

Reproducible *in vivo* characterization of multiple sclerosis lesions using multi-modal MRI

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Thesis submitted in partial fulfillment
of the requirements for the degree of
Docteur en sciences de l'ingénieur et technologie

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Abstract

Multiple sclerosis (MS) is the leading cause of non-traumatic neurological disability in young adults, driven by focal lesions in the central nervous system that trigger demyelination, axonal loss, and progressive clinical decline. While conventional magnetic resonance imaging (MRI) remains indispensable for diagnosis, its limited pathological specificity restricts its ability to capture the heterogeneous processes underlying MS evolution, such as chronic inflammation, neurodegeneration, and repair. Advanced quantitative MRI techniques, particularly diffusion-weighted imaging, offer indirect access to tissue microstructure and lesion dynamics *in vivo*. However, their clinical translation is limited by methodological variability, fragmented biomarker interpretation, and an incomplete understanding of how these metrics relate to underlying pathology.

This thesis addresses these challenges through three interconnected contributions, prioritizing reproducibility, biological interpretability, and clinical relevance. First, a user-friendly neuroimaging software platform, BMAT, was developed to facilitate standardized data processing and improve accessibility of advanced MRI analysis pipelines for clinical researchers, thereby promoting reproducibility and open scientific practices. Second, comparative investigations of diffusion MRI models and critical methodological evaluation of chronic active lesion biomarkers were conducted to bridge the gap between engineering implementation and clinical interpretation, clarifying the strengths, limitations, and biological relevance of commonly used imaging markers and methodology. Third, a longitudinal data-driven analysis of MS lesions dynamics was performed to characterize the relationship between chronic inflammatory activity, lesion volume dynamics, and clinical disability, providing new insights into the pathological mechanisms underlying lesion evolution.

Collectively, this thesis advances the methodological rigor and interpretability of quantitative MRI in MS research. By integrating standardized processing tools, validated microstructural models, and longitudinal clinical data, it provides a foundation for more precise *in vivo* characterization of MS pathology. These developments hold promise for refining disease monitoring, improving prognostic accuracy, and ultimately personalizing therapeutic strategies in multiple sclerosis.

Acknowledgments

Completing a PhD is far from a solitary journey, and looking back, I realize how many people have shaped, supported, and carried me through these years. I would like to take these few lines to express my deepest and most heartfelt gratitude to all of you.

First and foremost, I would like to thank my supervisors, Prof. Benoît Macq and Prof. Pietro Maggi, without whom this journey simply would not have been possible. Benoît, thank you for giving me the opportunity to embark on this PhD, which ultimately led me toward a research career I had not initially envisioned, but quickly grew to love. Thank you for believing in me, for your constant positivity, and for your remarkable ability to find solutions even in the most uncertain moments. Your guidance, as well as your role in building this collaboration with Prof. Maggi, has been instrumental in shaping this work and my path. Pietro, thank you for your daily support, your generosity with your time and knowledge, your incredible international network, and your openness to ideas, especially to my more creative and unconventional ones. Your enthusiasm, curiosity, and playfulness made the everyday experience of research lighter and genuinely enjoyable. Being part of your lab, and witnessing it grow, has been a true privilege.

I would also like to sincerely thank all the members of the jury for their time, their insightful feedback, and their thoughtful perspective on this work. In particular, I would like to express my gratitude to Prof. Martina Absinta and Dr. Laurence Dricot for their guidance as exceptional scientists. Martina, since the very beginning of my PhD, your presence has been a constant. Being able to collaborate with you on so many projects has been both an honor and a tremendous learning experience. Your expertise, your sharp feedback, and your openness to discussion have deeply shaped the quality of my work. Thank you, Laurence, for your expertise, your constant support, and your thoughtful advice, as well as for your kindness and empathy. Working alongside you has always felt easy and natural, and I have truly valued every moment of our collaborations, many of which are not represented in this manuscript. They remain, nonetheless, some meaningful and enjoyable parts of this journey.

A special acknowledgment goes to Gaëtan Duchene and Julie Poujol, who helped shape my growing interest in the field of MRI. Your willingness to take the time to explain this complex and fascinating technology was invaluable. It allowed me to better ground my research and inspired me to continue exploring this field further.

To all my colleagues and friends with whom I shared a workspace, collaborated, taught, and traveled to conferences, Nicolas, Quentin, Manon, Clément, Maxence, Karim, Pauline, Simon, Estelle, Anna, Serena, François, Elena, thank you for making everyday work both easier and more enjoyable. Your camaraderie, and your openness to discussing both research and life beyond it, created quite a positive and stimulating environment.

More broadly, I would like to thank all members of the PiLab and IoNS NEUR departments, and especially Lara, Claire, Auriane, Emilie, Yasmine, Flo, Tiffanie, Mélanie, Maxime, Benoît, for the many memorable moments we shared: lunch breaks, coffee breaks, badminton games, board games, raclette evenings, and countless conversations beyond work. Your presence and support made daily life during this PhD both richer and more motivating. I am truly grateful to have met each and every one of you.

I would like to express my heartfelt gratitude to my friends, especially Jacques, Louis, Tanguy, Guillaume, Lara, Bianca, and Zoé. You have been my constant support system throughout these past four years. Thank you for being there in every way that mattered: for listening when I needed to talk, for distracting me when I needed it most, and for celebrating both the big and the small moments along the way. Last but not least, I would also like to thank my family with all my love, and especially my parents, Dominique and Serge. Thank you for your unconditional support, your encouragement, and your belief in me, even at times when I doubted myself. I would not be where I am today without you.

Finally, to all of you, and to those I may not have explicitly mentioned, but whose presence has nonetheless left a mark, thank you for being part of this incredible journey.

Author's Publication List

Journal Papers

1. **Vanden Bulcke, C.**, Wynen, M., Detobel, J., La Rosa, F., Absinta, M., Dricot, L., Macq, B., Bach Cuadra, M., Maggi, P. (2022). BMAT: An open-source BIDS managing and analysis tool. *NeuroImage: Clinical*, 36, 103252. <https://doi.org/10.1016/j.nicl.2022.103252> —¹
2. **Vanden Bulcke, C.**, Stölting, A., Maric, D., Macq, B., Absinta, M., & Maggi, P. (2024). *Comparative overview of multi-shell diffusion MRI models to characterize the microstructure of multiple sclerosis lesions and periplaques*. *NeuroImage: Clinical*, 42, 103593. <https://doi.org/10.1016/j.nicl.2024.103593> —²
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4. Wynen, M.*, **Vanden Bulcke, C.***, Borrelli, S., Gordaliza, P. M., Stölting, A., Guisset, F., Cordier, C., Martire, M. S., Tamanti, A., Macq, B., Sati, P., Filippi, M., Calabrese, M., Absinta, M., Reich, D. S., Bach Cuadra, M.*, & Maggi, P.* (2026). *Machine learning-based combination of the central vein sign, cortical lesions and paramagnetic rim lesions: A web-based tool for the diagnosis of multiple sclerosis*. *Brain Communications*, 8(2), fcag079. <https://doi.org/10.1093/braincomms/fcag079> —⁴

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Conference Oral Presentation

1. Wynen, M.*, **Vanden Bulcke, C.***, Borrelli, S., Gordaliza, P. M., Stölting A., Guisset F., Cordier C., Martire M. S., Tamanti A., Macq B., Sati P., Filippi M., Calabrese M., Absinta M., Reich D. S., Cuadra M. B.*, & Maggi P. *Machine Learning-Based Combination of the Central Vein Sign, Cortical Lesions and Paramagnetic Rim Lesions: A Web-Based Tool for the Diagnosis of Multiple Sclerosis*. Oral presentation at France SEP conference (Paris, 2025)
2. **Vanden Bulcke, C.**, Stölting, A., Macq, B., Maggi, P. *Advanced diffusion imaging methods to assess the microstructure of multiple sclerosis lesions*. Oral presentation at International Society for Magnetic Resonance in Medicine (ISMRM) (Toronto, 2023). In: Proc. Intl. Soc. Mag. Reson. Med. 31 (2023) —¹⁵

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1. **Vanden Bulcke, C.**, Stölting, A., Absinta, M., Maggi, P. *Microstructural assessment of multiple sclerosis lesions and peri-plaques using advanced multishell diffusion MRI*. European Committee for Treatment and Research in Multiple Sclerosis (Milan, 2023). In: Multiple Sclerosis Journal, Vol. Volume 29, no.Issue 3_suppl, p. 394 - 649 (2023). doi:10.1177/13524585231196194. —¹⁶
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5. **Vanden Bulcke, C., Borrelli, S., Stölting A., Du Pasquier, R., Macq, B., Maggi, P.** *Synthetic DIR/PSIR contrasts computed with clinical sequences for cortical lesion assessment.* IEEE International Symposium on Biomedical Imaging (ISBI 2024) (Athens, 2024). <http://hdl.handle.net/2078.1/293909>

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Acronyms

AD	Axial Diffusivity
BBB	Blood-Brain Barrier
BIDS	Brain Imaging Datastructure
BMAT	BIDS Managing and Analysis Tool
CAL	Chronic Active Lesion
CEL	Contrast-Enhancing Lesion
CESPR	Contrast-Enhanced Spoiled-Gradient Recalled Echo
CNS	Central Nervous System
CPU	Central Processing Unit
CSD	Constrained Spherical Deconvolution
CSF	Cerebro-Spinal Fluid
CUSL	Cliniques Universitaires Saint-Luc (Saint-Luc University Hospital)
CVS	Central-Vein Sign
DIAMOND	DIstribution of Anisotropic MicrOstructural eNvironments in DWI
DICOM	Digital Imaging and Communications in Medicine
DIR	Double Inversion Recovery

DMT	Disease-Modifying Therapy
DSC	Dice Similarity Score
DTI	Diffusion Tensor Imaging
DWI	Diffusion-Weighted Imaging
EBV	Epstein-Barr Virus
EDSS	Expanded Disability Status Scale
EL	Expanding Lesion
EPI	Echo Planar Imaging
ERC	European Research Council
FA	Fractional Anisotropy
FID	Free Induction Decay
FLAIR	Fluid-Attenuated Inversion Recovery
FOV	Field Of View
Fig.	Figure
GE	General Electrics
GM	Gray Matter
GRE	Gradient-Echo
GUI	Graphical User Interface
HC	Healthy Control
HLADR	Human Leukocyte Antigen - DR isotope
HPC	High-Performance Computing
ID	Identification
IP	Internet Protocol
IR	Inversion Recovery
IoNS	Institute of NeuroScience

JI	Jaccard Index
JSON	JavaScript Object Notation
LMM	Linear Mixed-effects Model
MBP	Myelin Basic Protein
MD	Mean Diffusivity
MF	Microstructure Fingerprinting
MI	Mutual Information
MP2RAGE	Magnetization-Prepared 2 Gradient-Echo
MPRAGE	Magnetization-Prepared Gradient-Echo
MRI	Magnetic Resonance Imaging
MSE	Mean Square Error
MSSS	Multiple Sclerosis Severity Score
MS	Multiple Sclerosis
NAIMS	North American Imaging in Multiple Sclerosis
NAWM	Normal-Appearing White Matter
NFH	NeuroFilament Heavy chain
NIMA	Neuroimaging platform
NIfTI	Neuroimaging Informatics Technology Initiative
NMR	Nuclear Magnetic Resonance
NODDI	Neurite Orientation Dispersion and Density Imaging
OS	Operating System
PGSE	Pulsed Gradient Spin-Echo
PLP	ProteoLipid Protein
PMS	Progressive Multiple Sclerosis
PPMS	Primary Progressive Multiple Sclerosis

PPWM	PeriPlaque White Matter
PRL	Paramagnetic Rim Lesion
QSM	Quantitative Susceptibility Mapping
RD	Radial Diffusivity
RF	Radiofrequency
ROC	Receiver Operating Characteristic
ROI	Region Of Interest
RRMS	Relapsing-Remitting Multiple Sclerosis
SANDI	Soma And Neurite Density Imaging
SEL	Slowly Expanding Lesion
SE	Spin-Echo
SLURM	Simple Linux Utility for Resource Management
SL	Shrinking Lesion
SNR	Signal-to-Noise Ratio
SPMS	Secondary Progressive Multiple Sclerosis
SSH	Secure Shell
SSS	Secteur des Sciences de la santé (Health Science Sector)
SVM	Support Vector Machine
T1w	T1-weighted
T2*w	T2*-weighted
T2w	T2-weighted
TE	Echo Time
TI	Inversion Time
TR	Repetition Time
TSE	Turbo Spin-Echo

TSPO-PET	18-kDa Translocator Protein - Positron Emission Tomography
TSV	Tabular Separated Value
WM	White Matter
95% CI	95% Confidence Interval
auc	Area under the curve
cf.	<i>conferatur</i>
dMRI	Diffusion MRI
ecvf	Extra-axonal volume fraction
fODF	fiber Orientation Distribution Function
fcsf	Free Water volume fraction
fiso	Isotropic diffusivity volume fraction
fixel	<i>One fiber population per voxel</i>
fvf	Fiber volume fraction
icvf	Intra-axonal volume fraction
odi	Orientation dispersion index
R_s	Apparent soma radius
κ	Cohen's Kappa
d_{ear}	extra-axonal restricted diffusivity
f_{csf}	free water volume fraction
f_{ear}	extra-axonal restricted diffusion volume fraction
f_{ec}	extra-cellular volume fraction
f_{extra}	extra-axonal volume fraction
f_{fibers}	fiber volume fraction
f_{intra}	intra-axonal volume fraction

f_{iso}	isotropic diffusivity volume fraction
f_{is}	intra-soma volume fraction
$f_{neurite}$	neurite volume fraction

1

Introduction

1.1 Context and Thesis Motivation

Multiple sclerosis (MS) is the most common chronic inflammatory disease of the central nervous system (CNS) and the leading cause of non-traumatic neurological disability in young adults. More than two million people worldwide are affected by the disease, which remains incurable despite the advent of new high-efficacy therapies. MS is characterized by the formation of focal inflammatory lesions in the brain and spinal cord, leading to demyelination, axonal injury, and, over time, progressive and often irreversible neurological impairment.²⁰ This accumulation of disability severely impacts quality of life, representing a major personal and societal burden. Consequently, a central challenge in MS research is to understand how these lesions form, evolve, and drive long-term tissue damage, thereby enabling the development of more targeted and effective disease-modifying therapies.

The biological processes underlying MS lesion formation are highly complex, involving interactions between the peripheral immune system, resident CNS glial cells, and neurons. These interactions result in waves of inflammation, tissue injury, and, in some cases, partial repair.²⁰ Our current understanding of MS pathophysiology is largely derived from *post mortem* histopathological studies, which provide detailed cellular and molecular maps of lesions. These studies have revealed that MS lesions exist in distinct pathological states, including active inflammatory lesions, chronic active (or "smoldering") lesions with persistent inflammatory activity at their borders, chronic inactive lesions, and partially remyelinated lesions.²¹ Among these, active and chronic active lesions are considered the primary drivers of ongoing tissue damage as well as short-term²² and long-term disability.²³ However, histopathology captures the disease at a single time point; therefore, it cannot track how individual lesions evolve in the living brain, limiting our ability to characterize their dynamic behavior.

Magnetic resonance imaging (MRI) offers a unique opportunity to study MS *in vivo*. By leveraging its sensitivity to the physical and chemical properties of tissue at microscopic scales, MRI allows clinicians and researchers to visualize MS lesions non-invasively and monitor them longitudinally. Conventional MRI sequences enable the detection and quantification of lesions throughout the CNS, while specialized sequences can reveal aspects of lesion activity, such as the blood-brain barrier disruption characteristic of

active lesions (visualized using gadolinium-based contrast agents). More recently, advanced MRI techniques have made it possible to probe features more directly linked to underlying pathology. For instance, susceptibility-based MRI can detect iron accumulation in activated microglia at the edges of specific chronic active lesions, manifesting as *paramagnetic rim lesions* (PRLs).²⁴ In parallel, longitudinal MRI studies have identified *slowly expanding lesions* (SELs),²⁵ which increase in size over time and are thought to reflect ongoing inflammatory activity. While both PRLs and SELs are used as *in vivo* markers of chronic lesion activity, they do not always overlap^{26,27} and their relationship to underlying pathology remains incompletely understood, underscoring significant gaps in our current ability to characterize MS lesion dynamics.²⁸

Quantitative MRI represents a further step toward investigating MS lesions at the microstructural level. By measuring physical tissue properties — such as relaxation times or the random diffusive motion of water molecules — quantitative MRI captures changes in myelin content, cellular density, and tissue organization. Diffusion MRI (dMRI), in particular, is sensitive to the constraints placed on water motion by cellular structures (e.g., axons, glial cells) and the extracellular space, rendering it a powerful tool for studying MS-induced microstructural alterations. While these techniques have demonstrated clear differences between lesion types and normal-appearing tissue, they remain limited by their lack of specificity, i.e. diverse biological changes can produce similar diffusion MRI signals. Additionally, the heterogeneity of acquisition protocols and diffusion models complicates the comparison of results across studies and the identification of the most biologically informative measures.

In this thesis, we position our work in this complex and interdisciplinary framework with the following **Central Research Question**:

Central Research Question?

How can multimodal MRI be used to achieve accurate, reproducible, and biologically meaningful characterization of multiple sclerosis lesions?

A representative example of microstructural MS lesion imaging

At the start of this PhD, I contributed to a longitudinal MRI study led by Prof. Pietro Maggi, titled *B cell depletion therapy does not resolve chronic active multiple sclerosis lesions*.⁵ This study illustrates both the promises and the limitations of current MRI-based approaches to lesion microstructure. Using quantitative T_1 mapping and quantitative susceptibility mapping (QSM) in a multi-center cohort, the study investigated whether high-efficacy B-cell-depleting therapy could suppress chronic inflammatory activity in PRLs, serving as a proxy for chronic active lesions. Despite successful *in silico* modulation of inflammatory signaling pathways in microglia, chronic active lesions persisted *in vivo*, indicating that these lesions are resistant to current therapies and remain a major driver of tissue damage. Crucially, this work exposed several methodological and conceptual hurdles that affect many of the current *in vivo* microstructural studies of MS lesions:

Longitudinal, multi-center MRI studies are inherently complex, necessitating rigorous standardization of data acquisition, management, and processing to ensure robustness and reproducibility.

Quantitative and diffusion MRI rely on diverse acquisition protocols and mathematical models, making it difficult to compare findings across studies or determine which approaches maximize sensitivity and specificity to MS pathology.

Different MRI markers of chronic lesion activity, such as PRLs and SELs, are often used interchangeably despite an incomplete understanding of their interrelationship and their correspondence to histopathological definitions.

Lesion analyses commonly rely on the manual or semi-manual segmentation of a limited subsample of lesions. This labor-intensive process necessarily introduces selection bias and restricts the ability to characterize the full MS lesion spectrum.

This thesis addresses these challenges through the development of a structured approach for reproducible MRI-based characterization of MS lesions. By integrating advanced imaging techniques with thoughtful data processing and analysis strategies, this work aims to deepen our understanding of chronic active lesions and enhance the reliability of *in vivo* MS lesion characterization.

1.2 Thesis outline and contributions

To address this central research question, this thesis is structured around four key methodological, conceptual and clinical contributions, each presented in its own chapter and directly targeting one of the limitations identified above by defining specific sub *Research Questions* (see Figure 1.1). Together, they build toward a more robust, interpretable, and clinically grounded framework for MRI-based MS lesion characterization, with a particular focus on CALs, a key driver of irreversible neurological disability. A brief overview of each chapter is provided below.

Chapter 2: *Theoretical background*

This chapter establishes the conceptual and technical foundations required to understand the contributions of this thesis. It is designed to bridge two complementary domains: the physics of MRI, the pathophysiology of MS, and the link between the two provided by quantitative and diffusion imaging.

The first part introduces the physical principles underlying magnetic resonance imaging, from its historical development to the core mechanisms that govern signal generation and image formation. This section provides an intuitive understanding of how MRI encodes information about tissue properties and reviews the main classes of MRI sequences and contrasts used in MS neuroimaging. Particular emphasis is placed on quantitative MRI and diffusion MRI, which enable the measurement of tissue relaxation, susceptibility, and microstructural properties through a range of modeling approaches.

The second part of the chapter focuses on the pathophysiology of MS. It describes how the interplay between immune cells, glial cells, and neurons drives the formation, evolution, and heterogeneity of MS lesions. Special attention is given to the cellular and microstructural processes — such as inflammation, demyelination, axonal injury, and gliosis — that shape lesion development over time.

Finally, this chapter connects these two perspectives by explaining how pathological changes occurring at the cellular and tissue scale translate into measurable MRI signals. By linking MS biology to MRI observables, this chapter provides the framework needed to interpret *in vivo* imaging biomarkers of lesion activity and microstructural damage that are explored throughout the remainder of the thesis.

Chapter 3: *BMAT: an open-source BIDS Managing and Analysis Tool*

Research Question 1? Reproducibility & standardization

How to standardize multi-center MRI studies for robust and reproducible MS lesion characterization?

This chapter introduces the *BIDS Managing and Analysis Tool* (BMAT), an open-source, local software platform designed to make BIDS-based neuroimaging research accessible, efficient, and scalable. BMAT provides a graphical user interface that allows users to convert scanner data into BIDS, curate and manage BIDS datasets, as well as execute automated processing pipelines within a unified environment.

Beyond its technical features, this chapter demonstrates the successful deployment of BMAT in a real research environment over multiple years, showing how it enables standardized data handling, reproducible analyses, and collaborative workflows at the scale of an institute. By lowering the technical barrier to BIDS adoption, BMAT provides the infrastructure required to perform robust, multi-centre, and longitudinal microstructural investigations of MS lesions, thereby directly addressing one of the central limitations highlighted in the introduction.

Chapter 4: *Comparative overview of multi-shell diffusion MRI models to characterize the microstructure of multiple sclerosis lesions and periplaques*

Research Question 2? MS pathology modeling

Which diffusion MRI models most sensitively and specifically capture MS complex pathophysiology?

One of the central methodological challenges in microstructural imaging of multiple sclerosis is the strong dependence of biological interpretation on the choice of diffusion model. Although diffusion MRI is widely used to probe MS lesions, the field is fragmented by the coexistence of many competing models, each providing different and sometimes contradictory metrics of tissue damage.

This chapter provides a systematic, side-by-side comparison of several state-of-the-art multi-shell diffusion models, together with quantitative T1 mapping and a model-free diffusion approach, to characterize the microstructural architecture of MS lesions and their surrounding tissue. The analysis spans multiple lesion stages — contrast-enhancing, chronic active (paramagnetic rim), and chronic inactive lesions — as well as their periplaque white matter and the normal-appearing white matter.

By explicitly comparing diffusion models rather than treating them as interchangeable, this work demonstrates that MS-related tissue damage extends well beyond the MRI-visible lesion edge, forming a gradient of microstructural abnormality from the lesion core into the surrounding white matter. Importantly, while multi-compartment diffusion models with biophysically interpretable parameters can robustly capture these subtle and spatially complex pathological patterns, simpler signal-representation models, such as diffusion tensor imaging, consistently underperformed to discriminate lesion types and underestimate extra-lesional damage.

Therefore, this work provides both a biological and a methodological contribution: it refines our understanding of the microstructural signature of MS lesions and their interaction with their periplaque, and it establishes principled guidelines for selecting diffusion models that are more sensitive to neuroinflammatory processes *in vivo*.

Chapter 5: *Do slowly expanding lesions correspond to chronic active multiple sclerosis lesions? An integrated imaging analysis study*

Research Question 3? CALs Biomarker interpretation

What is the relationship between SELs and PRLs as MRI-defined biomarkers of chronic active lesions?

This chapter directly addresses one of the most pressing conceptual ambiguities in contemporary MS imaging: whether SELs truly represent histopathological CALs, and what their relationship with PRLs is.

Using a strictly controlled longitudinal dataset acquired on a single scanner with homogeneous protocols, this study performs a rigorous head-to-head comparison between SELs and PRLs, the latter being a histopathologically validated *in vivo* marker of CALs. Contrary to the prevailing assumption that chronic active lesions must expand over time, PRLs were found to include both shrinking and expanding lesions, indicating that volume expansion is not a defining feature of chronic activity.

At the methodological level, this chapter demonstrates that SEL detection is highly sensitive to image resolution, and segmentation algorithmic choices, leading to poor reproducibility across reasonable variations of the pipeline. Most critically, SELs showed essentially no lesion-level correspondence with PRLs once chance overlap was accounted for, and their association with clinical disability was weaker and less stable.

Together, these results challenge the use of SELs as a surrogate biomarker of chronic active pathology. This chapter therefore clarifies a major source of confusion in the field and argues for a conceptual separation between histopathological lesion subtypes (such as CALs) and MRI-based descriptors of longitudinal lesion evolution.

By disentangling these two dimensions — biological lesion identity and imaging-based longitudinal behavior — this work lays the foundation for more biologically grounded and reproducible biomarkers of chronic lesion activity.

Chapter 6: *In vivo* characterization of multiple sclerosis "MRI evolving lesions"

Research Question 4? Longitudinal MS lesions characterization

How does the microstructural evolution of chronic active lesions relate to irreversible disability accumulation in MS?

A central challenge in multiple sclerosis research is to understand why some lesions remain relatively quiescent while others drive irreversible tissue damage and long-term disability. Although CALs have emerged as key contributors to disease progression, their *in vivo* characterization remains incomplete. Current imaging markers — most notably PRLs and lesion expansion — capture important but only partial aspects of chronic lesion biology, and are often treated as interchangeable indicators of ongoing inflammation. This work is motivated by the need to move beyond this simplified view and to reconcile the apparent heterogeneity of chronic active lesions observed *in vivo* with their underlying microstructural and clinical consequences.

In this chapter, we leverage longitudinal quantitative MRI to dissect the complex relationship between lesion activity, microstructural tissue integrity, and clinical progression in MS. By jointly analyzing PRL status and lesion volumetric evolution with advanced diffusion-derived microstructural metrics, this work provides a unified framework for interpreting chronic active lesion heterogeneity. Rather than assuming that lesion expansion or PRL presence alone defines lesion aggressiveness, this study demonstrates that chronic active lesion behavior is best understood through their combined microstructural trajectories, revealing unexpected pathological patterns in shrinking lesions, traditionally considered as benign.

Beyond refining lesion classification, this work establishes a direct link between lesion-level microstructural evolution and subject-level clinical outcomes, highlighting which lesion phenotypes are most relevant for disability accumulation and neurodegeneration. As such, this contribution bridges the gap between mechanistic imaging biomarkers and clinically meaningful disease progression. More broadly, it illustrates how integrating complementary MRI contrasts and longitudinal modeling can uncover hidden dimensions of MS pathology, laying the groundwork for more biologically informed biomarkers and, ultimately, the potential development of more targeted therapeutic strategies.

Chapter 7: *Reflexive synthesis and discussion of my PhD journey*

This final chapter offers a more reflexive synthesis and discussion of the doctoral work, stepping beyond a conventional recapitulation of results to examine the intellectual and methodological trajectory of the thesis as a whole. It reflects on the central role of biomedical engineering in neuroimaging research and on creativity as a driving force in scientific discovery. By revisiting key contributions through these lenses, the chapter highlights how methodological choices, interdisciplinary dialogue, and data-driven reasoning shaped this research. This perspective aims to contextualize the scientific findings within a broader understanding of how complex neuroimaging research is conceived, developed, and interpreted.

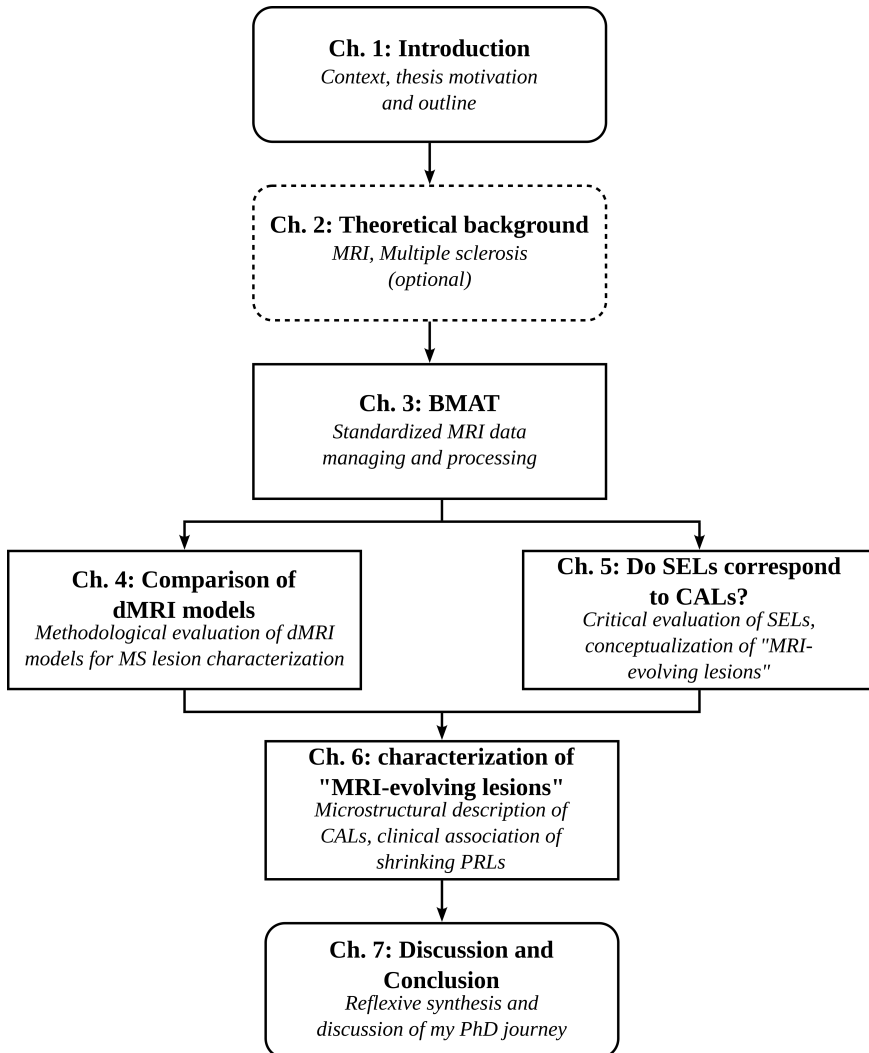


Fig. 1.1 Overview of the thesis outline

2

Theoretical Background

Preliminary Note:

This chapter provides a brief overview of the fundamental magnetic resonance imaging principles and multiple sclerosis lesion pathophysiology necessary for this thesis.

