all T1 lesions were higher in pwSPMS compared to pwRRMS in a cohort of 91 pwMS. A higher proportion of RALs was additionally observed in patients with an EDSS score ≥ 4.0 compared with those with a lower EDSS score (Nylund et al., 2022). Furthermore, in a cross-sectional cohort of 82 pwMS, high proportion of RALs and low proportion of inactive lesions associated with an increase in EDSS score during a 5-year follow-up. In the subgroup of patients free of relapses, high proportion of RALs at baseline predicted disease progression with an odds ratio (OR) of 34.8 (Polvinen et al., 2023). Recent studies have also found associations between these lesion phenotypes and serum biomarker (sNfL, sGFAP) levels (Saraste et al., 2023; Sjöros et al., 2025). Another lesion phenotyping method classifies WM lesions into homogenously active, rim-active, or nonactive based on second-generation TSPO-radioligand [^{18}F]-DPA-714 binding. With this lesion phenotyping method, the number of homogenously active lesions (high TSPO binding in the lesion center regardless of the classification of the corresponding rim) predicted subsequent clinical progression and brain atrophy during a 2-year follow-up (Hamzaoui et al., 2023).

TSPO-PET has also been used to evaluate the effects of currently available DMTs on microglial activation. Two small studies including pwRRMS/SPMS have demonstrated a decrease in TSPO binding in NAWM, perilesional NAWM, and within lesions following 6-12 months of natalizumab treatment (Kaunzner et al., 2017; Sucksdorff et al., 2019). In small patient cohorts, a decrease in TSPO binding has been observed after the initiation of fingolimod (Sucksdorff et al., 2017), and in pwMS treated with glatiramer acetate (Ratchford et al., 2012). Conversely, no longitudinal alterations in TSPO binding were observed in pwRRMS 12 months after initiation of teriflunomide treatment (Lehto et al., 2023a). Moreover, 18 months of treatment with rituximab led to a reduction in TSPO binding in the perilesional NAWM and thalamus in a single person with PPMS (Lehto et al., 2023b).

Overall, findings from TSPO-PET imaging studies strongly suggest that TSPO binding quantified with PET reflects harmful pathological changes driving disease progression and is associated with worse disease outcomes. Thus, TSPO-PET represents a potential imaging tool for monitoring *in vivo* pathology associated with progressive MS and may be used as an outcome measure in future treatment trials targeting microglia. Figure 3 demonstrates an MRI image and a respective [^{11}C]-PK11195 PET image with chronic active and chronic inactive T1 lesions.

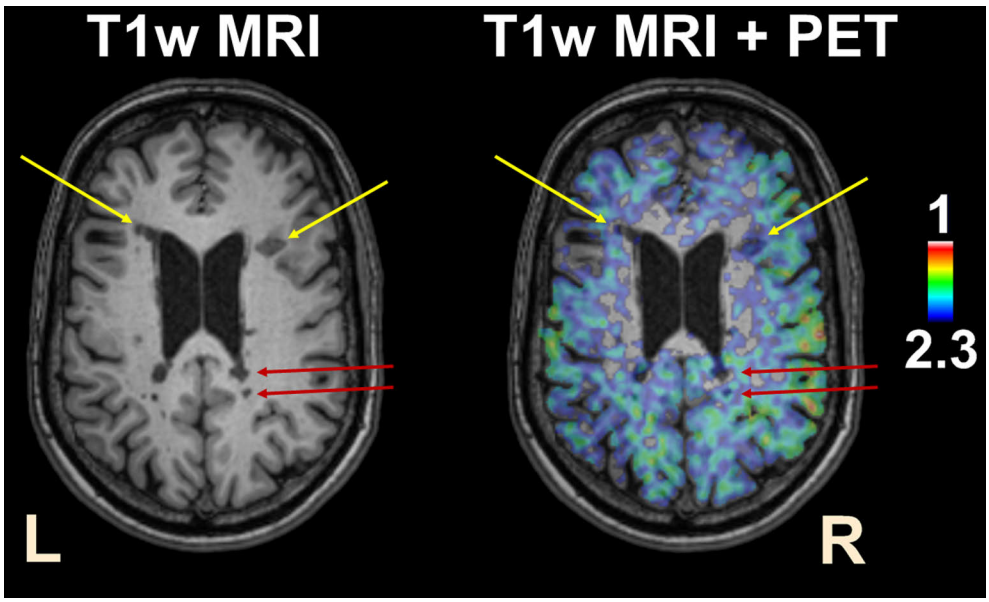

Figure 3. Visual demonstration of the axial T1 MR image (left) and respective [^{11}C]-PK11195 TSPO-PET image overlayed with the T1 image (right). Red arrows point to two chronic active lesions showing increased perilesional [^{11}C]-PK11195 binding whereas yellow arrows point to two chronic inactive lesions showing only minor perilesional [^{11}C]-PK11195 binding. In the parametric PET image, scaled color bar indicates the voxel wise ratio which represent the intensity of radioligand binding measured as distribution ratio volume. Abbreviations: MR = magnetic resonance; TSPO = translocator protein; PET = positron emission tomography.

2.4.6.3 Broad Rim Lesions

Broad rim lesions (BRL) are a recently introduced new pathological and imaging biomarker that show association with rapid disease progression. In a study published in April 2025 (Klotz et al., 2025), histological features and transcriptomic signatures were compared between pwMS exhibiting very rapid disease progression resulting in marked disability accumulation quickly after disease onset (n = 29) and pwMS exhibiting very slow disease progression (n = 33). A subset of MS lesions showed a remarkably broad rim of macrophages and microglia (at least 1mm in width; an average rim width 1,761 mm) with a specific transcriptomic profile linked to pro-inflammatory cytokine production, myeloid cell migration, enhanced protein turnover, and apoptosis, and were termed BRLs. In the study cohort, BRLs were identified predominantly in patients with rapid disease progression. In a larger autopsy cohort (n = 198), pwMS with at least one BRL reached EDSS scores 6 and 8 faster than those without BRLs. The findings were validated in another cohort of pwMS undergoing TSPO-PET imaging. Lesions with high TSPO binding extending up to 4 mm from the lesion edge were classified as radiological BRLs. The

proportion of radiological BRLs correlated with TSPO binding in NAWM, indicating association with diffuse microglial activation, as well as with T1 and T2 lesion volumes. Additionally, a higher proportion of radiological BRLs was associated with a higher proportion of TSPO-PET RALs and a lower proportion of TSPO inactive lesions. Patients who showed clinical progression after PET imaging showed a trend towards a higher number of radiological BRLs. Moreover, one year of natalizumab treatment reduced the number of radiological BRLs. (Klotz et al., 2025). Further studies are needed to confirm these preliminary findings.

2.4.7 Overlap Between TSPO-PET Detectable Chronic Active Lesions, PRLs and SELs

SELs are at least twice as common as PRLs as discussed above (Calvi et al., 2022b, 2022a; Martire et al., 2022). When the co-localization of SELs and PRLs was evaluated in a cohort of 61 pwCIS/RRMS, 92% of patients had at least one SEL, 56% at least one PRL, and 51% both a SEL and a PRL. Compared to patients with only SELs, patients with both SEL and PRL showed a greater increase in EDSS score during a 3.2-year follow-up (Calvi et al., 2023). Another study including 41 pwRRMS found that 39.5% of PRLs overlapped with a SEL, whereas only 17.2% of SELs overlapped with a PRL. Only PRLs co-localizing with SELs showed ongoing tissue damage suggesting that "expanding PRLs" might represent the most destructive lesion phenotype (Elliott et al., 2023).

Studies have also demonstrated co-localization of TSPO-positive lesions and PRLs. Increased [^{11}C]-PK11195 binding was observed in PRLs compared with non-PRLs identified by QSM MRI (Kaunzner et al., 2019). In a recent study using [^{11}C]-PK11195 binding based lesion classification and QSM MRI, PRLs did not co-localize reliably with TSPO-PET rim-active lesions (Lehto et al., 2023a). Furthermore, a study using 7T MRI and TSPO-PET imaging with [^{11}C]-PBR28 radioligand reported that 63% of detected PRLs were classified TSPO-PET active, but additionally 44% of non-PRLs were considered TSPO-PET active suggesting that TSPO-PET identifies a higher number of chronic active WM lesions (whole and peripherally active lesions) than 7T MRI PRL assessment (Treaba et al., 2024). To date, no studies assessing co-localization of TSPO-positive lesions and SELs have been published.

In summary, TSPO-positive lesions, PRLs, and SELs are all considered promising biomarkers of chronic active lesions, but no consensus exists on which of these three biomarkers is most specific and sensitive to chronic active lesions (Bagnato et al., 2024). The partial overlap of TSPO-positive lesions, PRLs, and SELs suggests that different imaging modalities capture distinct types of chronic active lesion and/or different developmental phases of these lesions, reflecting discrete

pathophysiological mechanisms (Bagnato et al., 2024; Dal-Bianco et al., 2024). Combined imaging and histopathological studies have shown that PRLs have the best histopathological correlation to chronic active lesions (Absinta et al., 2019). The biology underlying SELs, overlapping lesions, and TSPO-positive lesions that do not correspond to PRLs is only partially understood and requires further research.

2.5 Prognostic Factors in MS

Although several clinical, imaging and laboratory markers can be useful in assessing disease prognosis, predicting prognosis at the individual level remains challenging due to significant differences in disease activity and progression between individuals.

2.5.1.1 Clinical Prognostic Factors

Based on large epidemiological natural history studies, increasing disease duration and age are the two most important demographic factors predicting disease conversion from RRMS to SPMS (Koch et al., 2010; Leray et al., 2010; Tremlett et al., 2008; Tutuncu et al., 2013). Several studies have associated male sex with poor long-term disability outcomes, more rapid disability worsening and SPMS conversion (Leray et al., 2010; Ribbons et al., 2015; Scalfari et al., 2014; Sharmin et al., 2023; Tremlett et al., 2008; Vukusic and Confavreux, 2003). Other demographic and clinical factors associated with poor prognosis include older age at disease onset (Koch et al., 2007; Scalfari et al., 2014; Sharmin et al., 2023), higher disability at diagnosis (EDSS score), and early disability accumulation (Kappos et al., 2015; Rudick et al., 2010; Sharmin et al., 2023; Traboulsee et al., 2016; Uher et al., 2017). Relapses are associated with short-term disease worsening, and previous epidemiological studies have linked incomplete recovery from the first relapse, a short interval between first and second relapse, a high relapse rate, and certain relapse characteristics with long-term disability progression (Confavreux et al., 2003; Debouverie, 2009; Eriksson et al., 2003; Leray et al., 2010; Scalfari et al., 2014; Stewart et al., 2017; Vukusic and Confavreux, 2003). However, the contribution of relapses to long-term disability remains controversial. Additionally, cognitive impairment at the time of MS diagnosis is associated with disability progression and conversion to SPMS (Moccia et al., 2016; Pitteri et al., 2017). Importantly, several real-world registry-based studies have emphasized the importance of early high-efficacy treatment in delaying disability accrual (Buron et al., 2020; Cerqueira et al., 2025; Harding et al., 2019; He et al., 2020; Iaffaldano et al., 2021a; Spelman et al., 2021), also in Finnish pwMS (Hänninen et al., 2022). In addition, initial high-efficacy treatment was associated with a lower risk of SPMS

conversion compared with moderate-efficacy treatment in an international multicenter study including 1555 patients (Brown et al., 2019).

2.5.1.2 MRI Biomarkers

Conventional MRI plays a key role in the diagnosis and monitoring of MS, but its prognostic value is less remarkable. However, the number, location and evolution of T2 lesions may be informative when assessing the long-term disease prognosis. Previously, a high number of T2 lesions on diagnostic MRI in pwCIS/early RRMS has been shown to increase the risk of physical disability and SPMS 20 years later (Fisniku et al., 2008). Infratentorial lesions (Chung et al., 2020; Tintore et al., 2010) and spinal cord lesions (Brownlee et al., 2019; Rocca et al., 2022) also predict long-term physical disability. Spinal cord lesions are additionally associated with the development of SPMS after 15 years (Brownlee et al., 2019). Early increase in T2 lesion burden (particularly during the first 5 years) and gadolinium-enhancing lesions are associated with later physical disability (Brownlee et al., 2019; Fisniku et al., 2008; Popescu et al., 2013; Uher et al., 2017), cognitive impairment (Brownlee et al., 2019; Ziccardi et al., 2023) and SPMS (Brownlee et al., 2017; Fisniku et al., 2008).

Beyond lesion evaluation, conventional MRI allows measurement of changes in brain and spinal cord volumes, which reflect lesion-induced and neurodegenerative processes within the CNS. Earlier studies have demonstrated that whole brain atrophy associates with physical disability (Jacobsen et al., 2014; Popescu et al., 2013; Rocca et al., 2022), disability progression and PIRA (Cagol et al., 2022; Cree et al., 2019). Atrophy of GM structures appears to be more relevant for clinical outcomes, as multiple studies have repeatedly demonstrated associations between cortical and deep GM atrophy and both physical disability progression (Eshaghi et al., 2018; Hänninen et al., 2020; Rocca et al., 2022, 2010) and cognitive impairment (Rojas et al., 2018; Schoonheim et al., 2015). Several studies have also indicated that cervical cord atrophy is most pronounced in progressive MS forms (Lukas et al., 2013; Rocca et al., 2011), and a higher rate of cervical cord atrophy predicts physical disability and SPMS conversion (Bischof et al., 2022; Zeydan et al., 2018). Atrophy measurements are recommended in clinical practice by the Magnetic Resonance Imaging in MS (MAGNIMS) study group (Sastre-Garriga et al., 2020), but several technical challenges and lack of clear guidance hamper their use (Rocca et al., 2017).

