A multifaceted approach to understand cognitive impairment in MS: exploring the nonlinearity of cognition

Dam, M. van

1

General Introduction

MULTIPLE SCLEROSIS

Multiple sclerosis (MS) is a chronic disease of the central nervous system, i.e., the brain and spinal cord. MS is characterized by inflammation, demyelination and neurodegeneration in both white and grey matter tissue,[1] leading to a wide variety of symptoms. The most frequently reported symptoms include motor symptoms (muscle weakness, loss of coordination, and difficulty with balance or walking), sensory symptoms (numbness, tingling, and pain in various parts of the body), visual problems (blurred or double vision, or even vision loss), bladder and bowel dysfunction (problems with bladder control, urgency, or constipation), cognitive impairment (memory problems, reduced information processing speed, and difficulties with concentration and problem-solving), fatigue (extreme tiredness, reportedly described as "MS-fatigue"), and mood disturbances and anxiety.[2]

The unpredictability of MS

MS is notorious for its unpredictable disease course, not only because of the heterogeneity in how extensive the damage can become in the central nervous system, but also due to the diverse array of symptoms that can vary greatly between individuals and fluctuate over time.[3] This heterogeneity is also evident in the how the disease develops over time: while some people experience relapses where symptoms suddenly worsen and then (partially) recover (relapsing-remitting MS), others may have a more steady progression of symptoms with little to no clear relapses (primary and secondary progressive MS).[2] This uncertainty when symptoms might flare up or worsen, makes it difficult for people with MS to plan their lives. MS is often diagnosed in young adulthood,[4] a time when individuals are typically building careers, relationships, and families. As a result of the disease, many people with MS struggle to maintain employment, often due to factors like fatigue, cognitive impairment, and physical limitations.[5, 6] As the disease progresses, cumulative damage to the central nervous system leads to more severe and widespread symptoms.[7] In such later stages, neurodegeneration becomes more prominent and can cause further cognitive decline.[3] The unpredictable nature of MS, combined with its physical and cognitive symptoms, can lead to social isolation, anxiety, depression, and a reduced quality of life.[8, 9] While there are treatments available to slow inflammation and manage symptoms, there is currently no cure, and these treatments cannot reverse the neuronal damage that already has taken place. In fact, disease progression during effective treatment can still continue over time, resulting in a gradual accumulation of disability that remains difficult to treat.[10]

MS in statistics

MS affects approximately 2.8 million people worldwide, with estimates of 25.000 people being affected in the Netherlands.[4] In the Netherlands, the prevalence rate is estimated at 150 per 100.000 people.[4] The prevalence of MS is increasing

worldwide; since 2013, it has risen by more than 30%, which has been attributed to better detection and survival, such as the widespread access to Magnetic Resonance Imaging (MRI) scanners and modifications to diagnostic criteria enabling earlier disease identification.[11] The first symptoms of the disease typically appear in young adulthood, with the average age at diagnosis being 32 years.[4] Approximately 43% of people with MS stop working within three years after diagnosis, a percentage that increases to 70% after ten years.[12] Women are more often affected than men (ratio 3:1 in the Netherlands).[13] While the underlying cause of MS remains unknown, the risk of developing MS is increased by well-established environmental factors, such as vitamin D deficiency, smoking, and Epstein-Barr virus infection, as well as various genetic predispositions.[14, 15]

The pathological hallmarks in MS

MS primarily involves two key pathological processes: inflammation and neurodegeneration.[1] These processes manifest in varying degrees among people at the onset of the disease and may evolve within an individual over time.[16]

Inflammation. The immune system, activated by an unknown trigger, targets the myelin sheath surrounding axons, which is crucial for efficient transmission of information between neurons.[1] This immune-mediated inflammatory process causes demyelination, leading to a loss of myelin around the axons and resulting in inefficient communication between neurons.[13] There is ongoing debate about whether this trigger originates from within the central nervous system itself (the "inside-out" paradigm) or from an external event outside the brain (the "outside-in" paradigm).[17] The loss of myelin leads to the formation of lesions, or plaques, which primarily develop around blood vessels.[18] These lesions are marked by inflammation, scar tissue formation (gliosis), and axonal loss.[19] While lesions occur in both white and grey matter, they are more difficult to detect in grey matter (e.g., cortical lesions) with standard imaging techniques.[16, 20] Over time, some damaged areas may partially or fully recover through remyelination. Inflammation can be present throughout the disease course but tends to be more predominant in its earlier stages.[16]

Neurodegeneration. Neurodegeneration is the second significant pathological hallmark of MS. This process becomes more prominent as the disease progresses and serves as a major contributor to clinical disabilities, including cognitive impairment.[21-23] The loss of support by demyelination can cause degeneration of axons and neurons, and ultimately leads to tissue loss in both white and grey matter, a process known as atrophy.[24] Atrophy of the white matter is hypothesized to result from axonal shrinkage and loss following chronic demyelination.[21] Conversely, grey matter atrophy appears to arise from neuroaxonal shrinkage and loss, often due to degeneration along the axon, a process known as Wallerian degeneration.[25] The interaction between inflammation and neurodegeneration varies from person to

person and throughout the disease course, the underlying mechanisms of which are not yet fully understood.[23, 26]

Diagnosis of MS

Diagnosing MS involves the combination of a neurological examination, assessment of the patient's clinical history, and supporting tests such as MRI and cerebrospinal fluid (CSF) analysis. A description of typically acquired MRI sequences is explained in Box 1. When symptoms persist for at least 24 hours and are accompanied by observable lesions in the central nervous system, it is referred to as a clinical episode, commonly known as a relapse.[16] The 2017 revisions of the McDonald Criteria include the most recent diagnostic criteria,[27] and require evidence that the disease is developing over time (dissemination over time) and affecting more than one distinct anatomical region within the central nervous system (dissemination in space).

MS phenotypes

MS is currently classified into four main disease types based on the occurrence of relapses and the progression of disability:

1. ***Clinically isolated syndrome (CIS).*** CIS refers to the first clinical relapse with characteristics of inflammatory demyelination without fulfilling the accepted diagnostic criteria.[28]
2. ***Relapsing-remitting MS (RRMS).*** RRMS is the most common form of MS, affecting about 85% of people with MS. People with RRMS report episodes of new or worsening symptoms (relapses) followed by partial or complete recovery (remission).[2, 27]
3. ***Secondary progressive MS (SPMS).*** About two-thirds of people with RRMS eventually develop SPMS, where symptoms gradually worsen, and relapses become less frequent. During this phase, neurodegeneration becomes more pronounced, leading to a more rapid accumulation of disability and cognitive decline.[2, 29]
4. ***Primary progressive MS (PPMS).*** For some people with MS (between 10-15%), those diagnosed with PPMS, symptoms progressively worsen over time, typically in the absence of relapses.[2, 30]

Box 1. Structural Magnetic Resonance Imaging techniques.
Magnetic resonance imaging (MRI) serves as a cornerstone in the diagnosis and management of MS. It provides critical insights into the structural changes in the brain and spinal cord, aiding in the detection of lesions, monitoring disease progression, and investigating the mechanisms underlying cognitive impairment. Different MRI techniques and sequences are used to capture various aspects of MS pathology. This information box outlines the most commonly used structural MRI techniques and sequences for understanding cognitive impairment in MS.

T1-weighted imaging. T1-weighted imaging is a fundamental sequence for diagnosing MS. It provides clear images of (normal) anatomy, allowing for detailed measurements of brain volume and the detection of brain atrophy.[31] This type of sequence is also useful for identifying acute inflammatory lesions (enhanced with contrast), as well as areas with edema or axonal loss, known as "persistent black holes". Furthermore, T1-weighted imaging contributes to understanding cognition in MS by enabling the assessment of grey and white matter loss and brain atrophy, which are closely associated with cognitive decline.[3]

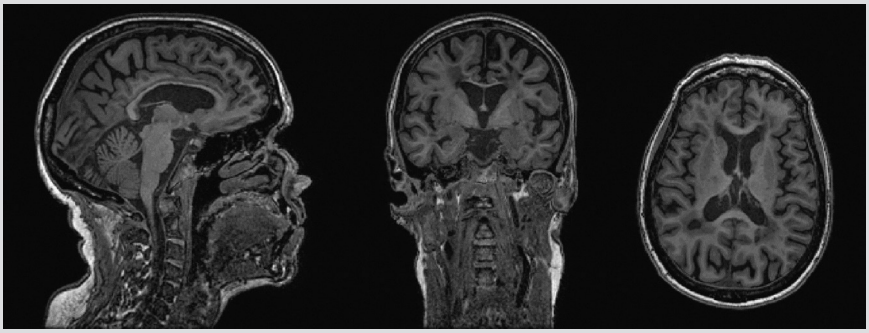

Figure 1. Example of a T1-weighted MRI sequence of an individual with MS, showing cortical atrophy.