Readers already familiar with the following concepts are invited to skip this chapter.

- *Magnetic Resonance Imaging*
 - *A brief history of MRI*
 - *An overview of MRI sequences*
 - *Diffusion-weighted MRI*
- *Multiple Sclerosis*
 - *Epidemiology and Etiology*
 - *MS Pathogenesis*
 - *MRI as a window into MS pathophysiology*

2.1 Magnetic Resonance Imaging

This section provides a theoretical overview of the physical principles and methodological developments that characterize modern magnetic resonance imaging (MRI). Beginning with the fundamental concepts of nuclear magnetic resonance and spin relaxation, it traces through its history how magnetic fields, radiofrequency excitation, and gradient encoding enable the acquisition of spatially resolved images as we know today. The evolution of MRI pulse sequences is then presented, highlighting how different acquisition strategies give rise to specific image contrasts and sensitivities. Particular emphasis is placed on diffusion MRI, which extends conventional MRI by probing the microscopic motion of water molecules to infer tissue microstructure. Finally, a range of diffusion models is introduced, from tensor-based representations to multi-compartment and numerical approaches, illustrating how increasingly sophisticated models allow MRI to access progressively richer biophysical information about biological tissues.

2.1.1 A brief history of MRI

Magnetic Resonance Imaging (MRI) is a relatively recent medical imaging modality, yet it has become one of the most powerful tools for the diagnosis and follow-up of disease, as well as for advancing our understanding of brain structure and function in neuroscience. Although the foundations of MRI rely on physical concepts developed over a century ago, its clinical adoption is the result of a series of recent key scientific breakthroughs, several of which were recognized by Nobel Prizes.

One of the earliest conceptual contributions can be traced back to Nikola Tesla, who introduced the concept of a rotating magnetic field in 1882. While far removed from medical imaging, this work laid part of the theoretical groundwork for modern electromagnetic technologies. In recognition of his contributions, the unit of magnetic field strength, the tesla (T), bears his name and is an essential measure unit in MRI. In the following sections, we address the challenges of understanding the fundamental principles of MRI by tracing its development through its major historical milestones.

Nuclear Magnetic Resonance (NMR)

The physical principles underlying MRI originate from nuclear magnetic resonance (NMR) and quantum spin theory. The first experimental demonstration of NMR was achieved by Isidor I. Rabi in 1938,²⁹ for which he was awarded the Nobel Prize in Physics in 1944. Rabi showed that atomic nuclei exposed to a magnetic field could absorb and emit radiofrequency (RF) energy at characteristic frequencies determined by their magnetic properties and the strength of the applied magnetic field.

In the late 1940s, Felix Bloch and Edward Purcell independently demonstrated that the same resonance phenomenon could be observed in bulk matter, including solids and liquids, rather than only in particle beams. Their work led to the formulation of the Bloch equations, which describe the dynamics of nuclear magnetization under the influence of magnetic fields and RF excitation, including the processes of precession and relaxation of quantum spins.^{30–32} Bloch and Purcell were jointly awarded the Nobel Prize in Physics in 1952 for these discoveries.

The relevance of NMR to medical imaging arises from the chemical composition of biological tissues, which consist largely of water molecules and therefore contain abundant hydrogen nuclei. A hydrogen nucleus is composed of a single proton and possesses a non-zero intrinsic angular momentum, or spin ($\text{spin}^{1/2}$), which gives rise to a magnetic dipole moment. As a consequence, hydrogen nuclei interact with external magnetic fields.

In the absence of an external magnetic field, nuclear spins are randomly oriented, resulting in a net macroscopic magnetization equal to zero. When placed in a strong static magnetic field (B_0), however, the magnetic moments of the hydrogen nuclei tend to align either parallel (low-energy state) or antiparallel (high-energy state) with respect to the field direction. Because the parallel configuration is energetically favored, a slight excess of spins occupies this state, giving rise to a net macroscopic magnetization vector ($\mathbf{M}$) aligned with B_0 (see Figure 2.1). By convention, this direction defines the longitudinal axis.

In addition to alignment, nuclear spins undergo a rotational motion, referred to as precession, around the direction of the applied magnetic field. This precession occurs at a characteristic angular frequency known as the Larmor frequency:

$$\omega_0 = \gamma B_0, \quad (2.1)$$

where γ denotes the gyromagnetic ratio, a constant specific to the nuclear species, and B_0 is the strength of the static magnetic field. The coherent precession of the spins gives rise to a time-varying transverse magnetization, which can be perturbed by RF pulses and detected as an NMR signal. This signal forms the physical basis upon which all MRI techniques are built.

NMR Signal and Relaxation

To generate an NMR signal, the system at thermal equilibrium can be perturbed by applying a RF pulse at the Larmor frequency, which allows for efficient energy transfer to the nuclear spin system. This excitation promotes a fraction of the spins from the lower-energy (parallel) state to the higher-energy (antiparallel) state, thereby disturbing the equilibrium magnetization. The RF excitation produces two key effects (see Figure 2.1):

1. The RF pulse tips the net magnetization vector $\mathbf{M}$ away from the longitudinal axis into the transverse plane. The flip angle depends on both the amplitude and duration of the RF pulse, giving rise to the conventional nomenclature of RF pulses (e.g., a 90° pulse rotates $\mathbf{M}$ entirely into the transverse plane). This reorientation in the transverse plane is essential for signal detection, as receiver coils are sensitive only to time-varying magnetic fields perpendicular to the main magnetic field B_0 . Changes in magnetization along the direction of B_0 cannot be directly detected because B_0 is several orders of magnitude larger than $\mathbf{M}$.
2. The RF pulse also induces phase coherence among the precessing spins, such that immediately after excitation, the spins precess synchronously and point in the same direction in the transverse plane. This coherent precession generates an oscillating transverse magnetic field, which induces a measurable voltage in the receiver coils.

Because RF excitation is applied as a short pulse, the detected signal following excitation takes the form of a *free induction decay* (FID). In the absence of further excitation, the system gradually returns to thermal equilibrium, a phenomenon first characterized by Hahn in 1950.³³ This return to equilibrium occurs through two fundamental relaxation processes (see Figure 2.1):

- **Longitudinal relaxation:** After the RF pulse is switched off, the excess energy stored in the spin system is transferred to the surrounding molecular environment (the lattice), partially in the form of heat. As a result, spins progressively return to the lower-energy parallel state, and the longitudinal component of the magnetization vector $\mathbf{M}$ recovers along the direction of B_0 . This recovery follows an exponential behavior and is characterized by the longitudinal relaxation time constant T_1 , defined as the time required for the longitudinal magnetization to recover 63% of its equilibrium value. Because this process depends on interactions between the spins and their molecular environment, it is also referred to as *spin-lattice* or T_1 relaxation.
- **Transverse relaxation:** Following RF excitation, transverse relaxation describes the loss of phase coherence among spins in the transverse plane. This process leads to a decay of the transverse magnetization and is characterized by two distinct time constants:
 - T_2 **relaxation** represents the irreversible loss of transverse phase coherence due to microscopic, time-dependent magnetic field fluctuations arising from *spin-spin* interactions between neighboring nuclei.
 - T_2^* **relaxation** represents the effective transverse relaxation time and combines true T_2 relaxation with additional reversible dephasing caused by static or slowly varying magnetic field inhomogeneities. These inhomogeneities may be macroscopic (e.g., imperfections in the main magnetic field or susceptibility differences at tissue–air or tissue–bone interfaces) or microscopic, such as those arising from paramagnetic substances (e.g., iron, deoxy-hemoglobin, ferritin) or diamagnetic substances (e.g., myelin, calcium), which generate local but effectively static magnetic field gradients.

Because hydrogen nuclei in biological tissues experience different molecular environments (e.g. free water, gray matter, or white matter) they exhibit distinct relaxation behaviors. Variations in molecular mobility, chemical composition, and local magnetic susceptibility lead to tissue-specific T_1 , T_2 , and T_2^* relaxation times. By recording the full FID signal following excitation and transforming it into the frequency domain using a Fourier transform, these differences can be quantified and visualized as a frequency

spectrum. This principle forms the basis of NMR spectroscopy, notably developed by Carr and Purcell in 1954.³⁴

During the same period, G. Lindström and E. Odeblad proposed the use of NMR to investigate the composition of biological tissues.³⁵ While these developments demonstrated the potential of NMR for probing microscopic tissue properties, the resulting signal remained spatially non-resolved, corresponding to the integrated response of the entire excited volume and yielding only a one-dimensional spectral measurement.

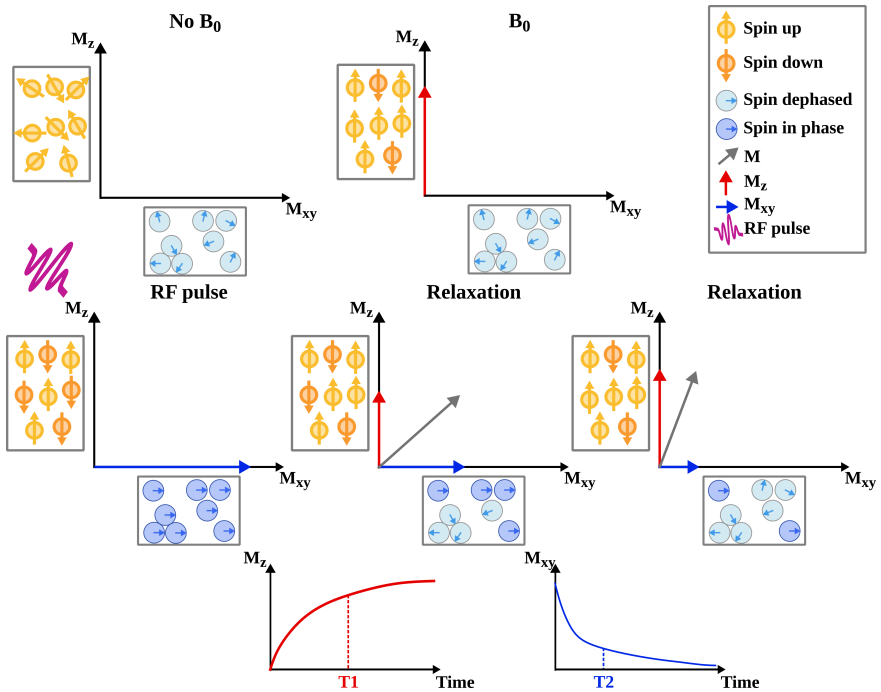


Fig. 2.1 Representation of the Magnetization process (top row) arising from an external B_0 magnetic field. The middle row represents the Relaxation process following a 90° radio-frequency (RF) pulse. The bottom row represents two M_z (in red) and M_{xy} (in blue) exponential relaxation over time, with their respective T_1 and T_2 time constant.

Spatial gradient encoding and first MRI images

A final essential ingredient required to produce magnetic resonance images is spatial encoding. In the situation described above, all spins within the static magnetic field B_0 are excited simultaneously by an RF pulse and subsequently contribute to the detected NMR signal. Because the receiver coils are located outside the object, they inherently record the superposition of signals emitted by all excited spins, without any intrinsic spatial discrimination. Without an additional spatial encoding mechanism, this signal contains no information about the spatial origin of the magnetization, thereby preventing image reconstruction.

The introduction of spatial encoding in magnetic resonance was achieved in the early 1970s by Paul Lauterbur, who demonstrated that controlled magnetic field gradients could be incorporated into NMR pulse sequences to encode spatial information and generate the first magnetic resonance images.³⁶ Magnetic field gradients are linear variations of the magnetic field strength superimposed on B_0 , causing the Larmor frequency to become position dependent along the direction of the applied gradient. An illustration of spatial encoding gradients is displayed in Figure 2.2.

By applying gradients along the three orthogonal spatial directions, spatial information can be encoded into the frequency and phase of the NMR signal. Following RF excitation, spins located at different positions experience different magnetic field strengths and therefore precess at different frequencies or accumulate different phases depending on the gradient amplitude and duration. As a result, the detected signal is no longer a single coherent oscillation but a superposition of spatially encoded frequency and phase components.

By systematically varying the strength and duration of these encoding gradients, the measured signal can be sampled in a multidimensional spatial-frequency domain known as *k-space*. Each point in *k-space* corresponds to a specific combination of gradient-induced spatial frequencies (see Figure 2.2), and the complete sampling of this domain contains all the information required to reconstruct an image. The spatial distribution of the magnetization is then obtained by applying an inverse Fourier transform to the acquired *k-space* data, yielding an image in spatial space.

Because the NMR signal is inherently complex-valued, reflecting both amplitude and phase information, the receiver coils are designed to detect two orthogonal components of the transverse magnetization, which are conventionally represented as the real and imaginary parts of the signal. In

most clinical applications, the final MRI image is derived from the magnitude of the complex reconstructed signal, although phase information can also be exploited in specific imaging techniques.

The development of spatial encoding using magnetic field gradients was further advanced by Peter Mansfield, who introduced key theoretical and practical innovations enabling rapid image acquisition and improved image quality.³⁷ In recognition of their foundational contributions to magnetic resonance imaging, Paul Lauterbur and Peter Mansfield were jointly awarded the Nobel Prize in Physiology or Medicine in 2003.

In parallel during the 1970s, Raymond Damadian proposed that NMR could be used as a non-invasive method for detecting pathological tissue, notably demonstrating differences in relaxation properties between healthy and cancerous tissues.³⁸ These findings highlighted the remarkable sensitivity of MRI to microscopic tissue properties, showing that even at macroscopic imaging resolutions (typically on the order of millimeters) MRI can probe cellular- and molecular-scale interactions and variations in tissue composition, provided that these differences are sufficiently pronounced.

2.1.2 An overview of MRI sequences

With the advent of spatial gradient encoding, the development of magnetic resonance imaging rapidly shifted from signal detection toward the design of pulse sequences capable of generating meaningful tissue contrast. MRI sequences define how RF pulses, magnetic field gradients, and signal readout are combined over time, thereby determining image contrast, spatial resolution, acquisition speed, and sensitivity to specific tissue properties. Among these sequence parameters, the repetition time (TR) defines the interval between successive excitation RF pulses, while the echo time (TE) corresponds to the delay between excitation and signal readout. These timing parameters play a central role in controlling how tissue relaxation properties contribute to image contrast. Early MRI development focused on exploiting relaxation phenomena to differentiate tissues, leading to the establishment of contrast-weighted imaging as a central feature of MRI.

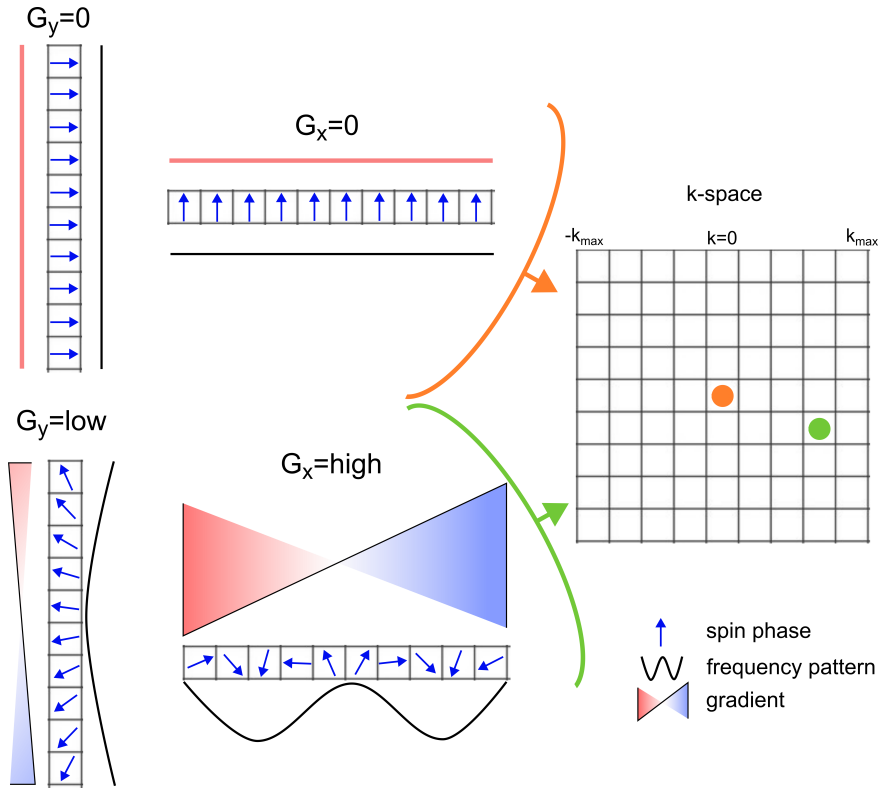


Fig. 2.2 Representation of the MRI spatial encoding gradients mechanisms to sample the k-space (on the right). The top left represents the situation where no spatial encoding gradients are applied, therefore sampling the central frequency ($k=0$). The bottom left represents an application of a low gradient in the y-axis and high gradient in the x-axis, resulting in the sampling of a different position (low y, high x) of the k-space.

Contrast mechanisms: T_1 , T_2 , and T_2^* weighting

The most fundamental MRI contrasts arise from differences in longitudinal (T_1) and transverse (T_2) relaxation times across tissues. By adjusting TR and TE, MRI sequences can be made preferentially sensitive to these relaxation processes. T_1 -weighted imaging emphasizes differences in longitudinal relaxation and is commonly used to depict anatomical detail, whereas T_2 -weighted imaging highlights transverse relaxation differences and is particularly sensitive to fluid content and pathological changes. In contrast, T_2^* -weighted imaging exploits both true transverse relaxation and susceptibility-induced dephasing, providing sensitivity to magnetic field

inhomogeneities caused by tissue composition, blood oxygenation, or paramagnetic substances.

Spin-echo and gradient-echo sequences

The spin-echo (SE) sequence represents one of the earliest and most robust MRI pulse sequences.³³ By incorporating a refocusing RF pulse, spin-echo sequences compensate for static magnetic field inhomogeneities, enabling reliable measurement of true T_2 contrast. Variants such as turbo spin-echo (TSE), also known as fast spin-echo, further improved acquisition efficiency by acquiring multiple echoes following a single excitation, significantly reducing scan times while preserving T_2 contrast.

Gradient-echo (GRE) sequences emerged as a faster alternative by replacing the refocusing RF pulse with gradient reversals. This simplification allows for shorter repetition times and rapid imaging but results in sensitivity to T_2^* effects rather than true T_2 relaxation. GRE sequences form the basis of many modern MRI techniques, including susceptibility-weighted imaging and functional MRI.

Magnetization-prepared gradient-echo (MPRAGE) sequences extend the GRE framework by introducing a preparation module prior to image readout. These preparation pulses manipulate the longitudinal magnetization to enhance specific contrasts, such as T_1 weighting, before rapid gradient-echo acquisition. This approach enables high-resolution, three-dimensional imaging with strong and flexible contrast control.

Echo-planar imaging

Echo-planar imaging (EPI), introduced by Mansfield,³⁷ marked a major technological advancement by enabling the acquisition of an entire image following a single RF excitation. By rapidly traversing k-space using oscillating readout gradients, EPI achieves extremely fast imaging at the cost of increased sensitivity to magnetic field inhomogeneities and geometric distortions. Despite these limitations, EPI remains a cornerstone of modern MRI due to its speed and is widely used in applications such as diffusion-weighted imaging and functional MRI.

Inversion recovery and tissue suppression

Inversion recovery (IR) sequences further expanded MRI contrast capabilities by introducing an inversion pulse prior to excitation. By selecting an appropriate inversion time, specific tissue signals can be selectively suppressed. A prominent example is fluid-attenuated inversion recovery (FLAIR), which suppresses cerebrospinal fluid signal while preserving T_2 contrast, thereby improving lesion conspicuity in neurological imaging. Double inversion recovery (DIR) extends this concept by suppressing two tissue components simultaneously (fluid and white matter), enabling enhanced visualization of cortical and juxtacortical structures.

Quantitative MRI and diffusion imaging

More recent developments have shifted MRI from qualitative contrast-weighted imaging toward quantitative characterization of tissue properties. Quantitative MRI aims to directly estimate intrinsic physical parameters, such as relaxation times, rather than relying on qualitative image contrast. The MP2RAGE sequence represents a notable example, providing robust and spatially homogeneous T_1 mapping while reducing sensitivity to radiofrequency field inhomogeneities.

Another example of quantitative MRI sequence is diffusion-weighted MRI (see next section 2.1.3) which rely on a shift of MRI sensitivity from physical spin relaxation to spin motion *in vivo*. Together, these sequence developments illustrate how MRI has evolved into a versatile imaging modality capable of probing tissue anatomy, composition, and microstructure through carefully designed pulse sequences.

2.1.3 Diffusion-weighted MRI

Diffusion-weighted imaging (DWI) exploits the sensitivity of MRI to the random thermal motion of water molecules, commonly referred to as Brownian motion. In free water, this motion is isotropic and occurs equally in all directions. In biological tissues, however, water diffusion is hindered and restricted by cellular membranes, macromolecules, and the overall microstructural organization of the tissue. As a result, diffusion properties vary across tissue types and reflect underlying microstructural features.

The most widely used method to sensitize the MRI signal to microscopic water motion is the pulsed gradient spin-echo (PGSE) sequence, originally introduced by Stejskal and Tanner in 1965.³⁹ This sequence extends the conventional spin-echo sequence by incorporating a pair of diffusion-sensitizing gradient pulses applied symmetrically around the 180° refocusing RF pulse. These gradients encode information about spin displacement along a specific spatial direction.

The first diffusion gradient imparts a position-dependent phase shift to the spins along the gradient direction. Following the refocusing RF pulse, which inverts the accumulated phase, an identical second diffusion gradient is applied. The effect of this second gradient depends on whether the spins have moved between the two gradient applications:

- **Stationary spins:** Spins that remain at the same spatial location experience equal and opposite phase shifts from the two gradients. As a result, their phase is fully refocused, and they contribute constructively to the spin-echo signal.
- **Moving spins:** Spins that have displaced along the gradient direction experience a mismatch between the two phase shifts. This incomplete refocusing leads to phase dispersion and a reduced contribution to the echo signal. Greater displacement results in stronger signal attenuation.

In free or weakly restricted environments, such as cerebrospinal fluid (CSF), water molecules can diffuse over relatively long distances during the diffusion time, leading to pronounced signal attenuation. In contrast, in highly structured tissues such as white matter, diffusion is directionally constrained by axonal membranes and myelin sheaths, resulting in anisotropic diffusion and signal attenuation that depends on the orientation of the applied diffusion gradient.

The sensitivity of the diffusion-weighted MRI signal to molecular motion is governed by the strength, duration, and timing of the diffusion gradients. These parameters are commonly summarized by a single scalar quantity known as the *b-value*, defined as:

$$b = \gamma^2 G^2 \delta^2 \left(\Delta - \frac{\delta}{3} \right), \quad (2.2)$$

where γ is the gyromagnetic ratio of hydrogen, G is the gradient amplitude, δ is the duration of each diffusion gradient pulse, and Δ is the time interval between the two gradient pulses. Higher b-values correspond to stronger diffusion weighting, increasing sensitivity to small displacements of water molecules, but at the expense of reduced signal-to-noise ratio (SNR).

Each diffusion-weighted acquisition is also associated with a *b-vector*, which specifies the direction of the applied diffusion gradient. In practice, diffusion MRI data are typically acquired in *shells*, consisting of multiple diffusion-weighted volumes with identical b-values but different gradient directions. Because diffusion encoding is insensitive to the sign of the gradient direction, the b-vectors are usually distributed uniformly over a half-sphere.

By analyzing diffusion-weighted signals acquired across multiple gradient directions — and, in more advanced protocols, across multiple b-values with multi-shell acquisition — it is possible to estimate tissue diffusion properties and, in some cases, infer aspects of tissue microstructure. This capability makes diffusion MRI a powerful tool for probing biological tissue organization beyond what is accessible with conventional contrast-weighted imaging. In the next paragraphs, we will explore different diffusion MRI signal representations and modeling to infer biological tissue microstructure.

Diffusion Tensor Imaging (DTI)

Diffusion Tensor Imaging (DTI)⁴⁰ provides a compact representation of the diffusion-weighted MRI signal that reduces its high dimensionality, arising from measurements acquired along multiple gradient directions, into a second-order tensor field that is easier to visualize and analyze. The diffusion tensor $\mathbf{D}$ is defined as

$$\mathbf{D} = \begin{bmatrix} D_{xx} & D_{xy} & D_{xz} \\ D_{yx} & D_{yy} & D_{yz} \\ D_{zx} & D_{zy} & D_{zz} \end{bmatrix} = \begin{bmatrix} \mathbf{v}_1 \\ \mathbf{v}_2 \\ \mathbf{v}_3 \end{bmatrix}^T \begin{bmatrix} \lambda_1 & 0 & 0 \\ 0 & \lambda_2 & 0 \\ 0 & 0 & \lambda_3 \end{bmatrix} \begin{bmatrix} \mathbf{v}_1 \\ \mathbf{v}_2 \\ \mathbf{v}_3 \end{bmatrix}, \quad (2.3)$$

where $\mathbf{D}$ is symmetric and positive definite. Its eigen-decomposition yields three eigenvalues ($\lambda_1, \lambda_2, \lambda_3$) and corresponding eigenvectors ($\mathbf{v}_1, \mathbf{v}_2, \mathbf{v}_3$), which describe the magnitude and orientation of diffusion along the princi-

pal axes of an ellipsoid (see Figure 2.3.A). This ellipsoid provides an intuitive geometric representation of diffusion anisotropy within each voxel.

Under the assumption of Gaussian diffusion, the diffusion-weighted MRI signal can be expressed as

$$\frac{S(b, \hat{\mathbf{g}})}{S_0} = \exp\left(-b \hat{\mathbf{g}}^T \mathbf{D} \hat{\mathbf{g}}\right), \quad (2.4)$$

where $S(b, \hat{\mathbf{g}})$ is the signal measured with diffusion weighting b applied along direction $\hat{\mathbf{g}}$, and S_0 is the non-diffusion-weighted signal. To estimate $\mathbf{D}$, at least six non-collinear diffusion-weighted measurements and one $b=0$ image are required, although in practice 20–30 or more directions are typically used to improve robustness and reduce noise sensitivity.^{41,42}

Several scalar indices derived from the eigenvalues are commonly used to characterize tissue microstructure (see Figure 2.3):

- *Mean diffusivity (MD)* measures the average diffusivity within a voxel:

$$MD = \frac{\lambda_1 + \lambda_2 + \lambda_3}{3} \quad (2.5)$$

- *Axial diffusivity (AD)* quantifies diffusion along the principal direction:

$$AD = \lambda_1 \quad (2.6)$$

- *Radial diffusivity (RD)* characterizes diffusion perpendicular to the principal direction:

$$RD = \frac{\lambda_2 + \lambda_3}{2} \quad (2.7)$$

- *Fractional anisotropy (FA)* measures the degree of diffusion anisotropy:

$$FA = \sqrt{\frac{3}{2} \frac{(\lambda_1 - MD)^2 + (\lambda_2 - MD)^2 + (\lambda_3 - MD)^2}{\lambda_1^2 + \lambda_2^2 + \lambda_3^2}} \quad (2.8)$$

Despite its wide adoption, DTI has important limitations. It assumes a single dominant fiber orientation per voxel, whereas crossing, kissing, or fanning fiber configurations occur in 60–90% of white matter voxels.^{43,44} In such regions, DTI produces an averaged representation that may lead to artificially reduced anisotropy and ambiguous biological interpretation. In

addition, the Gaussian diffusion assumption limits sensitivity to complex microstructural features such as axonal dispersion, branching, or restriction.

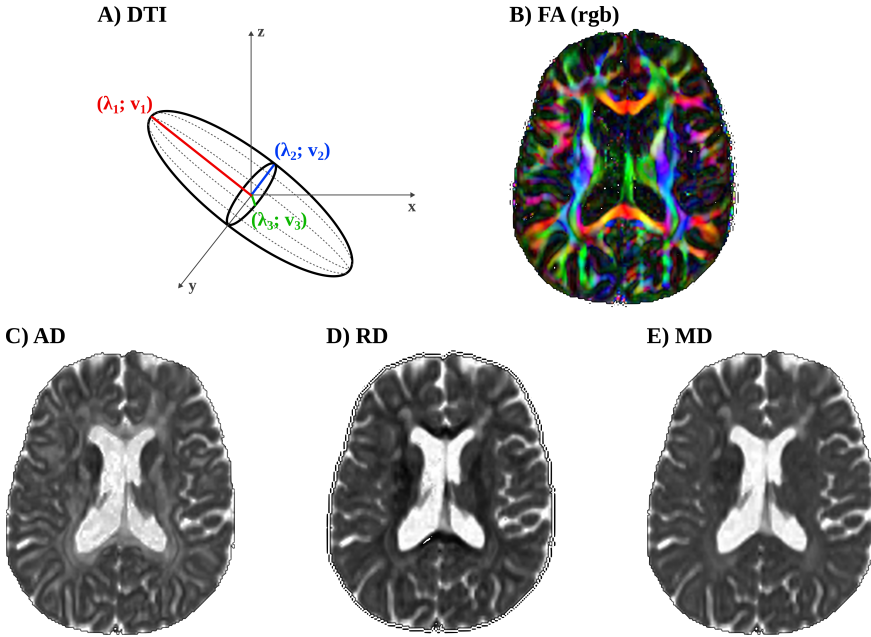


Fig. 2.3 Illustration of the diffusion tensor imaging (DTI) representation. (A) shows a representation of the diffusion tensor with its associated eigenvalues ($\lambda_1, \lambda_2, \lambda_3$) and eigenvectors (v_1, v_2, v_3). The output DTI maps are shown: (B) fractional anisotropy (FA) with a colored direction encoding (red – right-left; green – posterior-anterior; blue – inferior-superior); (C) axial diffusivity (AD); (D) radial diffusivity (RD); (E) mean diffusivity (MD).