Compared with conventional MRI, advanced MRI techniques are more helpful in predicting long-term disease outcomes and enable e.g., the detection and evaluation of cortical lesions (CL) and chronic active lesions. CLs can be detected with conventional 1.5/3.0 T MRI scanner but are better visualized using high field 7T MRI and specific MRI sequences (Jonkman et al., 2016; Kilsdonk et al., 2016).

Several previous studies have demonstrated that CLs are associated with physical disability and cognitive impairment, even at early disease stages (Calabrese et al., 2013; Nielsen et al., 2013; Ziccardi et al., 2023). Moreover, the volume of CLs is shown to predict SPMS conversion (Calabrese et al., 2013; Scalfari et al., 2018).

PRLs and SELs are considered MRI markers of chronic active lesions and therefore regarded as manifestations of chronic inflammation driving progression-related pathology. In accordance with this assumption, they may have prognostic value, as demonstrated in several studies over the past few years. Multiple studies have demonstrated that PRLs associate with more severe physical disability, disability accumulation, and PIRA (Absinta et al., 2019; Altokhis et al., 2022; Borrelli et al., 2024; Cagol et al., 2024a; Reeves et al., 2024b; Treaba et al., 2021), cognitive impairment (Absinta et al., 2019; Preziosa et al., 2024), different atrophy measurements (A. Dal-Bianco et al., 2021; Treaba et al., 2021; Weber et al., 2022), and sNfL levels (Maggi et al., 2021). Similarly, several studies have demonstrated associations between SELs and physical disability and disability worsening (Beynon et al., 2022; Calvi et al., 2022b, 2022a; Elliott et al., 2019a; Preziosa et al., 2022) and brain volume changes (Yokote et al., 2024). Both PRLs and SELs are discussed in more detail in Chapters 2.3.3.1 and 2.3.3.2.

The evaluation of PRLs, SELs and CLs is at least partly possible using MRI scanners that are already widely available. However, their assessment is not yet part of routine clinical practice. The incorporation of PRLs into the 2024 McDonald diagnostic criteria is likely to increase the use of this biomarker in the routine care of pwMS.

2.5.1.3 Fluid-based Biomarkers

A CSF sample is widely used in the diagnostic workup of MS. Traditionally, CSF evaluation has focused on the assessment of intrathecal IgG synthesis, a hallmark of humoral B cell-mediated immune activity. CSF-specific OCBs and/or elevated IgG index are present in majority of pwMS (Dobson et al., 2013b; Karrenbauer et al., 2021; Swane et al., 2026; Zheng et al., 2020). In addition to immunoglobulins, B cells and plasma cells in MS produce two isoforms (kappa and lambda) of free light chains. Increased synthesis of free light chains is not specific for MS as abnormally high concentrations of free light chains are also seen in patients with multiple myeloma and systemic amyloidosis. In pwMS, an elevated κ-FLC index in CSF appears to be as sensitive and specific as OCBs for differentiating MS from other diseases (Arrambide et al., 2022; Hegen et al., 2023a; Kaplan et al., 2011). Accordingly, the κ-FLC index was incorporated into the 2024 McDonald criteria as an alternative marker of immunopathology in CSF (Montalban et al., 2025).

The presence of CSF-specific OCBs remarkably increases the risk of developing MS in pwCIS (Dobson et al., 2013b; Kuhle et al., 2015). However, the prognostic value of OCBs and elevated IgG index is less remarkable in pwMS. Some studies have shown an association with disability worsening (Dobson et al., 2013b; Gasperi et al., 2019; Tintore et al., 2015; Zheng et al., 2020), while others have not (Becker et al., 2015; Lourenco et al., 2013; Tintoré et al., 2008). For example, in a longitudinal study including 673 newly diagnosed pwMS, a positive IgG index associated with a higher risk of EDSS score worsening over a 4-year-follow-up period (Gasperi et al., 2019). Studies examining the significance of CSF-specific OCBs have demonstrated an approximately 2-fold risk of disability progression in OCB-positive pwMS compared to pwMS without OCBs (Dobson et al., 2013a; Tintore et al., 2015) and also higher risk of SPMS conversion among OCB-positive pwMS (Karrenbauer et al., 2021). According to κ-FLC levels in CSF, recent studies have demonstrated an association between increased κ-FLC synthesis and early disease activity, PIRA, and cognitive decline (Hegen et al., 2023b; Rosenstein et al., 2023a, 2023b). In addition to intrathecal IgG production, pwMS have increased production of intrathecal IgM (indicated by IgM OCBs), which have also been associated with more a severe disease course (Capuano et al., 2021; Monreal et al., 2021).

Glial fibrillary acidic protein is a promising astrocyte marker which can be measured from CSF and serum. In addition to MS, elevated serum GFAP (sGFAP) levels are observed also in neuromyelitis optica spectrum disorder, another neuroinflammatory disease, as well as in other neurological conditions such as in traumatic brain injury, stroke and Alzheimer´s disease (Abdelhak et al., 2022). Additionally, sGFAP levels increase gradually with normal aging (Tybirk et al., 2022), higher average sGFAP levels have been observed in males compared with females (Tybirk et al., 2022) and BMI may also influence sGFAP levels (Yalachkov et al., 2022). In pwMS, higher sGFAP levels have been found in pwMS compared to HCs, and sGFAP levels are especially high among pwPMS (Abdelhak et al., 2018; Högel et al., 2020; Sjöros et al., 2025). Additionally, several studies have demonstrated an association of sGFAP levels with physical disability (Abdelhak et al., 2018; Högel et al., 2020; E. J. Lee et al., 2020; Monreal et al., 2024), disease progression (Barro et al., 2022; Madill et al., 2024; Meier et al., 2023; Monreal et al., 2024) and worse MRI outcomes (brain WM and GM volume loss, higher WM lesion load) (Ayrignac et al., 2020; Högel et al., 2020; Meier et al., 2023; Sjöros et al., 2025).

Neurofilament light chain is a biomarker of neuroaxonal injury, which is also measurable from CSF and serum. Similarly to GFAP, NfL lacks specificity to MS and elevated sNfL levels have been observed in various neurological conditions such as Alzheimer´s disease, frontotemporal dementia, amyotrophic lateral sclerosis, atypical parkinsonian disorders and traumatic brain injury (Gaetani et al., 2019).

Moreover, sNfL levels increase with aging, and sNfL values between aged healthy individuals and people with neurological diseases can overlap (Gaetani et al., 2019; Khalil et al., 2020). Other potential factors affecting sNfL levels include renal function (Korley et al., 2019) and BMI (Manouchehrinia et al., 2020). Due to the lack of specificity to MS, sNfL has only limited diagnostic value in MS. However, sNfL seems to be helpful in monitoring disease activity and treatment responses and may also have predictive value. Elevated sNfL levels have been demonstrated in all MS subtypes (Disanto et al., 2017), and sNfL may increase up to 6 years prior to the first clinical symptoms suggestive for MS (Bjornevik et al., 2020). Several studies have demonstrated an association of sNfL levels with clinical and radiological disease activity (Benkert et al., 2022; Disanto et al., 2017; Freedman et al., 2025; Kuhle et al., 2019). sNfL levels correlate also with short-term and long-term disability progression (Abdelhak et al., 2023; Cantó et al., 2019; Disanto et al., 2017; Freedman et al., 2025; Kuhle et al., 2019; Meier et al., 2023), and with brain and spinal cord atrophy (Barro et al., 2022; Buchmann et al., 2023; Cantó et al., 2019). Importantly, sNfL levels decrease after DMT initiation, and changes in sNfL levels after treatment initiation predict subsequent MRI and clinical outcomes (Benkert et al., 2024, 2022; Disanto et al., 2017; Freedman et al., 2025; Kuhle et al., 2019).

Studies comparing the prognostic value of sNfL and sGFAP have suggested that sGFAP is more strongly associated with disease progression and PIRA than sNfL. A recent study including over 1000 participants with progressive forms of MS reported that sGFAP levels were associated with disability worsening over a median follow-up time of 4.6 years, whereas sNfL levels predicted disability worsening only in pwPPMS (Abdelhak et al., 2025). In a cohort of 355 pwMS (RRMS or PMS), higher sGFAP levels were observed in worsening pwPMS compared to stable pwMS. Moreover, pwMS developing disability worsening had approximately 2-fold higher sGFAP levels than those with stable disability, whereas no difference was observed in sNfL levels between worsening and stable pwMS. However, combination of both serum biomarkers resulted in a 4- to 5-fold increased risk of future disability worsening and PIRA (Meier et al., 2023). Furthermore, a study following a cohort of 362 pwMS treated with anti-CD20 B cell depleting therapies found that sGFAP levels were more strongly associated with PIRA events one year after treatment initiation than sNfL levels. Higher sGFAP levels were observed in patients with PIRA compared with clinically stable patients. Additionally, sNfL levels remained high in PIRA patients, whereas sNFL levels decreased in stable patients (Benkert et al., 2024).

Currently, only the assessment of CSF immunopathology (IgG index/OCBs/ κ-FLC index) at the time of MS diagnosis is widely used in clinical practice. Other fluid-based biomarkers discussed above are used predominantly in research purposes, as several methodological challenges and the lack of established reference

values for different clinical settings limit their clinical implementation (Di Filippo et al., 2024). A combined assessment of sGFAP and sNfL may represent the most valuable approach when assessing disease prognosis.

2.5.1.4 Optical Coherence Tomography

OCT is a non-invasive imaging method allowing measurement of retinal layers. It was incorporated to the diagnostic workup of MS in the 2024 McDonald criteria to identify optic nerve involvement (Montalban et al., 2025). Several studies have demonstrated the prognostic potential of OCT measurements: the thickness of retinal layers (e.g., retinal nerve fiber layer and the ganglion cell-inner plexiform layer) correlates with and predicts brain atrophy, physical and cognitive disability, and disease progression (Cerdá-Fuertes et al., 2023; Mirmosayyeb et al., 2023; Rothman et al., 2019; Vidal-Jordana et al., 2020). Moreover, OCT measurements can also predict response to DMTs (Button et al., 2017; Lambe et al., 2021). OCT is already available for clinical practice and is a potential non-invasive tool to use in the assessment of disease prognosis. Limitations affecting the use OCT include for example need of specialized expertise (ophthalmologist) for interpretation, challenges related to history of optic neuritis, and some technical/methodological issues (Sergott et al., 2007).

2.5.1.5 PET Imaging Biomarkers

PET imaging allows the direct quantification of biological processes *in vivo* (inside a living body) using radioligands binding to different targets. As discussed earlier, radioligands binding to TSPO can be used to detect microglial activation, as TSPO is upregulated in neuroinflammation (Banati et al., 2000; Bodini et al., 2021). Multiple TSPO-PET studies have demonstrated that increased TSPO binding in several brain regions is associated with longer disease duration, physical disability, cognitive parameters, brain atrophy measures and progressive disease course (Banati et al., 2000; Datta et al., 2017; Giannetti et al., 2015; Misin et al., 2022; Politis et al., 2012; Polvinen et al., 2023; Rissanen et al., 2014; Sucksdorff et al., 2020). TSPO-PET can also help to predict disease progression (Misin et al., 2022; Polvinen et al., 2023; Sucksdorff et al., 2020) and treatment responses (Kaunzner et al., 2017; Lehto et al., 2023a; Ratchford et al., 2012; Sucksdorff et al., 2019, 2017). Moreover, TSPO-PET allows visualization of chronic active lesions (Hamzaoui et al., 2023; Nylund et al., 2022). Besides neuroinflammation, other important pathological processes involved in MS could be investigated with PET imaging using radioligands targeting, for example, myelin, astrocytes, neurodegeneration, or infiltrating leucocytes (Bodini et al., 2021). To date, several limitations such as logistical,

methodological, and safety concerns restrict the use of PET imaging in clinical practice and wide use in research field (Bodini et al., 2021; Gaitán et al., 2024). PET imaging is discussed in more detail in Chapter 2.4.6.

2.5.1.6 Summary of Prognostic Biomarkers

Despite recent improvements in the biomarker field, there is currently no reliable biomarker capable of predicting disease course. Therefore, there remains a great unmet need to identify biomarkers accurately reflecting the pathological mechanisms underlying different disease stages. Most likely, a combination of multiple biomarkers is most informative for assessment of individual patients' prognosis. Table 4 summaries the most promising current prognostic biomarkers.

Table 4. Summary of most promising prognostic biomarkers in MS. Modified from Chertcoff et al., 2024.

BIOMARKER	ADVANTAGES	LIMITATIONS
MRI measures of atrophy	Allow early prediction of long-term disability: greater atrophy associates with worse physical and cognitive outcomes	Availability of technical requirements Measurement variability related to technical and physiological factors Need for validation of software tools Lack of actionable clinical thresholds and guidance for timing of studies
Paramagnetic rim lesions	Allow early prediction of long-term physical and cognitive disability Association with atrophy measures	Special MRI sequences needed for visualization and detection Need for validation/ standardization of MRI protocols
Cortical lesions	Allow early prediction of long-term physical and cognitive disability	Difficult to detect on clinically available MRI sequences Suboptimal visualization even in ultra-high field MRI
Slowly expanding lesions	Thought to detect a proportion of chronic active lesions Association with disease progression, physical disability and atrophy measures	Lack of consensus on definition and optimal image algorithms Histopathological characteristics not systematically explored Need of multiple MRI scans

BIOMARKER	ADVANTAGES	LIMITATIONS
TSPO-PET imaging	Allow detection of microglia/macrophage activation *in vivo* Categorization of chronic lesions into active/inactive Association between increased TSPO binding in different brain regions with physical and cognitive disability and atrophy measures	High costs and limited availability Need for standardization of acquisition procedures Genotype may affect radioligand binding Limited specificity to microglial activation Risks of serial imaging (exposure to ionizing radiation)
Neurofilament light chain	Measurable in CSF and serum Sensitive to inflammatory neuroaxonal damage Prediction of short-term clinical and MRI disease activity Allow monitoring of disease activity and treatment response	Not specific to MS Role in progressive MS unclear Need to consider recent relapses, age, comorbidities and age Harmonization of methods across laboratories needed
Glial fibrillary acidic protein	Measurable in CSF and serum Likely reflects progressive disease biology May predict disability independent of acute disease activity	Not specific to MS Normative values still need to be established Need of adjusting for age, sex and BMI Harmonization of methods across laboratories needed
Optic coherence tomography	Allow early prediction of long-term disease course	Moderate cost and somewhat limited availability Need of specialized expertise for interpretation

Abbreviations: BMI = body mass index; CSF = cerebrospinal fluid; MRI = magnetic resonance imaging; MS = multiple sclerosis; PET = positron emission tomography; TSPO = translocator protein

3 Aims

Widespread microglial activation in the brain and at the rims of chronic active lesions is considered the major driver of disease progression in MS and can be measured *in vivo* using TSPO-PET imaging. The objectives of this thesis were to evaluate, which established risk factors of poor disease prognosis are associated with later TSPO-PET measured microglial activation, and to assess the usability of TSPO-PET imaging in predicting conversion to SPMS. Additionally, the effect of sex on [^{11}C]-PK11195 binding was studied in both pwMS and in healthy individuals to explore whether sex-related variability in microglial activation could contribute to the worse disease prognosis observed in men with MS.