Fluid attenuated inversion recovery (FLAIR) imaging. FLAIR imaging is particularly useful for detecting lesions in the brain's white matter, especially around the ventricles, where MS lesions often occur.[27] This technique suppresses the signal from CSF, enhancing the visibility of lesions by making them appear bright against a dark background. FLAIR is effective in highlighting chronic lesions that may not be visible on other types of MRI. Additionally, FLAIR imaging aids in understanding cognitive impairment in MS by visualizing lesion burden and distribution, which are linked to disruptions in neural connectivity and cognitive processing.[3]

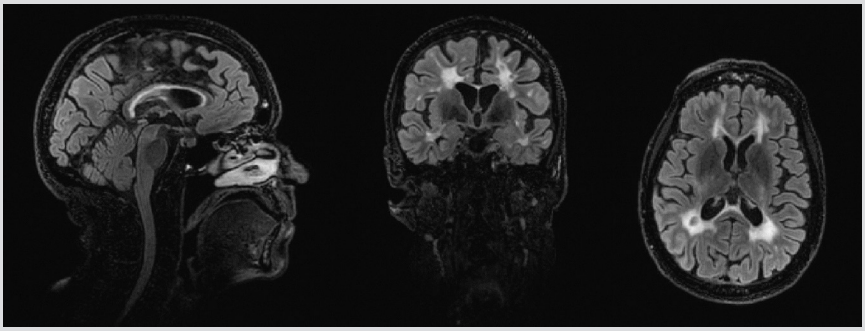

Figure 2. Example of a FLAIR sequence of an individual with MS. Hyperintensities are visible around the ventricles.

Double inversion recovery (DIR) imaging. DIR imaging is specifically designed to differentiate between the cerebral cortex and the white matter, making it possible to quantify lesions in the cortex. By suppressing the signals from white matter and CSF, only the signal originating from the grey matter becomes visible. This capability is particularly valuable for understanding cognitive impairment in MS, as cortical lesions are associated with cognitive dysfunction and can provide insights into the structural basis of cognitive deficits in affected individuals.[3]

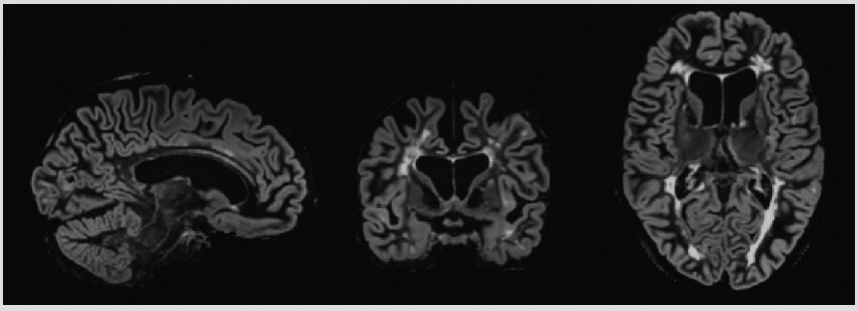

Figure 3. DIR imaging in an individual with MS, highlighting cortical lesions by suppressing white matter and CSF signals.

Diffusion tensor imaging (DTI). DTI focuses on the microstructural integrity of brain tissue, primarily white matter, but also grey matter to some extent.[31] It measures the movement of water molecules in the brain, which varies depending on the tissue type. In homogeneous tissues like grey matter, water molecules move equally in all directions. In white matter, water tends to diffuse along the direction of nerve fibers and is restricted in other directions due to cell membranes and axon walls. DTI contributes to understanding

cognitive impairment in MS by identifying microstructural changes in white matter tracts and grey matter, particularly within the thalamus, where altered diffusivity and atrophy are independently associated with cognitive dysfunction.[32]

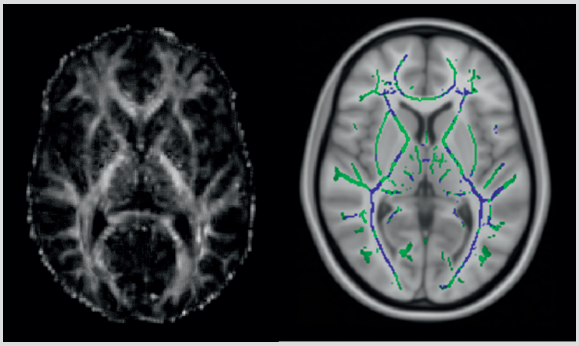

Figure 4. Example of a DTI sequence (left) and tract-based spatial statistics analysis results (right) in an individual with MS, illustrating white matter microstructural integrity measured by fractional anisotropy.

COGNITIVE IMPAIRMENT IN MS – A BACKGROUND

Cognition refers to the range of mental processes used by the brain to acquire, process, analyze, store, retrieve, and apply information from the external environment.[33] Derived from the Latin word "cognoscere", cognition refers to the ability to comprehend, or the mental processes of knowing.[34] Reports of cognitive problems in MS date back over 140 years ago when Jean-Martin Charcot, a pioneer in neurology, described people with MS with "marked enfeeblement of the memory" and "slow formation of conceptions".[35] However, it is only in recent decades that the cognitive aspects of MS have received significant attention.[7, 36, 37] This growing field has witnessed an increase in studies focusing on the prevalence and the manifestation of cognitive impairment in MS. Consequently, investigating biological mechanisms, developing diagnostic, screening and monitoring tools, as well as exploring treatments for this debilitating symptom have become increasingly important.[38]

Cognitive impairment affects a significant number of people with MS, with estimates ranging from 34% to 65% of all adults.[3] The variation in prevalence depends on factors such as the course of the disease, the setting of the particular study, and how impairment is defined.[3, 37] The various definitions of cognitive impairment are addressed in Box 2. Impairments in cognition can appear in all MS

phenotypes, sometimes even at the very early stages of the disease, in the absence of other neurological symptoms.[3] These impairments become more common in progressive forms of MS, affecting around 50-75% of those with SPMS compared to 30-45% in people with RRMS.[39] Aging and neurodegeneration further increase the likelihood of cognitive impairment in MS due to cumulative disease burden, accelerated neurodegeneration caused by MS-specific underlying processes, and the interaction of age-related changes with MS pathology.[40] These factors reduce the brain's resilience and exacerbate the damage, leading to more pronounced cognitive deficits.[3, 41] While many symptoms of MS are debilitating and warrant attention, cognitive impairment stands out for its profound impact on nearly every aspect of daily life, including clear thinking, decision-making, memory, and effective communication.[42] Cognitive impairment can disrupt practical, daily life activities like managing finances, driving, cooking, interpersonal relationships and overall wellbeing.[42] While physical symptoms can often be managed with aids, such as assistive devices, medication, or physical therapy, comparable solution for cognitive impairment are less available and typically require more complex and nuanced interventions, which remain underdeveloped.[43] Improving our understanding and treatment of cognitive impairment could profoundly enhance the overall quality of life for people with MS. Since cognitive impairment can affect a person's ability to manage their healthcare, such as remembering to take medications or follow treatment plans, it can significantly impact disease management and limit autonomy.[44]

Key areas of cognitive impairment in MS

As with other MS symptoms, cognitive impairment varies significantly between individuals and can manifest in different ways. The most common cognitive impairments, and often among the first observed, involve reduced information processing speed as well as visuospatial and verbal memory deficits.[3, 45] However, MS can impact several specific areas of cognition:[3, 46]

1. ***Visual perception.*** Visual perception involves the ability to interpret visual information from the environment, which is crucial for tasks like navigating a room, tying shoelaces, or reading.[33, 47] About 22% of people with MS experience difficulty in visuospatial processing.[46]
2. ***Memory.*** Memory is divided into short-term (temporary storage of information) and long-term (more permanent storage) memory.[33] Information is only transferred to long-term memory if it is encoded effectively, a process relying on sufficient attention.[33] Long-term memory can be categorized based on the type of information, such as verbal (verbal cues) and visuospatial memory (involving the spatial orientation of object presentation).[33] Deficits in visuospatial memory are estimated to be present in 54 to 56% of people with MS, while deficits in verbal memory are thought to occur in approximately 29 to 34%.[3] Both types

of memory are typically assessed based on the amount of information learned, recalled, or recognized, with impairments commonly observed in the learning and recalling phases.[46]