Multi-fixel diffusion models

To overcome the single-fiber limitation of DTI, multi-fixel approaches allow multiple fiber populations (fixels) to coexist within a voxel. These methods estimate the fiber orientation distribution function (fODF), which describes the angular distribution of diffusion orientations within a voxel, typically using constrained spherical deconvolution (CSD).⁴⁵ The peaks of the fODF correspond to dominant fiber orientations and can be interpreted as individual fixels.

Beyond orientation estimation, multi-fixel frameworks can also provide metrics related to the relative density or volume fraction of each fiber population. In this context, more advanced models may assign a separate

diffusion representation to each voxel. One example is the multi-tensor model (MTM), which models each voxel with its own diffusion tensor, allowing a more flexible representation of heterogeneous microstructural configurations within a voxel.

These approaches improve the characterization of complex white matter architecture, particularly in regions with crossing fibers, although they typically require high angular resolution diffusion imaging (HARDI) acquisitions and remain sensitive to noise and modeling assumptions.

Multi-compartment diffusion models

Multi-compartment models are designed to overcome some of the limitations of DTI, particularly partial volume effects and the inability to disentangle distinct tissue contributions, by representing the diffusion-weighted signal as a weighted sum of signals arising from multiple compartments:

$$S = \sum_{i=1}^C f_i S_i, \quad \sum_{i=1}^C f_i = 1, \quad (2.9)$$

where S_i denotes the signal from compartment i and f_i its corresponding volume fraction. These compartments are designed to reflect biophysically meaningful environments. A major challenge of such models is parameter degeneracy, as different parameter combinations may explain the same measured signal.

Neurite Orientation Dispersion and Density Imaging (NODDI)⁴⁶ is a widely used multi-compartment model that describes the diffusion-weighted signal as the sum of three compartments (see Figure 2.4):

$$A = (1 - v_{iso})(v_{ic}A_{ic} + (1 - v_{ic})A_{ec}) + v_{iso}A_{iso}. \quad (2.10)$$

Here, v_{ic} represents the intra-cellular (or intra-neurite) volume fraction, v_{iso} the isotropic (free water) volume fraction, and A_{ic} , A_{ec} , and A_{iso} denote the normalized signals of the intra-cellular, extra-cellular, and isotropic compartments, respectively.

The intra-cellular compartment models water diffusion within neurites (axons and dendrites), where diffusion is highly restricted perpendicular to the neurite axis due to cellular membranes, but relatively free along the longitudinal direction. This compartment is represented as an ensemble of zero-radius cylinders (often referred to as “sticks”), capturing the highly anisotropic nature of intra-neurite diffusion. Because neurites are

not perfectly aligned within a voxel, their orientations are modeled using a Watson distribution, which describes the dispersion of orientations around a dominant direction and is represented by the *Orientation Dispersion Index* (ODI).

The extra-cellular compartment represents the space surrounding neurites, where water diffusion is hindered by the presence of cellular structures but not fully restricted. This compartment is modeled as an anisotropic Gaussian diffusion process. To improve model stability and reduce the number of free parameters, NODDI introduces a tortuosity constraint linking the perpendicular diffusivity $d_{\perp}$ to the intra-cellular volume fraction v_{ic} and the parallel diffusivity $d_{\parallel}$:

$$d_{\perp} = d_{\parallel}(1 - v_{ic}). \quad (2.11)$$

This relationship reflects the intuitive idea that increasing neurite density leads to greater hindrance of diffusion perpendicular to their orientation.

The isotropic compartment accounts for free water diffusion, primarily associated with cerebrospinal fluid (CSF). This component is modeled as isotropic Gaussian diffusion with a fixed diffusivity corresponding to free water at physiological temperature.

Despite its advantages, NODDI relies on several simplifying assumptions. The use of a Watson distribution implies a single dominant fiber population and therefore limits the model in regions with crossing fibers. Additionally, the intra-cellular compartment assumes no radial diffusivity (i.e., zero-radius cylinders), which may not fully capture realistic axonal geometry. Finally, parameter estimation can remain sensitive to acquisition protocols and noise, particularly when the data do not sufficiently constrain the model.

While NODDI was primarily designed to characterize white matter microstructure, Soma and Neurite Density Imaging (SANDI)⁴⁷ extends this framework by introducing an additional compartment to account for water diffusion within cell bodies (soma). This compartment is modeled as restricted isotropic diffusion within spherical structures, allowing the model to capture contributions from neuronal and non-neuronal cell bodies. As a result, SANDI improves sensitivity to gray matter microstructure and non neuronal cell population, where soma represent a substantial fraction of the tissue volume. However, SANDI typically requires high b-values and strong gradients to ensure sensitivity to restricted diffusion within cell bodies.

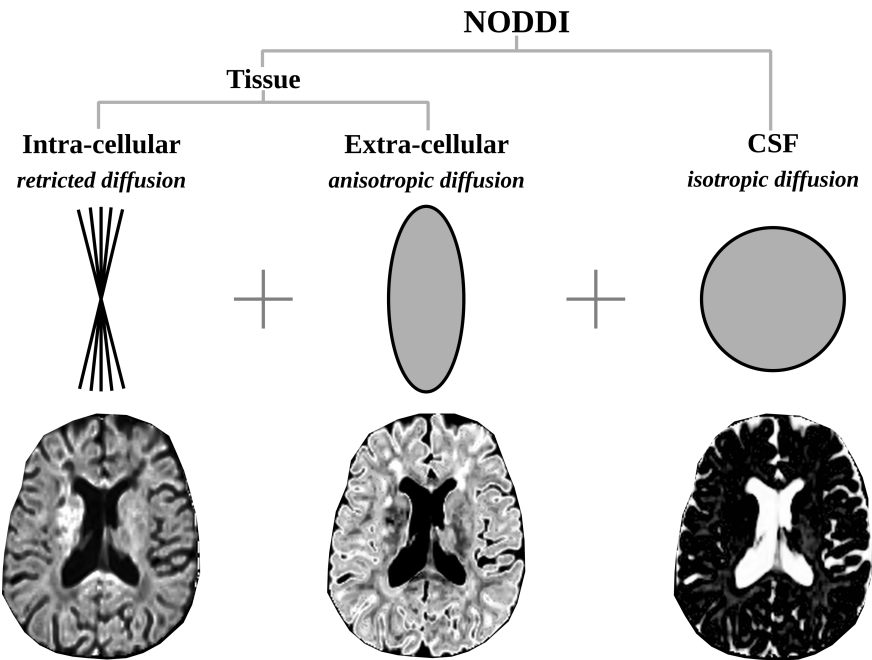


Fig. 2.4 Illustration and example metric maps of the Neurite Orientation Dispersion and Density Imaging (NODDI) diffusion model.

More generally, increasing model complexity must be balanced against the information content of the acquired data. When the diffusion acquisition does not provide sufficient sensitivity to the underlying compartments, parameter estimation may become unstable or non-identifiable, leading to reduced robustness and interpretability.

Numerical diffusion models

In contrast to analytical models, numerical diffusion models rely on Monte Carlo simulations of water diffusion in realistic microstructural environments. For instance, Microstructure Fingerprinting (MF)⁴⁸ uses a dictionary of simulated diffusion signals generated from virtual tissue substrates composed of cylindrical axons with varying radii, densities, and orientations. The measured diffusion signal is matched to this dictionary to infer microstructural parameters.

Such approaches can represent complex geometries and restrictions beyond analytical models, but their accuracy depends on the fidelity of the simulated substrates and the completeness of the dictionary.

2.2 Multiple Sclerosis

Multiple sclerosis (MS) is the most common chronic inflammatory disease of the central nervous system (CNS) and the leading non-traumatic cause of neurological disability in young adults. The disease typically manifests as recurrent episodes of neurological dysfunction that may be fully or partially reversible, including monocular visual loss due to optic neuritis, limb weakness or sensory deficits due to spinal cord involvement, diplopia due to brainstem lesions, and ataxia resulting from cerebellar pathology.⁴⁹ Pathologically, MS is characterized by a multifocal process involving the formation of focal demyelinated lesions within the brain and spinal cord.²⁰

Over time (approximately around 10-20 years), many patients transition from a relapsing–remitting course to a progressive disease phase marked by steadily accumulating largely irreversible neurological disability, while around 15% exhibit a progressive course from onset (primary progressive MS).²⁰ Although more than a dozen disease-modifying therapies (DMTs) have been approved to reduce relapse frequency and limit the accumulation of focal white-matter lesions on MRI, no currently available treatment can fully prevent or reverse progressive neurodegeneration.^{50,51}

2.2.1 Epidemiology and Etiology

According to the recent Atlas of Multiple Sclerosis, approximately 2.8 million people are affected by MS worldwide, with prevalence continuing to increase due to improved survival, population growth, enhanced diagnostic capabilities, and more comprehensive data collection.⁵² The global prevalence is estimated at approximately 36 cases per 100,000 inhabitants, but marked geographic heterogeneity exists.⁵³ The highest prevalence rates are observed in Europe and North America (111–300 per 100,000), whereas substantially lower rates are reported in Africa and the Western Pacific region (approximately 5 per 100,000).⁵² MS shows a pronounced sex bias, with an overall female-to-male ratio of approximately 3:1, consistent with its autoimmune nature. Disease onset typically occurs between 20 and 40 years of age, with a global mean age at diagnosis of approximately 32 years.⁵²

The etiology of MS is complex and multifactorial, and no single causative agent has been identified. Instead, disease susceptibility arises from the interaction of genetic predisposition with environmental exposures. Genome-wide association studies have identified more than 200 genetic variants

associated with increased MS risk, among which the strongest effect is conferred by the HLA-DRB1*15:01 haplotype.²⁰ Individuals with an affected first-degree relative have a 2–4% lifetime risk of developing MS, compared with approximately 0.1% in the general population, and concordance rates in monozygotic twins range from 30 to 50%.²⁰

Among environmental risk factors, a history of infectious mononucleosis, reflecting delayed primary infection with Epstein–Barr virus (EBV) is now considered a major and possibly necessary contributor to MS pathogenesis, due to its interaction with HLA-DRB1*15:01-mediated genetic susceptibility.²⁰ Additional established risk factors include increasing latitude, which is thought to reflect reduced ultraviolet exposure and lower serum vitamin D levels, as well as vitamin-D-related genetic variants. Other contributors include smoking, obesity, and hypertension. Several additional factors, including the gut microbiome, exposure to organic solvents, infections other than EBV, and psychosocial or lifestyle influences, are currently under investigation, although evidence remains mixed or emerging.⁵⁴

2.2.2 MS Pathogenesis: How MS lesions develop?

MS lesions are most readily identified in the white matter as focal regions of demyelination, inflammation, and gliosis, arising from a complex and dynamic interaction between the immune system, glial cells (oligodendrocytes and their precursors, microglia, and astrocytes), and neurons. Figure 2.5 displays a schematic representation of MS lesions pathophysiology. The earliest stages of lesion formation, referred to as *early active* lesions, are immunopathologically heterogeneous, although a single dominant effector mechanism typically predominates within an individual patient. These lesions evolve rapidly over weeks to months and are usually centered on small veins, reflecting the perivenular nature of inflammatory infiltration.²⁰

Early active lesions are characterized by extensive myelin loss and marked hypercellularity, with dense infiltration of CD68⁺ macrophages and activated microglia throughout the lesion.⁵⁵ These cells, many of which display a foamy active morphology,⁵⁵ arise from both resident microglia and blood-derived monocytes entering through a disrupted blood–brain barrier (BBB), and they play a central role in the phagocytosis of myelin debris.^{56–58} Both CD4⁺ helper and CD8⁺ cytotoxic T cells are present, with CD4⁺ T cells predominantly located in perivascular cuffs and CD8⁺ T cells more widely distributed within the lesion parenchyma. B cells and plasma cells are also detected and contribute to lesion development through antigen presenta-

tion, immunoglobulin production, and the secretion of pro-inflammatory cytokines. In some early active lesions, oligodendrocyte apoptosis is observed, further contributing to demyelination. Microglia play a dual role during this phase, mediating both tissue injury and, through the clearance of myelin debris, creating conditions permissive for subsequent repair.^{20,21}

Over a period of approximately 4–8 weeks, acute inflammatory lesions typically transition into a *post-demyelinating active* stage, accompanied by progressive restoration of BBB integrity. At this stage, most of the active myelin breakdown has ceased, and macrophages and microglia predominantly contain residual major myelin proteins, while minor myelin components become less abundant. As inflammatory activity subsides, blood-derived immune cells largely exit the lesion core, and the lesion evolves toward one of the possible chronic states.²¹

One possible outcome is the development of *mixed active/inactive* (or *chronic active*) lesions, which are characterized by persistent, compartmentalized inflammation behind an essentially closed BBB. These lesions exhibit a hypocellular, fully demyelinated core surrounded by a rim of activated microglia and macrophages at the lesion edge. Unlike early active lesions, the lesion center is largely devoid of phagocytic cells. The microglial rim does not necessarily form a complete ring around the lesion but marks the site of ongoing inflammatory activity. Hypertrophic astrocytes are prominent, and moderate numbers of T cells may be found both perivascularly and diffusely within the lesion. In a subset of chronic active lesions, macrophages and microglia at the rim contain iron-rich myelin basic protein (MBP) or proteolipid protein (PLP) degradation products, indicating ongoing, slow demyelination.²¹

Alternatively, lesions may evolve into *chronic inactive* plaques, which are sharply demarcated, hypocellular, and almost completely devoid of mature oligodendrocytes. Only sparse T cells and microglia are present, and the density of microglia is often lower than in the surrounding normal-appearing white matter.⁵⁹ Axonal loss is common,⁶⁰ and occasional axonal swellings suggest limited ongoing axonal injury, likely driven by non-inflammatory mechanisms. These lesions lack a microglial rim, and astrocytes within the lesion form a dense gliotic scar.⁶¹

Across all lesion subtypes, varying degrees of remyelination may occur. Remyelinated regions are often, but not exclusively, located at the lesion periphery and are characterized by thin myelin sheaths surrounding axons, resulting in lighter histological staining. Remyelination is mediated by

oligodendrocyte progenitor cells that migrate into lesions and differentiate into mature, myelinating oligodendrocytes.²¹

Beyond focal plaques, widespread pathological changes are also present in the so-called normal-appearing white matter (NAWM) and in the periplaque white matter (PPWM), which refers to the tissue immediately surrounding lesions. Both NAWM and PPWM exhibit diffuse microglial activation, while astrocytic hypertrophy is particularly prominent in the periplaque region, reflecting a gradient of tissue injury and inflammatory activity extending beyond lesion borders.²¹

2.2.3 MRI as a window into MS pathophysiology

Magnetic resonance imaging (MRI) is the central imaging modality for the diagnosis and monitoring of multiple sclerosis, owing to its exceptional sensitivity to microstructural and biochemical changes in soft tissues. In MS, inflammatory demyelination, edema, axonal injury, and gliosis alter the local magnetic and relaxation properties of brain tissue, thereby enabling the acquisition of image contrast that reflects underlying pathology (see Figure 2.6 for a representative example of MRI contrasts in MS). Demyelination leads to prolongation of both T_1 and T_2 relaxation times, while inflammatory edema associated with BBB disruption primarily increases T_2 and allows extravasation of gadolinium-based contrast agents. Consequently, post-contrast T_1 -weighted imaging enables the visualization of BBB permeability and the detection of *contrast-enhancing lesions* (CELs), which correspond to areas of active inflammation.

In early active lesions, contrast enhancement is typically homogeneous throughout the lesion, reflecting widespread BBB disruption. As lesions transition to a post-demyelinating stage and BBB integrity is progressively restored, enhancement often becomes ring-shaped, with reduced or absent signal in the lesion core. Contrary to contrast-enhanced imaging, T_2 -weighted sequences are sensitive to both acute and chronic inflammatory tissue changes and therefore depict the total burden of focal MS lesions. Among these, T_2 -weighted FLAIR imaging, which suppresses the high signal from cerebrospinal fluid, provides optimal lesion conspicuity, particularly in periventricular regions. Double inversion recovery (DIR) further suppresses both cerebrospinal fluid and normal-appearing white matter, improving the detection of cortical and juxtacortical lesions.

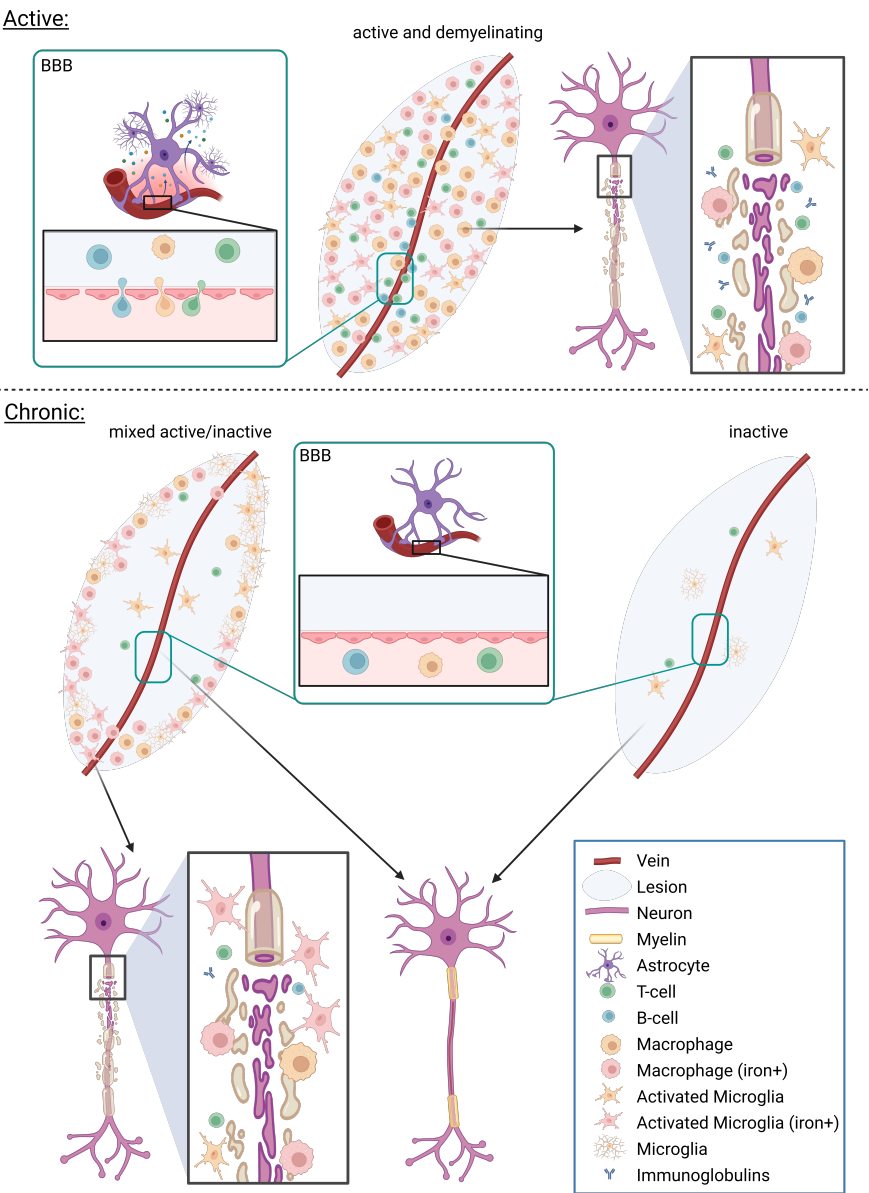


Fig. 2.5 Representation of multiple sclerosis pathophysiology in active, chronic mixed active/inactive and inactive lesions. Made with BioRender.

Axonal injury and severe tissue destruction, which are common in active and chronic lesions, lead to marked prolongation of T_1 relaxation. T_1 -weighted imaging, particularly with three-dimensional MPRAGE sequences, therefore provides greater specificity for irreversible tissue damage. For instance, chronic hypointense lesions on T_1 -weighted images, commonly referred to as *black holes*,⁶² correspond to areas of pronounced axonal loss and matrix destruction. By providing high gray–white matter contrast, T_1 -weighted MPRAGE also allows sensitive detection of brain atrophy, a key marker of neurodegeneration in MS.

Susceptibility-based MRI provides an additional window into MS pathophysiology by exploiting magnetic field perturbations caused by paramagnetic substances. The presence of iron-rich deoxyhemoglobin in venous blood renders veins visible on T_2^* -weighted susceptibility imaging, enabling the identification of the perivenular architecture of MS lesions. This forms the basis of the *central vein sign* (CVS),^{63,64} a highly specific imaging biomarker reflecting the perivascular origin of MS lesions. In addition, iron accumulation within activated microglia and macrophages during chronic inflammatory activity generates local magnetic field inhomogeneities that shorten T_2^* and shift the MRI phase. Although subtle, these effects allow the visualization of *paramagnetic rim lesions* (PRLs)^{24,65} on susceptibility-based unwrapped-filtered phase image, which correspond to chronic active lesions characterized by an iron-laden inflammatory rim surrounding a demyelinated core.

Quantitative and diffusion MRI for MS lesion characterization

The MRI contrasts described above provide predominantly qualitative information, enabling visual detection of lesions and their activity state. Quantitative MRI aims to convert these signal changes into physically meaningful and comparable tissue parameters. Quantitative T_1 , T_2 , and T_2^* mapping yields voxel-wise estimates of relaxation times, which are sensitive to myelin content, tissue water fraction, and overall tissue integrity. Quantitative susceptibility mapping (QSM) provides a measure of tissue magnetic susceptibility and thus enables the detection and quantification of paramagnetic and diamagnetic substances such as iron and myelin. In combination with proton density-based χ -separation techniques, QSM can help disentangle these opposing contributions, thereby improving the specificity of susceptibility-based biomarkers in MS. These quantitative maps are typically obtained by acquiring multiple images with systematically

varied sequence parameters, such as inversion time for T_1 or echo time for T_2 and T_2^* , and fitting appropriate signal models to estimate underlying tissue parameters.

Beyond relaxation and susceptibility properties, MS pathology profoundly alters the microscopic organization of brain tissue, thereby affecting the diffusion properties of water molecules. In normal-appearing white matter (NAWM), tightly packed, coherently aligned myelinated axons impose strong directional constraints on diffusion, resulting in highly anisotropic water motion.

During acute inflammation, lesion tissue becomes hypercellular owing to infiltration by blood-derived macrophages and activation of resident microglia and astrocytes. These glial cells undergo marked morphological and phenotypic changes, transforming from thin, ramified forms into enlarged, amoeboid cells with expanded somata and retracted processes. This shift increases the intracellular volume fraction, reduces the extracellular space, and introduces more isotropic cellular barriers, thereby altering both the magnitude and directional dependence of water diffusion. Concurrent demyelination and axonal injury (including swelling, beading, and fragmentation) further disrupt the coherent fiber architecture, reducing diffusion anisotropy and increasing diffusion heterogeneity.

As inflammation subsides, chronic lesions may develop a hypocellular core dominated by astrocytic gliosis, extracellular matrix deposition, and loss of axons and oligodendrocytes. Reactive astrocytes become hypertrophic and reorganize their cytoskeleton, forming a dense glial scar that modifies the size, shape, and tortuosity of extracellular compartments. In chronic active lesions, activated microglia accumulate at the lesion rim, forming a spatially confined inflammatory compartment with distinct diffusion properties compared to the lesion center. In addition, cellular senescence and chronic activation can alter intracellular viscosity and membrane permeability, further influencing water mobility even in the absence of overt changes in lesion size.

Even in chronic inactive lesions, persistent demyelination, axonal loss, and astroglial remodeling lead to reduced structural coherence and more isotropic diffusion. Diffusion MRI is therefore sensitive not only to axonal architecture but also to cellular density, morphology, and activation state, making it uniquely suited to probe the evolving microstructural and cellular landscape of MS lesions *in vivo*.

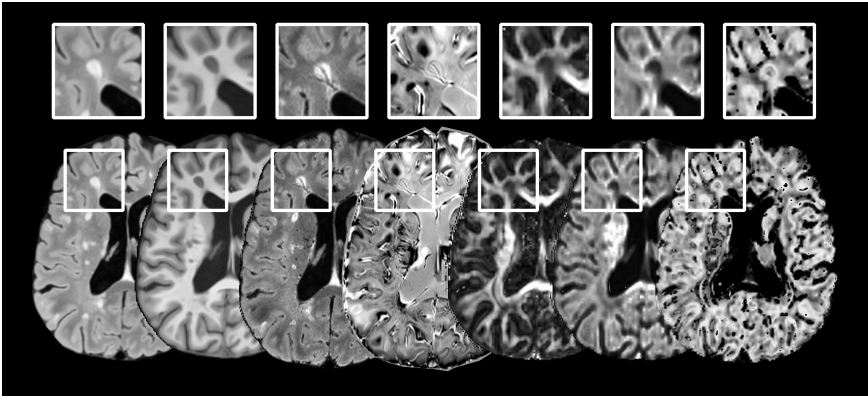


Fig. 2.6 Representative example of multi-modal MRI contrasts to observe MS lesion pathology in vivo. From left to right: T2w-FLAIR, T1w-MPRAGE, FLAIR, T2*w-EPI filtered-phase, DTI FA, NODDI f_{intra} , DIAMOND f_{eat}*

3

BMAT: an open-source BIDS Managing and Analysis Tool

Preliminary Note:

This chapter expands a full Journal paper:

Vanden Bulcke C., Wynen M., Detobel J., La Rosa F., Absinta M., Dricot L., Macq B., Bach Cuadra M., Maggi P., *BMAT: An open-source BIDS managing and analysis tool*. *NeuroImage Clinical*, 2022;36:103252. doi: 10.1016/j.nicl.2022.103252. Epub 2022 Nov 4. PMID: 36451357; PMCID: PMC9723304.

3.0 **Positioning within the thesis**

A central objective of this thesis is to improve the reliability and interpretability of multi-modal MRI for multiple sclerosis lesion characterization. This chapter focuses on a fundamental prerequisite to this objective: the standardization and reproducibility of MRI data processing in multi-center and longitudinal studies. This chapter specifically addresses the following research question:

Research Question 1? Reproducibility & standardization

How to standardize multi-center MRI studies for robust and reproducible MS lesion characterization?

To tackle this challenge, this work introduces BMAT (BIDS Managing and Analysis Tool), a user-friendly neuroimaging platform that integrates standardized data organization, curation, and automated processing within a single graphical environment. Critically, BMAT was not developed in isolation — it was deployed and validated in real multi-center research environments and within a research institute over the course of this PhD, directly supporting the acquisition and processing workflows underlying the studies presented in the following chapters, besides other high-impact studies.

Within the broader context of this thesis, this contribution provides the operational backbone for what follows. It enables the consistency and reproducibility of the multi-modal MRI analyses in Chapters 4, 5, and 6, as well as it supports the implementation and dissemination of the analysis pipelines implemented throughout this work, most notably the SEL detection and MS lesion splitting algorithm presented in Chapter 5 and Annex C. As such, BMAT plays a central role in ensuring the robustness, transparency, and reproducibility of the studies presented in this thesis.

3.1 **Introduction**

Magnetic Resonance Imaging (MRI) offers the unique opportunity to safely and non-invasively investigate brain pathology. The study of neurological

disorders, such as Multiple Sclerosis (MS), often requires the acquisition of multiple MRI sequences, producing complex data in different formats.^{66,67} Furthermore, MRI data sharing is common in large-scale multicenter neuroimaging studies, as it allows increasing the sample size and produces generalizable results.^{68,69} In this framework, a well-structured organization of neuroimaging data is required in order to facilitate data sharing, ensure generalizability and ease the adoption of automated image-processing pipelines.

The Brain Imaging Data Structure (BIDS)⁷⁰ format proposes a universal standard for naming and organizing neuroimaging data. Indeed, adopting the BIDS format ensures an organized and homogeneous structuring of neuroimaging data, eases data sharing in the context of multicenter studies and facilitates the development of automated neuroimaging tools.⁷¹

In a nutshell, the root directory of a BIDS dataset contains all raw data converted in an open file format such as Neuroimaging Informatics Technology Initiative (NIFTI) for images. These raw data are stored in specific subfolders according to the subject, session, and class of data. Metadata are stored in dictionary JavaScript Object Notation (JSON) files or Tabular Separated Value (TSV) files. Each file is named following a strict but explicit standardized convention following a *<key - value>* scheme (e.g. sub-001_ses-01_part-mag_T2starw.nii.gz).⁷² The root directory is also composed of two other folders, *sourcedata* and *derivatives*, containing the original and the processed data, respectively. Finally, the *dataset_description.json* is used to store information about the study dataset (e.g. dataset name, authors, version, etc.) while the *participants.tsv* contains information about the study subjects (e.g. ID, age, sex, etc.)^{70,72}

Although the BIDS format is a promising solution to organize neuroimaging datasets, its diffusion and applicability are limited by the high number of restrictive principles required to manage different types of neuroimaging data. Applying BIDS rules by hand is tricky, time-consuming and potentially associated with intra and inter-user errors. On the other hand, the use of shell scripts to automatically apply BIDS principles prevents non-informatics experts (such as medical neuroimaging scientists) to apply these principles by themselves. Therefore, software able to automatically implement the BIDS format are needed to broadcast its utilization. Furthermore, since several funding agencies, including the European Research Council (<https://erc.europa.eu>), now require a detailed Data Management Plan as part of new research proposals, we envision an increasing need and

a more straightforward application of the BIDS format to neuroimaging projects in the near future.