The specific aims were the following:

1. To explore which demographic, clinical and paraclinical MS disease related parameters are associated with later TSPO-PET measurable microglial activation (I).
2. To evaluate the effect of sex on [^{11}C]-PK11195 binding in pwMS and in healthy individuals (II).
3. To evaluate the usability of TSPO-PET imaging, MRI and soluble biomarkers in predicting conversion to SPMS after 5 years in a cohort of pwRRMS considered at risk of disease progression according to their age and disease duration (III).

4 Materials and Methods

4.1 Study Participants

This thesis consists of three separate original studies. All studies were performed as investigator initiated academic research projects. A total of 102 pwMS (75 RRMS and 27 SPMS) and 76 HCs were included in the studies and the study populations partially overlap. Table 5 presents a summary of the study participants included in the studies of this thesis.

All pwMS were recruited from the Neurocenter outpatient clinic of Turku University Hospital in Turku. Inclusion criteria for the study participants with MS in all studies were (1) a definite diagnosis of MS according to the diagnostic criteria valid at the time of MS diagnosis and (2) willingness to participate in a PET study. Studies I and III included only pwRRMS whereas in Study II both pwRRMS and pwSPMS were included. Additional inclusion criteria for Study I were age of 40–55 years and at least 5 years disease duration at the study enrolment, and for Study III age of 40–50 years, at least 5 years disease duration and moderate-efficacy or no DMT at the study enrolment. Exclusion criteria for pwMS in all the studies were clinical relapse and/or corticosteroid treatment within 30 days of evaluation, gadolinium contrast enhancement in MRI, active neurological or autoimmune disease other than MS or another notable comorbidity, pregnancy and inability to tolerate PET or MRI.

Inclusion criteria for the healthy volunteers were willingness to participate in a PET study and no known history of neurological disease. Exclusion criteria for healthy volunteers included neurological or other major illness, pregnancy and contraindication to PET or MRI.

Table 5. Summary of study participants.

	NUMBER OF STUDY PARTICIPANTS	AGE (YEARS)	FEMALES /MALES	DISEASE DURATION (YEARS)	EDSS SCORE AT PET IMAGING
Study I, Original publication I	37 RRMS 14 HCs	47.8 (3.8)* 45.8 (6.8)*	32/5 12/2	9.8 (5.4–16) NA	2.5 (2–3) NA
Study II, Original publication II	75 RRMS 27 SPMS 76 HCs	42.9 (8.9)* 51.7 (9.2)* 45.5 (14.5)*	56/19 17/10 46/30	9.6 (4.8–13.8) 19.2 (13.9–22.9) NA	2.5 (2–3) 6.0 (4.25–6.5) NA
Study III, Original publication III	23 RRMS 21 HCs	47 (3.9)* 45 (7.9)*	17/6 15/6	12.0 (6.7–14.0) NA	3 (2.5–3) NA

Values are presented as median (IQR) except for age which is presented as *mean (SD). Abbreviations: EDSS = Expanded Disability Status Scale; HC = healthy control; IQR = interquartile range; NA = not applicable; RRMS = relapsing-remitting multiple sclerosis; SD = standard deviation; SPMS = secondary progressive multiple sclerosis.

4.2 Study Procedures

4.2.1 Study I

Thirty-seven pwRRMS, aged 40–55 years, and with a disease duration of at least 5 years, were imaged at Turku PET Centre (TPC) between years 2013–2018 with TSPO-PET using [^{11}C]-PK11195 radioligand and MRI. Clinical evaluation for disability using EDSS score was performed at the time of PET imaging. Data of earlier MS disease-related clinical and paraclinical parameters was obtained from physical and/or electronic medical records, which were reviewed retrospectively until the time of each participant´s first MS related symptoms and examinations. Initial clinical MR-images from the time of MS diagnosis were re-evaluated by an experienced neuroradiologist when available. For nine patients (25%) original MR-images were not available, or image quality did not allow exact estimation of the number of T2 lesions, and in these cases, the written neuroradiologist report was used for estimation of the number of T2 lesions in lesion number subgroup analyses. A group of 14 age-matched HCs was imaged for comparison with TSPO-PET. The overall study design of Study I presented in Figure 4.

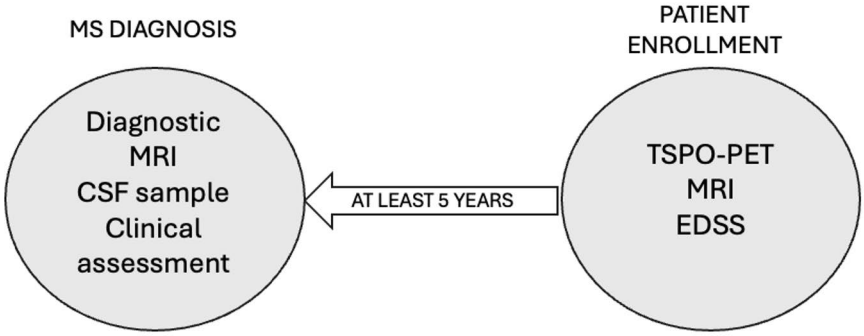

Figure 4. A flowchart presenting the study design of Study I. Modified from original publication I. Abbreviations: CSF = cerebrospinal fluid; EDSS = Expanded Disability Status Scale; MRI = magnetic resonance imaging; MS = multiple sclerosis; PET = positron emission tomography; TSPO = translocator protein.

4.2.2 Study II

A total of 102 pwMS were imaged in TPC between years 2009-2022 with TSPO-PET using [^{11}C]-PK11195 radioligand and MRI. Healthy individuals were similarly imaged either in TPC (37 HCs) or in Wolfson Molecular Imaging Centre in Manchester UK (WMIC, 39 HCs) between years 2007-2020. Additionally, at the time of PET imaging, a neurological examination was performed to evaluate EDSS score in pwMS.

4.2.3 Study III

Twenty-five pwRRMS, aged 40-50 years and with at least 5 years disease duration, were recruited during 2016-2017. Patients with high-efficacy DMT were not considered for the study. At baseline patients were imaged with TSPO-PET using [^{11}C]-PK11195 radioligand and MRI. Blood samples were obtained for measurement of serum NfL and GFAP concentrations, and neurological examination was conducted to evaluate EDSS score. A group of 21 age- and sex-matched HCs was imaged for comparison with TSPO-PET and MRI. Participants with MS were followed for five years. Brain MRI and EDSS score assessment were repeated yearly. MRI sequence allowing QSM and detection of PRLs was added to MRI protocol 2–4 years after baseline. At the final study visit, patients were evaluated clinically to determine possible conversion to SPMS using the Lublin diagnostic criteria (Lublin et al., 2014). Clinical evaluation included review of medical records, an interview including assessment of clinical symptoms of SAW (cognitive symptoms, fatigue, impaired balance, spasticity, limb weakness, and urinary problems), and neurological examination for assessment of EDSS score. The diagnosis of SPMS was made if there was an increase in EDSS score, significant

accrual of SAW-related symptoms, and an agreement between the patient and the evaluating neurologist regarding continuous worsening of neurological function. Two patients were lost during the follow-up and were excluded from the study. The overall study design of Study III is presented in Figure 5.

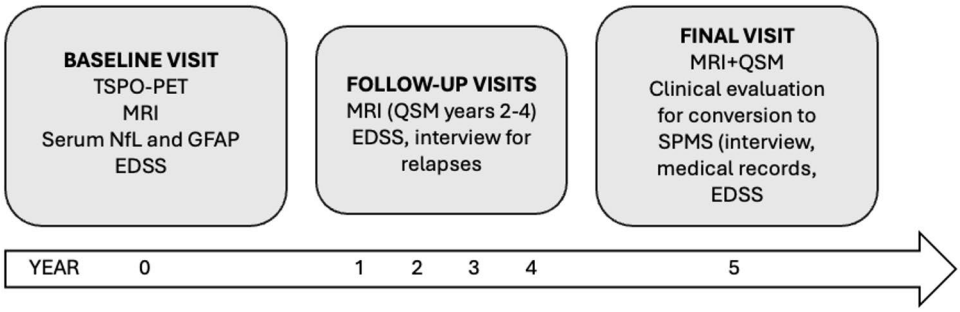

Figure 5. A flowchart presenting the study design of Study III. Modified from original publication III. Abbreviations: EDSS = Expanded Disability Status Scale; GFAP = glial fibrillary acidic protein; MRI = magnetic resonance imaging; NfL = neurofilament light chain; PET = positron emission tomography; QSM = quantitative susceptibility mapping: SPMS = secondary progressive multiple sclerosis; TSPO = translocator protein.

4.3 MRI Acquisition

Brain MRI was performed to acquire an anatomical reference for PET image analyses and for exclusion of participants with anatomical abnormalities. In pwMS, brain MRI was also used for evaluation of MS related pathology.

Different MRI scanners were used in the Studies I-III. MRI scanner used in Study I and Study III baseline imaging was 3T Ingenuity TF PET/MR scanner (Philips Healthcare, Cleveland, Ohio, USA). The follow-up MRI scans in Study III were obtained with either 3T Ingenuity TF PET/MR scanner or 3T Ingenia scanner (Philips Healthcare). In Study II MRI scanner used in TPC was either Gyroscan Intera 1.5T Nova Dual (Philips Healthcare, 25 participants) or 3T Ingenuity TF PET/MR scanner (Philips Healthcare, 114 participants). At WMIC, a 1.5T or 3T Philips Achieva scanner (Philips, Best, Netherlands) was used (25 participants and 14 participants, respectively).

The MR imaging protocol for pwMS in all studies included axial T2, 3D FLAIR, 3D T1 and gadolinium enhanced 3D T1 sequences (spatial resolution 1x1x1 mm). Additionally in Study III, a GRE sequence enabling detection of PRLs with QSM was added to MRI protocol 2–4 years after baseline: 2 years after baseline in 9 participants, 3 years after baseline in 11 participants and 4 years after baseline in 3 participants. MRI protocol for all healthy volunteers included at least a 3D T1-weigted sequence.

4.4 MR Image Processing and Analysis

MR images were processed and analyzed with similar methodology in all studies. First, MS lesions were identified from MR images using the Lesion Segmentation Tool (LST), a toolbox running within statistical parametric mapping (SPM, Wellcome Trust Center for Neuroimaging, London, UK; a software running in Matlab (MathWorks, Natick, MA, USA)). SPM version 8 (Schmidt et al., 2012) was used in Studies I and II, and SPM version 12 in Study III. Next, the preliminary lesion masks were manually corrected slice by slice in Carimas (Turku PET Centre, Finland). FLAIR sequence was used for identification of T2 hyperintense WM lesions and creation of T2 lesion masks, and correspondingly T1 sequence was used for identification of T1 hypointense lesions and creation of T1 lesion masks. The T1 lesion mask images were used to fill the corresponding T1 image with the lesion-filling tool in LST and the filled T1 lesion masks were then used for segmentation of cortical GM, WM and thalamus volumes with Freesurfer software (a neuroimaging toolkit for processing ana analyzing brain MR images, https://surfer.nmr.mgh.harvard.edu/). Freesurfer version 5.3 was used in Studies I and II, and Freesurfer version 7.2.3 in Study III. The lesion masks were utilized for both the volumetric analyses of the lesions, as well as for the corresponding ROI level analyses of the PET data.

The NAWM ROI for PET analyses was created in all studies by removing T2 lesion ROI from the WM ROI. In Study II the whole brain ROI included supratentorial brain parenchyma, cerebellum and brain stem. In Studies I and III lesion ROI and/or perilesional ROI were used. The perilesional ROI in Study I was created by dilating the T1 lesion ROI mask by 6 voxels (the size of voxel = 1mm x 1mm x 1mm) and then removing the lesion core and a 3-voxel area surrounding the core from it. PET analyses were also performed for alternative perilesional ROIs (the 3-voxel area and 6-voxel surrounding the lesion core), but results were not included to the publication. In Study III the lesion rim ROI was created by dilating the T1 lesion ROI by 2 voxels and removing then the lesion core from it, and the perilesional ROI was created by dilating the T1 lesion ROI by 6 voxels and then removing the lesion core and the lesion rim ROI from it.

In Study III, PRLs were analyzed using QSM methodology. First, the GRE images were postprocessed using MEDI (Morphology Enabled Dipole Inversion) Toolbox (Liu et al., 2018). Reconstructed QSM images were then co-registered with the T1 images using SPM12 and visually inspected by two experienced raters to determinate PRLs. Lesions which had a visually assessed complete or partial hyperintense bright rim relative to lesion core were considered as PRLs. Furthermore, in Study III, all the follow-up MR images of one study participant were co-registered with the baseline T1 MR image using SPM12. As important parameters regarding T1 sequences (such as repetition time, echo time, field of view, acquisition

matrix size), number of acquired slices, and flip angle were equal in both MRI scanners used, no normalization or correction for MRI scanner variability was applied while processing longitudinal volumetric data.