3. ***Attention and information processing speed.*** Attention is crucial in information processing by separating relevant information from irrelevant information.[33] The concept of attention is closely tied to the speed of information processing, as the brain's capacity to process information is limited.[33] Deficits in attention have been reported in less than 10% of the people with MS, with assessments primarily targeting sustained attention (i.e., the ability to focus on an activity or stimulus over a long period of time).[46, 48] Information processing speed refers to the time it takes to process information, compare it with information from memory, and select and execute responses.[33] In MS, information processing speed is typically assessed by the amount of work completed within a time limit. Impairment in information processing speed have been reported in 27-51% of people with MS.[46]
4. ***Executive function.*** This set of higher-level cognitive abilities includes planning, organizing, problem-solving, and regulating behavior.[33] Executive functions allow people to adapt to new situations and pursue relevant life goals effectively.[49] Executive functions, often referred to as "cold cognitive functions", are frequently studied in the field of MS across various sub-domains, including working memory, mental flexibility (shifting), inhibition, and verbal fluency. It is worth noting that working memory and verbal fluency are often categorized under the broader domain of "memory". In MS, deficits in executive function occur in about 15-28% of people, while problems with specific skills like verbal fluency are less common, affecting fewer than 10% of people.[46]

Treatment for cognition

There is currently no cure for cognitive impairment in MS. Available interventions, both pharmacological and non-pharmacological, aim to enhance or stabilize cognitive function and slow down cognitive decline.[3] While disease-modifying therapies primarily target inflammation and have shown some modest effects on neurodegeneration, no treatments are specifically approved for cognitive impairment in MS, and their cognitive benefits are generally small-to-medium.[50] Cognitive rehabilitation therapy is the gold standard non-pharmacological treatment.[51] It includes restorative approaches to improve specific cognitive skills and compensatory approaches to help individuals manage daily tasks despite cognitive limitations. However, establishing the transferability of the effects of cognitive rehabilitation therapy to daily life can be challenging, as many trials rely on neuropsychological tests (objectively assessed cognition) as outcome measures rather than daily functional outcomes.[51-53] Exercise has also shown promise in improving cognitive function in people with MS, [52, 54] though more research is needed to fully understand its benefits.

INTRODUCING A THEMATIC ORGANIZATION

Signaling and assessing cognitive impairment in MS

Changes in thinking, memory, or concentration noticeable in the daily lives of people with MS **signal** the need for a neuropsychological assessment to evaluate the extent and nature of cognitive impairment.[7, 36] In clinical practice, referrals for these assessments are usually based on the individual's ability or awareness to recognize and report these difficulties. The assessment process includes several components: individuals complete **patient-reported outcome measures** to report cognitive complaints, they undergo a series of neuropsychological tests, and their behavior is observed.[33] In research settings, these neuropsychological tests are considered the "gold standard" for **assessing** cognitive impairment in MS (see Box 2). Commonly used test batteries for MS include the Brief Repeatable Neuropsychological Test Battery (BRB-N)[55] and the Minimal Assessment of Cognitive Function in Multiple Sclerosis (MACFIMS),[46] both designed to address the most common cognitive deficits in people with MS. While comprehensive neuropsychological test batteries are the most thorough method for assessing cognitive function, they can be time-consuming and require trained personnel.[38] As a faster alternative, shorter test sets such as the Brief International Cognitive Assessment for Multiple Sclerosis (BICAMS)[56] may be used to screen for cognitive impairment, focusing on information processing speed and memory. However, caution is warranted when using the BICAMS in research contexts, particularly if the objective extends beyond mere screening for cognitive impairment. Given the complexity of cognition, it is recommended to include a range of tests to assess various cognitive functions, given the inherent complexity of cognition.[33]

The cycle of neuropsychological assessment, encompassing the processes of signaling and assessing described above, is illustrated in Figure 5 and serves as the thematic framework for this thesis.

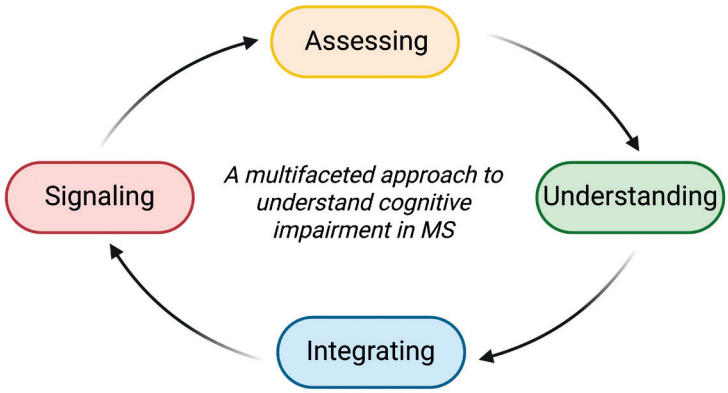

Figure 5. Cycle of neuropsychological assessment: a thematic framework for studying cognitive impairment in MS.

Understanding the results

There is no universally accepted standard for the **understanding** of neuropsychological test results (see Figure 5). Box 2 illustrates some of the common classification methods used to define cognitive impairment. Cognitive function is often interpreted by averaging scores from certain tests into broader categories or domains. For instance, one method considers a person to have cognitive impairment if they score 1.5 standard deviations below healthy individuals on approximately 20% of the tests.[57, 58] Another method may define impairment based on performance 2.0 standard deviations below healthy controls in at least two cognitive domains, such as memory and attention.[59] It is essential to adjust cognitive test scores for demographic factors, such as sex, age, and educational level, as these can significantly influence cognitive performance (see Box 2).[60] Additionally, to meet DSM-5 criteria, clinicians are advised to consider factors that may affect cognitive performance, including psychiatric comorbidities, medication side effects, and other MS-related symptoms.[3, 61]

Box 2. How is cognitive impairment in MS defined?

Comparison to healthy controls. To interpret cognitive performance using neuropsychological tests, common practice involves adjusting test scores for demographic factors such as age, sex, and educational level.[60] This adjustment allows for a meaningful comparison by benchmarking individual's performance against a normative sample or healthy controls.[60] From these comparisons, a ***z*-score** is calculated for each test score. This *z*-score represents where an individual's performance falls within a normal distribution, depicted by a bell-shaped Gaussian curve illustrating the probability distribution (see Figure 6).[62] The peak of this curve represents the mean, or average level of functioning. As one moves away from the mean in

either direction, the frequency of occurrences decreases, signifying cognitive functioning at the extremes. Approximately 68% of individuals are expected to fall within one standard deviation of the mean, and about 95% within two standard deviations. In practical terms, a score below the mean of healthy controls indicates poorer cognitive performance compared to the average healthy individual.

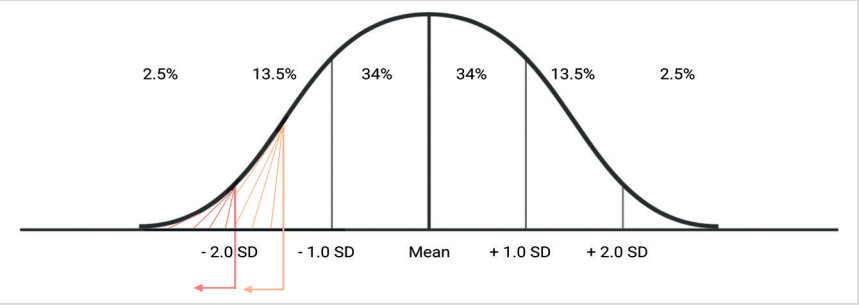

Figure 6. An illustration of a normal distribution with in yellow (-1.5 standard deviations) and pink (-2.0 standard deviations) the most often used cut-off scores to define low cognitive performance. *SD = standard deviation.*

Multiple definitions of cognitive impairment. In both this thesis and the broader literature, definitions of cognitive impairment in people with MS vary, with no agreed-upon specific cut-off scores. The definition typically depends on the neuropsychological test battery used and the specific research question being investigated.