Several solutions have already been proposed. The "BIDS manager" is a software designed to create and manage BIDS datasets.⁷³ However, the import of new MRI data within the dataset is performed one sequence at a time and requires the user to provide the name of the MRI sequences to the software every time, which make the software less user-friendly. "BIDS manager" is more restrictive than the BIDS specification and does not permit the importation or naming of every possible BIDS compliant sequences, potentially by lack of update. It does not allow either to work with research sequence not included in the BIDS specification yet. This ensures that the BIDS principles are well implemented but makes the software more restrictive and with limited flexibility.

"BrainLife" is another existing solution, a free online platform to perform neuroscience data analysis based on the BIDS format (<https://brainlife.io/>). BrainLife is an efficient data organizer and analysis tool, allowing to create and manage BIDS datasets as well as to run automated tasks (<https://brainlife.io/docs/>). BrainLife works exclusively online and offers cloud-computing resources to run extensive analysis. However, uploading MRI images online can raise relevant ethical questions for neuroimaging scientists, especially when working with sensitive patients' data.

We propose the BIDS Managing and Analysis Tool (BMAT), a local offline neuroimaging analysis tool based on the BIDS format. BMAT desktop application is a Graphical User Interface (GUI) wrapper working with existing command-line neuroimaging tools. BMAT can create and manage BIDS datasets, providing a user-friendly interface for running automated tasks on the dataset and for viewing analysis results. This software has been specifically developed and tested in the framework of multiple Sclerosis (MS) MRI research studies with multicentric data and dedicated analysis tools.

3.2 Materials and Methods

This section describes the proposed BMAT software in detail, starting with the Main Window structure, and then presenting more in depth some MS relevant BMAT analysis pipelines. Fig. 3.1 shows a view of the software's Main Window.

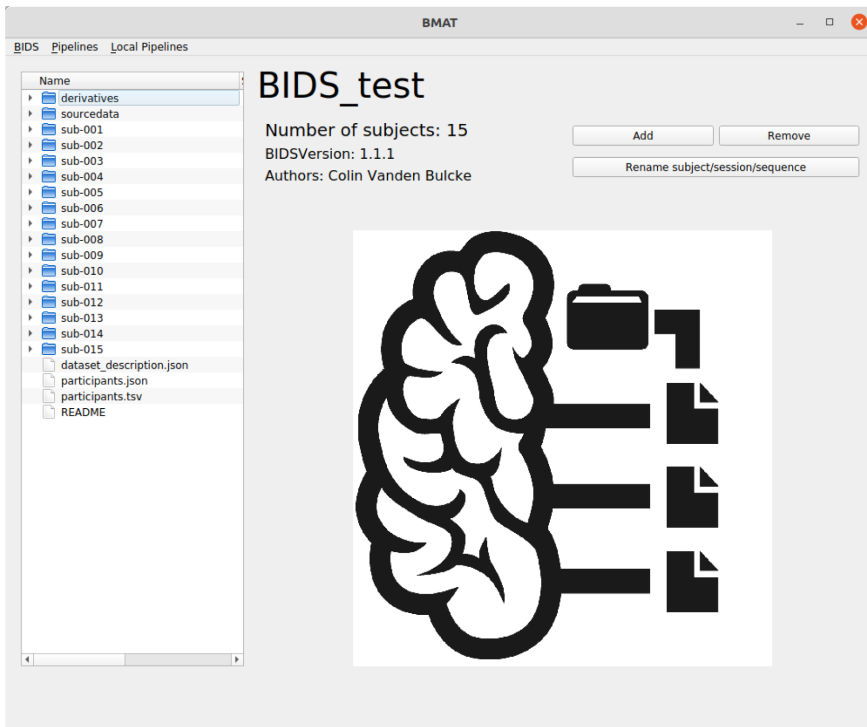


Fig. 3.1 A view of the software's Main Window. The top central widget displays metainformation about the dataset. The left widget allows to safely navigate the BIDS root directory. The bottom central widget is used to view files and the right widget are buttons to apply actions on the dataset. The BIDS and Pipelines drop-down menu are respectively used to apply less often used actions and run preprogrammed automated tasks on the dataset.

3.2.1 Download and installation

BMAT can be downloaded for Linux, Windows and MacOSX operating system (OS) directly from its GitHub repository (<https://github.com/ColinVDB/BMAT>), which contains the source code of the software as well as a detailed documentation describing how to install and use the software. As will be described hereafter, BMAT requires a number of additional dependencies and the overall installation process takes one to two hours, depending on the OS and the informatic background of the user. Here is a list of the required dependencies:

- Python (3.8): to run the software
- dcm2niix: used for the conversion of the DICOM files to NifTI
- ITK-SNAP: 3D viewer with annotation tools
- Docker: used to run several tools (most complex dependency to install especially on Windows and MacOSX)

3.2.2 Graphical user interface (GUI) and functions

After the software has been downloaded and installed, once launched, BMAT asks to select a folder corresponding to a BIDS directory. If there is no BIDS directory available, the user can select an empty folder to create a new dataset. The Main Window is composed of several widgets, including:

- Dataset Information: the top central widget of the Main Window displays a short description of the dataset with useful information:
 - Name of the BIDS directory
 - Number of subjects in the dataset
 - The BIDS version of the directory
 - The Authors of the dataset

Most of these pieces of information come from the previously described `dataset_description.json` file present in the directory.

- Directory Tree Viewer: placed on the left of the Main Window, this widget allows to view and navigate the BIDS directory in a safe manner. Indeed, its use is similar to a classic File Explorer, without the risk of modifying, moving or deleting files in the dataset.

- **Quick Viewer:** the central bottom widget allows the user to observe the files in the dataset. By default, it shows the icon of the software (like in Figure 3.1). When opening any non-image file in the Directory Tree viewer by double-clicking on it, the software will open a dedicated viewer in this widget to observe the file. For any NIfTI file, the user can completely open the image in a separated ITK-SNAP window, an efficient advanced viewer for 3D medical volumes (<http://www.itksnap.org/pmwiki/pmwiki.php>).⁷⁴
- **BIDS actions:** this corresponds to the right widget and contains buttons to trigger specific actions on the dataset such as importing data, removing subjects and sessions, and renaming subjects, sessions, or sequences. These actions automatically edit all the files that needs to be updated. BMAT also offers the possibility to automatically rename a sequence across the whole dataset. This is particularly useful if the user made a mistake in following the BIDS naming convention.
- **BIDS drop-down menu:** it provides the user with other, less often used, actions such as creating or selecting another BIDS directory, updating the `dataset_description.json` file, or checking the BIDS validity of a dataset. The software uses the BIDS Validator tool to provide useful information to the user about the quality of its BIDS dataset. BIDS Validator is a proven tool highlighted in the BIDS specification.⁷² This feature also provides the user with useful links towards the BIDS specification.
- **Pipelines drop-down menu:** this menu allows the user to run specific tasks on the dataset in a fully automatic way. The pipelines are coming from the BMAT community and can be downloaded directly through the GUI. Some already available pipelines will be described below.
- **Local Pipelines drop-down menu:** it provides the user to add his own pipeline locally. For that, the user will need to have informatics knowledge in python and PyQt5. The process to add locally a pipeline is further explained in the supplementary materials A.2.

Dataset management

The first objective of the software is to provide researchers with a user-friendly interface to create and manage BIDS datasets. This section focuses on the software's usability and convenience.

The first step after creating a BIDS dataset is to import new subjects. Neuroimaging data are nowadays exported from MRI devices in a DICOM format.⁷⁵ This format is universal and contains relevant information about the sequence but is dependent on the service provider and usually heavy and not convenient to manipulate.⁷⁶ Older formats, such as PAR/REC format from Philips, can also be used. In order to follow the BIDS guidelines, the images need to be converted into NIfTI format, which is an open-source format accessible by all and easy to manipulate (<https://NIfTI.nimh.nih.gov/>). In parallel, a JSON file containing the metadata is always associated with the image, and for some sequences such as diffusion MRI, additional specific files are created. BMAT uses *dicom2niix* to convert the DICOM files into NIfTI format.⁷⁷ The NIfTI file's name is mainly based on the ProtocolName and SeriesDescription attributes in the respective DICOM. Although these attributes are free-form and non-guaranteed to be unique, as a common practice, the same SeriesDescription attribute is generally not used for more than one sequence within the same study MRI protocol. Also, the choice of DICOM attributes for the naming of the NIfTI files is limited by *dcm2niix*. After this step, the NIfTI files are renamed following the BIDS specification and organized into the right folder of the BIDS dataset. The renaming strategy is user-dependent and based on a mapping between the NIfTI file name and the corresponding BIDS modality, described in the *sequences.csv* file in the source code of the software. The *sequences.csv* file should follow the BIDS specification⁷² and needs to be completed only once at the beginning of the study; the software will be then able to recognize and work with these specific MRI sequences. The renaming strategy is further detailed in the supplementary materials A.1.

Information about the subjects of the dataset is essential for neuroimaging studies and is contained in the *participants.tsv* file, completed automatically by BMAT. This file is composed of one row per subject and contains by default its *BIDS ID*, *age*, *sex*, and the *dates* of its different MRI sessions. As an additional feature, BMAT proposes the option for the user to automatically retrieve any information from the DICOM files of the subjects and store it in the *participants.tsv* file.

MS Image Analysis Tools

As previously mentioned, BMAT can also incorporate different image analysis tools. Here we present some image analysis pipelines which are of specific interest for MS MRI studies, but also for neuroimaging studies in general.

A viewer is essential when carrying out neuroimaging studies as it allows to observe the images, and the results of computations performed on the images. Another essential feature is the possibility to manually segment and annotate regions of interest. This is particularly true in the case of MS MRI studies, where the segmentation of White Matter (WM) lesions is a fundamental starting point in many research studies, including MRI-based clinical trials. Lesion segmentation can be performed automatically (see below "Brain lesion segmentation" pipeline) or manually. To perform this task manually, our tool works with ITK-SNAP, an open-source easy-to-use viewer that allows to annotate 3D images (<https://www.itksnap.org/pmwiki/p>).⁷⁴ It also allows to open multiple images at the same time and provides a synchronization option when several windows are open to concurrently observe and annotate pathological details on different MRI images. Files edited using ITK-SNAP needs to be manually saved by the user in the right BIDS folder via the save button in ITK-SNAP.

Neuroimaging studies often use automatic computational tools, called *pipelines*, to produce specific resources and results. Thanks to the BIDS structure, the pipeline can be scripted to perform the same automatic task on all subjects' images. For BMAT, the implementation of pipelines is community-driven and pipelines can be downloaded directly through the GUI from a dedicated GitHub organization (<https://github.com/BMAT-T-Apps>). Depending on the ongoing study, pipelines may differ between research groups. Here, we propose 4 representative BMAT pipelines, which can be used in the context of MS MRI studies:

- **Image registration:** this pipeline provides a tool to perform a rigid registration of two images from a single subject using ANTs Registration^{78,79} and to script this operation for any subject of the dataset. It also allows the possibility to apply the same transformation to another image by using the transformation matrix computed during the original registration. An image registration takes about 2 two three minutes to be computed.
- **Brain lesion segmentation:** Lesion segmentation is a fundamental task in MS MRI studies. The brain segmentation pipeline uses SAMSEG⁸⁰ to automatically segment WM lesions based on the Fluid Attenuated Inversion Recovery (FLAIR) and, if available, the Magnetization Prepared - Rapid Gradient Echo (MPRAGE) contrasts. SAMSEG can produce an output segmentation in around 15 to 20 min.

- **FLAIR* computation:** The FLAIR* contrast allows the simultaneous visualization of WM lesions and parenchymal veins in MS, and is a widely accepted technique to detect the central vein sign (CVS) diagnostic biomarker.^{63,81–83} The FLAIR* computation pipeline allows to compute a FLAIR* image by voxel-wise multiplication of the registered FLAIR and T2* images,⁸⁴ using a previously proposed docker container (Blakedewey/Flairstar Docker Image | Docker Hub, n.d.). FLAIR* computation can take up to around 20 to 25 min.
- **Phase Unwrapping:** this pipeline allows to unwrap and filter phase images obtained from susceptibility-based MRI using a previously proposed docker container (Blakedewey/Phase_unwrap - Docker Image | Docker Hub, n.d.)⁸⁵ The analysis of unwrapped phase images (in combination with other MRI contrasts)⁸⁶ allows to detect Paramagnetic Rim Lesions (PRL), an emerging neuroimaging prognostic biomarker of chronic active inflammation in MS.^{23,64,87} Phase unwrapping is computed in around 3 to 5 min.

Besides, the conception of the Local Pipelines drop-down menu allows the implementation of new pipelines to run specific tasks related to the study being conducted. To add a new pipeline locally to the software, the user must add its source code in python containing a dedicated graphical interface using PyQt5 and the computation code, as well as an associated JSON file containing metainformation about the pipeline, in the 'LocalPipelines' folder of the software source code. The computation part of the pipeline can use docker containers or can be implemented locally, allowing adaptability of the software to a wide range of neuroimaging applications. The steps for adding new local pipelines are explained in detail in the supplementary materials A.2. To use this feature, the user needs good programming skills, especially in python and PyQt5. Afterwards, the new pipeline can be shared with the community through the GitHub organization (<https://github.com/BMAT-Apps>).

3.2.3 BMAT performance and differences with existing BIDS managing solutions

BMAT performance against "BIDS manager" and "BrainLife" (presented in the Introduction) for the conversion of a representative MRI session containing 15 sequences (14210 DICOM files) was tested by a researcher with good experience in data processing and BIDS conversion (CVB).

Additionally, five independent researchers from four separate universities (Université Catholique de Louvain, Ecole Polytechnique Fédérale de Lausanne, Icahn School of Medicine at Mount Sinai, University of Berne) have tested BMAT and filled out a survey on the installation and utilization of the program. The full survey can be accessed in the supplemental materials A.3.

3.3 Results

This section presents the results of the utilization of BMAT, first through an illustrative example of a typical workflow when using the software for MS MRI studies and then by comparing the performance of BMAT with respect to "BIDS manager" and "BrainLife", to convert source DICOM data into the BIDS format.

The example consists of 1) managing MS patients MRI DICOM data following the BIDS principles, 2) performing image registration, 3) WM lesion segmentation as well as 4) FLAIR* and 5) phase unwrapping for the CVS and PRL diagnostic and prognostic work-up.

3.3.1 Dataset management

The first task BMAT proposes is to import the data to create a new BIDS dataset for a new study. The MRI data presented here were acquired on different MRI machines from 3 different vendors: General Electrics (GE), Siemens and Philips. For the import to work properly, the user must first edit the `sequences.csv` file with the names of the imaging sequences that were used in the study. For example, `"3DT2_0.65mmiso_20180824081221_11_nii.gz"`, and `"_3DT2_0.65mm_iso_20180824081221_12_ph.nii.gz"` are the names of the magnitude and phase images respectively obtained from a 3D segmented T2*-weighted echo-plan-imaging (EPI) sequence acquired on a Siemens Prisma scanner after conversion to NIfTI format.⁸⁸ For the renaming scheme, the first step is to select a specific keyword that captures the modality of the sequence ('3DT2'), search in the BIDS specification for the corresponding BIDS modality ('T2starw') and MRI type ('anat'). Now, the BIDS fields remain to be completed; the phase and the magnitude part needs to be differentiated thanks to the "part" field. The `"_ph"` keyword can differentiate the phase from the magnitude. Similarly, the names of the

Table 3.1 Representative section of a `sequences.csv` file to homogenize the BIDS-renaming of a 3D-EPI MRI sequence acquired on different scanners from different vendors. Abbreviations: *modality*, keyword from the sequence name identifying the type of MRI sequence; *modality_bids*, corresponding BIDS MRI modality; *part*, keyword from the sequence name identifying a specific part or a specific image derived from the MRI sequence (optional); *part_bids*, corresponding BIDS part (optional); *MRI_type*, BIDS MRI type value corresponding to the modality.

modality	modality_bids	part	part_bids	MRI_type
3DT2	T2starw	_ph	phase	anat
3DT2	T2starw	-	mag	anat
3D_EPI	T2starw	SWIphase	phase	anat
3D_EPI	T2starw	-	mag	anat
SWI_NEW_S2	T2starw	_ph	phase	anat
SWI_NEW_S2	T2starw	-	mag	anat

same 3D-EPI sequence acquired on a GE and on a Philips scanner are "_p_Sag_3D_EPI_20220628104007"/ "SWIphase_p_Sag_3D_EPI_20220628104007" and "SWI_NEW_S2_20201229103505"/ "_SWI_NEW_S2_20201229103505_ph" respectively. Following the same process as explained above, the `sequences.csv` file can be completed accordingly (Table 3.1).

At this point, the software can recognize three different names of the same 3D-EPI MRI sequence implemented on 3 different scanners (Siemens, GE, Philips) and rename them according to the BIDS specification (`sub-xxx_ses-xx_part-mag_T2starw.nii.gz` and `sub-xxx_ses-xx_partphase_T2starw.nii.gz`).

Once the renaming file has been updated, the process of importing new data can begin. Compared to other existing BIDS managing software which import MRI sequences one by one (BIDS Manager), BMAT can convert at once the whole MRI session (containing several sequences acquired in series). This reduces the time spent on importing new data and increases the comfort of use. Thanks to the user interface, one can select any DICOM folder, compressed (ZIP file) or uncompressed (folder), that correspond to an MRI session of the subject. The user must then tell the software which patient this session corresponds to, by providing the corresponding BIDS_ID (e.g. `sub-013`) and session (e.g. `ses-02`). If this information is not provided, BMAT will consider that this session corresponds to the first session of a new subject and select a new available BIDS_ID (i.e. `sub-001` and `ses-01` for the first MRI session). The software will then 1) convert all the files contained in the DICOM folder to the NIfTI format, 2) rename the files according to the renaming strategy provided (e.g. `sub-013_ses-01_part-mag_T2starw.nii.gz`) and 3) create the directories to store these files. The

DICOM folder is copied in the BIDS dataset following the corresponding subject and session folders within the sourcedata directory. This ensures that the original DICOM are stored unmodified in the dataset, so that the user can always retrieve this information and have a backup of the images in case an error is made. Finally, it will also modify the participants.tsv file to add the information relative to this new session.

When the import is complete, the user may assess the BIDS validity of its dataset by running the BIDS quality control tool and manually edit the dataset based on the feedback provided and on the BIDS specification.

3.3.2 MS image analysis tools

The next step corresponds to the image analysis part of the study performed using one or more of the different image analysis tools provided by the software (cf. Materials and methods - Image analysis tools). Here we present a representative BMAT MS MRI study analysis. The results of pipelines computations are shown in Fig. 3.2.

The first step is to segment WM lesions which can be automatically performed by the SAMSEG pipeline, using as input 3D FLAIR alone or in combination with 3D-MPRAGE images (see Fig. 3.2.a). Then, to perform the CVS and PRL biomarkers work-up, a 3D-EPI MRI sequence, giving magnitude and phase images, is required. In order to have all the different MRI contrast images in the same space, images are registered together using the registration pipeline (and the image transformation matrix can be easily applied to lesion masks if needed). FLAIR* and unwrapped phase images can be obtained using the corresponding BMAT pipelines and the segmented lesions can be evaluated for the presence of the PRL (Fig. 3.2.b) and CVS (Fig. 3.2.c) imaging biomarkers. Thanks to use of ITK-SNAP, the user is able to manually edit an existing lesion mask or to create new lesion masks.

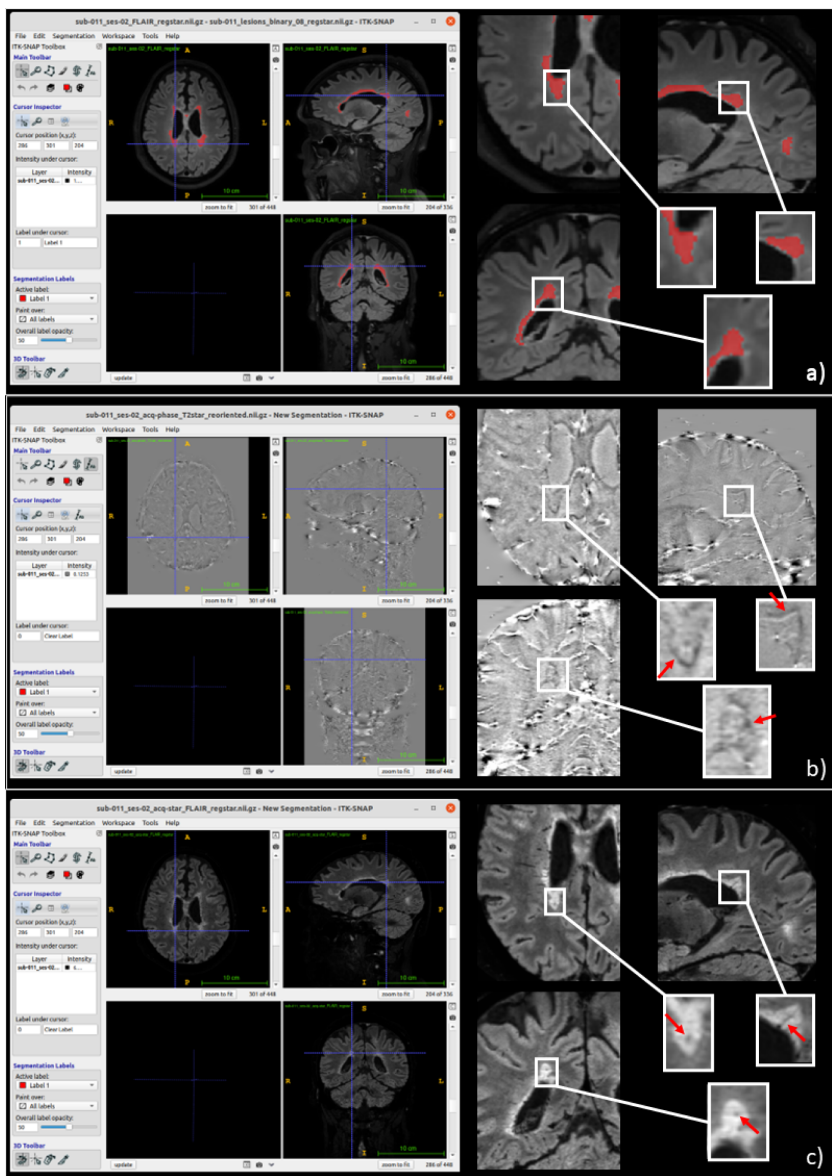


Fig. 3.2 Results of the processing pipelines for the detection of the PRL and the CVS imaging biomarkers in MS viewed in ITK-SNAP (left) and in details (right). Lesion segmentation is performed automatically with the SAMSEG pipeline (a). A PRL (arrow) can be observed on unwrapped phase images (b), computed using the Phase Unwrapping pipeline; while the CVS work-up is performed on FLAIR* images, obtained with the FLAIR* computation pipeline (c). Abbreviations: PRL, paramagnetic rim lesions; CVS, central vein sign; MS, multiple sclerosis.

3.3.3 BMAT performance and differences with existing BIDS managing solutions

The main differences and the performance of BMAT with respect to "BIDS manager" and "BrainLife" (presented in the Introduction) are summarized in Table 3.2. Both BIDS Manager and BMAT require the user to manually provide the name of the MRI sequences to the software (by filling in the requirements.json and sequences.csv file, respectively), see "Preprocessing before conversion Time" in Table 3.2. While this process is inevitably time consuming, it needs to be done only once at the beginning of each new imaging study (assuming that the MRI protocol does not change during the study) and makes the overall renaming step accurate using BMAT. As a drawback, BIDS Manager requires the user to import the whole session sequence by sequence and to rename by hand each sequence once imported, considerably slowing down the overall BIDS conversion process. By contrast, BrainLife automatically recognize and rename MRI sequences. However, the different MRI sequences within a session can be wrongly renamed (especially research MRI sequences), requiring the researcher to check and eventually fix the sequence name every time a new session is imported. Moreover, BrainLife requires to upload online the entire session before BIDS conversion, considerably slowing down the overall process. We believe that the major difference between BMAT and previous BIDS managing solutions lies in the ability of BMAT to convert DICOM to BIDS independently. Indeed, once the sequences.csv file is completed, the BMAT user can launch the conversion of several MRI sessions at a time, while both BIDS Manager and BrainLife requires the user intervention at some stage of the conversion.

Table 3.2 Comparison of BMAT against "BIDS manager" and "Brainlife" for the conversion of an MRI session containing 15 sequences (14210 DICOM files).
 Abbreviations: Platform, platform on which operating system the software is available; Analysis possibility, possibility to run additional post-processing analysis on a BIDS dataset; Image viewer, presence/absence of an integrated complete 3D viewer; MRI import, possibility to import a new MRI session sequence by sequence or entirely; Preprocessing before conversion time, time needed to complete a possible preprocessing step before conversion; Conversion time; time needed to perform the complete conversion of an MRI session; Total time (first conversion), total time for the first conversion of the study; Total time: total time of conversion for a conversion; Scriptable conversion, does the software allow the conversion of several MRI session in one go; Flexibility, is the software more restrictive (<), equivalent (=) or less restrictive (>) than the BIDS specification.

	BIDS Manager	BrainLife	BMAT
Platform	Windows, Linux	Online	Linux, Windows, MacOSX
Analysis possibility	No	Yes	Yes
Image viewer	No	Yes	Yes
MRI import	Sequence by sequence requirements, json: 1'39"38 (once)	Entire Session Upload: 38'30"	Entire Session sequences, csv: 4'40"26 (once)
Preprocessing before conversion time	6'26"09	3'57"02	1'06"39
Conversion time	8'05"47	44'27"02	5'46"65
Total time (first conversion)	6'26"09	44'27"02	1'06"39
Total time	No	No	Yes
Scriptable conversion	< BIDS	= BIDS	> BIDS
Flexibility			

Finally, five independent researchers from four separate universities (Université Catholique de Louvain, Université de Lausanne, Icahn School of Medicine at Mount Sinai, University of Berne) have tested BMAT and gave their feedback on the installation and utilization of the program. Based on the BMAT survey, the installation process was rated 4.4/5. Indeed, although some issues were encountered during this step, all users had a medium-good level of informatics expertise that allowed them to solve basic problems and successfully complete the installation. The overall utilization of BMAT was rated at 4.8/5 with 5/5 for the GUI design and usability, but 4.2/5 for the format conversion with difficulties experienced in completing the sequences.csv file. Finally, all users were favorable to recommend BMAT to fellow researchers, describing it as having an intuitive GUI and being at least equivalent to other existing tools for data conversion into the BIDS format (see supplementary material A.3 for the full survey results).

3.4 Discussion and conclusion

BMAT is a complete local and open-source neuroimaging analysis tool that uses the BIDS format to organize and process brain MRI data for MS imaging research studies. The software enables the creation of well-structured datasets and the import of new data from different MRI scanners and vendors, including GE, Philips and Siemens MRI scanners. The quality of the BIDS dataset is ensured by using a BIDS validator tool, giving instantaneous feedback to the user about the validity of its dataset. BMAT also enables the use of pipelines, downloadable as BMATApps, to automatically perform specific post-processing and image analysis tasks on the entire dataset. Here we present four relevant pipelines for MS imaging studies: image registration, image segmentation, FLAIR* computation, and phase-unwrapping. Many other MS and non-MS relevant image analysis pipelines can be implemented and shared open-source via the BMAT-Apps GitHub organization with the scientific community. Being based on the BIDS format, BMAT eases the sharing of datasets and automated MRI analysis tools between multiple institutions.

When compared to other available solutions, BMAT stands out by being at least equivalent or better than the competition, faster for conversion and more intuitive and complete. Part of its intuitiveness is due to the freedom it gives to its users to manage their dataset, by being less restrictive than

the BIDS specification. A significant advantage of this feature is to allow the use of research imaging sequences not present in the BIDS specification. However, the software does not entirely enforce BIDS principles and lets the responsibility to the user, while providing the tools, to follow the specification.

The renaming scheme offers the possibility to use the software with any MRI sequences. BMAT allow users to add new analysis pipelines implemented by the community as well as adding personalized scripts to compute ad-hoc analysis on the dataset, thanks to the implementation of the local pipelines feature. These three options make the software generalizable to any kind of neuroimaging study.

As it has been implemented as an intuitive GUI, BMAT would especially be useful for non-computer experts, such as medical neuroimaging researchers, to implement the BIDS format and have a complete analysis tool for their studies. It also allows to run image processing pipelines through a simple GUI. This software can also be very useful for graduate students to take in hand the BIDS format that could be otherwise overwhelming, especially for early-stage researchers.

The principal limitations of this software are its installation and setting up. Since this software is integrated with several other tools (e.g. ITK-SNAP, Docker, etc.), these dependencies make the installation tedious. The use of Docker makes the software available on every OS. However, Docker performance is influenced by the OS, specifically Mac with an M1 chip can considerably slow Docker compared to Linux. As another limitation, the renaming scheme requires the user to complete the `sequences.csv` file following the BIDS specification before starting a new MRI study. However, this is only needed once at the beginning. Finally, as the conversion from DICOM to NIfTI is performed using `dicom2niix`, possible new issues can arise from the conversion of new specific research sequences that have not yet been implemented in this tool.

In the future, we aim to integrate the software with DataLad. DataLad is a free and open-source distributed data management system that keeps track of your data, creates structure, ensures reproducibility, supports collaboration, and integrates with widely used data infrastructure.⁸⁹ This will allow the versioning and sharing of datasets with other researchers. This last point raises some ethical question that needs to be addressed when implementing this feature.