4.5 PET Imaging and [^{11}C]-PK11195 Radioligand Production

All PET examinations in TPC and WMIC were performed using a brain dedicated ECAT High-Resolution Research Tomograph scanner (CTI, Siemens Medical Solutions, Knoxville, TN, USA) with an intrinsic spatial resolution of approximately 2.5 mm (De Jong et al., 2007). The radioligand used in all PET imaging studies was [^{11}C]-PK11195 ((R)-[N-methyl-11C]-1-(2-chlorophenyl)-N-(1-methylpropyl)-3-isoquinolinecarboxamide), the radiochemical synthesis of which was performed as described detailly in Rissanen et al., 2014. The target radioactivity dose of administered radioligand in all studies was 500 MBq.

PET imaging was initiated with a 6-minute transmission scan using a ^{137}Cs point source for attenuation correction of PET data. Thereafter, a 60-min dynamic imaging was started simultaneously with an injection of intravenous bolus of the radioligand. A thermoplastic mask was used to minimize head movements in TPC.

4.6 PET Image Processing, Modelling, and Analysis

PET images in all studies were processed, reconstructed and analyzed similarly. PET images were reconstructed using 17 time frames (2x15, 2x30, 3x60, 7x300 and 2x600; total of 3600 seconds) and possible displacements between frames due to head movement were corrected using mutual information realignment. Then, the PET images were co-registered with the 3D T1 MR image in their original anatomic space acquired at the same time point in SPM and dynamic emission data was smoothed using a Gaussian 2.5-mm post-reconstruction filter before statistical analysis or the individual lesion DVR analysis.

The specific binding of [^{11}C]-PK11195 radioligand in the prespecified ROIs was quantified using DVR. A supervised cluster algorithm with four predefined kinetic tissue classes (Super-PK-software) (Turkheimer et al., 2007; Yaqub et al., 2012) was used to determine reference a region devoid of specific TSPO binding, and DVR values were calculated using a reference tissue-input Logan method with time interval from 20 to 60 minutes (see also Chapter 2.4.3.) (Logan et al., 1996).

Besides DVR values, the proportion of active voxels in prespecified ROIs was used in Study III. The threshold for active voxel was calculated as follows: DVR + 1.96 x standard deviation (SD) (95% confidence interval threshold) was calculated

for all WM voxels for each HC, and the average of these individual values was used as a threshold for abnormally active voxel (Nylund et al., 2022). Additionally in Study III, T1 hypointense lesions larger than 27 mm^3 were classified into three subtypes according to the proportion of active voxels in the lesion core and lesion rim: rim-active, overall active, or inactive. A lesion was considered rim-active, if the proportion of active voxels at the lesion rim (0-2 mm) was considerably higher than in the lesion core (less than 5% active voxels in the lesion core and at least 5% point higher proportion of active voxels at the rim compared to the core, or 5–20% active voxels in the lesion core and at least double the proportion of active voxels at the rim concurrently). In inactive lesions, no active voxels were observed in the lesion core nor in the lesion rim. Lesions with activity in lesion core and rim not fulfilling the criteria of the other two categories were classified as overall-active lesions.

4.7 Measurement of Soluble Biomarkers

In Study III, blood was collected for the measurement of serum NfL and GFAP levels. After venous blood sampling, the blood was allowed to clot for 30 minutes at room temperature, then centrifugated (2000g, 10min), and the serum was stored at −80°C in the Auria Biobank (Turku, Finland). Samples were shipped on dry ice to Basel, Switzerland, where s NfL and sGFAP concentrations were measured with the Neurology 2-Plex B Kit (Quanterix, Billerica, MA, USA) and single-molecule array technique. Besides absolute sNfL and sGFAP levels, z-scores measuring the deviation of absolute sNfL and sGFAP value from HCs were calculated. Age- and BMI-adjusted sNfL *z*-scores were calculated using a large reference database and a Generalized Additive Model for Location, Scale and Shape (GAMLSS model). (Benkert et al., 2022). Similarly, the GAMLSS model and a large reference database including 4297 samples from control persons (Maleska Maceski et al., manuscript under preparation) was used to calculate age-, BMI- and sex-adjusted sGFAP z-scores.

4.8 Statistical Analyses

All statistical analyses were conducted using R (Study I version 4.1.1., Study II version 4.2.1. and Study III version 4.4.0.). Variables were reported as mean (SD) unless otherwise stated. A p-value < 0.05 from a two-tailed test was considered significant for all analyses.

4.8.1 Study I

Associations between different retrospectively collected clinical and paraclinical parameters at the time of MS diagnosis or regarding early disease course, and DVR

values in the prespecified ROIs an average of 12.2 years later were analyzed. Group associations between categorical variables and DVR values were evaluated using Wilcoxon-rank sum test due to the non-normal distribution of variables. If three or more groups were compared, Holm adjustment was used for p-values. Group comparisons between continuous/ordinal variables and DVR values were conducted using Spearman correlation. Results from initial group comparisons were adjusted for potential confounding factors using either multiple linear regression model (for continuous variables) or analysis of covariance (ANCOVA, for categorical variables). Confounding factors considered in modelling were time until treatment initiation (less than or at least 3 years), treatment before imaging (no treatment or moderate-efficacy treatment, high-efficacy treatment), age and disease duration at imaging, and sex. Tukeys's method was used to adjust initial p-values from pairwise comparisons (multiple linear regression model, ANCOVA).

4.8.2 Study II

DVR values of the prespecified ROIs were analyzed between groups of HCs, all pwMS, pwRRMS, and pwSPMS, and stratified by sex. In pwMS, associations between DVR values of different ROIs and EDSS score at the time of PET imaging were also studied. Wilcoxon rank-sum test was used for initial group comparisons between categorical variables and DVR values. A false discovery rate (FDR) was used to correct multiple comparisons. Associations between EDSS score and DVR values were conducted using Spearman correlation. Results from initial analyses were adjusted for potential confounding factors with multiple linear regression models in pwMS. Linear mixed model (conducted using R software package lmerTest version 3.1.3) was used while analyzing HC data, and the imaging center was used as a random effect to control the possible effect of two imaging centers. Confounding factors considered in the modelling were age, sex, brain volume (cm^3), and BMI. The model assumption was checked using Shapiro-Wilk test (for evaluation of the normality of the residuals) and variance inflation factors (for detection of multicollinearity). Additionally, Cook's distance was used to identify possible influential observations, and in case of influential observations, analyses were repeated without such observations to confirm the results.

4.8.3 Study III

Associations between the phenotype of conversion to SPMS an average of 5 years later, and the baseline imaging parameters (TSPO-PET and brain volumetric data) and serum biomarkers were analyzed. Shapiro-Wilk test was used to evaluate the data distribution. Depending on the normality of the variable, group comparisons

were performed with Welch's test (for normally distributed variables) or Wilcoxon rank-sum test (for non-normally distributed variables). Differences between the two time points were analyzed with paired t-test when variables were normally distributed and with Wilcoxon signed-rank test when data was not normally distributed. Fisher's exact test was used for comparisons of categorical variables.

4.9 Ethical Considerations

The study protocols of Studies I-III were approved by the Ethics Committee of the Hospital District of Southwest Finland and the Local Research Ethics Committee. The study protocol of Study II was additionally approved by the Administration of Radioactive Substances Advisory Committee in the United Kingdom. All studies were conducted in accordance with the principles of the Declaration of Helsinki. People with MS and healthy individuals participating in the studies provided written informed consent. The study participants voluntarily exposed themselves to ionizing radiation, pain and other potential harms related to cannulation, and discomfort during PET imaging without direct personal benefit, in order to promote MS research. The total radiation dose from the PET scan was 2.2 mSv, which corresponds to the average effective radiation dose received in Finland over approximately four months and is only slightly higher than radiation dose from a head CT scan (1.8 mSv).

5 Results

5.1 T2 Lesion Burden on Diagnostic MRI, Magnitude of IgG Index at MS Diagnosis and Early Clinical Disability Associate with Later TSPO-PET Measurable Microglial Activation (Study I)

Demographic and clinical characteristics of the 37 pwRRMS and 14 HCs participating in Study I at the time of PET imaging are presented in Table 5. The median time from diagnostic MRI to PET imaging was 13.2 (range 5.6-25.6) years. During the disease course prior to PET imaging, patients had used different DMTs, predominantly moderate-efficacy DMTs. Only four patients (11%) had been treated with high-efficacy DMTs (all natalizumab) (Scolding et al., 2015). At the time of PET imaging, all patients were either treated with moderate-efficacy DMTs or were untreated (11 patients, 30%).

Compared with HCs, pwMS had higher DVR values reflecting the specific [^{11}C]-PK11195 binding in NAWM and the thalamus, whereas no difference was observed in cortical GM DVR values.

Initial diagnostic MR images were available for evaluation in 27 patients. The median (interquartile range, IQR) number of T2 lesions on diagnostic brain MRI was 10 (range 6-18). A higher number of T2 lesions at diagnosis correlated with higher later [^{11}C]-PK11195 binding in the perilesional area, T1 lesions, and the thalamus (Figure 6). Initially, no correlation between the number of T2 lesions and later [^{11}C]-PK11195 binding in NAWM was found. However, after modelling for confounding factors, a higher number of T2 lesions on diagnostic MRI correlated with higher later [^{11}C]-PK11195 binding in NAWM. Instead, the correlation between number of T2 lesions and T1 lesion DVR value did not remain significant after modelling.

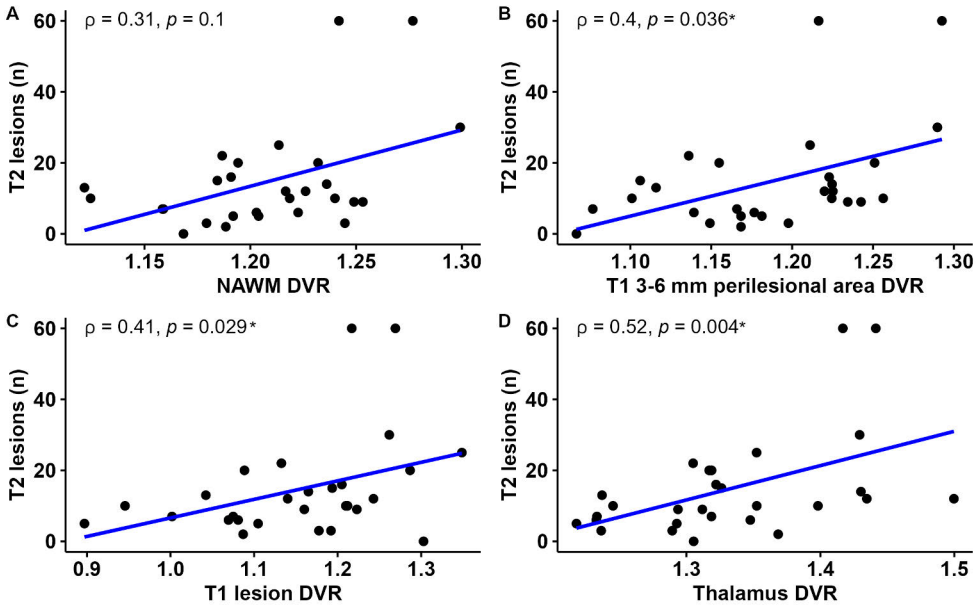

Figure 6. Correlation between number of T2 lesion at the time of MS diagnosis and later [^{11}C]-PK11195 distribution volume ratio (DVR) values in (**A**) normal-appearing white matter (NAWM), (**B**) the perilesional area, (**C**) T1 lesions, and (**D**) the thalamus. *Significant at the level of p<0.05. Modified from original publication I.

Patients were additionally divided into groups based on the number of T2 lesions on the diagnostic brain MRI: 14 patients (39%) had less than 10 lesions, 16 patients (41%) had 10-20 lesions, and 6 patients (17%) had more than 20 lesions. In the initial comparisons, the only difference between subgroups was observed in [^{11}C]-PK11195 binding in the thalamus. Higher [^{11}C]-PK11195 binding was observed in patients with more than 20 lesions and in patients with 10-20 lesions compared with patients with less than 10 lesions. However, after modelling for confounding factors, several additional significant differences between subgroups were observed. Patients with more than 20 lesions had higher [^{11}C]-PK11195 binding in NAWM, the perilesional area and the thalamus compared with patients with less than 10 lesions. Likewise, compared with patients with 10-20 lesions, patients with more than 20 lesions had higher [^{11}C]-PK11195 binding in NAWM and the perilesional area, but not in the thalamus. The only difference between patients with 10-20 lesions and less than 10 lesions was observed in [^{11}C]-PK11195 binding in the thalamus, which was higher in the patients with 10-20 lesions compared with patients with less than 10 lesions. No differences between the groups were observed in [^{11}C]-PK11195 binding in T1 lesions, T2 lesions, or cortical GM (Figure 7).