Cognitive performance is typically assessed at multiple levels:

1. ***Neuropsychological test level.*** The *z*-scores of individual subtests are averaged to represent performance on the corresponding test. *Example: For the California Verbal Learning Test, z-scores for short-term retrieval, long-term retrieval, and recognition are averaged to produce a single score that reflects overall verbal learning and memory performance.*
2. ***Cognitive domain level.*** *Z*-scores from tests measuring similar cognitive functions are averaged to represent overall performance in a specific cognitive domain. *Example: Z-scores for the Symbol Digit Modalities Test and the Paced Auditory Serial Addition Test are averaged to produce a single score that reflects overall performance in information processing speed.*
3. ***Cognitive status level.*** Determining cognitive impairment involves assessing the *z*-scores of all administered tests. However, there is no consensus on exact cut-off points. Commonly used criteria include a cut-off of 1.5 or 2.0 standard deviations below the scores of healthy controls or a normative sample across a certain number of tests (see Figure 6).

a. When using the **BRB-N** battery, cognitive impairment is often defined at the test level, requiring at least two tests to have a *z*-score below -2.0 (illustrated by the pink line in Figure 6).[59]
b. When using the **MACFIMS** battery, impairment is frequently defined as 20% of the test scores falling below -1.5 standard deviations compared to healthy controls or normative data (illustrated by the yellow line in Figure 6).[57, 58]

Differences between the BRB-N and the MACFIMS. The differences in scoring cognitive impairment between the BRB-N and the MACFIMS may be related to their design (test composition), focus, and intended use.[63, 64] The MACFIMS is a more comprehensive battery that assesses a broader range of cognitive domains, including information processing speed, memory, executive function, and visuospatial skills, often using MS-specific normative data to enhance sensitivity to impairments in the MS population.[46] In contrast, the BRB-N is shorter, focusing primarily on information processing speed, memory, and verbal fluency, with fewer tests and broader normative data, making it more practical for routine clinical use.[55]

Integrating the underlying mechanisms of cognitive impairment in MS

The pathological mechanisms underlying cognitive impairment remain unclear. Understanding these mechanisms by integrating diverse methods and perspectives is essential for improving diagnosis, personalizing treatment, predicting disease progression, developing new therapies, deepening our understanding of MS, and enhancing monitoring efforts (a theme referred to as "**integrating**" in Figure 5).

MRI, as detailed in Box 1, has significantly advanced our understanding of cognitive impairment in MS. Cortical and deep grey matter atrophy, driven by neuro-axonal degeneration, are among the most promising neurobiological markers linked to cognitive impairment, showing a moderate association with cognitive decline.[22, 65, 66] However, MRI-derived brain measures alone cannot fully account for the wide spectrum of cognitive deficits observed in MS, a phenomenon known as the **clinical-radiological paradox**.[67] For example, individuals with significant brain atrophy may exhibit no cognitive impairments, while others with severe cognitive deficits may show minimal atrophy. To address this paradox, researchers are increasingly investigating both structural brain changes and how different brain regions communicate with one another, as measured using functional imaging. These studies aim to quantify altered brain function and evaluate the impact of structural damage on brain activity. A description of commonly used functional imaging techniques and their associated analyses is provided in Box 3.

The brain as a complex network. The brain comprises numerous regions interconnected either directly or indirectly and can be conceptualized as a complex network, where connections are not random but follow specific principles assessed using graph theory.[68] In this framework, a network is defined as a collection of nodes (e.g., brain regions) linked by edges (e.g., structural pathways). Analogously, brain regions can be compared to a train or road map, where cities (nodes) are connected through train tracks or roads (edges). In the brain, these roads are referred to as structural connections composed of white matter fibers, which can be measured with a technique called diffusion MRI (see Box 1). The quality of these structural connections is called "structural connectivity". The transport along these roads, representing the use of structural connections, is known as "functional connectivity" and is present when regions show similar activation patterns (see Box 3). Interestingly, the brain's network achieves an optimal balance or configuration to facilitate information flow in a cost-effective manner.[69] This configuration, or "wiring", varies from person to person, which may explain why some are susceptible to cognitive impairment than others.

Network abnormalities in MS. MS is increasingly recognized as a network disease, where lesions and diffuse damage disrupt structural and functional connectivity within the brain.[70] These disruptions lead to a less optimal configuration of the brain's network, which is associated with cognitive impairment.[71] Indeed, a cascade of network-level changes in MS contributes to various symptoms, including cognitive impairment.[72, 73] Altered functional connectivity in grey matter structures, such as the thalamus, hippocampus, and cerebral cortex, has been linked to distinct activation patterns in cognitively impaired people with MS.[74] For instance, increased functional connectivity between the cerebellum and regions of the default-mode network has been associated with poorer working memory, verbal memory, and reduced information processing speed (see Box 3 for more details on resting-state networks).[75] Additionally, cognitively impaired people with MS showed reduced functional dynamics within the default-mode network.[76, 77]

Box 3. Functional Magnetic Resonance Imaging techniques.

Resting-state networks. Spontaneous, low-frequency fluctuations in neuronal activity that are temporally correlated between spatially distinct brain regions are called resting-state networks and are associated with healthy cognitive functioning.[79] These networks include the default-mode, frontoparietal, dorsal attention, ventral attention, somatomotor, visual, limbic and deep grey matter networks.[80] Dynamic interactions among these networks are thought to be essential for preserving cognitive abilities, allowing for a diverse functional repertoire of the brain. [81, 82]

When using fMRI, researchers differentiate between:

1. ***Static connectivity.*** With static connectivity, the amount of information transfer between two brain regions is measured, typically by analyzing the correlation between their activity signals.
2. ***Dynamic connectivity.*** With dynamic connectivity it is assessed how this information transfer varies over time, providing a more comprehensive understanding of how different regions interact under various conditions.[75, 78]

Resting-state functional MRI (fMRI). Functional imaging examines brain function by measuring changes in blood flow and oxygen levels, providing insights into how different brain regions communicate.[33] With task-based fMRI, neural activity is measured while an individual performs specific cognitive tasks inside the scanner. In contrast, resting-state fMRI assesses brain connectivity by analyzing statistical coherence between different brain regions while the person is at rest, not engaged in any particular task. When different regions show similar patterns of activations, it is assumed that these fluctuations in oxygenated blood levels reflect synchronized neuronal processes. This overlap in activation patterns is referred to as "functional connectivity".

ADVANCING OUR UNDERSTANDING OF COGNITIVE IMPAIRMENT IN MS

Potential diagnostic biomarkers for cognition

To advance our understanding of cognitive impairment in MS, this thesis will begin at the molecular level by exploring fluid biomarkers. These biomarkers, measurable indicators within the body, provide insights into the diagnosis, progression, and treatment of diseases like MS.[83, 84] In the context of cognitive impairment, fluid biomarkers hold potential for revealing underlying mechanisms and signaling cognitive deficits, although their role in MS remains understudied.[85]

This thesis will specifically focus on diagnostic (indicating the presence of cognitive impairment) and prognostic (predicting the future course of cognitive function or the progression of decline) biomarkers for cognitive impairment in MS. Two promising fluid biomarkers in this context are **neurofilament light (NfL)** and **glial fibrillary acidic protein (GFAP)**.[85, 86] These biomarkers quantify specific proteins that are released into the CSF or bloodstream following neuronal damage or glial activation, respectively. By directly assessing neuronal injury and gliosis, they provide insights into brain pathology that are not fully captured by MRI. Moreover, fluid biomarkers offer a dynamic view of ongoing neurodegenerative processes, reflecting real-time changes that may correlate with disease progression.[87]

1. ***NfL.*** As a key structural protein in the neuronal and axonal cytoskeleton, NfL plays an important role in maintaining structural support in the central nervous system.[88] Elevated levels of NfL reflect axonal damage, independent of the underlying cause, although absolute values and their temporal dynamics may vary between etiologies.[89] Preliminary studies have linked elevated NfL levels to reduced information processing speed, but findings have been inconsistent due to small sample sizes and limited cognitive testing.[85]
2. ***GFAP.*** While research on GFAP is more exploratory, GFAP is a major intermediate cytoskeletal protein of astrocytes and is thought to reflect ongoing astrocytic reactivity.[90, 91] Associations have been observed between GFAP levels and clinical disability severity, suggesting it may also be relevant for cognitive functioning in MS.[92] Given the role of neurodegeneration in cognitive impairment, GFAP could provide additional insights into the mechanisms driving cognitive decline.