The BMAT software is a research software completely open-source and will be maintained through a GitHub forum to report issues. Other con-

tributors are welcomed to join the project or give feedback and ideas for improvement.

Overall, BMAT is a complete and local software based on the BIDS format to carry out neuroimaging studies. It provides a user-friendly framework to create and manage as well as run automated tasks on BIDS dataset. BMAT is convenient to use and customizable by the user to adapt to any kind of neuroimaging study.

3.5 BMAT 1.1.1

Since the first implementation and publication of BMAT in 2022, the software has undergone continuous development and maintenance, leading to the release of BMAT version 1.1.1 in July 2025. This supplementary section describes the main evolutions of the software, including newly added features, architectural extensions, and its increasing adoption within the local research community.

BMAT compilation

One of the main limitations of the original BMAT software was the complexity of its installation procedure, particularly for users without a strong informatics background. The installation required manual installation of multiple dependencies followed by downloading and setting-up the Python source code. To address this limitation, a compiled version of BMAT, based on the *PyInstaller* framework, has been released for Linux and Windows operating systems (OS) and is available through the BMAT GitHub releases. Users can now download a precompiled package (either a tar .gz archive containing the binary or a setup .exe installer for Windows) and install the software using standard operating-system-specific procedures.

Although several external dependencies still need to be downloaded separately, these are relatively straightforward to install on all supported OS. The availability of a compiled version substantially lowers the barrier to entry, allowing researchers to install and test BMAT within a few minutes, thereby improving dissemination, reproducibility, and collaborative development.

At present, a compiled version for MacOS is not provided. Indeed, MacOS requires applications to be signed and registered through Apple's proprietary developer infrastructure in order to be distributed without being blocked by the OS's security mechanisms. Since this requirement is not compatible with the open-source and open-access distribution model adopted for BMAT, the software is currently distributed for MacOS only in source-code form, as in the original implementation.

BMAT server compatibility

A second major extension of BMAT is the implementation of server compatibility, designed to enable the execution of data conversion and processing pipelines on high-performance computing (HPC) infrastructures. This functionality was developed to interface with the secure storage server of the health science sector (server SSS) at UCLouvain, which combines a secure data storage system with a dedicated HPC cluster.

The storage component of the SSS server is based on a shared Samba file system, which can be mounted on the user's local computer to access authorised folders through the standard file explorer. In parallel, users can connect to the server via secure shell (SSH) for command-line access. The HPC component is managed using the SLURM scheduler and the EasyBuild package manager.

Within this architecture, BMAT can now submit and manage conversion and processing jobs directly on the HPC cluster by submitting SLURM jobs through SSH. To enable this functionality, BMAT relies on a dedicated configuration file, `server_info.json`, located at the root directory of the software. This file contains the information required to establish the connection, including the server Internet Protocol (IP) address, the path to the user's SSH private key, and the mapping between local and remote Samba-mounted directories.

Each processing or conversion task is further defined by a dedicated JSON configuration file specifying the Python script to be executed, the optional virtual environment, the location of log files, the EasyBuild modules to load, the SLURM job parameters (number of CPUs, memory, etc.), and a list of static command-line arguments passed to the pipeline. Dynamic arguments, such as BIDS subject ID, can be provided interactively through the BMAT pipeline GUI.

This server compatibility significantly streamlines large-scale MRI data processing and enables efficient execution of BIDS-based pipelines on secure HPC infrastructures. Although this functionality was initially developed for the specific architecture of the UCLouvain SSS server, the underlying implementation is openly available on the BMAT GitHub repository and can be adapted to other HPC environments. The use of standard technologies such as SSH, SLURM, and EasyBuild facilitates generalization to a wide range of server architectures. Users are encouraged to fork the BMAT repository and tailor the server interface to their institutional infrastructure. In addition, the *PyInstaller* specification file is also provided, allowing advanced users to adapt, recompile, and redistribute customized versions of BMAT if required.

New available processing pipelines

Since the version of BMAT presented in the original publication, several new processing pipelines have been developed and are now available through the BMAT-Apps GitHub organisation, from which they can be downloaded directly via the BMAT interface. The newly available pipelines include:

- **Denoise:** a pipeline based on the adaptive non-local means denoising algorithm implemented in ANTs,⁷⁸ applicable to MRI data of any contrast.
- **N4BiasFieldCorrection:** a pipeline implementing the N4 bias field correction from ANTs⁷⁸ to compensate for low-frequency intensity inhomogeneities in MRI images.
- **mri_synthstrip:** a skull-stripping pipeline based on the *mri_synthstrip* tool from FreeSurfer,⁹⁰ enabling robust brain extraction across multiple MRI contrasts.
- **FreeSurfer:** a pipeline for anatomical brain volumetry based on the *recon-all* workflow from FreeSurfer.⁹¹
- **SEL:** a pipeline for the identification of slowly expanding lesions (SELs) in multiple sclerosis patients (described in detail in Chapter 5.2.3).

- **MS lesion splitting:** a pipeline designed to separate confluent multiple sclerosis lesions in binary segmentation masks using combined T1w and T2w information (described in Annex C.1).

Adoption in the Institute of Neuroscience (IoNS)

Over three years of deployment within the Institute of Neuroscience (IoNS) at UCLouvain, and in particular within the Neuroimaging (NIMA) platform, BMAT has become an increasingly key component of the local neuroimaging research infrastructure. In collaboration with Quentin Des-sain and Nicolas Delinte, and with the support of Laurence Dricot, Gaëtan Duchênes, and Julie Poujol, a complete MRI research workflow was established for the acquisition, management, and processing of MRI data.

At the Cliniques universitaires Saint-Luc (CUSL), a lightweight graphical interface was implemented directly on the GE MRI console, allowing researchers to export DICOM sessions as compressed archives via secure SSH transfer to the SSS server, where a research PACS is maintained by the NIMA platform. This step greatly facilitates the secure transfer of sensitive clinical imaging data and standardises the export procedure across studies.

BMAT can then be executed directly on the HPC infrastructure of the SSS server to convert these raw DICOM archives into BIDS-compliant datasets. The use of an institute-wide `sequences.csv` file further ensures consistent and standardised renaming of MRI sequences across studies and laboratories. Once converted to BIDS, the neuroimaging pipelines described above can be executed directly on the same HPC infrastructure, enabling harmonised processing workflows at the institutional level.

This shared processing framework prevents unnecessary duplication of pipeline development across laboratories and provides newly established research groups with immediate access to a proofed toolbox of neuroimaging pipelines. While not all laboratories have yet fully adopted BMAT, new research groups have generally embraced it, and an increasing number of existing laboratories are progressively transitioning toward both BMAT and the BIDS standard, despite the initial hurdle associated with restructuring legacy datasets. This trend is expected to continue as additional features and pipelines are deployed at the institutional level.

Final Conclusion

This three-year experience of deploying BMAT within the IoNS at UCLouvain demonstrates the strong potential of such a platform for standardising data management and processing at the scale of an entire research institute. Interest from external laboratories, together with the inclusion of

BMAT on the official BIDS website, further supports the long-term relevance of the software.

The establishment of a dedicated development and maintenance team would substantially accelerate the evolution of BMAT toward becoming a widely adopted international neuroimaging platform. In the present context, however, I have mainly implemented, maintained and developed BMAT, in parallel with my doctoral research activities, which necessarily limits the pace at which new features can be implemented. Despite these constraints, the current maturity of BMAT already demonstrates its value as a robust and scalable solution for BIDS-based neuroimaging research.

4

Comparative overview of multi-shell diffusion MRI models to characterize the microstructure of multiple sclerosis lesions and periplaques

Preliminary Note:

This chapter expands a full Journal paper:

Vanden Bulcke C, Stölting A, Maric D, Macq B, Absinta M, Maggi P. *Comparative overview of multi-shell diffusion MRI models to characterize the microstructure of multiple sclerosis lesions and periplaques*. NeuroImage: Clinical. 2024 Jan 1;42:103593.

4.0 Positioning within the thesis

While a substantial body of literature exists on MS lesion characterization, these studies often provide a fragmented view of the disease. Most investigations rely on a limited number of quantitative MRI metrics — particularly in diffusion MRI — thereby offering only a single perspective on lesion microstructure. As a result, this makes comparisons across studies difficult and ultimately limits our ability to build a coherent and comprehensive understanding of MS pathology *in vivo*.

This issue is particularly critical in diffusion MRI, where microstructural interpretation strongly depends on the chosen model — a choice that is often driven more by convenience or convention than by biological relevance. This chapter aims to address this limitation by tackling the following question:

Research Question 2 — MS pathology modeling

Which diffusion MRI models most sensitively and specifically capture MS complex pathophysiology?

To tackle this question, this work provides a systematic comparison of several state-of-the-art multi-shell diffusion models, alongside quantitative T1 mapping and a model-free diffusion approach, to characterize MS lesions across different stages and their surrounding tissue. By explicitly confronting these models and interpreting them jointly, this study shows that MS-related tissue damage is best captured by multi-compartment models with biophysically meaningful metrics, particularly those modeling the extra-axonal compartment which is deemed highly relevant for MS. In contrast, simpler signal representations, such as diffusion tensor imaging, consistently underestimate the complexity and spatial extent of pathological changes.

In the context of this thesis, this chapter plays a key methodological role. It provides practical guidelines on how to choose and interpret diffusion-derived metrics, and directly informs the choice of microstructural metrics used in the longitudinal MS lesion characterization study presented in Chapter 6. In that sense, it helps ensure that the patterns observed in the following chapter are not only statistically robust, but also biologically meaningful.

4.1 Introduction

Multiple Sclerosis (MS) is the most prevalent chronic inflammatory-mediated disease of the central nervous system (CNS), mostly characterized by recurring neurological symptoms and associated with focal demyelinated lesions in the brain and spinal cord.²⁰ Up to this time, microstructural investigation of MS-associated brain tissue damage remains challenging, primarily because of the lack of accurate methods able to depict the heterogeneous nature of MS pathology *in vivo*. MS lesions are indeed heterogeneous in terms of inflammatory response, myelin and axonal damage.⁹² In their initial stage, they are usually characterized by opening of the blood-brain barrier, causing perivascular and parenchymal acute inflammation.²⁰ During this acute-early phase, lesions typically appear as contrast-enhancing on gadolinium-based magnetic resonance imaging (MRI).⁹³ Once this initial phase of acute inflammation resolves (lasting about 4-8 weeks), newly formed lesions can either evolve into a chronic inactive stage, associated with variable degrees of remyelination, or into a chronic active stage, which is characterized by a continuous smoldering inflammatory process at the lesion edge. Indeed, as seen on *ex vivo* histopathology, chronic active lesions feature an inactivated core and a peripheral rim of activated microglia/macrophages containing paramagnetic iron and are detectable on susceptibility-based MRI as paramagnetic rim lesions (PRL).^{64,94} These lesions are characterized by extensive tissue damage and are associated with increased levels of neuro-filament light chain in the peripheral blood⁹⁵ as well as with lower brain volumes.^{23,96} From a clinical point of view, chronic active lesions are correlated with disease severity and progression.^{5,23,64} Beyond focal tissue damage, advanced MRI techniques can also depict *in vivo* diffuse pathological changes in the normal-appearing white matter (NAWM) of MS patients.⁹⁷ *Ex vivo* histopathological studies suggest that these extra-lesional pathological changes are even more pronounced in the immediate periplaque of MS lesions.⁹⁸ At present, only few studies have investigated *in vivo* the microstructural damage of the MS lesions periplaque⁹⁹⁻¹⁰¹ and, to our knowledge, comparative analyses of active, chronic active and chronic inactive MS lesions periplaques are lacking.

MRI offers invaluable insight into the neuropathological aspects of multiple sclerosis within living patients. Quantitative longitudinal relaxation (T1) time has been widely used in the past to describe MS lesions, given its high

correlation with myelination and axonal density in MS.¹⁰² Additionally, multi-shell diffusion MRI stands out as a widely used method to quantify *in vivo* the microstructure of MS tissue, showing enhanced pathological specificity and correlation to clinical outcomes.^{101,103,104} However, various diffusion models have been implemented, each with unique intrinsic characteristics derived from assumptions and specific optimization processes. This results in distinct metrics which reflect the diffusion of water molecules and/or biophysically interpretable brain compartments, exhibiting varying degrees of correlation with *in vivo* biology.¹⁰⁵ This study employs four different diffusion models to characterize the microstructural damage patterns in different MS lesion types, in their periplaque and in the surrounding NAWM. Moreover, we conduct a comparative analysis of these diffusion models, relative to a model-free diffusion signal representation, revealing their strengths and weaknesses in the context of MS.

4.2 Materials and methods

4.2.1 Participants

Imaging and clinical data were consecutively collected under an institutional review board-approved protocol in adults with a diagnosis of MS followed at Cliniques Universitaires Saint-Luc (CUSL). Specific patient inclusion criteria were the following: (1) age ≥ 18 years, (2) diagnosis of MS based on the 2017 McDonald criteria,¹⁰⁶ and (3) availability of 3-Tesla (3T) 3-dimensional (3D) postcontrast T1-weighted image (for active lesion assessment), T2*-weighted echo-planar imaging (EPI)⁸⁸ (for chronic active lesion assessment), T2-weighted fluid-attenuated inversion recovery (FLAIR) (for lesion segmentation), T1-weighted magnetization prepared rapid gradient echo image (MPRAGE) (for automatic NAWM assessment), quantitative T1map derived from a sagittal 3D magnetization-prepared 2 rapid gradient echo (MP2RAGE) and multishell diffusion-weighted images (for microstructural investigations). Imaging data of adults presenting no clinical/MRI evidence of CNS disease, hereafter healthy controls (HC), were also additionally collected. Overall, a total of 83 MS participants (56 relapsing-remitting — RRMS —, 18 secondary-progressive — SPMS — and 9 primary-progressive — PPMS) and 23 age and gender-matched healthy controls (HC) were included in the study. This study was approved by

the local ethical committee of CUSL and all participants provided written informed consent prior to the study.

4.2.2 Human neuropathology

Seven formalin-fixed paraffin-embedded brain tissue samples from 7 progressive MS (PMS) autopsy cases (5 women, average age 50 years old, age range 43-61 years), including lesions at different pathological stages (active, chronic active and chronic inactive lesions), were provided by the Netherlands Brain Bank. Neuropathological analysis included multiplex immunofluorescence⁸⁷ and evaluation of myelin basic protein (MBP) — a marker for myelin and oligodendrocytes —, neurofilament heavy chain (NFH) — a marker for axonal integrity — and of human leukocyte antigen - DR isotope (HLADR) — a marker for myeloid antigen-presenting cells. Using ImageJ/Fiji software, we performed a semi-quantitative immunostaining analysis based on the *z-score* normalization of specific areas compared to the whole tissue. The post-mortem human brain tissue samples were used as an illustrative aid to better interpret the diffusion MRI results.

4.2.3 MRI imaging protocol

MRI acquisition was performed on a 3T whole-body MR scanner (GE SIGNA™ Premier research scanner, General Electric, Milwaukee, WI) equipped with a 48-channel head coil. The MRI protocol for MS participants included: (i) sagittal 3D MPRAGE sequence (repetition time (TR) = 2186 ms, echo-time (TE) = 3 ms, inversion time (TI) = 900 ms, field of view (FOV) = 256 mm, number of slices (#slices) = 156, voxel size = $1.0 \times 1.0 \times 1.0$ mm), (ii) sagittal 3D FLAIR sequence (TR = 5000 ms, TE = 105 ms, TI = 1532 ms, FOV = 256 mm, #slices=170, voxel size = $1.0 \times 1.0 \times 1.0$ mm), (iii) a sagittal high-resolution 3D EPI⁸⁸ sequence (TR = 80.2 ms, TE = 35 ms, flip angle = 18° , FOV = 256 mm, #slices = 355, voxel size = $0.67 \times 0.67 \times 0.67$ mm), (iv) quantitative T1map derived from a sagittal 3D MPRAGE sequence (TR = 1925 ms, TE = 3 ms, TI = 700 and 2500 ms, FOV = 256 mm, #slices=156, voxel size = $1.0 \times 1.0 \times 1.0$ mm), (v) multi-shell diffusion-weighted sequence (TR =4842 ms, TE = 77 ms, Δ = 35.7 ms, δ = 22.9 ms, 64 gradients at b = 1000, 32 at b = 2000, 3000, 5000 and 7 b = 0 s/mm², FOV = 220, #slices = 68, voxel size = $2.0 \times 2.0 \times 2.0$) with reversed phase encoding, and (vi) a post-gadolinium T1-weighted spoiled

gradient recalled echo (CESPGR) sequence (TR = 6.9 ms, TE = 2.1 ms, flip angle = 12°, FOV = 240, #slices = 150, voxel size = 0.69 × 0.69 × 1.0 mm). MRI protocol for HC only included the same MPAGE and multi-shell diffusion-weighted sequence.

4.2.4 Region of interest identification and segmentation

We investigated the following WM regions of interest (ROI): (i) acute contrast-enhancing lesions (CEL) and non-enhancing lesions bearing or not a paramagnetic rim (respectively, PRL+ and PRL-), (ii) the periplaque of the above-mentioned lesions (respectively, peri-CEL, periPRL+, periPRL-), and (iii) the NAWM of MS participants and HC (respectively, NAWM-MS and NAWM-HC). To this end, 3D FLAIR and post-gadolinium CE-SPGR images were rigidly coregistered to the high-resolution 3D EPI scan using ANTs.⁷⁸ CEL were identified as lesions showing contrast enhancement on the postgadolinium CE-SPGR image. Besides, a chronic non-enhancing MS lesion was defined as a PRL+ if it exhibited no enhancement on the post-gadolinium CE-SPGR image and a paramagnetic rim on unwrapped-filtered phase images¹⁰⁷ according to the latest consensus statement²⁸ with the following features as previously described:⁵ (i) colocalization with the edge of an MS lesion on FLAIR image, (ii) visibility on at least two planes, and (iii) paramagnetic rim covering at least 2/3 of the lesion WM edge on the slice of maximum visibility. PRL assessment was determined by consensus of 2 trained raters (AS and PM), and manually segmented on the coregistered FLAIR image by AS using ITK-SNAP (<https://www.itknap.org>).⁷⁴ PRL- was defined as chronic non-enhancing lesions showing no visible paramagnetic rim on unwrapped-filtered phase images. Lesions with a volume smaller than 24 mm³ (corresponding to a minimum of three voxels in the diffusion resolution) and paramagnetic rim lesions not fulfilling the PRL+ criteria²⁸ were excluded from the analysis. Overall, 587 lesions (317 PRL+, 232 PRL-, and 38 CEL) were included in the analysis. NAWM mask was automatically computed based on FreeSurfer brain segmentation⁹¹ and corrected by subtracting a lesion mask segmented using SAMSEG software⁸⁰ and a ventricle mask computed using an *in-house* algorithm based on Otsu thresholding.¹⁰⁸ The segmentation of the periplaque was automatically computed by expanding the lesion boundary by 3 mm into the surrounding NAWM using an *in-house* algorithm. NAWM areas surrounding more than one lesion type were excluded from the periplaque analysis. Periplaque segmentations were then subtracted from the NAWM

segmentation mask for the final analysis. An illustration of every ROI and their segmentation is displayed in Fig. 4.1. Data management, registration and segmentation were managed using the BIDS Managing and Analysis Tool.¹

4.2.5 Microstructural investigation

Microstructural tissue integrity was investigated using two quantitative imaging methods: quantitative longitudinal relaxation time (T1) derived from the MP2RAGE sequence (MP2RAGE-derived T1map) used as an established marker of MS tissue integrity¹⁰² and multi-shell diffusion MRI, in order to further investigate microstructural alterations. The multi-shell diffusion data were first preprocessed with denoising,¹⁰⁹ top-up,¹¹⁰ motion and eddy-currents correction,¹¹¹ and skull-stripping using FSL BET.¹¹² It was then processed in one model-free approach and by four different diffusion models of WM detailed hereunder:

- The model-free approach consists of computing the average diffusion signal on each shell. This approach was employed to inspect the sensitivity differences between multi-shell and single-shell diffusion schemes, as well as the sensitivity of each b -value within the scheme for characterizing MS lesion pathology.
- Diffusion Tensor Imaging (DTI)¹¹³ is a robust single-compartment and widely used diffusion model that characterizes the diffusivity of water molecules within tissue as a single fiber population per voxel. DTI was computed using the multi-shell diffusion scheme with a weighted linear least squares estimation of the diffusion parameters.¹¹⁴ This model was used to study axial diffusivity (AD_{DTI}), radial diffusivity (RD_{DTI}), mean diffusivity (MD_{DTI}), and fractional anisotropy (FA_{DTI}) which are correlated to axonal integrity, myelination, fiber integrity, and tissue integrity respectively. However, DTI suffers from low biological interpretability of its diffusion metrics, as they are influenced by multiple microstructural features.
- DIstribution of Anisotropic MicrOstructural eNvironments in DWI (DIAMOND)¹¹⁵ model is a diffusion model capable of describing multiple crossing fiber populations. DIAMOND metrics correspond to a fiber population-weighted version of the DTI metrics (AD_{DMD} ,

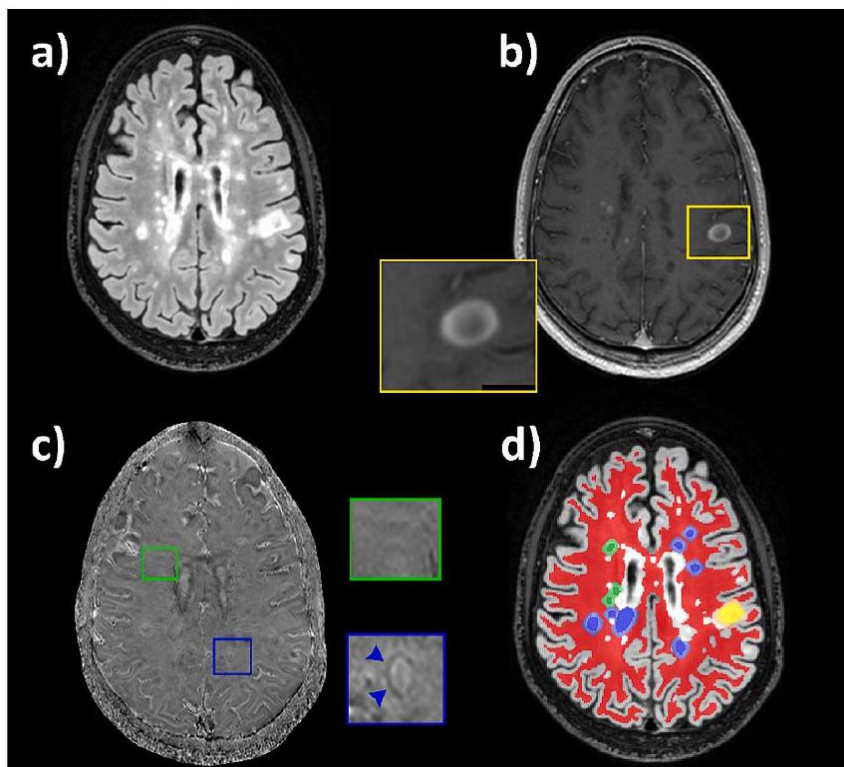


Fig. 4.1 (a, d) Axial FLAIR image of a 25-year-old man with RRMS showing different types of white-matter lesions, their periplaque and the normalappearing white matter (NAWM). (b) A typical contrast-enhancing lesion (CEL - yellow box) is visible on the post-gadolinium CE-SPGR. (c) Chronic non-enhancing paramagnetic rim lesions (PRL+) and non-paramagnetic rim lesions (PRL-) can be observed on the axial filtered unwrapped-phase image (respectively blue and green boxes). In (d), the NAWM is segmented in red while CEL, PRL+, and PRL- are respectively segmented in yellow, blue, and green; lesions' periplaques are segmented and displayed using similar desaturated colors.

RD_{DMD} , MD_{DMD} and FA_{DMD}) with a supplementary free water compartment ($fcsf_{DMD}$). Therefore, DIAMOND overcomes some limitations of DTI but somehow suffers from the same lack of metrics interpretability.

- Neurite Orientation Dispersion and Density Imaging (NODDI)⁴⁶ is a multi-compartment model aiming at characterizing neurite orientation dispersion. Contrarily to DTI, NODDI computes a set of biologically interpretable metrics as the fraction of intra-axonal volume ($icvf$), the fraction of extra-axonal cell volume ($ecvf$), the fraction of isotropic diffusivity ($fiso$), and the orientation dispersion index of the fibers (odi). NODDI's limitations come from its two assumptions: (i) one fiber population per voxel and (ii) fixed diffusivity for all the voxels in the brain tissue.
- Different from the three previously described models which are analytical diffusion models, making assumptions about the tissue's diffusion processes often digressing from the biophysical reality, Microstructure Fingerprinting (MF)¹¹⁶ is a novel numerical diffusion model that uses pre-computed Monte Carlo simulations of water molecules random-walk in complex 3D configurations representative of WM tissue, in order to estimate biophysically interpretable microstructural parameters. MF was designed to be specific to the fiber volume fraction (fvf) correlated to axonal volume fraction, and the fraction of free water ($fcsf_{MF}$).

4.2.6 Statistical analysis

Statistical analysis was performed using *R* and Jamovi (<https://www.jamovi.org>). Age comparisons between MS participants and HC as well as between RRMS and PMS participants were performed by independent samples *t*-tests, and Fischer's exact test was used for gender comparisons. Non-parametric Mann-Whitney *U*-test was used to compare lesions population between RRMS and PMS participants. The different methods used for the microstructural tissue investigation were compared for area under the curve (auc), accuracy, sensitivity and specificity using a support vector machine (SVM) classifier through the computation of a receiver operating characteristic (ROC) curve. For each model, the input vector used corresponds to the computed metrics of the model, and the performance reported

are the mean values of a 10-fold cross-validation. When evaluated on multiple differentiating tasks, the mean auc and the variance of auc — as a proxy of the model’s stability across several differentiation tasks — were used to compare the different models between one another. Finally, Welch’s ANOVA and Games-Howell post-hoc tests were used to compare the MRI metrics among the different investigated ROI and p-values < 0.05 , < 0.01 , < 0.001 , were considered significant (*), very significant (**), and strongly significant (***) respectively.

4.3 Results

4.3.1 MS phenotype comparison

Table 4.1 summarizes the MS cohort characteristics. The MS cohort was composed of 56 participants diagnosed with an RRMS phenotype and 27 with a PMS phenotype (18 SPMS, 9 PPMS). PMS participants were older ($p < 0.001$) and had longer disease duration ($p < 0.001$) than RRMS participants, but no statistical difference in sex distribution was found between the two groups. Compared to RRMS, no PMS participants born any CEL, and no statistical differences were found in terms of number and total volume load of PRL+ and PRL- between the two MS phenotypes.

A complete comparison of the microstructural metrics of PRL+ and PRL-core between the RRMS and PMS participants is described in the supplementary materials (Table B.1). In summary, when compared to PRL+ in RRMS, PRL+ in PMS participants were bigger ($p < 0.01$) and had longer T1 ($p < 0.001$), suggesting more tissue damage. On the contrary, PRL- in PMS participants were characterized by lower MD_{DMD} ($p < 0.01$), RD_{DMD} ($p < 0.01$) and $ecvf$ ($p < 0.01$), potentially reflecting timedependent recovery/repair in chronic inactive MS lesions.

Table 4.1 Multiple Sclerosis cohort characteristics.

	RRMS	PMS (SPMS, PPMS)	Statistical analysis
Participants, n	56	27(18,9)	n/a
Women, n (%)	39 (69.64 %)	15 (55.56 %)	p = 0.207
Age, mean (range) [years]	39.5 (19.3 – 67.3)	52.8 (32.7 – 72.0)	p<0.001
Disease duration, mean ± std [years]	5.46 ± 6.63	14.9 ± 10.8	p<0.001
PRL+, n (mean ± std)	239 (4.27 ± 11.54)	78(2.89 ± 3.99)	p = 0.8382
PRL-, n (mean ± std)	157 (2.8 ± 3.98)	75 (2.78 ±4.07)	p = 0.7929
CEL, n (mean ± std)	38 (0.68 ± 1.65)	0	n/a
PRL+, volume load (mean ± std) [mm ³]	696 ± 1931.15	845.4 ± 1539.65	p = 0.8109
PRL-, volume load (mean ± std) [mm ³]	204.34 ± 335.42	306.27 ± 611.51	p = 0.583
CEL, volume load (mean ± std) [mm ³]	192.9 ± 563.96	n/a	n/a

Table 4.2 Receiver operating characteristic (ROC) curve analysis comparing the ability of the average diffusion signal computed on different shells ($b = 0, 1000, 2000, 3000, 5000 \text{ s/mm}^2$) and the whole multi-shell scheme to differentiate between chronic active (PRL+) and chronic inactive (PRL-) lesions. The table displays a summary of the full performance (area under the curve (auc), accuracy, sensitivity, specificity) from the ROC curve analysis.

	$b = 0$	$b = 1000$	$b = 2000$	$b = 3000$	$b = 5000$	Multi-shell
auc	0,6826	0,6842	0,6171	0,6497	0,6943	0,7292
accuracy	0,6007	0,6259	0,5737	0,5773	0,6421	0,6457
sensitivity	0,4489	0,582	0,4644	0,4768	0,7059	0,5666
specificity	0,8112	0,6867	0,7253	0,7167	0,5536	0,7554

4.3.2 Multi-shell vs. single-shell model-free analysis

Fig. 4.2 presents an axial view of average diffusion-weighted MRI images acquired with different b -values. From visual inspection, we can observe that higher b -values (typically $b=5000 \text{ s/mm}^2$) are more sensitive to compartments with high restrained diffusivity such as those found in the white matter microstructure (e.g. axon bundle). However, higher b -values are also more sensitive to noise. In addition, certain lesions are nearly invisible at certain b -values ($b=2000$ or $b=3000 \text{ s/mm}^2$).