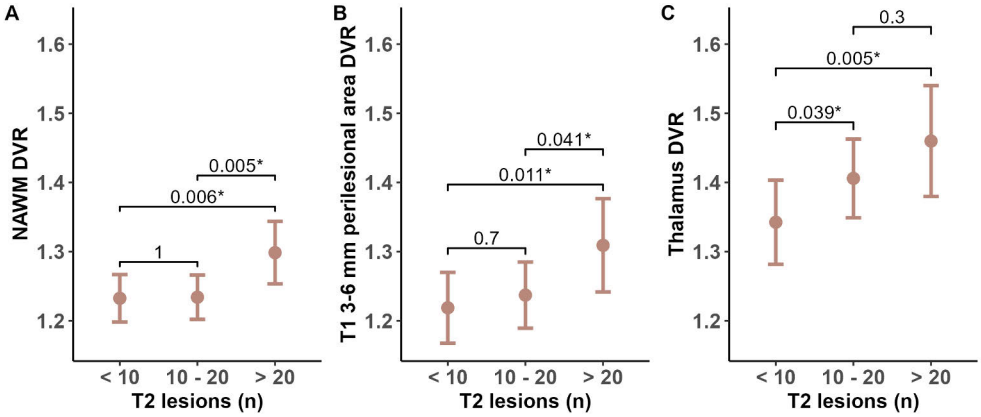

Figure 7. Association of grouped number of T2 lesions at the time of MS diagnosis and estimated means of later [^{11}C]-PK11195 distribution volume ratio (DVR) in (**A**) normal-appearing white matter (NAWM), (**B**) the perilesional area, and (**C**) the thalamus. *Significant at the level of $p<0.05$. Modified from original publication I.

Elevated (> 0.6) IgG index in CFS was observed in 29 (83%) patients at the time of MS diagnosis. Initially, higher IgG index correlated with higher later [^{11}C]-PK11195 binding in NAWM (Figure 8), but this association was abrogated when confounding factors were considered in modelling.

Mean (SD) EDSS score collected from medical records five years after MS diagnosis was 2.0 (1.2). Patients were categorized into two subgroups based on the 5-year EDSS score: EDSS score less than 2.0 (13 patients, 45%) and EDSS score at least 2.0 (16 patients, 55%). An EDSS score 2.0 was selected as the cutoff value, as it was considered to represent early disability accrual and is defined as minimal disability in one FS (Kurtzke, 1983). Patients with EDSS score at least 2.0 had higher later [^{11}C]-PK11195 binding in the perilesional area compared with patients having lower EDSS score five years after MS diagnosis. This difference remained significant after modelling for confounding factors (Figure 8).

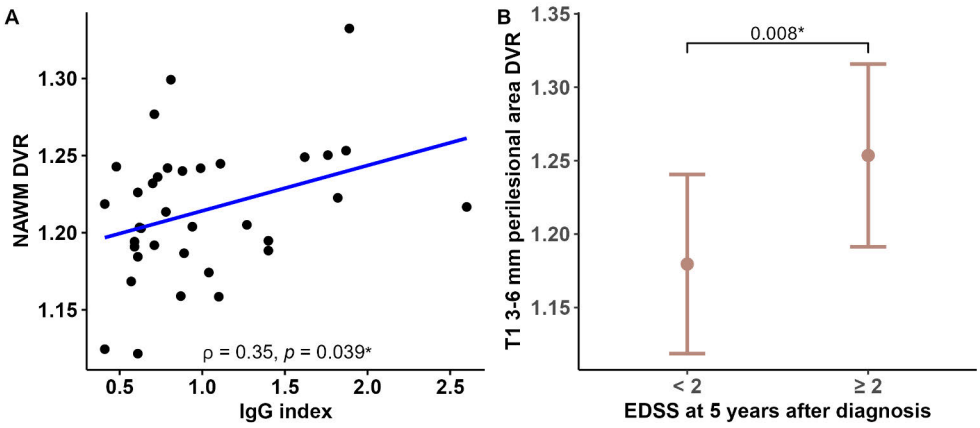

Figure 8. (**A**) Correlation between immunoglobulin G (IgG) index at the time of MS diagnosis and later [^{11}C]-PK11195 distribution volume ratio (DVR) value in normal appearing white matter (NAWM), and (**B**) Expanded Disability Status Scale (EDSS) score five years after MS diagnosis and later [^{11}C]-PK11195 distribution volume ratio (DVR) value in the perilesional area in patients with EDSS score < 2 compared to patients with EDSS score ≥ 2.0. *Significant at the level of p<0.05. Modified from original publication I.

5.2 TSPO-PET Measurable Microglial Activation is More Prominent in Men with MS and Healthy Men Compared with Women (Study II)

The main demographic and clinical characteristics of HCs (n = 76), all pwMS (n = 102), pwRRMS (n = 75), and pwSPMS (n = 27) included in Study II, as well as comparisons between subgroups at the time of PET imaging, are presented in Table 6. No significant differences in age, sex distribution, or BMI were observed between HCs and pwMS. Compared with pwRRMS, pwSPMS were older, had a longer disease duration, a higher EDSS score, less relapses, and a lower BMI. Additionally, pwSPMS were more often treated with moderate-efficacy DMTs or were untreated compared with pwRRMS. No significant differences in clinical parameters were observed between male and female pwMS.

Compared with HCs, pwMS had smaller brain, NAWM, cortical GM, and thalamic volumes (measured as parenchymal fraction, PF). Also, larger brain, NAWM, and cortical GM volumes (PF) were observed in pwRRMS compared with pwSPMS (PF). A larger total brain volume (cm^3) was observed in men compared with women in all studied subgroups, but when brain volume was normalized to total intracranial volume (PF), no differences were observed between sexes. MRI volumetric parameters and group comparisons are presented in Table 6.

Table 6. Demographic, clinical and MRI volumetric parameters of the study cohort. Modified from original publication II.

	HC	MS	HC vs. MS, p-value	RRMS	SPMS	RRMS vs. SPMS, p-value	MALE MS	FEMALE MS	MALE vs. FEMALE MS, p-value
Subjects, n	76	102	–	75	27	–	29	73	–
Female, n (%)	46 (61)	73 (72)	0.15	56 (75)	17 (63)	0.3	–	–	–
Age (years)	45.5 (14.5)	45.3 (9.7)	0.8	42.9 (8.9)	51.7 (9.2)	**< 0.001**	45.9 (9.8)	45.0 (9.8)	0.9
BMI* (kg/m^2)	25.2 (4.0)	26.5 (5.8)	0.3	27.2 (5.8)	24.8 (5.8)	**0.024**	26.5 (5.3)	26.6 (6.1)	0.6
Disease duration (years), median (IQR)	–	12.2 (7.0–17.2)	–	9.6 (4.8–13.8)	19.2 (13.9–22.9)	**< 0.001**	9.8 (3.2–16.9)	12.2 (7.7–17.3)	0.3
EDSS, median (IQR)	–	3.0 (2.0–3.9)	–	2.5 (2.0–3.0)	6.0 (4.25–6.5)	**< 0.001**	3.0 (2.0–3.5)	3.0 (2.0–4.0)	0.9
ARR, median (IQR)	–	0.31 (0.22–0.53)	–	0.4 (0.2–0.6)	0.24 (0.18–0.40)	**0.010**	0.4 (0.2–0.6)	0.3 (0.2–0.5)	0.6
DMT, n (%)									
No DMT	–	48 (47)	–	25 (33)	23 (85)		14 (48)	34 (47)	
ME-DMT	–	51 (50)	–	47 (63)	4 (15)	**< 0.001**	13 (45)	38 (52)	0.3
HE-DMT	–	3 (3)	–	3 (4)	0 (0)		2 (7)	1 (1)	
Brain volume	0.855 (0.04)	0.828 (0.05)	**< 0.001**	0.834 (0.04)	0.810 (0.05)	**0.034**	–	–	–
NAWM volume	0.35 (0.03)	0.33 (0.04)	**< 0.001**	0.33 (0.03)	0.32 (0.05)	**0.035**	–	–	–
Cortical GM volume	0.33 (0.02)	0.31 (0.03)	**< 0.001**	0.32 (0.02)	0.30 (0.03)	**0.003**	–	–	–
Thalamic volume	0.011 (0.001)	0.010 (0.001)	**< 0.001**	0.010 (0.001)	0.010 (0.002)	0.059	–	–	–

Values are presented as mean (SD) unless otherwise stated. Wilcoxon rank-sum test was used for group comparisons. Significant p-values are bolded. Disease duration is years from first MS symptoms. ARR is calculated from disease onset to PET imaging. Moderate-efficacy DMT includes interferon beta, glatiramer acetate, dimethyl fumarate, teriflunomide and fingolimod. High-efficacy DMT includes natalizumab and rituximab. MRI volumetric parameters are presented as parenchymal fractions (PF). *BMI has one missing value in MS (female, RRMS) and two missing values in HCs (male and female). Abbreviations: ARR = annual relapse rate; BMI = body mass index, DMT = disease modifying treatment; EDSS = Expanded Disability Status Scale; HC = healthy control; HE-DMT = high-efficacy DMT; IQR = interquartile range; ME-DMT = moderate-efficacy DMT; MS = multiple sclerosis; RRMS = relapsing remitting MS; SPMS = secondary progressive MS.

Compared with HCs, DVR values reflecting the specific [^{11}C]-PK11195 binding were higher in the whole brain, NAWM, the thalamus, and cortical GM in pwMS (Figure 9). In pwRRMS and pwSPMS, higher [^{11}C]-PK11195 binding in the whole brain and NAWM was observed compared with HCs. Additionally, pwRRMS had higher [^{11}C]-PK11195 binding in cortical GM and pwSPMS in the thalamus compared with HCs. All results in the whole brain and NAWM survived FDR correction, but results in the thalamus and cortical GM did not.

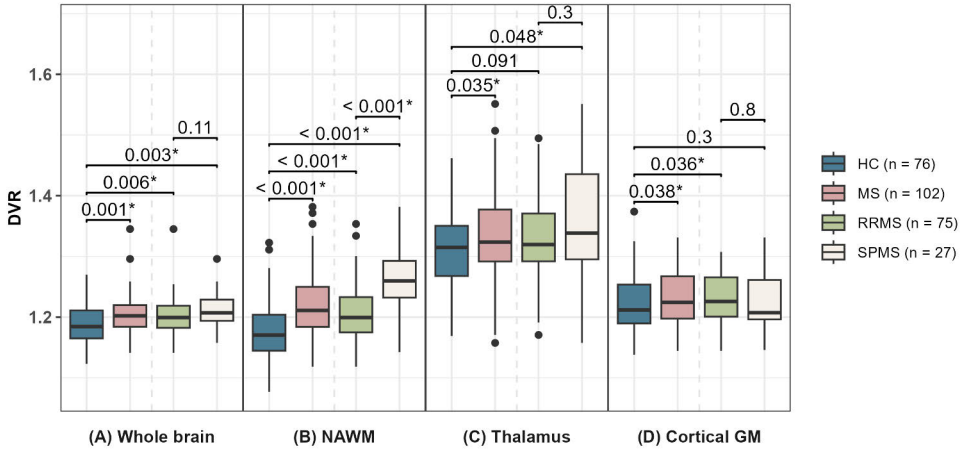

Figure 9. [^{11}C]-PK11195 distribution volume ratio (DVR) values of pwMS, healthy controls (HC), patients with relapsing-remitting MS (RRMS), and patients with secondary progressive MS (SPMS) in (**A**) the whole brain, (**B**) normal appearing white matter (NAWM), (**C**) the thalamus, and (**D**) cortical gray matter (GM). *Significant at the level of p<0.05. Modified from original publication II.

When [^{11}C]-PK11195 binding was compared between men and women, higher [^{11}C]-PK11195 binding in the whole brain, NAWM/WM, and the thalamus was observed in men in both pwMS and HCs (Figure 10). These differences in [^{11}C]-PK11195 binding between sexes remained significant after adjusting for confounding factors (the whole brain: $p = 0.006$ in pwMS and $p = 0.03$ in HCs; NAWM: $p < 0001$ in pwMS and $p = 0.006$ in HCs; the thalamus: $p = 0.006$ in pwMS and $p = 0.007$ in HCs). Male pwMS had also initially higher [^{11}C]-PK11195 binding in cortical GM compared with females, but this difference was abrogated when confounding factors were considered in modelling.

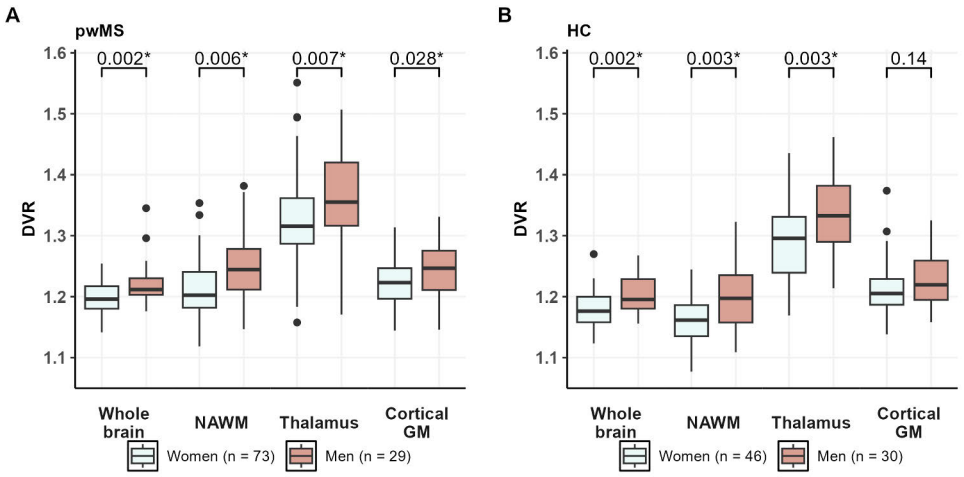

Figure 10. [^{11}C]-PK11195 distribution volume ratio (DVR) values in (**A**) all pwMS and (**B**) healthy individuals by sex in the whole brain, normal appearing white matter (NAWM), the thalamus, and cortical gray matter (GM). *Significant at the level of p<0.05. Modified from original publication II.

When pwRRMS and pwSPMS were analyzed separately, differences in [^{11}C]-PK11195 binding between sexes were more prominent in pwSPMS compared with pwRRMS. In both subgroups, men had higher [^{11}C]-PK11195 binding in the whole brain than women. Compared to women, male pwSPMS, had higher [^{11}C]-PK11195 binding in NAWM and male pwRRMS higher [^{11}C]-PK11195 binding in cortical GM. However, after adjustment for confounding factors, higher NAWM [^{11}C]-PK11195 binding in male compared with female pwSPMS was the only statistically significant difference ($p = 0.004$). After modelling, higher [^{11}C]-PK11195 binding in the thalamus was also observed in male compared with female pwSPMS ($p = 0.040$), a difference not observed in the initial analyses.