In this thesis, we will investigate the combined utility of NfL and GFAP in both serum and CSF alongside conventional imaging measures, such as brain atrophy and lesion load. By integrating fluid and imaging biomarkers, we aim to explore their added value in detecting cognitive impairment in MS and to determine whether fluid biomarkers can provide a complementary and more direct assessment of neurodegenerative processes.

Key questions for signaling cognitive impairment in MS

- What is the potential of fluid biomarkers (i.e., NfL and GFAP in both serum and CSF) for detecting cognitive impairment in MS?
- What is the added diagnostic potential of these fluid biomarkers in comparison with conventional imaging markers?

Key questions for integrating information on cognitive impairment in MS

- What do variations in fluid biomarkers (e.g., NfL and GFAP in both serum and CSF) reveal about the underlying pathological mechanisms of cognitive impairment in MS, specifically the interplay between axonal damage, glial activation and disease progression?

The importance of network changes for cognition

Fluid biomarkers, such as NfL and GFAP, might have the potential to serve as diagnostic markers by providing molecular insights into the neuronal and glial processes underpinning cognitive impairment in MS. However, bridging these molecular changes to a comprehensive understanding of disrupted cognitive

functioning requires shifting focus to the imaging level, examining the brain as an interconnected network of regions. As mentioned above, cognitive functions depend not only on the integrity of individual brain regions but also on the complex coordination between them.[93] This highlights the need to focus on network-level disruptions in MS to better understand cognitive impairment. Recent studies on functional connectivity, have revealed both increased and decreased network reorganization, or adaptability, in relation to cognitive functioning in MS.[94, 95] Despite growing interest in this area, only few studies have investigated how structure and function directly interact in MS.[82, 95-97] Understanding this interplay, measured as **structure-function coupling**, could clarify how network disruptions contribute to cognitive impairment. In this thesis, we will investigate whether jointly analyzing structural and functional connectivity provides new insights into the mechanisms underlying cognitive impairment in MS.

Key questions for integrating information on cognitive impairment in MS

· How does structure-function coupling relate to cognitive impairment, and what can fluctuations in coupling tells us about cognitive functioning in MS?

A fine-grained take on cognition

Building on the concept of structure-function coupling, which emphasized the interplay between brain structure and function in cognitive impairment, it is also important to understand how these disruptions manifest at the behavioral level. While MS is known to result in deficits across multiple cognitive domains at the group level, there is still limited understanding of the variability at the individual level, as well as the timing of such deficits. Some individuals may have **isolated deficits** in a single cognitive domain, whereas others experience **multidomain** impairments.[36] This variability suggests that cognitive impairment in MS might not follow an uniform trajectory, making it crucial to understand these individual differences to develop more precise diagnostic and treatment strategies.[98] To capture this heterogeneity, research has increasingly focused on identifying distinct **cognitive profiles**, or phenotypes, which reflect shared patterns of cognitive strengths and weakness on cognitive tests among subgroups of individuals.[98] Studies employing various methods to identify cognitive profiles have revealed varying numbers of subgroups, depending on the approach.[99, 100] Interestingly, these profiles often align along a continuum, ranging from preserved to impaired cognitive function.

In this thesis, we will employ two advanced, complementary methodological approaches to study cognitive impairment. First, we will examine homogeneous isolated deficits to determine whether this approach offers novel insights into

specific impairments. Second, we will consider the full spectrum of cognitive performance, treating cognition as a latent construct (an underlying construct inferred from measurable test scores) and aiming to identify cognitive profiles. Together, these approaches will provide a more comprehensive view of cognitive impairment in MS, advancing our understanding of its variability and progression.

Key questions for <u>understanding</u> cognitive impairment in MS

- What patterns of isolated cognitive impairments can be identified and how do they inform our understanding of the progression of cognitive impairment in MS?
- How do distinct cognitive profiles in MS, identified through latent profile analysis, capture individual variability in cognitive impairment, and what insights do they offer into the progression and heterogeneity of cognitive decline?

Cognition from a multidimensional perspective

In this part of the thesis, we shift from viewing cognition as a latent variable to understanding it as a dynamic network of interacting and co-occurring symptoms. This perspective recognizes that cognitive performance arises from the interplay between objective and self-reported cognitive functioning, as well as psychological factors, and aligns with the view of MS as a network disease.

Self-reported measures, such as the Multiple Sclerosis Neuropsychological Questionnaire,[101] often do not align with **objective neuropsychological test results**.[102-104] This discrepancy highlights the complexity of cognition and the various factors influencing how individuals perceive their cognitive abilities, including:

1. ***Fatigue.*** Reported by up to 83% of people with MS, fatigue can range from temporary episodes to chronic conditions, and often negatively impacts cognitive performance.[105]
2. ***Mood disorders.*** Depression and anxiety are also common comorbidities in MS, with prevalence rates of about 31% and 22%, respectively.[106] These mood changes can significantly affect how individuals evaluate their cognitive abilities, potentially inflating or deflating their self-assessment.
3. ***Awareness and premorbid IQ.*** Some people with MS may notice subtle cognitive changes in their daily life before they become apparent in objective assessments. This awareness may be influenced by their premorbid IQ (cognitive abilities before the onset of MS).[107]

In this thesis, we aim to use a novel framework "**symptom network analysis**" to better understand the interplay between these factors.[108] In this symptom network,

nodes represent symptom data (e.g., cognitive test scores and patient-reported outcome measures), and edges depict associations between symptoms at the group level. Although previously used in neuro-oncology and psychiatry, its application in MS is relatively new and promising for understanding symptom co-occurrence.

Key questions for integrating information on cognitive impairment in MS

- How do objective cognitive performance, self-reported cognitive difficulties, and psychological factors interact within a symptom network in MS, and what insights does symptom network analysis offer into the multidimensional nature of cognitive impairment and its contributing factors?

The impact of cognitive impairment on daily life

Having explored the molecular, structural, functional, and network-level perspectives of cognitive impairment, we now turn to its most tangible manifestation: its impact on daily life. Cognitive impairment is particularly evident in tasks requiring complex thinking and decision-making, known as **instrumental activities of daily living (IADL)**.[109] In MS, difficulties in IADL have been reported in areas such as employment, social engagement, driving, making medical decisions, adhering to treatment plans, and financial management.[38] While neuropsychological assessments are typically conducted in quiet, controlled environments to minimize distractions, real-life situations often present more challenges, which may not be fully captured in a clinical setting.[36] This discrepancy raises concerns about the **ecological validity** of these assessments, i.e., how well they reflect the difficulties individuals face in their everyday environments. The potential gap between clinical test results and real-world performance limits the generalizability of findings and highlights the need for more contextually relevant tools.[110] Even more so, provided that self-reported cognitive functioning does not align very well with objective cognitive testing as reported before.

Understanding how cognitive impairment affects daily life requires a distinction between challenges stemming from cognitive difficulties and those caused by physical limitations, as both can impact the same activities.[111] For instance, cooking involves cognitive skills, such as remembering steps and managing timing, alongside physical abilities, like handling utensils and mobility. Currently, there is no specific tool or questionnaire that fully captures this distinction, leaving an important gap in our ability to assess and address the real-world implications of cognitive impairment in MS. In this thesis, our aim will be to develop and validate a new tool, the **Multiple Sclerosis Instrumental Activities of Daily Living Questionnaire (MS-IADL-Q)**, to more accurately assess how cognitive impairment influences everyday tasks for people with MS.

Key questions for assessing cognitive impairment in MS

- Can we develop and validate a tool to assess the impact of cognitive functioning in daily life by focusing on IADL?
- By differentiating between cognitive and physical difficulties in this tool, can we better understand the cognitive impact of MS in everyday functioning?

AIMS AND OUTLINE OF THESIS

The overarching aim of this thesis is to enhance the understanding of cognitive impairment in people with MS through a multifaceted approach. By integrating diverse methodologies and perspectives, we seek to contribute to earlier diagnosis, improved prognosis, and better management strategies, ultimately improving the quality of life for people with MS.