As a more quantitative analysis, the performance of each shell to differentiate between PRL+ and PRL- lesions is reported in Table 4.2. Multi-shell acquisition scheme showed the best performance (auc = 0.7292) to differentiate between PRL+ and PRL- lesions. When using only one shell, b -value = 5000 s/mm^2 showed the best performance (auc = 0.6943), followed by b -value = 1000 s/mm^2 (auc = 0.6842), b -value = 0 s/mm^2 (auc = 0.6826), b -value = 3000 s/mm^2 (auc = 0.6497), and b -value = 2000 s/mm^2 (auc = 0.6171).

4.3.3 Comparison of the different diffusion models

The relative performance of each diffusion model to differentiate between the different types of lesions, and their WM periplaque is reported in Table 4.3. Overall (i.e., across all analyzed lesional and periplaque ROI) NODDI showed the best performance (mean auc=0.8002) followed by MF (mean auc = 0.7488), DIAMOND (mean auc = 0.7445), T1 (mean auc = 0.73375), and DTI (mean auc = 0.6942). Besides, DIAMOND appeared to be the most stable model (variance = 0.000239) followed by MF (variance = 0.001160), NODDI (variance = 0.001764), T1 (variance = 0.002605) and DTI (variance = 0.003188). Finally, the mean rank of each model across all applications is 1.16 for NODDI, 2.83 for DIAMOND, 3 for MF, 3.83 for T1 and 4.16 for DTI.

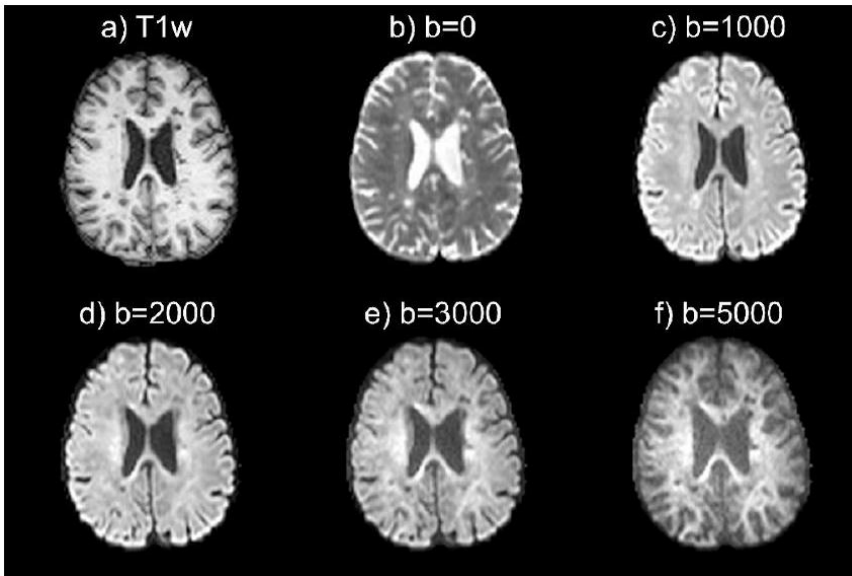


Fig. 4.2 Axial T1- (a) and average diffusion-weighted MRI (b-f) images of a 28-year-old woman with RRMS showing the influence of b -value in the sensitivity for specific brain tissue. Higher b -values ($b = 3000, b = 5000 \text{ s/mm}^2$) are more sensitive to white matter and white matter alteration (i.e. lesions), but noisier compared to lower b -values ($b = 1000 \text{ s/mm}^2$).

Table 4.3 Receiver operating characteristic (ROC) curve analysis comparing the ability of the different diffusion models and quantitative Tmap to differentiate between different regions of interest (ROI). The table displays a summary of the full performance (auc, accuracy, sensitivity, specificity) of each model for differentiating ROI between one another and their mean performance across all applications. Abbreviations: area under the curve (auc); periplaque of paramagnetic rim lesion (pPRL+); periplaque of non-paramagnetic rim lesion (pPRL-); periplaque of contrast-enhancing lesion (pCEL); Diffusion Tensor Imaging (DTI); Distribution of Anisotropic Microstructural Environments in DWI (DIAMOND); Neurite Orientation Dispersion and Density Imaging (NODDI); Microstructure Fingerprinting (MF); Quantitative longitudinal relaxation time (T1).

		PRL+-PRL-	pPRL+	PRL+-CEL	pPRL+-pCEL	CEL-PRL-	pCEL-pPRL-	mean	variance
DTI	auc	0.6922	0.6152	0.735	0.7095	0.7785	0.6349	0.6942	0.003188
	accuracy	0.6284	0.6029	0.6141	0.6275	0.7259	0.7168	0.6526	-
	sensitivity	0.5268	0.3576	0.6025	0.6203	0.7368	0.4878	0.5996	-
	specificity	0.7672	0.9286	0.7105	0.6829	0.7241	0.7563	0.7616	-
DIAMOND	auc	0.7302	0.7265	0.7507	0.7329	0.7608	0.7661	0.7445	0.000239
	accuracy	0.652	0.6352	0.6875	0.5892	0.7037	0.7986	0.6777	-
	sensitivity	0.5	0.4601	0.6879	0.5687	0.6316	0.575	0.5706	-
	specificity	0.8578	0.8655	0.6842	0.75	0.7155	0.8361	0.7849	-
NODDI	auc	0.8167	0.765	0.8419	0.7369	0.8556	0.7852	0.8002	0.001764
	accuracy	0.7468	0.6787	0.7859	0.6919	0.7963	0.681	0.7301	-
	sensitivity	0.7571	0.6139	0.795	0.6994	0.6842	0.8293	0.7298	-
	specificity	0.7328	0.7647	0.7105	0.6341	0.8147	0.6555	0.7187	-
MF	auc	0.7786	0.7126	0.7199	0.7141	0.7955	0.7721	0.7486	0.00116
	accuracy	0.6995	0.6047	0.6	0.6891	0.7778	0.7061	0.6795	-
	sensitivity	0.7161	0.4082	0.5868	0.6994	0.7368	0.5854	0.6221	-
	specificity	0.6767	0.8655	0.7105	0.6098	0.7845	0.7269	0.729	-
T1	auc	0.8422	0.7241	0.7018	0.694	0.7001	0.7403	0.73375	0.002605
	accuracy	0.7596	0.6552	0.6535	0.7423	0.7259	0.6918	0.7047	-
	sensitivity	0.7161	0.6013	0.6562	0.7785	0.5789	0.6341	0.6609	-
	specificity	0.819	0.7269	0.6316	0.4634	0.75	0.7017	0.6821	-

4.3.4 MRI analysis of lesions' microstructural damage

The main results of the lesions' *in vivo* MRI microstructure characterization are reported in Fig. 4.3. Additional diffusion models' parameters and statistical analysis results are reported in Figure B.1 and Table B.2 of the supplementary materials (see Annex B).

Lesions

When compared to PRL-, PRL+ were characterized by a more pronounced tissue damage. Specifically, PRL+ were characterized by an overall decreased tissue integrity with longer T1 ($p < 0.001$), higher MD_{DTI} and MD_{DMD} ($p < 0.001$), and lower FA_{DTI} and FA_{DMD} ($p < 0.001$), by increased axonal damage with lower icvf and fvf ($p < 0.001$), by more demyelination with higher RD_{DTI} and RD_{DMD} ($p < 0.001$), and by more pronounced cellular infiltration with higher ecvf ($p < 0.001$). CEL lesions, with respect to PRL-, showed similar values of MD_{DTI} (vs. PRL-: $p = 0.913$; vs. PRL+: $p < 0.01$) and icvf (vs. PRL-: $p = 0.998$; vs. PRL+: $p < 0.05$), whereas they resembled PRL+ in terms of RD_{DMD} (vs. PRL-: $p < 0.05$; vs. PRL+: $p = 1$), fvf (vs. PRL-: $p < 0.01$; vs. PRL+: $p = 1$), ecvf (vs. PRL-: $p < 0.05$; vs. PRL+: $p = 0.315$), FA_{DTI} (vs. PRL-: $p < 0.001$; vs. PRL+: $p = 0.262$) and FA_{DMD} (vs. PRL-: $p < 0.001$; vs. PRL+: $p = 0.219$). CEL showed T1 relaxation times in between PRL- ($p < 0.05$) and PRL+ ($p < 0.05$) and pronounced edema with a tendency towards higher $fcsf_{MF}$ values when compared to PRL- ($p = 0.078$).

Lesions' periplaque

The comparison between the periplaque of PRL+ and PRL- yielded similar, although more subtle results, to the ones obtained for the lesion parenchyma. Indeed, compared to peri-PRL-, peri-PRL+ were characterized by less tissue integrity with longer T1 ($p < 0.001$), higher MD_{DTI} and MD_{DMD} ($p < 0.001$), and lower FA_{DTI} ($p < 0.05$) and FA_{DMD} ($p < 0.001$), by increased axonal damage with lower icvf and fvf ($p < 0.001$), by more demyelination with higher RD_{DTI} and RD_{DMD} ($p < 0.001$), and by more cellular infiltrate with higher ecvf ($p < 0.001$). The periplaque of CEL, resembled the peri-PRL+ in terms of ecvf (vs. peri-PRL-: $p < 0.001$; vs. peri-PRL+: $p = 1$) and fvf (vs. peri-PRL-: $p < 0.05$; vs. peri-PRL+: $p = 0.993$).

NAWM vs. periplaque

Compared to the NAWM of HC, the NAWM of MS subjects showed a slight decrease in overall tissue integrity with higher MD_{DTI} and MD_{DMD}

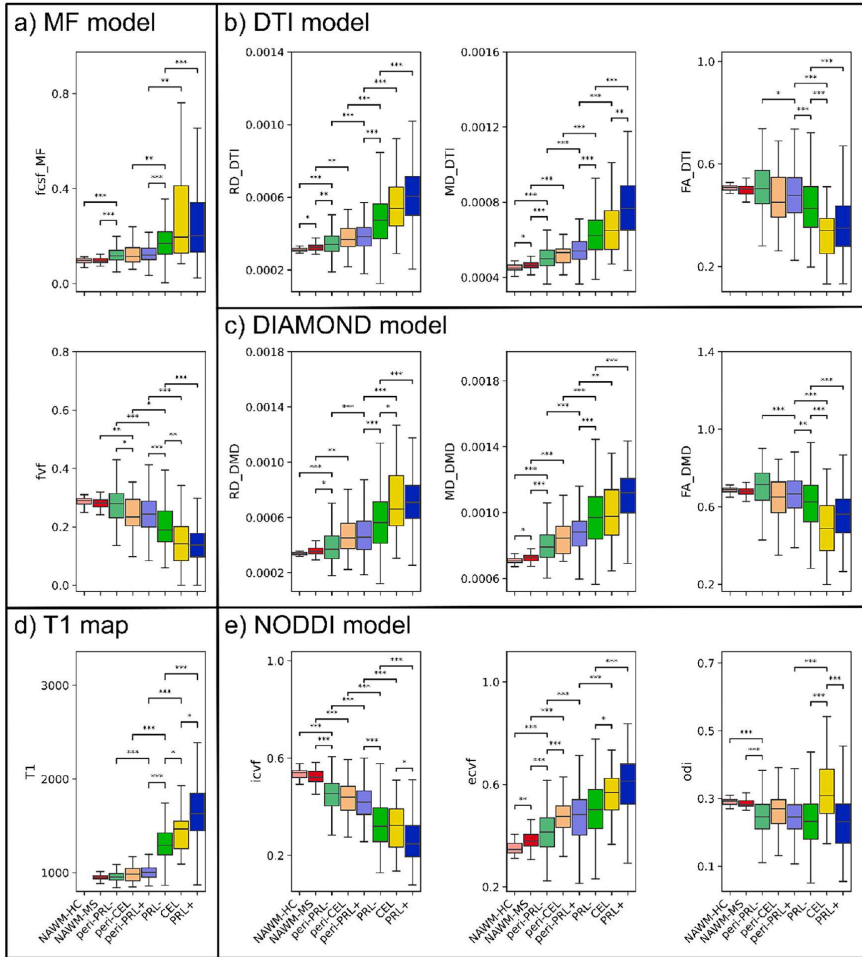


Fig. 4.3 Microstructure of different regions of interest (ROI) according to T1 (d) and 4 diffusion models; MF (a), DTI (b), DIAMOND (c), NODDI (e). On each graph, the different ROI are; NAWM-HC (desaturated red), NAWM-MS (red), peri-PRL- (desaturated green), peri-CEL (desaturated orange), peri-PRL+ (desaturated blue), PRL (green), CEL (yellow), PRL+ (blue). The ROI are ordered by order of microstructural damage according to histopathological analysis. Statistical differences are only noted between ROI within a range of 2, with * ($p < 0.05$), ** ($p < 0.01$), *** ($p < 0.001$).

($p < 0.05$), more demyelination with higher RD_{DTI} ($p < 0.05$), and increased cellular infiltration with higher $ecvf$ ($p < 0.01$). When the NAWM-MS was compared to the periplaque of each type of lesions, the first was characterized by more tissue integrity with lower MD_{DTI} and MD_{DMD} ($p < 0.001$) and lower T1 value than peri-PRL+ ($p < 0.001$), by more axonal integrity with higher $icvf$ ($p < 0.001$) and higher fvf than peri-CEL ($p < 0.01$) and peri-PRL+ ($p < 0.001$), by more myelination with lower RD_{DTI} and RD_{DMD} (vs. peri-PRL-: $p < 0.01$; vs. peri-CEL: $p < 0.01$; vs. peri-PRL+: $p < 0.001$), by less cellular infiltrate with lower $ecvf$ ($p < 0.001$), and by lower $fcsf$ MF than peri-PRL- and peri-PRL+ ($p < 0.001$). Finally, when compared with the core of all lesion types, the NAWM-MS was clearly characterized by an overall less pathological tissue microstructure according to all microstructural metrics ($p < 0.001$).

Periplaque vs. lesions core

For all the different lesion types, the periplaque vs lesion core was characterized by an increase in tissue integrity with shorter T1, lower MD_{DTI} and MD_{DMD} , and higher FA_{DTI} and FA_{DMD} , by more axonal integrity with higher $icvf$ and fvf , by increased myelination lower RD_{DTI} and RD_{DMD} , and by less cellular infiltrate with lower $ecvf$. Interestingly, peri-CEL and peri-PRL+ were not statistically different from the core of PRL- in terms of cellular infiltrate with somewhat similar proportion of $ecvf$ ($p = 0.499$ for peri-CEL vs. PRL-; $p = 0.064$ for peri-PRL+ vs. PRL-).

4.3.5 Histological illustration of MS lesions and periplaques' microstructural damage

In Fig. 4.4, representative histopathological examples of MS lesions at different stages are shown. Three main pathological features were evaluated: (1) myelin content (MBP immunostaining), (2) axonal density (NFH immunostaining) and (3) HLADR+ activated microglia/macrophage infiltrate in three distinct locations; within the lesion core/edge, close periplaque (1; 0-2 mm) and distal periplaque (2; 2-4 mm).

The active lesion (Fig. 4.4.a) shows accumulation of myeloid cells in the center of the lesion, extending in the close periplaque, as well as demyelination and minimal axonal degeneration inside the lesion, with very subtle axonal damage in the close periplaque.

The chronic active lesion (Fig. 4.4.b) shows a gradient of demyelination and axonal loss between the core of the lesion and the periplaque, with infiltration of activated myeloid cells mostly taking place at the edge of the lesion.

The chronic inactive lesion (Fig. 4.4.c) shows scarce infiltration of activated myeloid cells and a less evident gradient of axonal degeneration between the core of the lesion and the periplaque, with no apparent demyelination in the periplaque.

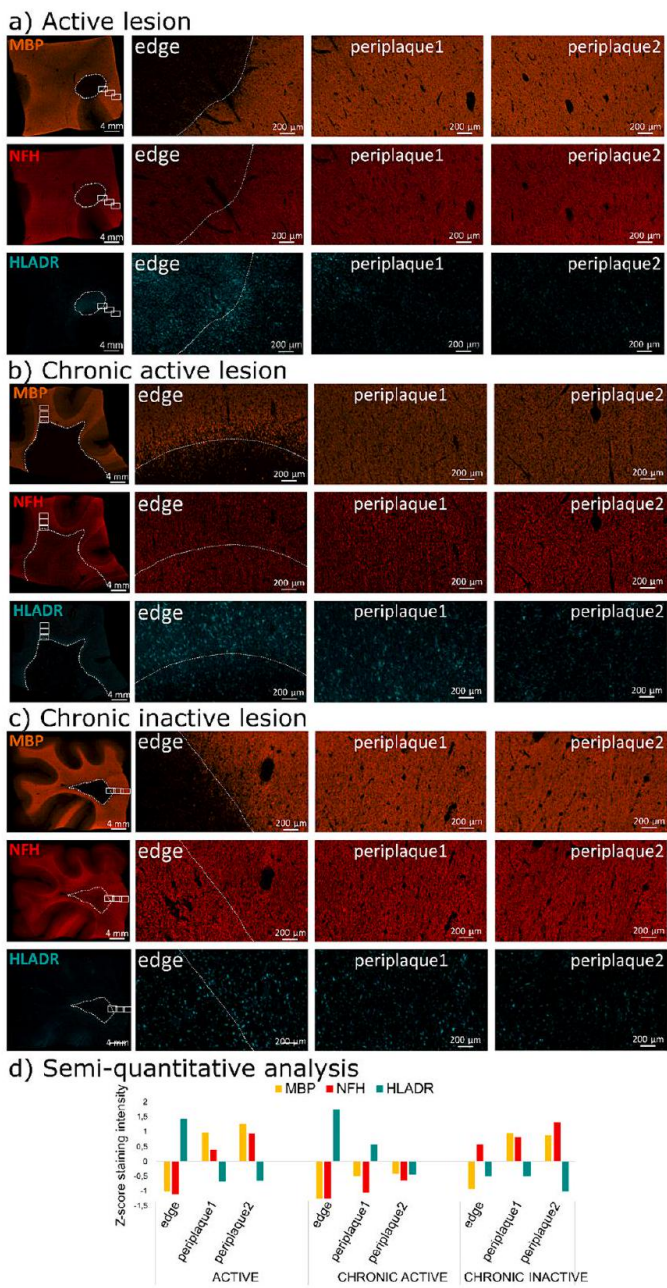


Fig. 4.4 Histological illustration of the microstructural tissue damage in the core and WM close periplaque (1; 0-2 mm) and distal periplaque (2; 2-4 mm) of (a) an active, (b) a chronic active and, (c) a chronic inactive MS lesion. The white dotted line denotes the edge of the lesion. The first row shows immunofluorescent staining of the myelin basic protein (MBP), found in myelin and oligodendrocytes, the second row shows immunofluorescent staining of neurofilament heavy chain (NFH), a marker for axonal integrity, whereas the third row shows immunofluorescent staining of human leukocyte antigen-DR isotope (HLADR), a marker of antigen presenting myeloid cells (activated microglia/macrophages). A semi-quantitative analysis, based on a z-score normalized intensity of the region observed compared to the whole tissue intensity, is shown in d. See the text for the histopathological description.

4.4 Discussion

In this study, we employed one model-free approach and five distinct quantitative imaging techniques, including MP2RAGE-derived T1map and four diffusion models (DTI, DIAMOND, NODDI and MF), to investigate the microstructure of the principal types of MS WM lesions and their corresponding periplaque. In our cohort, PRL+ lesion core exhibited higher tissue damage in PMS participants when compared to RRMS participants, suggesting a continuous process of progressive tissue damage associated with longer disease duration (in PMS vs. RRMS). Interestingly, a less pathologic PRL- core in PMS participants could imply a potential time-dependent process of tissue repair in inactive lesions. Our model-free approach revealed the superiority of multi-shell over single-shell diffusion MRI, while highlighting the significance of b value selection in diffusion MRI scheme, as different b -values exhibit varying sensitivity to brain microstructure. While high b -values (e.g. $b=5000 \text{ s/mm}^2$) are particularly valuable for describing MS lesions, it is important to note that achieving such high b -values may be limited by hardware constraints, including gradient coil capabilities, scanner performance, and signal-to-noise ratio considerations, limiting the generalizability of this method in a clinical setting. Finally, NODDI model demonstrated the best performance, highlighting the ability of advanced multi-shell diffusion models to depict the unique microstructural pattern of CEL, and the severe tissue damage present in PRL+ core and periplaque areas.

4.4.1 Comparison of diffusion models

NODDI's performance is linked to its interpretable parameters, most notably $ecvf$ (see supplementary analysis in Table B.3). This parameter characterizes the extra-neuronal space,⁴⁶ a specific feature not found in the other diffusion models and deemed relevant to depict the neuropathology of MS lesions. In this study, this parameter is interpreted as cellular tissue infiltration. However, it should be noted that this parameter lacks histopathological validation, and it could also be interpreted as the result of a shift in extracellular diffusivity.¹¹⁷

When comparing the performance of the different diffusion models, following NODDI, both MF and DIAMOND exhibited comparable performance, each presenting distinct strengths and weaknesses. MF demon-

strated a higher mean auc and better peak performance, while DIAMOND showed greater stability with lower variance and a superior mean rank. However, DIAMOND mainly provides diffusion metrics corresponding to a representation of the multi-shell diffusion signals, with only a marginal gain (auc=0.7302 vs. 0.7292) when compared to the model-free average multi-shell diffusion signals for the differentiation of PRL+ from PRL-. In contrast, MF features biophysically interpretable parameters, offering more informative insights (auc=0.7786) for characterizing the microstructure of MS lesions core.

When comparing the parameters for free water content derived from NODDI (fiso), MF (fcsf_{MF}), and DIAMOND (fcsf_{DMD}), significant disparities become apparent, particularly between MF and the other two models. Notably, fiso and fcsf_{DMD} exhibit a similar trend across ROI, which differs from the behavior observed with fcsf_{MF}. This discrepancy arises from the fact that both NODDI and DIAMOND are analytical diffusion models, describing the free water compartment as a Gaussian isotropic diffusion with a fixed diffusion coefficient of $3.10^{-3} \text{ mm}^2/\text{s}$. This representation appears suboptimal, given that the results for fiso and fcsf_{DMD} suggest a decreased water content in CEL (vs other lesion types), a trend inconsistent with physiological expectations.⁹³ Additionally, fiso and fcsf_{DMD} display a somewhat erratic distribution of free water content throughout the NAWM of HC (see Figure B.2 (a) and (b) in the supplementary materials B). In contrast, fcsf_{MF} from MF reveals a trend more consistent with physiological expectations. It shows a tendency towards increased water content in CEL (albeit not statistically significant) and indicates reduced water content in the NAWM of HC, as illustrated in Figure B.2 (c) in the supplementary materials. In order to characterize MS brain pathology, the numerical representation of free water diffusion appears to be a more suitable choice.

In contrast, DTI performance not only fell behind the other diffusion models, but also underperformed compared to the use of T1map, a technique characterized by shorter acquisition and processing times. For the differentiation of PRL+ from PRL-, DTI underperformed as well against the model-free average multi-shell diffusion signals (auc=0.6842 vs. 0.7292), and was marginally better than the model-free average single-shell ($b=1000 \text{ s/mm}^2$) diffusion signal (auc=0.6842). The primary limitations of DTI stem from its model assumptions, its inability to represent multi-shell diffusion signals with high b -values, and the biologically uninterpretable nature of its metrics.¹⁰⁵ These constraints restrict its capability to capture the intricacies of MS lesion microstructural damage. Our findings align with previous

studies, reporting a drop in DTI-biology correlation in the context of brain neuroinflammation.¹¹⁸ Nevertheless, compared to T1map, DTI still offers the possibility to perform additional analyses such as MRI tractography.

Given the heterogeneous and subtle microstructural changes characterizing MS lesional and extra-lesional brain pathology, our results suggest adopting a combination of diffusion models with interpretable parameters, such as NODDI and MF.

4.4.2 Pathological interpretation of diffusion analysis

In agreement with our histopathological results, the diffusion analysis revealed extensive demyelination, edema and extra-neuronal cell infiltration in the core of acute lesions and extensive microstructural damage in the periplaque of chronic active lesions (when compared to the surrounding NAWM), extending previous evidence on chronic active lesions microstructure^{99–101} and confirming their destructive phenotype. Combining the parameters of the different diffusion models (based on their respective performance discussed above), we provide a comprehensive qualitative pathological interpretation of our diffusion analysis, portraying acute, chronic active and chronic inactive lesions microstructure relative to NAWM based on four main compartments: axonal (derived from $icvf$), myelin (derived from RD_{DMD}), free water (derived from $fcsf_{MF}$) and extra-neuronal cell (derived from $ecvf$) fraction (Fig. 4.5). These parameters have been adjusted to provide a visually interpretable overview, rather than pure quantitative results. Overall, the diffusion analysis accurately depicts the evolution of a lesion from an acute to a chronic inactive stage, exhibiting reduced edema, similar moderate axonal damage, remyelination (with similar level of myelination in peri-PRL- than NAWM) and reduced concentration of extra-neuronal cells. Instead, when evolving into a chronic active stage, our diffusion data suggest ongoing axonal damage, demyelination, and increase of extraneuronal cells both in the lesion core and periplaque. This microstructural pattern observed in the core and periplaque of PRL+ suggests, consistent with prior studies,^{23,64,95} that chronic active inflammation is a major driver of neuroaxonal damage in MS. While this qualitative illustration captures the key features of MS lesion composition, it also emphasizes the limitation of non-interpretable diffusion metrics such as RD to correlate with actual biological compartment, especially in neuroinflammation.¹¹⁸

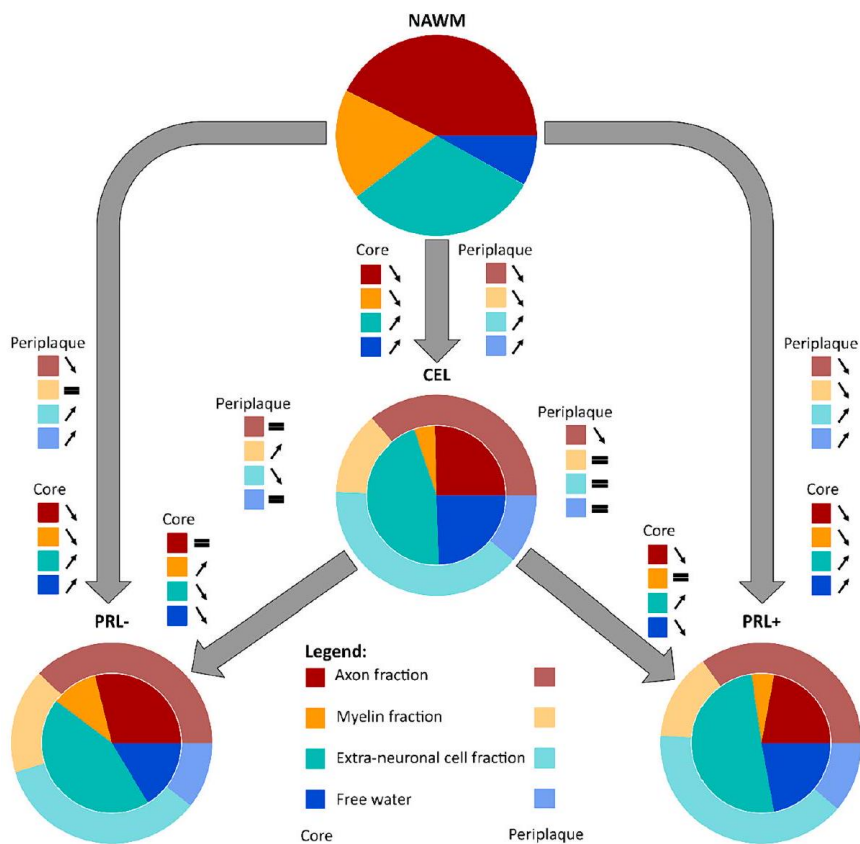


Fig. 4.5 Graphical representation of the evolution of active lesions (CEL) into chronic inactive lesions (PRL-) or chronic active lesions (PRL+), relative to the normal appearing white matter (NAWM), based on 4 pathologically relevant microstructural compartments: axonal (derived from $icvf$; red), myelin (derived from RD_{DMD} ; orange), free water (derived from $fcsf_{MF}$; blue) and extra-neuronal cell (derived from $ecvf$; turquoise) fraction. Active lesions are characterized by the presence of edema and pronounced cellular infiltration, with moderate axonal damage and high demyelination. When evolving into a chronic inactive stage, inflammation tends to resolve (less cellular infiltration and edema), potentially contributing to remyelination. On the other hand, when CEL evolve into chronic active lesions, both axonal and myelin damage are prominent, while the edema resorbs itself and the fraction of extra-neuronal cells increases.

4.4.3 Limitations

A substantial limitation in our study was CEL sample size, consisting of only 38 lesions, in contrast to the larger pools of 317 PRL+ and 232 PRL- lesions. This significant discrepancy in sample size severely impacted the statistical power to discern meaningful differences between CEL and other types of chronic MS lesions. However, due to the transient nature of acute inflammation which typically occurs over a brief timeframe of around 4-8 weeks, the frequency of CEL on routine MRI sessions is relatively low. Additionally, the small size and the incomplete MRI protocol adopted for the HC cohort may have influenced the MS-HC NAWM comparison presented here. Finally, our study encountered limitations stemming from the relatively poor resolution of diffusion MRI. This limited resolution hindered our ability to investigate the immediate periplaque area, especially at the lesion edges, and potentially introduced a bias due to partial volume effect in the analysis of smaller ROI, corresponding to few voxels on the diffusion images. These small voxel populations were susceptible to noise in both acquisition and processing, potentially affecting the accuracy of our results. To mitigate this bias, lesions included in the analysis had a minimum size of 24 mm³.