The association between [^{11}C]-PK11195 binding and disability was investigated. In all pwMS, higher [^{11}C]-PK11195 binding in NAWM, and the thalamus correlated with a higher EDSS score ($R = 0.42$, $p < 0.001$, and $R = 0.2$, $p = 0.04$; respectively). When the association between [^{11}C]-PK11195 and disability was investigated by sex, the correlation between [^{11}C]-PK11195 binding in NAWM and EDSS score appeared somewhat stronger in male pwMS compared with females ($R = 0.57$ vs. $R = 0.39$). Moreover, the correlation between [^{11}C]-PK11195 binding in the thalamus and EDSS score was observed only in male pwMS ($R = 0.46$, $p = 0.012$).

Finally, specific [^{11}C]-PK11195 binding was compared between healthy women and female pwMS, and between healthy men and male pwMS, to explore whether sex differences observed in pwMS were also present when comparing healthy individuals of the same sex. Higher [^{11}C]-PK11195 binding in the whole brain,

NAWM, and the thalamus was observed in female pwMS compared with healthy women. By contrast, in men, [^{11}C]-PK11195 binding was higher only in NAWM in male pwMS compared with healthy men, and no differences were observed in [^{11}C]-PK11195 binding in the whole brain or the thalamus (Figure 11).

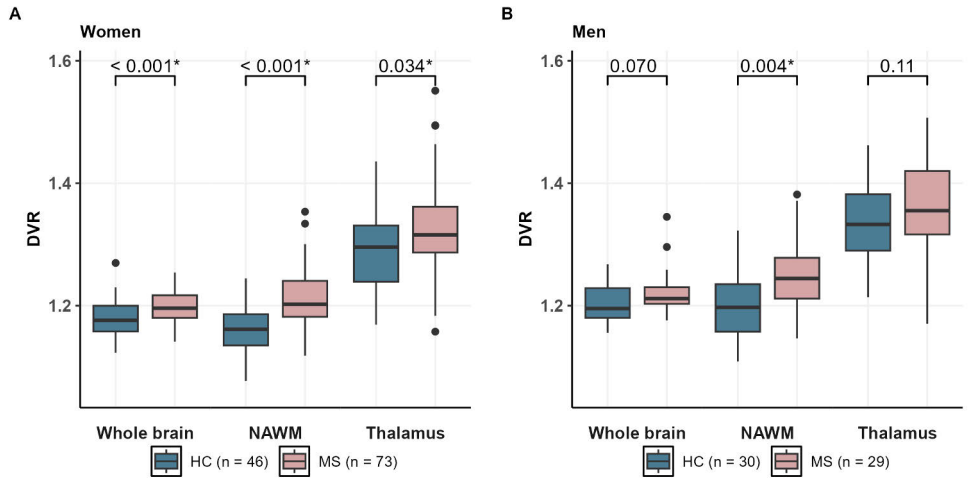

Figure 11. [^{11}C]-PK11195 distribution volume ratio (DVR) values in (**A**) female and (**B**) male healthy controls (HC) and pwMS in the whole brain, normal appearing white matter (NAWM), and the thalamus. *Significant at the level of p<0.05. Modified from the Original publication II.

5.3 TSPO-PET, Serum GFAP and Thalamic Atrophy Associate with Conversion to SPMS (Study III)

A summary of the demographic and clinical characteristics of 23 pwRRMS and 21 HCs participating in Study III is presented in Table 5. Patients were followed for a mean (SD) of 5.0 (0.4) years. At the final study visit, eight patients (35%) were evaluated to have converted to SPMS. The main demographic, clinical, and MRI volumetric characteristics of patients who converted to SPMS (SPMS convertors) and those who did not convert (SPMS non-convertors) at baseline and after the 5-year follow-up, as well as comparisons between variables at baseline and at follow-up, are presented in Table 7. At baseline, the majority of the patients (87%) were treated with moderate-efficacy DMTs. DMT was escalated to high-efficacy DMT in seven (30%) patients during the follow-up due to clinical or radiological disease activity, or disease worsening. SPMS convertors had a higher EDSS score and lower thalamic volume at baseline compared with SPMS non-convertors.

Table 7. Demographic clinical, and MR imaging features of SPMS convertors and SPMS non-convertors, and comparisons between variables at baseline and after the 5-year follow-up. Modified from original publication III.

	SPMS CONVERTORS			SPMS NON-CONVERTORS			CONVERTED VS. NOT
	BL	5Y FU	Change p-value	BL	5Y FU	Change p-value	Comparison, p-value
Females, n (%)	7 (88)			10 (67)			0.4
Follow-up time (y)	5.0 (0.6)			5.1 (0.2)			0.6
Age (y)	47 (3.8)	52 (3.7)	**< 0.001**	47 (4.1)	52 (4.1)	**< 0.001**	0.8
Disease duration (y), median (IQR)	13.6 (11.8–20.5)	18.8 (17.2–25.4)	**0.014**	9.5 (5.8–13.6)	14.5 (11.0–18.7)	**< 0.001**	0.056
EDSS, median (IQR)	3.3 (2.9–4)	4 (3.8–4.9)	**0.013**	2.5 (2.3–3)	3 (2.2–3.5)	**0.008**	**0.028**
DMT, n (%)							
No DMT	1 (13)	1 (13)		2 (13)	3 (20)		
ME-DMT	7 (88)	3 (38)	0.077	13 (87)	9 (60)	0.17	1
HE-DMT	0	4 (50)		0	3 (20)		
Brain volume (cm^3)	1085 (120)	1052 (114)	**< 0.001**	1175 (98)	1146 (94)	**< 0.001**	0.096
NAWM volume (cm^3)	417 (73)	410 (71)	0.15	474 (57)	469 (56)	**0.004**	0.080
Cortical GM volume (cm^3)	417 (40)	394 (37)	**< 0.001**	444 (32)	422 (28)	**< 0.001**	0.12
Thalamic volume (cm3)	12.4 (1.8)	11.9 (1.5)	**0.011**	14.2 (1.8)	13.8 (1.7)	**0.015**	**0.040**
T1 lesion load (cm^3), median (IQR)	7.0 (3.0–15.5)	7.2 (4.5–15.9)	**0.021**	2.7 (1.4–4.2)	2.9 (1.5–4.8)	**0.001**	0.087
T2 lesion load (cm^3), median (IQR))	12.6 (7.1–26.3)	13.4 (7.8–26.3)	0.059	4.2 (3.7–8.5)	4.7 (3.9–9.4)	**0.010**	0.065
Serum GFAP (pg/ml), median (IQR)	106 (67–112)			73 (63–104)			**0.034**

Values are presented as mean (SD) unless otherwise stated. Disease duration is from MS-diagnosis. Moderate-efficacy DMTs include interferons, glatiramer acetate, teriflunomide and fingolimod. High-efficacy DMTs include cladribine, rituximab and natalizumab. Significant p-values are bolded. *Number of PRLs is from QSM-MRI 2-4 years after baseline. Abbreviations: BL = baseline; DMT = disease modifying therapy; EDSS = Expanded Disability Status Scale; FU = follow-up; GFAP = glial fibrillary acidic protein; GM = grey matter; HE-DMT = high-efficacy DMT; IQR = interquartile range; ME-DMT = moderate-efficacy DMT; NAWM = normal appearing white matter; PRL = paramagnetic rim lesion; y = year.

Compared with age- and sex-matched HCs, pwMS had higher DVR values reflecting the specific [^{11}C]-PK11195 binding in NAWM and the thalamus ($p = 0.030$ and $p = 0.06$; respectively) at baseline PET imaging. However, no differences were observed in [^{11}C]-PK11195 binding in cortical GM.

At baseline, higher [^{11}C]-PK11195 binding in NAWM, the thalamus, and the perilesional area was observed in SPMS convertors compared with SPMS non-convertors (Figure 12). Moreover, the proportion (percentage) of active voxels in the thalamus was higher in SPMS convertors compared with SPMS non-convertors (34 (6.7) vs. 25 (9.6); $p = 0.023$). No differences in the proportion of active voxels in NAWM or the perilesional area were observed.

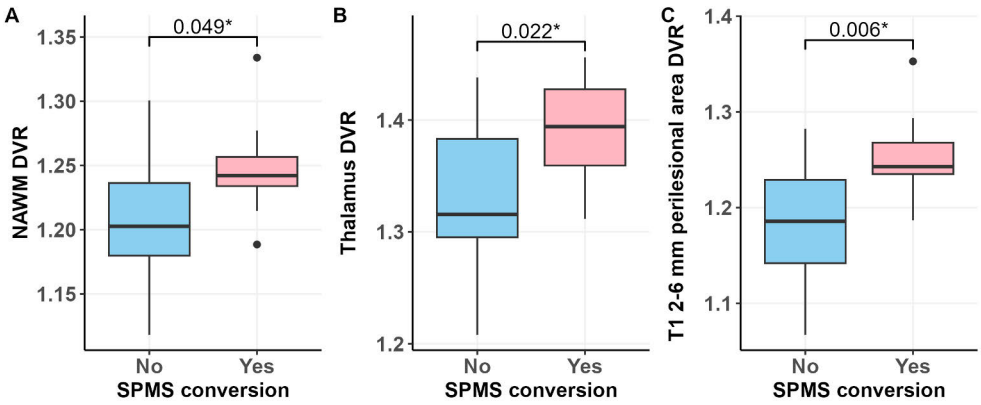

Figure 12. Comparison of [^{11}C]-PK11195 distribution volume ratio (DVR) values between SPMS convertors (n=8) and SPMS non-convertors (n=15) at baseline in (**A**) normal-appearing white matter (NAMW), (**B**) the thalamus, and (**C**) the perilesional area. *Significant at the level of $p<0.05$. Modified from original publication III.

TSPO-PET rim-active lesions were observed in 16 patients. The number and the proportion of RALs of all chronic T1 lesions at baseline was higher in SPMS convertors compared with SPMS non-convertors (Figure 13). The median proportion of inactive lesions did not differ between the two groups (25 (22–33) vs. 33 (27–65); $p = 0.18$).

PRLs were observed in 12 patients. No difference in the median number of PRLs 2-4 years after baseline was observed between SPMS convertors and SPMS non-convertors (2 (0–3) vs. 0 (0–1); $p = 0.14$).

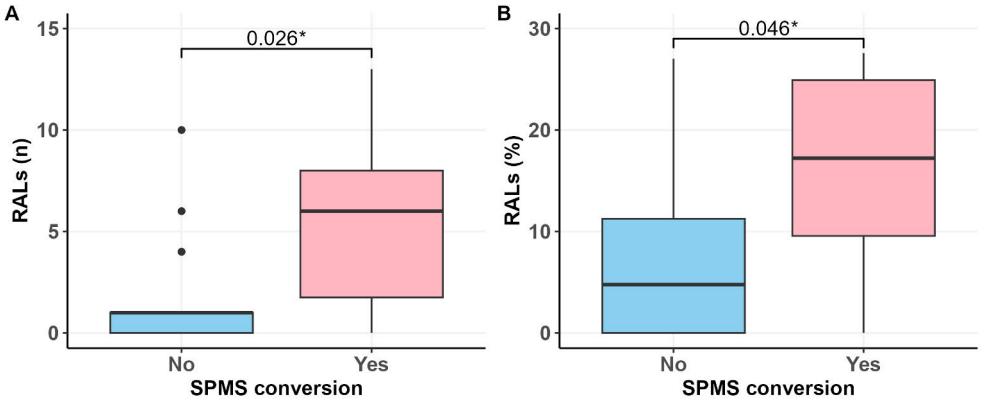

Figure 13. Number of TSPO-PET rim-active lesions (RALs) and proportion of TSPO-PET rim-active lesions in SPMS convertors (n=8) and SPMS non-convertors (n=15). *Significant at the level of p<0.05. Modified from original publication III.

SPMS convertors had higher median sGFAP concentrations compared with SPMS non-convertors (106 (100–115) vs. 73 (63–104) pg/ml), but no significant differences were observed in age-, sex-, and BMI-adjusted sGFAP z-scores (Figure 14). In addition, slightly higher absolute median sNfL concentrations were observed in SPMS convertors (11 (8.4–15) vs. 8 (6.9–8.8). However, the difference between groups was not statistically significant (p = 0.076). No difference between the groups was observed in sNfL z-scores (p = 0.11) either.

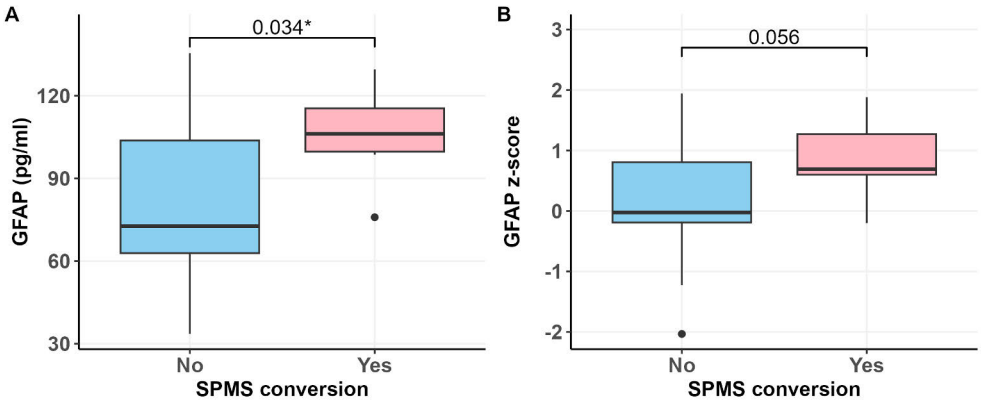

Figure 14. Serum glial fibrillary acidic protein (GFAP) concentration and z-scores in SPMS convertors (n=8) and SPMS non-convertors (n=15). *Significant at the level of p<0.05. Modified from original publication III.