The thesis employs three key strategies:

- **Integration of multiple data sources:** Combining imaging and fluid biomarkers, structural and functional MRI sequences, and objective cognitive test results with patient-reported outcomes in a symptom network.
- **Advanced statistical techniques.** Applying methods such as latent profile analysis to identify cognitive profiles, symptom network analysis to explore the interplay between cognitive and psychological symptoms, and item response theory to validate the MS-IADL-Q.
- **Development of a novel tool.** Developing the MS-IADL-Q to assess the cognitive and physical impact of MS on daily life.

The outline of this thesis is displayed in Figure 7. In **chapter 2**, we will investigate the potential of fluid biomarkers (NfL and GFAP) to detect cognitive impairment in MS and their added value over conventional imaging markers. We will also develop and investigate a composite multi-modal marker. In **chapter 3**, we will focus on network changes underlying cognitive impairment, specifically structure-function coupling. In **chapter 3.1**, we will introduce the theoretical framework, whereas in **chapter 3.2**, we will examine structure-function coupling at both the whole-brain and network levels. In **chapter 4**, we aim to explore isolated impairments and cognitive profiles. In **chapter 4.1**, we will examine isolated cognitive impairments in terms of frequency, progression, and MRI correlates, while in **chapter 4.2**, we will use latent profile analysis to identify cognitive profiles and compare them to cognitive status. In **chapter 5**, we will investigate the interplay between self-reported and objective cognitive functioning using symptom network analysis. In **chapter 5.1**, we will study how mood, anxiety, fatigue, and cognitive complaints interact with objective cognitive scores, comparing networks between those with

and without information processing speed impairments and people with low or high cognitive complaints. In **chapter 6**, we will develop and validate the MS-IADL-Q to assess the cognitive and physical impact of MS on daily life. In **chapter 6.1**, we will detail the development process involving people with MS, proxies, and healthcare professionals, while in **chapter 6.2**, we will evaluate its psychometric properties. In **chapter 7**, we will summarize the findings of the thesis and discuss the implications.

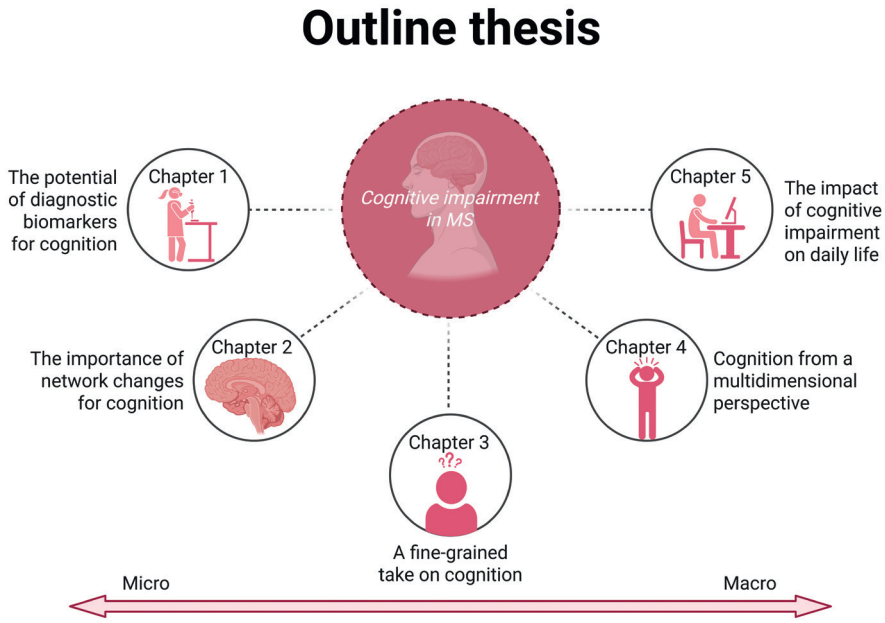

Figure 7. The outline of this thesis.

REFERENCES

1. Thompson AJ, Baranzini SE, Geurts J, Hemmer B, Ciccarelli O. Multiple sclerosis. The Lancet 2018;391:1622-1636.
2. Brownlee WJ, Hardy TA, Fazekas F, Miller DH. Diagnosis of multiple sclerosis: progress and challenges. The Lancet 2017;389:1336-1346.
3. Benedict RHB, Amato MP, DeLuca J, Geurts JJG. Cognitive impairment in multiple sclerosis: clinical management, MRI, and therapeutic avenues. The Lancet Neurology 2020;19:860-871.
4. Walton C, King R, Rechtman L, et al. Rising prevalence of multiple sclerosis worldwide: Insights from the Atlas of MS. Multiple Sclerosis Journal 2020;26:1816-1821.
5. Clemens L, Langdon D. How does cognition relate to employment in multiple sclerosis? A systematic review. Multiple sclerosis and related disorders 2018;26:183-191.
6. Campbell J, Rashid W, Cercignani M, Langdon D. Cognitive impairment among patients with multiple sclerosis: associations with employment and quality of life. Postgraduate Medical Journal 2017;93:143-147.
7. Benedict RH, DeLuca J, Enzinger C, Geurts JJ, Krupp LB, Rao SM. Neuropsychology of multiple sclerosis: looking back and moving forward. Journal of the International Neuropsychological Society 2017;23:832-842.
8. Rao SM, Leo GJ, Ellington L, Nauertz T, Bernardin L, Unverzagt F. Cognitive dysfunction in multiple sclerosis. II. Impact on employment and social functioning. Neurology 1991;41:692-696.
9. Motl RW, McAuley E. Symptom cluster and quality of life: preliminary evidence in multiple sclerosis. The Journal of neuroscience nursing: journal of the American Association of Neuroscience Nurses 2010;42:212.
10. Lublin FD, Häring DA, Ganjgahi H, et al. How patients with multiple sclerosis acquire disability. Brain 2022;145:3147-3161.
11. Wallin MT, Culpepper WJ, Campbell JD, et al. The prevalence of MS in the United States: a population-based estimate using health claims data. Neurology 2019;92:e1029-e1040.
12. Foundation MI. Global MS Employment report. 2016.
13. International TMS, Federation. Atlas of MS – 3rd Edition, part 2: clinical management of multiple sclerosis around the world 2021.
14. Ascherio A. Environmental factors in multiple sclerosis. Expert review of neurotherapeutics 2013;13:3-9.
15. Ward M, Goldman MD. Epidemiology and pathophysiology of multiple sclerosis. CONTINUUM: Lifelong Learning in Neurology 2022;28:988-1005.
16. McGinley MP, Goldschmidt CH, Rae-Grant AD. Diagnosis and treatment of multiple sclerosis: a review. Jama 2021;325:765-779.
17. 't Hart BA, Luchicchi A, Schenk GJ, Stys PK, Geurts JJ. Mechanistic underpinning of an inside–out concept for autoimmunity in multiple sclerosis. Annals of Clinical and Translational Neurology 2021;8:1709-1719.
18. Karussis D. The diagnosis of multiple sclerosis and the various related demyelinating syndromes: a critical review. Journal of autoimmunity 2014;48:134-142.
19. Popescu BFG, Pirko I, Lucchinetti CF. Pathology of multiple sclerosis: where do we stand? CONTINUUM: Lifelong Learning in Neurology 2013;19:901.
20. Lucchinetti CF, Popescu BF, Bunyan RF, et al. Inflammatory cortical demyelination in early multiple sclerosis. New England Journal of Medicine 2011;365:2188-2197.
21. Mahad DH, Trapp BD, Lassmann H. Pathological mechanisms in progressive multiple sclerosis. The Lancet Neurology 2015;14:183-193.