4.5 Conclusion

In conclusion, multi-shell diffusion MRI can accurately characterize the heterogeneous architecture of MS lesional and extra-lesional pathology, further extending the potential of this technique to detect microstructural alterations *in vivo*. Although the qualitative lesion microstructural model presented here recapitulates the key features of MS lesion pathology (Fig. 5), it also emphasizes the limitation of non-interpretable representative diffusion metrics to correlate with actual biological compartments, especially in neuroinflammation. We believe that the choice of multi-shell diffusion MRI schemes and appropriate diffusion models is paramount for achieving precise microstructural estimation of subtle and specific damage patterns. In order to achieve accurate results in the context of MS, we recommend the use of multi-compartment diffusion models based on biophysically interpretable metrics.

5

Do slowly expanding lesions correspond to chronic active multiple sclerosis lesions? An integrated imaging analysis study

Preliminary Note:

This chapter expands a full Journal paper:

Vanden Bulcke, C., Stolting, A., Borrelli, S., Macq, B., Bach Cuadra, M., Absinta, M., & Maggi, P. (2025). Do slowly expanding lesions correspond to chronic active multiple sclerosis lesions? An integrated imaging analysis study. medRxiv, 2025.12.03.25341192. <https://doi.org/10.64898/2025.12.03.25341192> (under review in *Imaging Neuroscience*)

5.0 Positioning within the thesis

A large part of our current understanding of MS lesion pathology originates from detailed histopathological studies, which provide a precise description of the cellular processes occurring within lesions. However, these *post mortem* observations remain inherently cross-sectional and cannot capture the dynamic evolution of lesions *in vivo*. Understanding these dynamics is crucial, as they are likely key drivers of long-term disability and represent potential targets for more effective disease-modifying therapies.

In this context, the identification of meaningful *in vivo* MRI biomarkers is essential. Yet, important ambiguities remain regarding the interpretation of chronic active lesion (CAL) MRI-derived biomarkers, particularly paramagnetic rim lesions (PRLs) and slowly expanding lesions (SELs). These two biomarkers capture different aspects of lesion pathology but are often used interchangeably, despite important conceptual and methodological differences. PRLs provide a direct, cross-sectional proxy of a well-defined histopathological feature, but only represent a subset of CALs and offer limited insight into their temporal evolution. Conversely, SELs aim to describe lesion dynamics through expansion, but rely on complex detection procedures, show limited reproducibility, and focus on a single aspect of longitudinal behavior, potentially overlooking other relevant trajectories such as lesion shrinkage. Against this background, this chapter aims to clarify these ambiguities by addressing the following question:

Research Question 3 — CAL biomarker interpretation

What is the relationship between SELs and PRLs as MRI-defined biomarkers of chronic active lesions?

From a practical perspective, the first contribution of this chapter is the implementation and open distribution (via BMAT) of a SEL detection pipeline¹ and a lesion-splitting algorithm (see Annex C.1). Beyond these technical aspects, the core contribution of this work lies in a rigorous methodological evaluation of MRI-derived CAL biomarkers. The results highlight fundamental limitations of SEL detection, question its biological

¹The SEL detection algorithm is critically evaluated in this chapter and should be used with caution

interpretability, and demonstrate a lack of meaningful correspondence with PRLs.

Importantly, this work also leads to a broader conceptual shift. By revisiting lesion volumetric evolution, it challenges the assumption that expansion alone defines chronic activity and introduces the concept of *MRI-evolving lesions* — a more flexible framework to describe lesion dynamics *in vivo*, without relying strictly on cross-sectional histopathological definitions.

Within the context of this thesis, this chapter plays a pivotal role in refining how chronic lesion activity is defined and interpreted. It provides a necessary conceptual foundation for the longitudinal microstructural analyses developed in Chapter 6, where these revised definitions are explored in relation to tissue damage and clinical disability.

5.1 Introduction

Multiple sclerosis (MS) is the most prevalent chronic inflammatory disease of the central nervous system (CNS). It is characterized by recurrent neurological symptoms and the formation of focal demyelinating lesions in the brain and spinal cord.²⁰ In their early stage, acute inflammatory MS lesions are marked by blood–brain barrier disruption,¹¹⁹ a key feature associated with MS relapses and predictive of short-term disease outcomes.²² After this acute phase resolves, newly formed MS lesions may evolve into either chronic active lesions (CALs)¹²⁰ or chronic inactive lesions, the latter showing variable degrees of remyelination.¹²¹

CALs represent focal areas of compartmentalized inflammation, confined within the CNS behind a relatively intact blood–brain barrier.¹²² Histopathologically, CALs are typically defined by a hypocellular core containing few macrophages or microglia, surrounded by a peripheral rim of iron-laden, chronically activated microglia/macrophages.⁹⁴ Both *ex vivo* and *in vivo* studies have demonstrated that CALs are associated with extensive tissue damage^{2,95} and are considered strong predictors of disease severity.^{5,23,64,96}

Two principal approaches are currently accepted for identifying CALs *in vivo* using magnetic resonance imaging (MRI): (i) paramagnetic rim lesions (PRLs),⁹⁴ detected on optimized susceptibility-sensitive MRI sequences,⁸⁸ and (ii) slowly expanding lesions (SELs), defined on longitudinal clinical T1-weighted (T1w) and T2-weighted (T2w) MRI.²⁵ Existing MRI-

histopathological evidence supports the identification of PRLs as the *in vivo* correlate of histopathologically defined CALs.²⁸ However, the sensitivity and specificity of PRL detection can vary across studies, and are affected by multiple factors, including the density of iron+ cells, the specific type of MRI sequence used, the MRI field strength, as well as the rater's expertise. To mitigate this variability, rigorous and conservative PRL detection guidelines have been recently established,²⁸ and this biomarker has now been proposed for the routine clinical work-up of MS patients.¹²³

In contrast, SELs are characterized by concentric, gradual expansion of chronic MS lesions.²⁵ Their association with disability progression^{124–126} and ongoing tissue damage^{25,124} has led to the hypothesis that SELs may also serve as an *in vivo* surrogate of CALs.^{25,124} Unfortunately, direct MRI-histopathological validation of SELs is not feasible due to their inherently longitudinal imaging definition. Moreover, only a limited number of studies have investigated the relationship between SELs and PRLs, reporting at best a moderate overlap between these two biomarkers.^{26,127} This highlights a persistent knowledge gap regarding the pathological validation of SELs as markers of CALs, as recently highlighted in the North American Imaging in Multiple Sclerosis (NAIMS) cooperative consensus statement.²⁸

The objectives of this study are to: (i) evaluate the longitudinal volumetric and microstructural dynamics in PRLs, (ii) assess the robustness of SEL identification against input data heterogeneity, and (iii) investigate the relationship between SELs and PRLs.

5.2 Materials and methods

5.2.1 Participants

Imaging and clinical data were consecutively collected under an institutional review board-approved protocol in adults with a diagnosis of MS followed at Cliniques Universitaires Saint-Luc (CUSL). We performed (i) a longitudinal study to investigate SEL in 56 MS patients (46 relapsing remitting (RRMS) and 10 primary or secondary progressive (PMS) with available 3 longitudinal/yearly MRI timepoints (see below); and, (ii) a cross-sectional study performed in 73 MS patients (54 RRMS and 19 PMS) in order to compare the performance of the different segmentation tools tested as variable inputs for the SEL detection algorithm. All patients were (1) ≥ 18

years old, had (2) an MS diagnosis according to the 2024 McDonald criteria,¹²³ (3) and a clinical expanded disability status state (EDSS) and multiple sclerosis severity score (MSSS) evaluation at baseline. The longitudinally homogeneous 3-Tesla (3T) MRI protocol for the longitudinal study included a 3-dimensional (3D) T2*-weighted echo-planar imaging (EPI)⁸⁸ (for PRL assessment), T2-weighted fluid-attenuated inversion recovery (FLAIR) and T1-weighted magnetization prepared rapid gradient echo image (MPRAGE) (for lesion segmentation and SEL assessment), and a quantitative T1map derived from a sagittal 3D magnetization-prepared 2 rapid gradient echo (MP2RAGE) (for microstructural investigations). Patients included in the cross-sectional study had available FLAIR and MPRAGE images, and a whole-brain manual lesion segmentation by an experienced rater (AS). This study was approved by the local ethical committee of CUSL, and all participants provided written informed consent according to the declaration of Helsinki prior to the study.

5.2.2 MRI imaging protocol

MRI acquisition was performed on a 3T whole-body MR scanner (GE SIGNATM Premier research scanner, General Electric, Milwaukee, WI) equipped with a 48-channel head coil. The MRI protocol for MS participants included: (i) sagittal 3D MPRAGE sequence (repetition time (TR)=2186 ms, echo-time (TE)=3 ms, inversion time (TI)=900 ms, field of view (FOV)=256 mm, number of slices (#slices)=156, voxel size=1.0x1.0x1.0 mm), (ii) sagittal 3D FLAIR sequence (TR=5000 ms, TE=105 ms, TI=1532 ms, FOV=256 mm, #slices=170, voxel size=1.0x1.0x1.0 mm), (iii) a sagittal high-resolution 3D EPI⁸⁸ sequence (TR=80.2 ms, TE=35 ms, flip angle=18°, FOV=256 mm, #slices=355, voxel size=0.67x0.67x0.67 mm), (iv) quantitative T1map derived from a sagittal 3D MP2RAGE sequence (TR=1925 ms, TE=3 ms, TI=700 and 2500 ms, FOV=256 mm, #slices=156, voxel size=1.0x1.0x1.0 mm), and (v) a post-gadolinium T1-weighted spoiled gradient recalled echo (CE-SPGR) sequence (TR=6.9 ms, TE=2.1 ms, flip angle=12°, FOV=240, #slices=150, voxel size=0.69x0.69x1.0 mm).

5.2.3 Slowly expanding lesions algorithm

This section describes the implementation of the SEL detection algorithm and outlines the experiments conducted to test its underlying assumptions,

evaluate the robustness of its implementation, and evaluate PRL-SEL colocalization.

Longitudinal volume assessment of PRLs

The detection of SELs relies on the assumption that CALs expand concentrically-outward over time due to the presence of persistent smoldering inflammation at their edges. This lesional expansion can be identified *in vivo* using clinically available MRI sequences. To test this assumption, we systematically screened our cohort to identify all PRLs at baseline, in order to investigate their longitudinal volumetric and microstructural behavior.

For this purpose, 3D FLAIR and CE-SPGR images were rigidly coregistered using mutual information to the high-resolution 3D EPI scan using ANTs.⁷⁸ Chronic non-enhancing MS lesions were classified as PRL if they showed no enhancement on post-gadolinium CE-SPGR images and demonstrated a paramagnetic rim on unwrapped filtered phase images,¹⁰⁷ in accordance with the latest consensus criteria²⁸ (see Figure 5.1). PRL identification required the following features, as previously described:⁵ (i) colocalization with the edge of an MS lesion on FLAIR, (ii) visibility in at least two planes, and (iii) a paramagnetic rim covering at least two-thirds of the lesion's white matter border on the slice of maximum visibility. PRL assessment was performed by consensus of two trained raters (AS and PM).

Whole-brain lesion segmentation was performed using FLAMEs¹²⁸ applied to the FLAIR image for semantic binary segmentation, combined with an in-house algorithm to track longitudinal lesion volume changes (detailed in supplementary materials C.1). Lesion volume trajectories were quantified by fitting a Huber^{129,130} linear regression model to normalized annual lesion volume changes. Lesions were classified as expanding if the slope exceeded +9%, shrinking if below -9%, or stable if between -4% and +4%, based on the distribution of our data. Lesions not meeting the $\pm 9\%$ criteria were defined as having a tendency to expand (or shrink). Finally, longitudinal PRL and non-PRL volume dynamics were assessed using a repeated-measures linear mixed-effects model of log-transformed lesion volume over time, including a random subject and lesion effect. In addition, the microstructural evolution of mean lesion T1 values was investigated using repeated-measures linear mixed-effects models (with random intercepts for subjects and lesions), to test the effects of time and its interactions with PRL status (PRL, non-PRL), lesion volume evolution (shrinking, expanding, stable), and their combination. Pairwise comparisons were performed using

Tukey-adjusted post-hoc tests (emmeans), and the corresponding contrast estimates (β) and confidence intervals are reported.

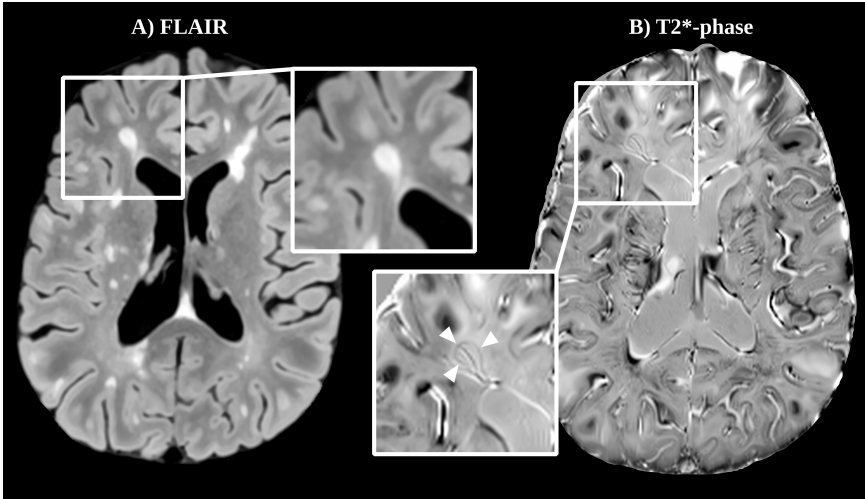


Fig. 5.1 Axial fluid-attenuated inversion recovery (FLAIR — A) and T2*-weighted unwrapped-filtered phase (B) images of an individual in their 20's with relapsing-remitting multiple sclerosis (RRMS) showing a typical example of a paramagnetic rim lesion (PRL — white arrowheads).

Implementation of SEL detection

The SEL detection algorithm (Figure 5.2) implemented in this work was designed as a faithful re-implementation of the original method described by Elliott et al.²⁵ Since the original algorithm is not open-source, certain methodological aspects required interpretative implementation choices based on the descriptions provided in the publication. The final implementation has been released as an open-source BMAT-App within the BMAT software.¹

The algorithm requires FLAIR and MPRAGE images as inputs acquired at three timepoints per subject (Figure 5.2.A and B). Both modalities are first denoised using a non-local means adaptive filter,¹³¹ and skull-stripped with SynthStrip.⁹⁰ Finally, the FLAIR image is rigidly registered using mutual information to the MPRAGE space using ANTs.⁷⁸ Following preprocessing, deformation fields are computed between the baseline (first timepoint) and the subsequent second and third timepoints. To compute this deformation field, the MPRAGE images from each pair of timepoints are registered in a halfway space using *mri_robust_register* from FreeSurfer¹³² with the dedicated halfway option. The corresponding FLAIR images and brain

masks are then transformed into the same halfway space using the resulting transformation matrix. A non-rigid diffeomorphic registration algorithm from ANTs⁷⁸ (step size = 0.7; Gaussian $\sigma = 2$) is subsequently applied to the MPRAGE and FLAIR image pairs in the halfway space to compute the deformation field between two timepoints. Finally, the Jacobian determinant of the deformation field is calculated with ANTs,⁷⁸ resulting in a scalar map that quantifies local expansion and shrinkage (Figure 5.2.D).

SEL candidates are identified using a binary lesion segmentation (Figure 5.2.C) in combination with the Jacobian determinant of the deformation field between the first and last timepoints. The Jacobian map is first normalized to a one-year interval. A SEL candidate (Figure 5.2.E) is then defined as a connected region within the lesion mask exhibiting a peak local expansion of at least 12.5%, surrounded by adjacent voxels showing a minimum of 4% expansion,²⁵ as determined using a hysteresis thresholding approach.¹³³ If multiple clusters of local expansion fulfilling these criteria occur within the same connected lesion area, each cluster is treated as an independent SEL candidate, consistent with previous methodology.¹²⁵

As originally described by Elliott et al.,²⁵ SEL candidates are then defined as definitive SELs based on two supplementary criteria: *concentricity* and *constancy* criteria. Concentricity was defined as an inside-out expansion pattern (Figure 5.2.E), where the Jacobian determinant is greatest at the center of a SEL candidate and decreases towards the edge. Quantitatively, the SEL candidate mask was subdivided into concentric voxel-wise bands, and concentricity was measured as the slope of the best Huber^{129,130} linear regression of mean Jacobian values in each concentric band as a function of radial distance from the mask edge. Constancy was defined as the longitudinal stability of lesion expansion across different timepoints. Specifically, for each SEL candidate, the mean Jacobian-derived expansion was modeled as a Huber^{129,130} linear regression over time, and constancy was quantified as the mean normalized squared residual error between the observed expansion values and the fitted linear trajectory, with lower residuals indicating more constant expansion over time. The concentricity and constancy metrics were then standardized into z-scores compared to the SEL candidates cohort and combined into a single scalar score S , defined as the difference between the z-score normalized concentricity and the z-score normalized constancy. Finally, a SEL candidate is defined as definite SEL (Figure 5.2.E) if its scalar score $S \geq 0$. SEL candidates with a score $S < 0$ are considered as possible SEL (SELpos).

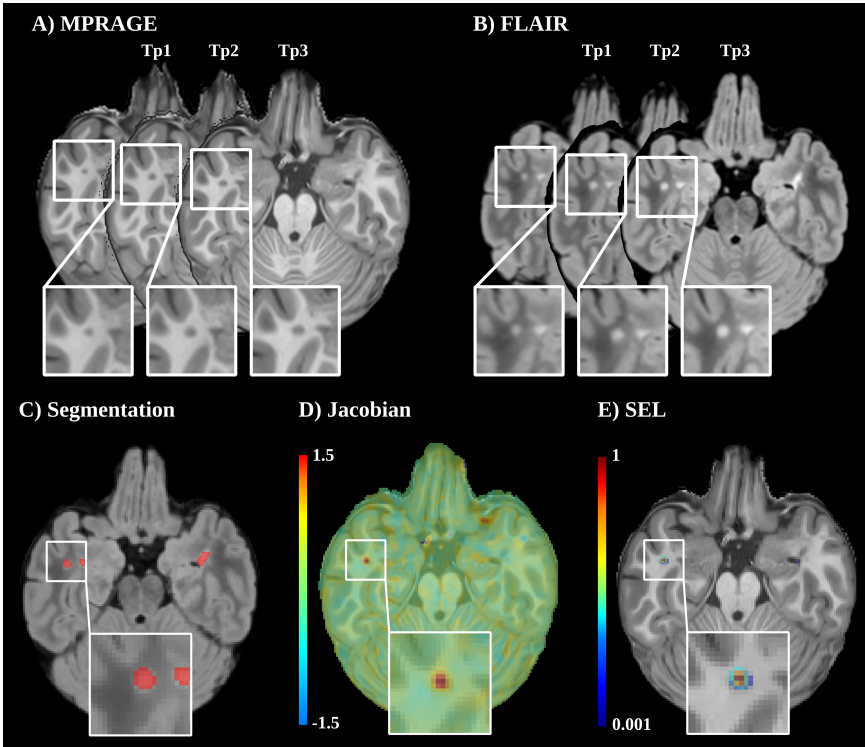


Fig. 5.2 Representation of a slowly expanding lesion (SEL) found in an individual in their 20's with relapsing-remitting multiple sclerosis (RRMS). The SEL is detected using a Jacobian-based analysis of the deformation field computed via a non-rigid registration between (A) magnetization prepared rapid gradient echo (MPRAGE) and (B) fluid-attenuated inversion recovery (FLAIR) images of three different timepoints (Tp1, Tp2, Tp3). (C) represents an automatic binary lesion segmentation, which is combined with the Jacobian determinant of the deformation field between Tp1 and Tp3 (D), to identify SEL (E; here displayed as an overlay between the MPRAGE image and the Jacobian map).

Evaluation of the SEL detection algorithm

In this study, in order to assess the reproducibility and stability of the SEL detection algorithm relative to its inputs, we systematically evaluated its performance under varying imaging and segmentation conditions. First, to simulate a routine clinical imaging protocol, the isotropic 1 mm³ FLAIR image was degraded to a 1×1×3 mm axial FLAIR and subsequently resampled back to 1 mm³ isotropic resolution, as originally described by Elliott et al.²⁵ Hereafter, this condition is referred to as *2D* throughout the paper, while the original isotropic image is referred to as *3D*. For both 2D and 3D images, binary lesion segmentations were generated using either FLAMEs¹²⁸ or SAMSEG⁸⁰ (referred to as *_flames* or *_samseg*), in order to evaluate the robustness of the SEL detection algorithm across lesion segmentation methods of varying performance.

The segmentation performance of FLAMEs- and SAMSEG-derived segmentations was evaluated against an expert manual segmentation (AS) using the Dice Similarity Coefficient (DSC),¹²⁸ and group-level differences were assessed using a Mann–Whitney *U*-test. To compare the effect of segmentation and image resolution on SEL detection, we performed an ANOVA on the number and volume of SELs at the subject level. The spatial overlap of SEL masks across conditions (2D vs. 3D; FLAMEs vs. SAMSEG) was further quantified using the DSC.

Association with clinical disability

The association between clinical disability scores and SEL burden was investigated by fitting linear regression models with EDSS as the outcome and log-transformed SEL volume (log_vSEL) as the predictor, adjusting for baseline *age*, *sex assigned at birth*, and *normalized brain volume* at baseline (computed using FreeSurfer and normalized by the intracranial volume).¹³⁴ To investigate lesion-specific tissue characteristics, we compared T1 values between SEL and non-SEL lesions at baseline, as well as longitudinal T1 changes between baseline and the third MRI timepoint, using a linear mixed-effects model with a random subject and lesion intercept. Finally, to evaluate the predictive value of SEL-derived volume for disability measures, we performed a random forest analysis (R, randomForest package)¹³⁵ with 10-fold cross-validation, using EDSS and MSSS as outcome variables. Candidate predictors included normalized values (scaled 0–1) of log_vSEL_3D_flames, log_vSEL_2D_flames, log_vSEL_3D_samseg, log_vSEL_2D_samseg, normalized brain volume, log_vLesions, log_vPRL, age (only for EDSS prediction), and sex.

Lesion-level assessment of PRL-SEL overlap

Lastly, spatial lesion-level overlap between PRLs and SELs was assessed. PRLs were manually and systematically assessed by two expert raters (AS and PM), as detailed in the *Longitudinal volume assessment of PRLs* section 5.2.3. For each tested input condition (described above), we recorded at the subject level: (i) the total number of SELs, (ii) the number of PRLs located within the binary lesion segmentation mask of the condition (to ensure methodological consistency), and (iii) the number of overlapping SEL – PRL pairs. From these counts, we computed lesion-level prevalence values for SELs ($P(SEL)$) and PRLs ($P(PRL)$), as well as the conditional probability ($P(SEL|PRL)$ & $P(PRL|SEL)$) across the cohort. To more rigorously characterize the relationship between SELs and PRLs, we further derived four complementary overlap metrics, designed to account for the overlap expected by chance under the assumption of two independent distributions drawn from the same lesion population:

- **Joint probability:** $P(SEL \cap PRL) = P(PRL) \cdot P(SEL | PRL) = P(SEL) \cdot P(PRL | SEL)$, representing the probability that a lesion is classified as both a SEL and a PRL.
- **Cohen's Kappa (κ):** $\kappa = \frac{P_o - P_e}{1 - P_e}$, where P_o is the observed agreement and P_e is the agreement expected by chance; 0 indicates no agreement beyond chance, and 1 indicates perfect agreement between the two biomarkers.
- **Jaccard Index (JI):** $\frac{SEL \cap PRL}{SEL \cup PRL}$, quantifying the proportion of shared lesions between SELs and PRLs relative to the total number of unique lesions identified by either biomarker.
- **Mutual Information (MI):** $I(X; Y) = \sum_{x \in X} \sum_{y \in Y} p(x, y) \log \left(\frac{p(x, y)}{p(x)p(y)} \right)$, quantifying the amount of shared information between SEL and PRL biomarkers, with higher values indicating greater correspondence.

As a supplementary analysis, we conducted a re-analysis of the two previous studies that have explored the overlap between SELs and PRLs.^{26,127} While both studies have reported the raw overlap between these two MRI biomarkers, they did not compute any further agreement metrics. Therefore, we propose to re-analyze the results of these studies with the same framework described above. Since the second study from Elliott et al.²⁶ did not report the total number of lesions in their study, thus preventing us to

compute the lesion-level biomarker prevalence, we used values computed in external studies for the prevalence of PRL¹³⁶ and prevalence of SEL,¹²⁴ introducing a certain bias to take into consideration.

5.3 Results

5.3.1 Demographics

Fifty-six participants were included in the SEL detection experiment. The baseline demographics, clinical and MRI characteristics are reported in Table 5.1. The median EDSS and MSSS at baseline were 2 (0 – 7) and 5.26 (0.62 – 9.46) respectively. Thirty-seven out of fifty-six (66.1%) of participants had at least one PRL and the mean total PRL volume was 1840.23 mm³.

Table 5.1 Demographics, clinical and MRI characteristics of the participants included in the SEL detection experiment. RMS: Relapsing-remitting MS; PMS: Primary progressive MS; EDSS: Expanded Disability Status Scale; MSSS: Multiple Sclerosis Severity Scale; PRL: paramagnetic rim lesions.

Demographics and clinical metrics	Values
Number of patients (RMS, PMS)	56(46, 10)
Age, mean (range) [years]	42.36 (19-72)
Female, N (%)	36 (64.3%)
Baseline disease duration, mean (range) [years]	7.98 (0-37.24)
EDSS at baseline, median (range)	2 (0-7)
MSSS at baseline, median (range)	5.26 (0.62-9.46)
Normalized brain volume, mean ± std	0.745 ± 0.029
Normalized gray matter volume, mean ± std	0.413 ± 0.026
Normalized white matter volume, mean ± std	0.243 ± 0.021
Number of PRL, mean (range)	5.84 (0-91)
PRL volume, mean ± std [mm ³]	1840.23 ± 4521.49
≥ 1 PRL, N (%)	37 (66.1%)

5.3.2 Longitudinal volume assessment of PRLs

Out of the 498 PRLs identified at baseline, 361 (72.5%) were classified as shrinking, 51 (10.3%) as expanding, and 46 (9.2%) as stable. Among the remaining lesions, 24 (4.8%) demonstrated a trend toward shrinkage and 16 (3.2%) a trend toward expansion, though neither group met the predefined 9% classification threshold. Group analyses confirmed that shrinking PRLs

continued to significantly decrease in volume over time ($\beta=-1.37\text{e-}04$ [95% CI: $-1.70\text{e-}04$; $-1.04\text{e-}04$], $p < 0.001$), whereas expanding PRLs continued to increase in volume ($\beta=2.48\text{e-}04$ [95% CI: $1.19\text{e-}04$; $3.77\text{e-}04$], $p < 0.001$). Moreover, shrinking PRLs displayed a significantly different volumetric trajectory compared to expanding PRLs ($p < 0.001$). Taken all together, PRLs exhibited a significant longitudinal decrease in log-transformed volume ($\beta=-7.88\text{e-}05$ [95% CI: $-1.10\text{e-}04$; $-4.78\text{e-}05$], $p < 0.001$).

Similarly, non-PRL lesions also demonstrated a significant overall tendency to shrink in log-transformed volume over time ($\beta=-4.12\text{e-}05$ [95% CI: $-5.56\text{e-}05$; $-2.69\text{e-}05$], $p < 0.001$). Within this group, subsets of lesions were identified as shrinking, expanding, or stable, each showing significant longitudinal trends consistent with their classification ($p < 0.001$) and different from the other sub-groups ($p < 0.001$).

Regarding the microstructural evolution of T1, PRLs exhibited higher baseline T1 values than non-PRLs ($\beta=+165$ ms [95% CI: $+143.64$; $+186.36$], $p < 0.001$), as well as a stable T1 evolution over time ($\beta=-0.0152$ [95% CI: -0.0482 ; 0.0177], $p=0.36$) compared to a significant decrease in T1 for non-PRL ($\beta=-0.1497$ [95% CI: -0.1643 ; -0.1352], $p < 0.001$). No significant T1 differences were observed at baseline between shrinking, stable, and expanding lesions; however, shrinking lesions exhibited a significantly ($p < 0.05$) steeper T1 decline ($\beta=-0.160$ [95% CI: -0.177 ; -0.1435], $p < 0.001$) compared to expanding lesions ($\beta=-0.1$ [95% CI: -0.139 ; -0.0606], $p = 0.203$). When considering the interaction between PRL status and lesion volume trajectory, only shrinking lesions showed a differential T1 evolution with regards to PRL status, with shrinking PRLs demonstrating a T1 increase over time ($\beta=0.017$ [95% CI: -0.018 ; 0.053]), significantly different ($p < 0.001$) from shrinking non-PRLs exhibiting a T1 decrease ($\beta=-0.211$ [95% CI: -0.229 ; -0.192], $p < 0.001$). No significant T1 changes were observed across PRL subgroups (shrinking, stable, or expanding; all $p > 0.05$). In contrast, shrinking non-PRLs showed a significantly steeper decrease in T1 compared to expanding non-PRLs ($p < 0.001$).

5.3.3 Evaluation of SEL detection

At baseline, FLAMeS segmentation outperformed SAMSEG, with higher overlap against the manual reference standard (DSC = 0.54 ± 0.16 vs. 0.45 ± 0.15 , $p < 0.01$).