6 Discussion

The prediction of MS disease course is challenging, and the prognosis of MS varies remarkably between individuals. Several clinical and imaging prognostic markers are widely used in clinical practice to guide treatment decisions. However, to date, no reliable prognostic biomarkers exist that can predict disease course, monitor disease progression, or assess treatment effects at the individual level. Thus, there is a need for new, personalized prognostic biomarkers. In particular, a deeper understanding of the mechanisms underlying disease progression is of interest for the development of new biomarkers capable of measuring progression-related smoldering pathology. As therapies targeting progressive CNS pathology are likely to become available in the near future (Fox et al., 2025b), tools for identifying patients at risk of disease progression with ongoing smoldering inflammation are urgently needed. In this thesis, the usability of TSPO-PET and other recent imaging and serum biomarkers in predicting progressive MS course was investigated in a homogenous cohort of late-stage pwRRMS. In addition, the associations between previously identified risk factors for poor disease prognosis and subsequent TSPO-PET measurable microglial activation were studied, as well as the effect of sex on [^{11}C]-PK11195 binding.

6.1 The Effect of Early Prognostic Factors on Later TSPO-binding (Study I)

We found that the number of T2 lesions on diagnostic brain MRI was associated with increased TSPO-PET measurable microglial activation in several brain regions important for MS progression on average 12 years later. Also, the magnitude of IgG index in the diagnostic CSF sample was associated with later increased TSPO binding in NAWM. Moreover, pwMS who experienced early disability accumulation (defined as EDSS score at least 2.0 five years after MS diagnosis) had increased perilesional TSPO binding compared with those with lower EDSS scores at the same time point.

Previous studies have demonstrated that early T2 lesion load on brain MRI associates with later physical disability and the risk of SPMS conversion (Fisniku et al., 2008; Popescu et al., 2013; Uher et al., 2017). Similarly, higher EDSS score at

diagnosis and early disability accumulation have been identified as risk factors of poor prognosis (Kappos et al., 2015; Rudick et al., 2010; Sharmin et al., 2023; Traboulsee et al., 2016; Uher et al., 2017). Therefore, the associations between the number of T2 lesions on diagnostic MRI and early disability with later higher TSPO binding were not unexpected, as TSPO-PET measurable microglial activation has been shown to correlate with worse long-term disease outcomes and clinical disease progression in several studies reviewed earlier (Banati et al., 2000; Datta et al., 2017; Giannetti et al., 2015; Misin et al., 2022; Politis et al., 2012; Polvinen et al., 2023; Rissanen et al., 2014; Sucksdorff et al., 2020). These findings reflect towards a disease trajectory where increased microglial activity is strongly linked with preceding and subsequent clinical and paraclinical disease activity.

In addition, a correlation between higher IgG index at diagnosis and later TSPO-PET measurable microglial activation was observed. Previous studies have suggested that IgG index has moderate predictive value for the long-term disease course (Akaishi et al., 2020; Gasperi et al., 2019; Izquierdo G et al., 2002). In a recent study, a correlation between the IgG index and increased CSF NfL levels was observed, suggesting an association between intrathecal IgG synthesis and neuro-axonal damage that is more prominent in patients with worsening MS. In the same study, an increased number of IgG-positive B cells was observed in the meninges of postmortem brain samples from pwSPMS, proposing an association between intrathecal IgG-producing B cells and microglial activation (Rodriguez-Mogeda et al., 2024). Other studies have also suggested that CSF-derived factors may drive microglial activation (Fadda et al., 2019; Mahajan et al., 2020). Increased TSPO binding in NAWM surrounding ventricles (as well as in cortical GM in Study II of this thesis) might be explained by these findings.

Altogether, the results from this study propose a link between early adaptive immune cell activation and the development of later progression-promoting pathology (Calabrese et al., 2024; Giovannoni et al., 2022; Kuhlmann et al., 2023; Scalfari et al., 2024). This can be hypothesized because the formation of acute T2 lesions associates with focal adaptive immune cell activation, an elevated IgG index can be interpreted as a manifestation of widespread intrathecal adaptive immune cell activation, and early disability accrual may relate to incomplete recovery from relapses (another manifestation of acute focal inflammatory activity) or may also reflect neurodegenerative mechanisms and PIRA already present from early disease stages (Cree et al., 2019). As several real-world registry-based studies have highlighted the potential of early high-efficacy treatments (that attenuate effectively acute inflammation) to delay disability accumulation (Buron et al., 2020; Harding et al., 2019; He et al., 2020; Iaffaldano et al., 2021b), the results from this study suggest that patients with a high number of T2 lesions on diagnostic MRI, an elevated IgG

index at diagnosis, and/or early increase in EDSS score may particularly benefit from treatment with high-efficacy DMTs to modulate their disease course.

6.1.1 Methodological Considerations and Limitations

Study participation was limited by age and disease duration to create a homogenous cohort of patients with late stage RRMS. This selection was intended to ensure potential variability in glial cell expression, thereby enabling the detection of possible differences in TSPO binding between participants despite the rather small study cohort. The rationale for the inclusion criteria was based on observations from large epidemiological studies indicating that increasing disease duration and age are the two most important demographic factors predicting conversion to SPMS, which typically occurs at the age of 40-50 years and after 15-20 years of disease duration (Koch et al., 2010; Leray et al., 2010; Tremlett et al., 2008; Tutuncu et al., 2013).

Several limitations should be considered when interpreting the results. First, missing data in some parameters collected from medical records may have impaired the statistical power of certain analyses. Variability in the MRI scanners used may have influenced the estimation and reported number of T2 lesions because the quality and resolution of MR images differs depending on the MRI camera used. Moreover, as original diagnostic MR images were not available for all patients, we estimated the number of T2 lesions from neuroradiologist´s report when classifying patients into T2 lesion subgroups. This may have caused under- or overestimation of the number of T2 lesions. Additionally, it is acknowledged that lesion sizes can vary significantly. However, due to the study design, we were unable to calculate total lesion load or determine size of individual lesions on the initial clinical MR images. As this was a real-world cohort of pwMS, participants had received various DMTs during their disease course, which may have influenced the observed TSPO binding, given that DMTs can affect microglial activity (Kaunzner et al., 2017; Lehto et al., 2023b; Ratchford et al., 2012; Sucksdorff et al., 2019, 2017). However, the variability in the use of DMTs and other potential confounding factors was carefully considered in the statistical analyses.

6.2 The Effect of Sex on [^{11}C]-PK11195 Binding (Study II)

Higher TSPO binding in several brain regions was found in male pwMS compared with female pwMS. A similar sex difference was observed in healthy individuals, with men having higher [^{11}C]-PK11195 binding compared with women in the whole brain, WM, and the thalamus. Additionally, TSPO binding associated more strongly with disability (EDSS score) in male pwMS compared with female pwMS. As male

sex is a known risk factor for faster disability progression and SPMS conversion (Leray et al., 2010; Ribbons et al., 2015; Scalfari et al., 2014; Sharmin et al., 2023; Tremlett et al., 2008; Vukusic and Confavreux, 2003), the results from this study suggest that inherent sex-variability in microglia predisposes male pwMS to worse long-term disease outcomes.

Potentially, differences in the function and phenotype of microglial cells between men and women could explain the observed sex difference in [^{11}C]-PK11195 binding. Previous animal and cell culture studies have demonstrated clear sex differences in microglial density and morphology (Guneykaya et al., 2018; Schwarz et al., 2012), gene expression (Barko et al., 2022; Villa et al., 2018), and functional properties such as migration capacity, phagocytic activity, and responsiveness to IFN (Thion et al., 2018; Weinhard et al., 2018; Yanguas-Casás et al., 2020, 2018) throughout the life.

A protective role of sex hormones on microglia in women could also explain the observed increased [^{11}C]-PK11195 binding among men. Previous studies have demonstrated that microglia from adult male mice express ERs (Baker et al., 2004; Sierra et al., 2008), and estrogen seems to have an anti-inflammatory effect on microglia as reviewed earlier (Baker et al., 2004; Bruce-Keller et al., 2000; Ghisletti et al., 2005; Vegeto et al., 2003, 2001). In supplementary analyses of Study II, we did not observe differences in [^{11}C]-PK11195 binding between pre- and postmenopausal women. This suggests that protective role of estrogen can only partly explain the lower [^{11}C]-PK11195 binding among female pwMS and healthy women.

Sex differences in [^{11}C]-PK11195 binding were also studied in subgroups of pwSPMS and pwRRMS, and greater sex differences were observed among pwSPMS. Among all subgroups, the highest [^{11}C]-PK11195 binding was observed in male pwSPMS, whereas healthy women had the lowest TSPO binding. This finding is in line with previous MRI studies suggesting that male pwMS are more susceptible to neurodegeneration (linked to microglial activation), as more prominent brain atrophy and ventricular enlargement have been observed in male pwMS compared with female pwMS (Jakimovski et al., 2020; Pozzilli et al., 2003; Schoonheim et al., 2012). Previous neuropathological studies have also demonstrated a higher proportion of chronic active lesions in male pwMS than female pwMS, suggesting more prominent and persistent microglial activation surrounding chronic active lesions in men (Frischer et al., 2015; Luchetti et al., 2018). Sex differences in TSPO rim-active lesions or perilesional [^{11}C]-PK11195 binding were not studied in the present study.

As far as I know, this was the first study exploring sex differences in [^{11}C]-PK11195 radioligand binding in pwMS and healthy individuals. One previous study assessing sex differences in second-generation TSPO-ligand [^{11}C]-PBR28 binding

in healthy individuals has been published. In contrast to our findings of male predominance, that study found higher [^{11}C]-PBR28-binding in healthy women compared with men in several cortical GM regions, was well as in the thalamus, and hippocampus. Results from these studies suggest that in the future TSPO-PET studies, sex differences in TSPO binding should be considered while designing, analyzing and interpreting the results. Additionally, sex-related inherent increase in microglial activation in men may explain the greater likelihood of disease progression among male pwMS. However, further studies are needed to confirm these results.

6.2.1 Methodological Considerations and Limitations

The large sample size was a strength of the study, as the sample size in PET imaging studies is typically much smaller. Healthy individuals were imaged at two different centers, which may have caused variability in DVR values, even though similar PET scanners were used. As in Study I, pwMS were using and had used different DMTs prior to PET imaging, which may have influenced microglial activation and the observed [^{11}C]-PK11195 binding. However, no differences in DMT use at the time of PET imaging were observed between male and female pwMS.

6.3 TSPO-PET as a Prognostic Tool for MS Disease Progression (Study III)

We found that several baseline TSPO-PET imaging related parameters were associated with conversion to SPMS phenotype after 5 years. SPMS convertors had higher TSPO binding in NAWM compared with patients remaining in RRMS phenotype. The proportion of active voxels and TSPO binding in the thalamus were also higher in SPMS convertors. Additionally, increased TSPO binding in the perilesional area, a higher number of TSPO-PET-defined chronic active lesions (RALs), and a higher proportion of T1 lesions classified as RALs were observed in SPMS convertors compared with SPMS non-convertors. When different studied prognosticators were compared, perilesional TSPO binding was a strong separator of SPMS convertors and SPMS non-convertors. Results from our study demonstrate, for the first time, an association between TSPO-PET measurable microglial activation and later SPMS conversion in a longitudinal study setting. This finding is in line with previous studies demonstrating an association between increased TSPO binding and MS disease progression (Misin et al., 2022; Polvinen et al., 2023; Sucksdorff et al., 2020).

Several earlier MRI studies have shown that atrophy of GM structures is linked to MS disease progression (Cagol et al., 2022; Eshaghi et al., 2018; Hänninen et al.,

2020; Rocca et al., 2022, 2010). Consistent with findings from larger patient cohorts, we found lower thalamic volume on baseline MRI in SPMS convertors. Numerous previous studies, reviewed in detail earlier, have also demonstrated an association between PRLs and more severe disease outcomes and disease progression (Absinta et al., 2019; Altokhis et al., 2022; Borrelli et al., 2024; Cagol et al., 2024a; Assunta Dal-Bianco et al., 2021; Preziosa et al., 2024; Reeves et al., 2024b; Weber et al., 2022). However, differences in the number of PRLs were not observed between SPMS convertors and SPMS non-convertors in this study. This may be due to the relatively small cohort size, as PRLs were detected in only 12 pwMS.

Higher sGFAP levels were observed in SPMS convertors compared with SPMS non-convertors, whereas sNfL levels did not differ significantly between the groups. This finding is in line with recent studies suggesting that sGFAP is more accurate predictor of disease progression than sNfL (Abdelhak et al., 2025; Benkert et al., 2024; Meier et al., 2023). Additionally, we replicated the previously and consistently demonstrated finding that higher EDSS scores are associated with an increased long-term risk of disease progression and SPMS conversion (Kappos et al., 2015; Rudick et al., 2010; Sharmin et al., 2023; Traboulsee et al., 2016; Uher et al., 2017).

In summary, TSPO-PET imaging parameters, sGFAP levels, thalamic volume, and EDSS score were biomarkers predicting SPMS conversion in this study cohort. Brain volume measurements, serum biomarkers, and clinical patient characteristics are already applicable in clinical practice. However, several technical and methodological challenges must be resolved before wider clinical implementation of thalamic volume or sGFAP measurements. TSPO-PET imaging is currently more feasible for research purposes, including phase 2 clinical trials evaluating treatments targeting progressive disease mechanisms. Additionally, the identification of blood-based or other biomarkers correlating with TSPO-PET measures could provide clinically applicable tools for monitoring smoldering inflammation. Altogether, the results of this study suggest that in patients presenting with signs or symptoms suggestive for SAW and disease progression, the measurement of thalamic volume and sGFAP could help stratify the risk of progressive disease and may be considered in clinical practice, despite the lack of validated and standardized methods.