22. Eijlers AJ, Dekker I, Steenwijk MD, et al. Cortical atrophy accelerates as cognitive decline worsens in multiple sclerosis. Neurology 2019;93:e1348-e1359.
23. Lie IA, Weeda MM, Mattiesing RM, et al. Relationship between white matter lesions and gray matter atrophy in multiple sclerosis: A systematic review. Neurology 2022;98:e1562-e1573.
24. Calabrese M, Magliozzi R, Ciccarelli O, Geurts JJ, Reynolds R, Martin R. Exploring the origins of grey matter damage in multiple sclerosis. Nature Reviews Neuroscience 2015;16:147-158.
25. Baecher-Allan C, Kaskow BJ, Weiner HL. Multiple sclerosis: mechanisms and immunotherapy. Neuron 2018;97:742-768.
26. Correale J, Marrodan M, Ysrraelit MC. Mechanisms of neurodegeneration and axonal dysfunction in progressive multiple sclerosis. Biomedicines 2019;7:14.
27. Thompson AJ, Banwell BL, Barkhof F, et al. Diagnosis of multiple sclerosis: 2017 revisions of the McDonald criteria. The Lancet Neurology 2018;17:162-173.
28. Miller DH, Chard DT, Ciccarelli O. Clinically isolated syndromes. The Lancet Neurology 2012;11:157-169.
29. Huijbregts S, Kalkers N, De Sonneville L, De Groot V, Reuling I, Polman C. Differences in cognitive impairment of relapsing remitting, secondary, and primary progressive MS. Neurology 2004;63:335-339.
30. Miller DH, Leary SM. Primary-progressive multiple sclerosis. The Lancet Neurology 2007;6:903-912.
31. Filippi M, Rocca M, Benedict R, et al. The contribution of MRI in assessing cognitive impairment in multiple sclerosis. Neurology 2010;75:2121-2128.
32. Benedict RH, Hulst HE, Bergsland N, et al. Clinical significance of atrophy and white matter mean diffusivity within the thalamus of multiple sclerosis patients. Multiple Sclerosis Journal 2013;19:1478-1484.
33. Kessels RPC, Eling P, Ponds R, Spikman J, van Zandvoort M, Noorderlicht V. Clinical neuropsychology: Boom, 2017.
34. Bayne T, Brainard D, Byrne RW, et al. What is cognition? Current Biology 2019;29:R608-R615.
35. CnARCOT J. Lectures on the Diseases of the Nervous System (Vol. 1). Trans by G Sigerson, London: New Sydenham Society 1877.
36. Sumowski JF, Benedict R, Enzinger C, et al. Cognition in multiple sclerosis: State of the field and priorities for the future. Neurology 2018;90:278-288.
37. Chiaravalloti ND, DeLuca J. Cognitive impairment in multiple sclerosis. The Lancet Neurology 2008;7:1139-1151.
38. Kalb R, Beier M, Benedict RH, et al. Recommendations for cognitive screening and management in multiple sclerosis care. Multiple Sclerosis Journal 2018;24:1665-1680.
39. Johnen A, Landmeyer NC, Bürkner P-C, Wiendl H, Meuth SG, Holling H. Distinct cognitive impairments in different disease courses of multiple sclerosis—a systematic review and meta-analysis. Neuroscience & Biobehavioral Reviews 2017;83:568-578.
40. Filippi M, Preziosa P, Barkhof F, et al. The ageing central nervous system in multiple sclerosis: the imaging perspective. Brain 2024;147:3665-3680.
41. Ruano L, Portaccio E, Goretti B, et al. Age and disability drive cognitive impairment in multiple sclerosis across disease subtypes. Multiple Sclerosis Journal 2017;23:1258-1267.
42. Goverover Y, Genova HM, DeLuca J, Chiaravalloti ND. Impact of multiple sclerosis on daily life. Changes in the Brain: Springer, 2017: 145-165.
43. Cohen BA. Identification, causation, alleviation, and prevention of complications (ICAP) An approach to symptom and disability management in multiple sclerosis. Neurology 2008;71:S14-S20.

44. Bruce JM, Hancock LM, Arnett P, Lynch S. Treatment adherence in multiple sclerosis: association with emotional status, personality, and cognition. Journal of behavioral medicine 2010;33:219-227.

45. Wojcik C, Fuchs TA, Tran H, et al. Staging and stratifying cognitive dysfunction in multiple sclerosis. Multiple Sclerosis Journal 2022;28:463-471.

46. Benedict RH, Cookfair D, Gavett R, et al. Validity of the minimal assessment of cognitive function in multiple sclerosis (MACFIMS). Journal of the International Neuropsychological Society: JINS 2006;12:549.

47. Trés ES, Brucki SMD. Visuospatial processing: A review from basic to current concepts. Dementia & neuropsychologia 2014;8:175-181.

48. Rao SM, Leo GJ, Bernardin L, Unverzagt F. Cognitive dysfunction in multiple sclerosis.: I. Frequency, patterns, and prediction. Neurology 1991;41:685-691.

49. Burgess PW, Simons JS, Halligan P, Wade D. 18 Theories of frontal lobe executive function: clinical applications. The effectiveness of rehabilitation for cognitive deficits 2005;211.

50. Landmeyer NC, Buerkner P-C, Wiendl H, et al. Disease-modifying treatments and cognition in relapsing-remitting multiple sclerosis: A meta-analysis. Neurology 2020;94:e2373-e2383.

51. Chen MH, Chiaravalloti ND, DeLuca J. Neurological update: Cognitive rehabilitation in multiple sclerosis. Journal of Neurology 2021;268:4908-4914.

52. DeLuca J, Chiaravalloti ND, Sandroff BM. Treatment and management of cognitive dysfunction in patients with multiple sclerosis. Nature Reviews Neurology 2020;16:319-332.

53. das Nair R, Lincoln NB. The effectiveness of memory rehabilitation following neurological disabilities: a qualitative inquiry of patient perspectives. Neuropsychological rehabilitation 2013;23:528-545.

54. Sandroff BM, Motl RW, Scudder MR, DeLuca J. Systematic, evidence-based review of exercise, physical activity, and physical fitness effects on cognition in persons with multiple sclerosis. Neuropsychology review 2016;26:271-294.

55. Rao S. A manual for the brief repeatable battery of neuropsychological tests in multiple sclerosis. Milwaukee: Medical College of Wisconsin 1990;1696.

56. Langdon D, Amato M, Boringa J, et al. Recommendations for a brief international cognitive assessment for multiple sclerosis (BICAMS). Multiple Sclerosis Journal 2012;18:891-898.

57. Benedict R. Standards for sample composition and impairment classification in neuropsychological studies of multiple sclerosis. Multiple Sclerosis Journal 2009;15:777.

58. Fischer M, Kunkel A, Bublak P, et al. How reliable is the classification of cognitive impairment across different criteria in early and late stages of multiple sclerosis? Journal of the neurological sciences 2014;343:91-99.

59. Eijlers AJ, van Geest Q, Dekker I, et al. Predicting cognitive decline in multiple sclerosis: a 5-year follow-up study. Brain 2018;141:2605-2618.

60. Parmenter BA, Testa SM, Schretlen DJ, Weinstock-Guttman B, Benedict RH. The utility of regression-based norms in interpreting the minimal assessment of cognitive function in multiple sclerosis (MACFIMS). Journal of the International Neuropsychological Society 2010;16:6-16.

61. Sachdev PS, Blacker D, Blazer DG, et al. Classifying neurocognitive disorders: the DSM-5 approach. Nature Reviews Neurology 2014;10:634-642.

62. Field A. Discovering statistics using IBM SPSS statistics: sage, 2013.

63. Gómez-Melero S, Caballero-Villarraso J, Escribano BM, Galvao-Carmona A, Túnez I, Agüera-Morales E. Impact of Cognitive Impairment on Quality of Life in Multiple Sclerosis Patients—A Comprehensive Review. Journal of Clinical Medicine 2024;13:3321.

64. Grossi P, Portaccio E, Bellomi F, et al. The minimal neuropsychological assessment of MS patients (MACFIMS): normative data of the Italian population. Neurological Sciences 2020;41:1489-1496.

65. Houtchens M, Benedict R, Killiany R, et al. Thalamic atrophy and cognition in multiple sclerosis. Neurology 2007;69:1213-1223.

66. Eijlers AJ, Meijer KA, van Geest Q, Geurts JJ, Schoonheim MM. Determinants of cognitive impairment in patients with multiple sclerosis with and without atrophy. Radiology 2018;288:544-551.

67. Barkhof F. The clinico-radiological paradox in multiple sclerosis revisited. Current opinion in neurology 2002;15:239-245.

68. Douw L, van Dellen E, Gouw AA, et al. The road ahead in clinical network neuroscience. Network Neuroscience 2019;3:969-993.

69. Bullmore E, Sporns O. Complex brain networks: graph theoretical analysis of structural and functional systems. Nature reviews neuroscience 2009;10:186-198.

70. Fleischer V, Radetz A, Ciolac D, et al. Graph Theoretical Framework of Brain Networks in Multiple Sclerosis: A Review of Concepts. Neuroscience 2019;403:35-53.

71. Schoonheim MM, Meijer KA, Geurts JJ. Network collapse and cognitive impairment in multiple sclerosis. Frontiers in neurology 2015;6:82.