6.3.1 Methodological Considerations and Limitations

To ensure a sufficient number of participants converting to SPMS, the study cohort was enriched for patients at risk of disease progression through selected inclusion criteria. Age enrichment most likely increased the proportion of patients converting to SPMS and enabled the detection of differences in the studied parameters between SPMS convertors and non-convertors, despite the small cohort size. Such age enrichment could also be utilized in clinical trials evaluating treatments targeting to

prevent or slowdown MS disease progression, thereby allowing smaller study cohorts. Notably, due to this age enrichment, these results are potentially applicable in pwMS aged 40-50 years with a longer disease duration. Additionally, we used the clinical Lublin criteria for SPMS diagnosis (Lublin et al., 2014) and evaluated systematically SAW-related symptoms (including increase in cognitive symptoms and fatigue) when evaluating possible conversion to SPMS. This likely enabled us to detect progression to the SPMS phase more sensitively, while still maintaining reliability, compared with purely EDSS-based SPMS classifications (Kappos et al., 2018a; Lorscheider et al., 2016; Manouchehrinia et al., 2018). Unfortunately, 9HPT or T25FW were not included in the study protocol, although they could have provided more objective information on disease progression. Overall, the unique longitudinal study design with a 5-year systematic follow-up and simultaneous evaluation of multiple biomarkers was a strength of this study.

The relatively small cohort size is a critical limitation of the study and prevented adjustment of the results for confounding factors. Therefore, these results should be confirmed in a larger patient cohort. Likewise in other studies, participants were treated with various DMTs during the study, which may have influenced the results, The effect of DMTs on studied biomarkers or disease progression during the study could not be considered in statistical analyses. However, no difference in the time on DMT before baseline was observed between SPMS convertors and non-convertors, and time on DMT before baseline did not correlate with any studied biomarkers at baseline. Additionally, differences in the MRI scanners used during the study may have influenced the volumetric results, despite the MRI scanners being largely similar. Finally, the consensus statement for the radiological definition of PRLs (Bagnato et al., 2024) was not yet published, and thus this definition was not applied to the data of Study III. In the future, automated methods for detecting PRLs and tools for quantifying PRL features, such as rim intensity, are likely replace the current expert visual assessment.

6.4 General Methodological Considerations and Limitations (Studies I-III)

The radioligand used in Studies I-III was the first generation TSPO-radioligand [^{11}C]-PK11195, which has several known shortcomings (see also Chapter 2.4.6.2). Nonetheless, several benefits support the use of the [^{11}C]-PK11195 radioligand, including long experience in PET imaging of neuroinflammation, well-validated post-processing and image analysis methods, and insensitivity to genetic polymorphism (rs6971), which requires patient genotyping when using second-generation TSPO-radioligands (Bodini et al., 2021b; Giannetti et al., 2014; Politis et al., 2012; Rissanen et al., 2018, 2014; Salerno et al., 2024; Sucksdorff et al., 2020;

Turkheimer et al., 2007b). Additionally, controversies remain regarding the source of TSPO signal and consequently, the interpretation of TSPO-PET findings. As reviewed earlier, TSPO expression is not specific to microglia (Guilarte et al., 2022; Lavisse et al., 2012; Nutma et al., 2019). Moreover, recent studies have suggested that instead of activated/altered phenotype, TSPO binding reflects microglial density (Nutma et al., 2021b, 2019; Owen et al., 2017; Wijesinghe et al., 2025). However, regardless of the exact cellular source of the TSPO signal in PET imaging, studies in the context of MS have repeatedly demonstrated the harmful nature of increased TSPO binding in the MS brain, which correlates with important clinical and MRI outcomes, treatment responses, and disease progression, as reviewed in detail earlier (Banati et al., 2000; Debruyne et al., 2003; Giannetti et al., 2014; Herranz et al., 2016; Pitombeira et al., 2022; Rissanen et al., 2018, 2014; Sucksdorff et al., 2019).

In neuropathological studies the rims of chronic active lesions containing activated microglia are typically less than 1 mm in width (BRLs at least 1mm) (Klotz et al., 2025). Thus, the source of the TSPO signal detected in the perilesional area (up to 6 mm in this thesis) may be questioned. However, in addition to the rims of chronic active lesions, activated microglia are present also in the WM surrounding chronic active lesions in MS brain samples (Hendrickx et al., 2017). TSPO signal may also partly arise from activated astrocytes, which also express TSPO (Nutma et al., 2019). Moreover, the resolution of the HRRT PET scanner used in this thesis was approximately 2.5 mm, which may have resulted in partial overlap of the detected TSPO signal between perilesional and lesional ROIs.

General limitations related to PET imaging are reviewed earlier in Chapter 2.4.4. Globally, high costs and limited accessibility substantially limit the widespread use of TSPO-PET imaging. In addition, exposure to ionizing radiation limits sample sizes and may result in unpowered studies reporting non-reproducible results. For the same reason, serial scans should be avoided. PET modelling and quantification processes are complex, and the applied models are only approximations of the underlying biological processes. Reference tissue models may be biased due to specific binding in the selected reference region, as MS pathology diffusively influences the whole brain. During image processing, all lesion masks used in the studies were checked and manually corrected slice by slice, which may have caused intra- and interrater variability. Comparison of TSPO-PET studies performed at different imaging centers – particularly when different types of scanners are used – is challenging, as the methodologies used in TSPO-PET image processing lack standardization. Therefore, careful validation and harmonization of methodologies will be necessary to allow multicenter studies with larger patient cohorts in the future.

6.5 Future Directions

Currently available MS treatments control the acute inflammatory activity effectively and have improved the long-term disease prognosis remarkably but fail to target the neurodegenerative disease mechanisms leading to progressive MS (Brown et al., 2019; Buron et al., 2020; Cree et al., 2016; Fambiatos et al., 2020; Harding et al., 2019; He et al., 2020; Iaffaldano et al., 2021b; Tedeholm et al., 2022). Consequently, the lack of treatment options for progressive MS has been one of the greatest unmet needs in the field, and MS continues to be one of the leading causes of neurological disability among young adults worldwide (Walton et al., 2020). Tolebrutinib will likely be the first DMT approved for the treatment of non-relapsing MS in the near future (Fox et al., 2025b). However, there remains a need for a wider repertoire of treatments with different mechanisms of action for pwPMS. A deeper understanding of the molecular processes underlying smoldering inflammation and neurodegenerative mechanisms may reveal new targets for future treatments, especially for progressive MS. TSPO-PET imaging enables *in vivo* evaluation of pathology related to disease progression and could be considered as an imaging outcome measure in future clinical trials, allowing evaluation of treatment effects on both diffuse and lesional microglial activation. Additionally, TSPO-PET enables the enrichment of patient cohorts for smoldering inflammation, hence allowing more efficient trials when outcomes are specific to the target of therapy. In addition to evaluating of microglial activation and smoldering inflammation, PET imaging can also assess other biological processes – such as demyelination, remyelination, neurodegeneration, and synaptic density – using radioligands specific to these processes (Bodini et al., 2021; Leoma et al., 2025; Ullrich Gavilanes et al., 2025; van der Weijden et al., 2022). The evaluation of chronic active lesions with advanced MRI techniques is increasingly being incorporated into clinical trials and post-marketing studies to assess the effects of treatments on non-progressive biology (Bagnato et al., 2024; Dal-Bianco et al., 2024).

An improved understanding of the pathophysiological mechanisms underlying progressive MS has highlighted the importance of progression-related disease mechanisms already in the early stages of the disease. This has led to the concept of MS as a single disease continuum from relapsing to progressive forms, rather than distinct clinical phenotypes (Kuhlmann et al., 2023; Lassmann, 2018). Identifying patients at risk of disease progression or with prominent smoldering inflammation may become valuable in the future to enable the allocation of therapies targeting progressive biology to those most likely to benefit. Moreover, it is probable that future treatment of pwMS will be multimodal, combining efficient suppression of acute inflammation, early attenuation of smoldering inflammation, and the use of neuroprotective and/or remyelinating agents.

In recent years, numerous potential biomarkers of disease progression have been examined. These include advanced MRI techniques, such as detection of PRLs (Absinta et al., 2019; Altokhis et al., 2022; Reeves et al., 2024b), brain volume measures (Cagol et al., 2022; Cree et al., 2019; Eshaghi et al., 2018), soluble biomarkers including sGFAP and sNfL (Abdelhak et al., 2025; Meier et al., 2023), and TSPO-PET imaging (Bodini et al., 2021). The evaluation of PRLs is already feasible in clinical practice, as they can be visualized using 1.5T and 3T MRI scanners, which are already widely available. The inclusion of PRLs in the 2024 McDonald criteria (Montalban et al., 2025) will likely increase the use of PRLs in clinical practice and provide additional information on the prognostic value of PRLs. Measurement of global brain volume and cervical cord atrophy is recommended by the MAGNIMS study group for clinical practice (Sastre-Garriga et al., 2020). However, technical challenges and the lack of clear guidance limit the routine use of atrophy measures (Rocca et al., 2017). Similarly, methodological challenges and the lack of established reference values delay the implementation of sNfL and sGFAP in clinical practice (Di Filippo et al., 2024). Recent studies suggest that combination of sNfL and sGFAP may have the greatest predictive value (Benkert et al., 2024; Meier et al., 2023). TSPO-PET imaging is not currently suitable for widespread clinical use due to its limited availability and other shortcomings. However, it can be beneficial for research purposes. Additionally, when available, even a single TSPO-PET scan may help identify pwMS at high risk of disease worsening (Polvinen et al., 2023; Sucksdorff et al., 2020).

Furthermore, additional biomarkers reflecting cell- and pathology-specific processes across various stages of MS are currently under investigation. Examples of potential future CSF biomarkers for progressive MS include markers of microglia and astrocyte activation, such as CHI3L1 and chitinase 1 (CHIT1) (Beliën et al., 2024; Floro et al., 2022; Oldoni et al., 2020). Additionally, blood and CSF biomarkers reflecting glial dysfunction, as well as axonal and neuronal damage, demyelination, inflammation, and immunomodulation, have been investigated (Di Filippo et al., 2024; Gill et al., 2023; Yang et al., 2022). In addition to PRLs, other advanced MRI techniques such as diffusion weighted imaging methods enable the detection of tissue-specific microstructural and functional changes and may help capture early tissue injury, thus improving the prediction of disease prognosis (Filippi et al., 2001; Granziera et al., 2021; Schiavi et al., 2021). Moreover, broad rim lesions may emerge as a novel PET imaging biomarker in the future (Klotz et al., 2025), alongside radioligands targeting different biological processes relevant to progressive MS (Bodini et al., 2021; Luoma et al., 2025; Ullrich Gavilanes et al., 2025; van der Weijden et al., 2022). Finally, artificial intelligence (AI) and machine learning may further enhance MRI analysis together with other automated quantification tools, such as lesion segmentation and quantification of brain

volumes, to improve the sensitivity (Rocca et al., 2024). Moreover, AI-based methods that integrate clinical, imaging, and soluble biomarker data may support the differential diagnosis of MS, as well as the monitoring and prediction of treatment response, and evaluation of long-term disease prognosis (Amin et al., 2024; Andorra et al., 2024; Eshaghi et al., 2021; Ganjgahi et al., 2025; Pontillo et al., 2022; Yousef et al., 2024).

The remarkable variability in disease activity and progression in pwMS poses significant challenges for MS clinicians. Despite improvements in the biomarker field, there is currently no reliable marker predicting disease progression and treatment response in an individual patient, and novel biomarkers for monitoring disease evolution and treatment response are still needed. Considering the complex pathogenesis of MS, it is likely that a combination of different biomarkers will provide the greatest benefit for assessing individual prognosis and designing personalized treatment strategies.

7 Summary/Conclusions

This study explored which demographic, clinical, and paraclinical factors are associated with later microglial activation measured by TSPO-PET imaging, and whether sex affects the binding of [^{11}C]-PK11195 radioligand, which is widely used in PET imaging studies of neuroinflammation. The prognostic value of TSPO-PET imaging, MRI measures, and serum biomarkers for disease progression was also evaluated.

1. Study I demonstrated that a higher number of WM lesions on diagnostic brain MRI, a higher IgG index at MS diagnosis, and early disability progression (EDSS score ≥ 2.0 five years after diagnosis) were associated with increased microglial activation in several brain regions approximately 12 years later. These findings suggest a link between early inflammatory activity and the development of later progression-related pathology.
2. In Study II sex-difference in the binding of [^{11}C]-PK11195 radioligand was demonstrated in a large cohort of 102 pwMS and 76 HCs. Higher [^{11}C]-PK11195 binding was observed in WM areas and the thalamus in healthy men and male pwMS compared with women. In the subgroup analyses, male pwSPMS had the highest [^{11}C]-PK11195 binding, whereas the lowest [^{11}C]-PK11195 binding was observed in healthy women. These findings suggest that men may have inherently higher levels of microglial activation or density, which potentially predisposes male pwMS to faster disease progression and worse disease outcomes compared with women.
3. In Study III, increased TSPO-PET measurable microglial activation in several brain regions, elevated serum GFAP, and lower thalamic volume predicted SPMS conversion after 5 years in late-stage pwRRMS, highlighting the utility of these biomarkers for assessing disease progression.

In conclusion, this thesis demonstrates that early clinical and paraclinical factors are associated with later microglial activation that may drive disease progression. It further suggests that TSPO-PET, together with serum GFAP levels and thalamic volume, can be useful for assessing disease prognosis in pwMS. Future research with

larger patient cohorts is needed to validate these findings. Moreover, sex should be considered as a potential confounding factor in TSPO-PET imaging studies. Finally, identifying patients at risk of disease progression or with ongoing smoldering inflammation could enable therapies targeting progressive biology to be directed effectively to those most likely to benefit.

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