72. Zhang J, Cortese R, De Stefano N, Giorgio A. Structural and functional connectivity substrates of cognitive impairment in multiple sclerosis. Frontiers in neurology 2021;12:671894.

73. Eijlers AJ, Meijer KA, Wassenaar TM, et al. Increased default-mode network centrality in cognitively impaired multiple sclerosis patients. Neurology 2017;88:952-960.

74. Meijer KA, Eijlers AJ, Douw L, et al. Increased connectivity of hub networks and cognitive impairment in multiple sclerosis. Neurology 2017;88:2107-2114.

75. Schoonheim MM, Douw L, Broeders TA, Eijlers AJ, Meijer KA, Geurts JJ. The cerebellum and its network: Disrupted static and dynamic functional connectivity patterns and cognitive impairment in multiple sclerosis. Multiple Sclerosis Journal 2021;27:2031-2039.

76. Eijlers AJ, Wink AM, Meijer KA, Douw L, Geurts JJ, Schoonheim MM. Reduced network dynamics on functional MRI signals cognitive impairment in multiple sclerosis. Radiology 2019;292:449-457.

77. Broeders TA, Douw L, Eijlers AJ, et al. A more unstable resting-state functional network in cognitively declining multiple sclerosis. Brain Communications 2022;4:fcac095.

78. Lurie DJ, Kessler D, Bassett DS, et al. Questions and controversies in the study of time-varying functional connectivity in resting fMRI. Network neuroscience 2020;4:30-69.

79. Van Den Heuvel MP, Pol HEH. Exploring the brain network: a review on resting-state fMRI functional connectivity. European neuropsychopharmacology 2010;20:519-534.

80. Yeo BT, Krienen FM, Sepulcre J, et al. The organization of the human cerebral cortex estimated by intrinsic functional connectivity. Journal of neurophysiology 2011.

81. Huiskamp M, Eijlers AJ, Broeders TA, et al. Longitudinal network changes and conversion to cognitive impairment in multiple sclerosis. Neurology 2021;97:e794-e802.

82. Koubiyr I, Besson P, Deloire M, et al. Dynamic modular-level alterations of structural-functional coupling in clinically isolated syndrome. Brain 2019;142:3428-3439.

83. Strimbu K, Tavel JA. What are biomarkers? Current Opinion in HIV and AIDS 2010;5:463-466.

84. Group BDW, Atkinson Jr AJ, Colburn WA, et al. Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. Clinical pharmacology & therapeutics 2001;69:89-95.

85. Rademacher T-D, Meuth SG, Wiendl H, Johnen A, Landmeyer NC. Molecular biomarkers and cognitive impairment in multiple sclerosis: state of the field, limitations, and future direction–A systematic review and meta-analysis. Neuroscience & Biobehavioral Reviews 2023:105035.

86. Abdelhak A, Hottenrott T, Morenas-Rodríguez E, et al. Glial Activation Markers in CSF and Serum From Patients With Primary Progressive Multiple Sclerosis: Potential of Serum GFAP as Disease Severity Marker? Frontiers in Neurology 2019;10.

87. Jakimovski D, Dwyer MG, Bergsland N, Weinstock-Guttman B, Zivadinov R. Disease biomarkers in multiple sclerosis: current serum neurofilament light chain perspectives. Neurodegenerative disease management 2021;11:329-340.

88. Bittner S, Oh J, Havrdová EK, Tintoré M, Zipp F. The potential of serum neurofilament as biomarker for multiple sclerosis. Brain 2021;144:2954-2963.

89. Bridel C, van Wieringen WN, Zetterberg H, Tijms BM, Teunissen CE, Group atN. Diagnostic Value of Cerebrospinal Fluid Neurofilament Light Protein in Neurology: A Systematic Review and Meta-analysis. JAMA Neurology 2019;76:1035-1048.

90. Högel H, Rissanen E, Barro C, et al. Serum glial fibrillary acidic protein correlates with multiple sclerosis disease severity. Multiple Sclerosis Journal 2020;26:210-219.

91. Barro C, Healy BC, Saxena S, et al. Serum NfL but not GFAP predicts cognitive decline in active progressive multiple sclerosis patients. Multiple Sclerosis Journal 2023;29:206-211.

92. Abdelhak A, Foschi M, Abu-Rumeileh S, et al. Blood GFAP as an emerging biomarker in brain and spinal cord disorders. Nature Reviews Neurology 2022;18:158-172.

93. Kupis L, Goodman ZT, Kornfeld S, et al. Brain dynamics underlying cognitive flexibility across the lifespan. Cerebral Cortex 2021;31:5263-5274.

94. Meijer K, Van Geest Q, Eijlers A, Geurts J, Schoonheim M, Hulst H. Is impaired information processing speed a matter of structural or functional damage in MS? NeuroImage: Clinical 2018;20:844-850.

95. Koubiyr ID, Mathilde; Brochet, Bruno; Besson, Pierre; Charré-morin, Julie; Saubusse, Aurore; Tourdias, Thomas; Ruet, Aurélie. Structural constraints of functional connectivity drive cognitive impairment in the early stages of multiple sclerosis. Multiple Sclerosis Journal 2020.

96. Koubiyr I, Deloire M, Besson P, et al. Longitudinal study of functional brain network reorganization in clinically isolated syndrome. Multiple Sclerosis Journal 2020;26:188-200.

97. Kulik SD, Nauta IM, Tewarie P, et al. Structure-function coupling as a correlate and potential biomarker of cognitive impairment in multiple sclerosis. Network Neuroscience 2022;6:339-356.

98. De Meo E, Portaccio E. It is time to define cognitive phenotypes in multiple sclerosis. SAGE Publications Sage UK: London, England, 2023: 489-491.

99. De Meo E, Portaccio E, Giorgio A, et al. Identifying the distinct cognitive phenotypes in multiple sclerosis. JAMA neurology 2021;78:414-425.

100. Podda J, Ponzio M, Pedulla L, et al. Predominant cognitive phenotypes in multiple sclerosis: Insights from patient-centered outcomes. Multiple sclerosis and related disorders 2021;51:102919.

101. Benedict RH, Cox D, Thompson LL, Foley F, Weinstock-Guttman B, Munschauer F. Reliable screening for neuropsychological impairment in multiple sclerosis. Multiple Sclerosis Journal 2004;10:675-678.

102. Benedict RH, Munschauer F, Linn R, et al. Screening for multiple sclerosis cognitive impairment using a self-administered 15-item questionnaire. Multiple Sclerosis Journal 2003;9:95-101.

103. Hughes AJ, Bhattarai JJ, Paul S, Beier M. Depressive symptoms and fatigue as predictors of objective-subjective discrepancies in cognitive function in multiple sclerosis. Multiple sclerosis and related disorders 2019;30:192-197.

104. Davenport L, Cogley C, Monaghan R, et al. Investigating the association of mood and fatigue with objective and subjective cognitive impairment in multiple sclerosis. Journal of Neuropsychology 2022;16:537-554.

105. Manjaly Z-M, Harrison NA, Critchley HD, et al. Pathophysiological and cognitive mechanisms of fatigue in multiple sclerosis. Journal of Neurology, Neurosurgery & Psychiatry 2019;90:642-651.

106. Boeschoten RE, Braamse AM, Beekman AT, et al. Prevalence of depression and anxiety in multiple sclerosis: a systematic review and meta-analysis. Journal of the neurological sciences 2017;372:331-341.

107. Stein C, O'Keeffe F, McManus C, et al. Premorbid cognitive functioning influences differences between self-reported cognitive difficulties and cognitive assessment in multiple sclerosis. Journal of Neuropsychology 2023.

108. Borsboom D, Cramer AO. Network analysis: an integrative approach to the structure of psychopathology. Annual review of clinical psychology 2013;9:91-121.

109. Sikkes SA, de Lange-de Klerk ES, Pijnenburg YA, et al. A new informant-based questionnaire for instrumental activities of daily living in dementia. Alzheimer's & Dementia 2012;8:536-543.

110. Weber E, Goverover Y, DeLuca J. Beyond cognitive dysfunction: Relevance of ecological validity of neuropsychological tests in multiple sclerosis. Multiple Sclerosis Journal 2019;25:1412-1419.

111. Allataifeh E, Khalil H, Almhdawi K, et al. The clinical correlates of participation levels in people with multiple sclerosis. NeuroRehabilitation 2020;47:153-160.