When comparing SEL detection across different input segmentations and image dimensions at a lesion-level, DSC between SEL masks was weak to moderate but comparable across segmentation methods and input resolution. More specifically, 3D_flames versus 2D_flames achieved a DSC of 0.37 ± 0.28 , while 3D_samseg versus 2D_samseg yielded a DSC of 0.36 ± 0.30 . When comparing FLAMeS to SAMSEG, the DSC was 0.41 ± 0.32 for 3D and 0.36 ± 0.32 for 2D. At a subject-level in contrast, minor differences were observed with the ANOVA analysis, as presented in Table 5.2. The number of SELs per subject ranged from 2.57 (2D_samseg) to 3.50 (2D_flames), with significantly more SELs detected using 2D_flames than 2D_samseg. Total SEL volume per subject ranged from 69.48 mm³ (3D_flames) to 102.48 mm³ (2D_flames), with significantly greater volumes detected in 2D_flames compared to 3D_flames. The rest of the pairwise comparisons were non-significant.

Table 5.2 SEL-derived metrics at a subject level computed for the different inputs images 3D vs 2D FLAIR and segmentation (FLAMeS vs SAMSEG). Abbreviations: Slowly Expanding Lesion (SEL); Proportion of SEL (pSEL); volume of SEL (vSEL)

	3D_flames	3D_samseg	2D_flames	2D_samseg
# T2 lesions, N (range)	43.36 (3 - 186)	28.18 (3 - 97)	28.7 (3 - 116)	20.29 (3 - 69)
# nonSEL, N (range)	37.20 (2 - 185)	24.43 (2 - 97)	23.70 (1 - 115)	17.55 (2 - 68)
# SELpos, N (range)	11.32 (1 - 69)	7.68 (0 - 51)	10.09 (0 - 47)	8.29 (0 - 37)
# SEL, N (ranges)	3.16 (0 - 16)	2.77 (0 - 10)	3.5 (0 - 16)	2.57 (0 - 13)
pSEL, %	7.29%	10.19%	12.20%	12.67%
vSEL, mean $\pm$ std (mm ³)	69.48 $\pm$ 113.87	82.36 $\pm$ 106.48	102.48 $\pm$ 145.99	83.61 $\pm$ 120
≥ 1 SEL, N (%)	41/56 (73.21%)	43/56 (76.79%)	50/56 (89.29%)	44/56 (78.57%)

At the lesion level, SELs showed a non-significant trend toward higher T1 values compared to non-SELs. However, SELs exhibited a greater increase in T1 between baseline and last follow-up, significant for 3D_flames ($p < 0.001$), 3D_samseg ($p < 0.05$), and 2D_samseg ($p < 0.05$), but not for 2D_flames ($p = 0.106$).

Association with clinical disability

Overall, in our cohort, no significant associations were observed between log-transformed SEL volume (log_vSEL) and EDSS. Random forest modeling identified normalized brain volume, age, log-transformed lesion volume (log_vLesions), and log_vPRL as the variables with the strongest predictive value in explaining EDSS (see Figure 5.3.A). More specifically, log_vSEL_3D_flames and log_vSEL_2D_flames showed moderate to weak importance, about 40% and 74% less important than log_vPRL respectively. Specifically, predictive performance of log_vSEL varied substantially depending on input type, ranging from 3.499 in percent increase in mean squared error (%In-

cMSE) ($\log_vSEL_3D_flames$) to -2.84 %IncMSE ($\log_vSEL_3D_samseg$), the latter even showing no predictive value/importance. Compared to EDSS, $\log_vLesions$, $\log_vPRL$, and $brain_volume$ were the strongest predictors of MSSS (see Figure 5.3.B). Once more, $\log_vSEL$ predictive values varied substantially depending on inputs, with a reduction of about 30% and 59% of importance for $\log_vSEL_2D_flames$ and $\log_vSEL_3D_flames$ when compared to $\log_vPRL$, and with $\log_vSEL_3D_samseg$ showing again no predictive value of disease severity.

5.3.4 Lesion-level assessment of PRL-SEL overlap

Finally, table 3 shows the lesion-level overlap of SELs against PRLs under the different input conditions. The mean prevalence of SELs across inputs was 9.11%, compared to 19.65% for PRLs. The conditional probability of a lesion being a SEL given it was a PRL was 7.73%, whereas the probability of a lesion being a PRL given it was a SEL was 16.85%. Overall lesion-level agreement between SELs and PRLs was essentially nonexistent ($P(SEL \cap PRL) = 1.57\%$, Cohen's $\kappa = -0.022$; JI = 0.056; MI = 0.00079).

5 | Do SELs correspond to CALs?

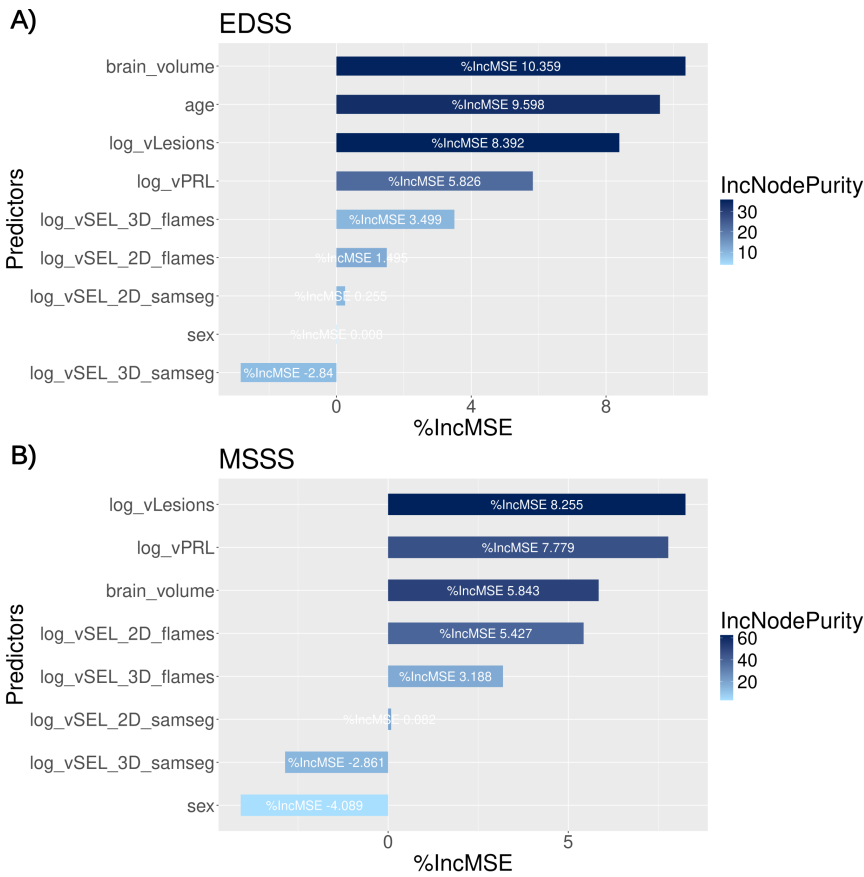


Fig. 5.3 Random Forest analysis of the predictive value of different imaging and clinical values on expanded disability status scale (EDSS, A) and on multiple sclerosis severity score (MSSS). The predictors are normalized brain volume, age, sex, log-transformed total T2 lesion volume (log_vLesions), PRL volume (log_vPRL), SEL_3D_flames volume (log_vSEL_3D_flames), SEL_3D_samseg volume (log_vSEL_3D_samseg), SEL_2D_flames volume (log_vSEL_2D_flames), SEL_2D_samseg volume (log_vSEL_2D_samseg). The x – axis shows the percent increase in mean squared error (%IncMSE) — relating to the overall importance of the predictor in explaining the clinical score —, while the color describe the Increase in Node Purity (IncNodePurity), a value indicating how much a feature contributes to producing cleaner, more homogeneous subsets.

Table 5.3 Lesion-level correspondence of SELs, computed with different input images (3D vs 2D FLAIR) and segmentation (FLAMeS vs SAMSEG), and PRLs. Abbreviations: prevalence of SEL [P(SEL)], prevalence of PRL [P(PRL)], conditional probability of being a SEL if a lesion is a PRL [P(SEL | PRL)], conditional probability of being a SEL if a lesion is a PRL [P(PRL | SEL)], joint probability of SEL and PRL [P(SEL $\cap$ PRL)], Cohen's agreement Kappa (Cohen's κ), jaccard index (JI), and mutual information (MI), 95% confidence interval [95% CI].

	3D_flames	3D_samseg	2D_flames	2D_samseg	mean
#Lesions	2428	1522	1607	1136	-
#SEL [P(SEL)]	151 [6.22%]	124 [8.15%]	169 [10.52%]	131 [11.53%]	9.11%
#PRL [P(PRL)]	341 [14.04%]	295 [19.38%]	331 [20.6%]	279 [24.56%]	19.65%
#SEL & PRL	19	25	25	26	-
P(SEL PRL)	5.57%	8.47%	7.55%	9.31%	7.73%
P(PRL SEL)	12.58%	20.16%	14.79%	19.85%	16.85%
P(SEL $\cap$ PRL)	0.78%	1.64%	1.56%	2.29%	1.57%
Cohen's κ , mean, [95% CI]	-0.0098 [-0.044; 0.025]	0.0052 [-0.04; 0.05]	-0.046 [-0.086; -0.005]	-0.036 [-0.085; 0.014]	-0.022
JI	0.04	0.063	0.053	0.068	0.056
MI	0.00009	0.00002	0.00187	0.00117	0.00079

The first study from Calvi et al. (2022) reported these results: out of 1492 lesions, 616 were SELs, 80 were PRLs, and 43 were both PRLs and SELs.¹²⁷ Re-analyzing these data using the same framework as our study yields a joint probability of 2.9%, a Cohen's κ of -0.04, a JI of 0.066, and a MI of 0.004 — all results consistent with our study. The second study, from Elliott et al. (2023), reported that 47/119 PRLs (39.5%) corresponded to SELs while 46/267 (17.23%) SELs corresponded to PRLs, but they did not report the total number of lesions in their cohort, preventing a direct prevalence-based calculation.²⁶ Using external prevalence estimates (29% for SELs¹²⁴ and 9.8% for PRLs¹³⁶), we obtain a joint probability of 11.5%, a Cohen's κ of 0.52, a JI of 0.421, and MI of 0.21 — suggesting a moderate overlap. However, using prevalences from different studies undermines the validity of this estimate as the prevalence of these biomarkers varies substantially between studies. More specifically, the computed conditional probability $P(SEL | PRL)$ based on the computed joint probability of 11.5% and $P(PRL)$ of 9.8% exceeded 100% (117%), an impossibility that indicates a sizeable overestimation of these computed overlap metrics.

5.4

Discussion

The objective of this study was to evaluate the SEL detection algorithm, assessing its consistency across heterogeneous imaging inputs (including variation in image resolution and lesion segmentation) and disentangling the relationship between SELs and PRLs, the latter representing a pathologically validated biomarker of CALs.

5.4.1 Rethinking volumetric dynamics of CALs

The SEL algorithm aims to identify concentric, gradual expansion of chronic MS lesions using clinically available MRI sequences.²⁵ The core assumption of SEL as a biomarker of CAL is that CALs would expand concentrically over time due to persistent smoldering inflammation at their edges. While this idea is conceptually plausible, it likely oversimplifies the underlying MS lesions' pathophysiology and is increasingly challenged by emerging *in vivo* evidence.¹³⁷ Direct histopathological validation is not feasible, since lesion expansion cannot be assessed *post mortem*, leaving longitudinal MRI as the only available tool for this evaluation.

Following the resolution of gadolinium enhancement (generally lasting 1-2 months), chronic MS lesions can follow different pathological trajectories, which in turn affect their short- and medium-term volumetric changes. Both remyelination and tissue loss are likely to drive lesion shrinkage, while smoldering edge inflammation with ongoing demyelination can lead to expansion. In this study, we identified distinct subsets of expanding, shrinking, and stable PRLs, confirming the heterogeneity of CALs in terms of lesional volume trajectories, and in line with previous work showing that PRLs in MS are highly heterogeneous in terms of lesional volume and tissue damage.⁶ Importantly, and in agreement with previous evidence,¹³⁷ we found that contraction is the predominant pattern of MS lesions' volumetric evolution, with the majority of chronic lesions (both PRL and non-PRL) significantly shrinking over time. From a microstructural standpoint, both PRLs and expanding lesions exhibited a more pronounced accumulation of lesional tissue damage than their respective non-PRL and shrinking counterparts, with PRL status exerting the strongest effect. Interestingly, shrinking PRLs showed a significantly greater progression of lesional tissue damage compared to shrinking non-PRLs, likely reflecting tissue loss driven by compartmentalized-inflammation-induced cell senescence.¹⁰ This supports the hypothesis that lesion shrinkage in chronic active and inactive lesions may be primarily driven by distinct pathological processes — likely tissue loss and remyelination, respectively.¹³⁷ In line with these observations, recent works investigating lesion volume change and quantitative tissue damage metrics have shown that shrinking lesions may display more severe baseline tissue damage than expanding ones^{19,138} (detailed in the Annex E). All these observations critically challenge the concept of longitudinal radial expansion as a biomarker of CALs, suggesting instead that CALs undergo multiple volumetric evolution phases throughout their lifespan.

5.4.2 Limitations of SEL as a reliable biomarker of CALs

The SEL algorithm, as introduced by Elliott et al.,²⁵ relies on non-rigid registration to estimate local tissue deformation, specifically expansion and contraction, using Jacobian determinant analysis. A first limitation regards reproducibility; the original implementation being not publicly available, calculating SELs necessarily requires parameter re-implementation. In this study, we evaluated the robustness of the algorithm under two main sources of input heterogeneity: (i) image quality, by comparing degraded 2D FLAIR (1×1×3 mm resampled to isotropic 1 mm) with native 3D FLAIR (clini-

cal vs. research sequence), and (ii) lesion segmentation, using FLAMeS (state-of-the-art) versus SAMSEG (less performant).¹²⁸ At the subject level, the average number and total volume of SELs remained relatively consistent across input conditions. However, voxel-wise comparison revealed poor overlap between SEL masks across varying input conditions (average DSC of about 0.375). This discrepancy suggests that while the overall SEL burden is relatively stable, the exact spatial localization of SELs is highly input-dependent, likely a consequence of the way SEL detection criteria are defined (further discussed below). At the lesion level, SELs showed a trend to higher baseline T1 and significantly greater longitudinal T1 increase between first and last timepoints, consistent with prior reports.^{25,124,125} This effect is plausibly explained by the Jacobian-based detection being directly influenced by intensity changes in the underlying T1-weighted images. Despite these associations, in our cohort we did not observe any significant association between log-transformed SEL volumes ($\log_vSEL$) and EDSS. Finally, in a random forest cross-validation analysis of EDSS prediction, SEL volume demonstrated moderate predictive value and its performance in predicting disability scores (EDSS and MSSS) was consistently outperformed by total T2 lesion load, and total PRLs' volume.

Beyond the lack of robustness demonstrated in this study, several conceptual limitations exist in the original implementation of the SEL algorithm as presented by Elliott et al.²⁵ A first limitation is the use of a binary lesion mask as input, rather than an instance-level lesion mask with pre-selected individual lesions. While the binary mask approach offers computational efficiency and automation, it introduces a fundamental ambiguity: SELs may not correspond to singular MS lesions as detected by MRI and potentially validated by MRI-histopathological studies. Indeed, previous studies have noted that if the same T2 hyperintense lesion includes two or more clusters of Jacobian expansion, this may reflect the presence of different lesions that became confluent,¹²⁵ therefore using the Jacobian analysis to separate confluent lesion. However, it was acknowledged in the original paper that T1 intensity changes exclusively localized at the edge of a lesion results in a concentric inside-out Jacobian expansion pattern (more pronounced expansion in the center of the lesion compared to the edge).²⁵ This illustrates that Jacobian-based expansion is not directly linked to voxelwise image intensity changes. Therefore, the hypothesis that Jacobian expansion can reliably differentiate individual lesion instances within confluent lesions lacks support.

A second major issue lies in the implementation of the concentricity and constancy criteria. Although each criterion is conceptually reasonable, their practical use introduces important limitations. Both criteria are z-score normalized against all SEL candidates in the study population, which makes SEL classification inherently population-dependent rather than lesion-specific. As a result, the very same lesion could be labeled SEL in one population but not in another. Moreover, z-score normalization enforces a distribution where a fixed proportion of lesions will have positive values, regardless of their raw criterion scores. This means that even a lesion with a raw negative concentricity value could end up with a positive z-score normalized concentricity score if the overall cohort has an even lower average. Such population-driven scaling likely explains why the number and volume of SELs at the subject level appeared rather consistent across different inputs, while lesion-level overlap remained poor. Finally, the decision to combine the normalized values of concentricity and constancy into a single abstract metric strips these measures of any biological meaning. Taken together, these limitations challenge the pathological value and utility of the SEL detection algorithm in MS. While SELs are presented as lesions with consistent concentric expansion, the actual implementation detects regions within lesion masks (not necessarily distinct lesions) using a population-normalized heuristic with no direct histopathological relevance.

5.4.3 Divergence between SELs and PRLs

A major concern with SELs is the absence of rigorous biological validation as markers of CALs. By design, SELs are purely longitudinal imaging constructs and therefore cannot be directly validated through histopathology. This makes comparisons with established *in vivo* biomarkers of CALs essential. Currently, two such biomarkers exist: PRLs, detectable with susceptibility-based MRI, and CALs identified through positron emission tomography (PET) imaging using 18-kDa translocator protein (TSPO) binding radioligands (TSPO-PET).²⁸ To our knowledge, no study has investigated lesion-level overlap between SELs and TSPO-PET-defined CALs, and only limited work has explored the correspondence between SELs and PRLs.^{26,127}

In our study, lesion-level correspondence between SELs and PRLs was essentially absent, with a mean joint probability of 1.57%, a mean Cohen's κ of -0.022, a mean JI of 0.056, and a mean MI of 0.00079, suggesting no meaningful relationship between PRLs and SELs. Although two other stud-

ies reported raw overlap between SELs and PRLs, neither accounted for chance-level overlap expected when comparing two biomarkers drawn from the same lesion population. Re-analyzing the available data from these studies showed similar results to our study, suggesting that SELs and PRLs are essentially independent. Given that PRLs, but not SELs, have been histopathologically validated as CALs, the lack of a correspondence/association between the two biomarkers suggests that SELs, as currently defined, may not reliably identify CALs *in vivo*.

5.4.4 Limitations

The principal limitation of this study lies in the relatively small sample size (56 subjects) and the specific characteristics of the cohort, a limitation driven by the conservative inclusion criteria for all participants to have a homogeneous advanced MRI protocol on the same 3T scanner for three consecutive timepoints. Notably, most PRLs in our dataset exhibited a reduction in volume over time, a pattern that diverges from trends commonly reported in the literature,^{98,139,140} albeit in line with long-term observation.¹³⁷ Nonetheless, this does not affect the central conclusion: even if a biomarker demonstrates a consistent group-level trajectory, individual lesions can still display heterogeneous behaviors, with sub-groups of PRL in our cohort showing significantly different patterns of volumetric evolution. The limited cohort size also likely reduced statistical power, preventing the detection of a potential association between SEL volume with clinical disability score. Importantly, these limitations should not alter the broader conceptual issues of the SEL algorithm highlighted in the discussion.

5.5 Conclusion

In conclusion, our findings challenge the central assumptions underlying SELs: all CALs are not associated with volume expansion. Furthermore, the SEL detection algorithm lacks robustness against heterogeneous imaging acquisition and lesion segmentation, while the Jacobian-based methodology shows limited biological interpretability. The reliance on binary lesion masks instead of lesion-level instances, combined with population-dependent heuristic thresholds for SEL selection, further undermines the histopathological correspondence of this biomarker. Most critically, in vali-

dation experiments against a histopathologically-validated biomarker of CALs (i.e. PRLs), SELs demonstrated near complete independent correspondence, strongly suggesting that SELs, as currently defined, may not represent CALs.

Nevertheless, the longitudinal volumetric dynamics of MS lesions — whether expansion or shrinkage — remain of high interest, as they provide valuable insights into ongoing lesional activity *in vivo*. Future research should prioritize reproducible and open-source implementations that allow for independent verification and methodological transparency. Beyond expansion, lesion shrinkage should also be systematically evaluated, as both shrinking and expanding PRLs likely capture distinct aspects of chronic activity and tissue remodeling, with both showing evidence of longitudinal increased tissue damage. Furthermore, new methodological approaches should move away from heuristic population-based criteria and instead incorporate lesion-level features with stronger biological interpretability, possibly leveraging multi-contrast MRI. Finally, “MRI evolving lesions” could be conceptualized as a separate class of imaging biomarkers: informative about longitudinal dynamics and potentially relevant for disease monitoring, but distinct from histopathological lesion subtypes, whose longitudinal changes cannot be validated *post-mortem*.

6

***In vivo* characterization of multiple sclerosis "MRI evolving lesions"**

Preliminary Note:

This chapter contains an exploratory/preliminary still unpublished analysis (with the aim of organizing these results in a scientific paper to be submitted for peer-review as soon as possible).

6.0 Positioning within the thesis

This final chapter represents the culmination of the thesis. Building upon the methodological and conceptual developments introduced in the previous chapters, it aims to provide a comprehensive and minimally biased *in vivo* characterization of the full spectrum of MS lesions. In doing so, it addresses the *Central Research Question* of this work while focusing on the following specific question:

Research Question 4 — Longitudinal MS lesion characterization

How does the microstructural evolution of chronic active lesions relate to irreversible disability accumulation in MS?

This study explicitly integrates all prior contributions of the thesis. BMAT (Chapter 3) provides the standardized framework for data management and processing. The selection of diffusion models is directly guided by the comparative analysis performed in Chapter 4, ensuring sensitivity and biological interpretability of the microstructural metrics. The conceptual definition of lesion activity builds upon the critical insights developed in Chapter 5, including the introduction of *MRI-evolving lesions*, while the lesion-splitting strategy enables an unbiased exploration of the full lesion spectrum.

Within this framework, this chapter provides a detailed longitudinal characterization of MS lesions by jointly analyzing their activity status, volumetric dynamics, and microstructural evolution. Beyond refining the *in vivo* description of chronic active lesions, this work reveals distinct and sometimes unexpected trajectories, most notably identifying shrinking paramagnetic rim lesions as markers of pronounced and irreversible tissue damage, likely driven by structural collapse of the surrounding tissue. Importantly, this specific lesion phenotype also shows a strong association with clinical disability and disease severity.

More broadly, this study reinforces the relevance of considering lesion dynamics beyond simple expansion and supports the use of *MRI-evolving lesions* as a meaningful framework for investigating MS pathology *in vivo*. By leveraging the sensitivity of carefully selected diffusion models, it further enables the proposal of a hypothesized life-cycle of chronic active lesions,

offering new future perspectives for understanding their evolution *in vivo*, while acknowledging the speculative nature of this model.

In the context of this thesis, this chapter provides the integrative link between methodological rigor, biological interpretation, and clinical relevance. It demonstrates how combining standardized processing, informed model selection, and refined biomarker definitions can lead to a more coherent and clinically meaningful understanding of MS lesion evolution, ultimately opening new avenues for improved disease monitoring and targeted therapeutic strategies.

6.1 Introduction

Multiple sclerosis (MS) is the most prevalent chronic inflammatory disease of the central nervous system (CNS), characterized by recurrent neurological symptoms and the accumulation of focal demyelinating lesions in the brain and spinal cord.²⁰ Lesion formation is initiated by an acute inflammatory phase marked by blood–brain barrier (BBB) disruption,¹¹⁹ which is closely associated with clinical relapses and predictive of short-term disease activity.²² However, acute inflammatory activity alone has limited predictive value for long-term disability accumulation and neurodegeneration.¹⁴¹ In contrast, the subsequent evolution of lesions toward a chronic active or smoldering state has led to the definition of chronic active lesions (CALs), which have emerged as robust biomarkers of ongoing tissue damage and aggressive disease in MS.^{120,121}

CALs are thought to represent sites of compartmentalized inflammation persisting behind a relatively restored BBB.¹²² Histopathologically, they are characterized by a hypocellular core with limited inflammatory infiltrates, surrounded by a rim of chronically activated, iron-laden microglia and macrophages.⁹⁴ *Ex vivo* studies have demonstrated that CALs are associated with extensive demyelination, axonal loss, and neurodegeneration, and are strongly linked to disability accumulation and disease severity in MS.^{23,87} While histopathological investigations provide unparalleled cellular and microstructural detail, their cross-sectional nature limits the ability to investigate lesion dynamics longitudinally *in vivo*.

Magnetic resonance imaging (MRI) offers a unique opportunity to study MS lesion pathology longitudinally in living patients. To date, two principal MRI-based approaches have been used to identify CALs *in vivo*. The

first relies on the detection of *paramagnetic rim lesions* (PRLs), visualized using susceptibility-sensitive T2*-weighted MRI sequences optimized to detect iron accumulation at lesion borders.^{88,94} The second approach is based on the longitudinal assessment of lesion expansion on conventional T1- and T2-weighted MRI, under the assumption that progressive lesion growth reflects ongoing inflammatory activity²⁵ (see Chapter 5). Substantial MRI–histopathological evidence supports PRLs as a robust *in vivo* correlate of histologically defined CALs.²⁸ Accordingly, PRLs have been associated with severe axonal loss, demyelination, and neurodegeneration,^{2,95} as well as with increased disease severity and disability accumulation in MS.^{5,23,64,96}

Despite their established relevance, PRLs exhibit considerable heterogeneity. Recent work using quantitative T1 mapping has identified distinct PRL subtypes, ranging from relatively young and mildly destructive lesions to older, highly destructive lesions, the latter showing a markedly stronger association with clinical disability.⁶ These findings highlight an incomplete understanding of PRL biology and suggest that PRL status alone may not fully capture the spectrum of chronic lesion pathology.

A complementary approach to identifying CALs relies on longitudinal lesion expansion. While lesion growth likely reflects persistent inflammatory activity, recent studies have highlighted important methodological limitations of currently accepted definitions of *slowly expanding lesions* (SELs)³ (see Chapter 5). In particular, lesion expansion alone does not uniquely identify chronic active pathology. Notably, emerging evidence suggests that shrinking lesions — traditionally considered benign or reparative — may also follow destructive microstructural trajectories^{19,138} (detailed in the Annex E), especially when PRL features are present.³ These observations challenge the simplistic dichotomy between expanding and non-expanding lesions and indicate that PRL status and volumetric evolution capture complementary but non-redundant aspects of lesion biology. This interpretation is further supported by the demonstrated independence between PRLs and SELs³ (see Chapter 5).

Beyond qualitative imaging, quantitative MRI provides powerful tools to investigate MS lesion microstructure *in vivo*, with increased specificity to cellular processes and tissue remodeling. Quantitative T1 relaxation time has been widely used as a marker of lesion severity due to its strong association with myelin content and axonal density in MS.¹⁰² In parallel, multi-shell diffusion MRI enables a more refined characterization of tissue microstructure and has demonstrated enhanced sensitivity to pathological

heterogeneity and clinical disability.^{2,101,103,104} This is particularly evident when using advanced multi-compartment diffusion models with biophysically interpretable compartments, especially those explicitly modeling intra-axonal and extra-axonal environments, which are central to MS-related cellular pathology² (see Chapter 4).

Against this background, the present study aims to disentangle the interplay between PRL status, lesion volumetric evolution, and microstructural dynamics in a large longitudinal cohort of MS patients. Specifically, we seek to (i) characterize baseline microstructural signatures of chronic lesion phenotypes defined by PRL status and volumetric behavior, (ii) quantify their longitudinal microstructural trajectories using complementary diffusion models and T1 mapping, and (iii) assess their clinical relevance through associations with disability and neurodegeneration measures. By integrating PRL status, lesion expansion behavior, and multi-modal quantitative MRI, this work provides a comprehensive framework for understanding chronic lesion heterogeneity and its contribution to disease progression in multiple sclerosis.

6.2 Materials and methods

6.2.1 Participants

Imaging and clinical data were consecutively collected under an institutional review board-approved protocol in adults with a diagnosis of MS followed at Cliniques Universitaires Saint-Luc (CUSL). Specific patient inclusion criteria were the following: (1) ≥ 18 years old, (2) diagnosis of MS according to the 2024 McDonald criteria,¹²³ (3) and a clinical expanded disability status state (EDSS) and multiple sclerosis severity score (MSSS) evaluation at baseline, and (4), the availability of a longitudinally homogeneous 3-Tesla (3T) MRI protocol including a 3-dimensional (3D) T2*-weighted echoplanar imaging (EPI)⁸⁸ (for PRL assessment), T2-weighted fluid-attenuated inversion recovery (FLAIR) and T1-weighted magnetization prepared rapid gradient echo image (MPRAGE) (for brain and lesion segmentation, and lesion expansion assessment), and a quantitative T1map derived from a sagittal 3D magnetization-prepared 2 rapid gradient echo (MP2RAGE) and multi-shell diffusion weighted (DWI) (for microstructural investigations). Overall, a total of 122 MS participants (95 relapsing-remitting — RMS, 27

progressive — PMS) were included in the study. This study was approved by the local ethical committee of CUSL, and all participants provided written informed consent according to the declaration of Helsinki prior to the study.

6.2.2 MRI imaging protocol

MRI acquisition was performed on a 3T whole-body MR scanner (GE SIGNA™ Premier research scanner, General Electric, Milwaukee, WI) equipped with a 48-channel head coil. The MRI protocol included: (i) sagittal 3D MPRAGE sequence (repetition time (TR) = 2186 ms, echo-time (TE) = 3 ms, inversion time (TI) = 900 ms, field of view (FOV) = 256 mm, number of slices (#slices) = 156, voxel size = $1.0 \times 1.0 \times 1.0$ mm), (ii) sagittal 3D FLAIR sequence (TR = 5000 ms, TE = 105 ms, TI = 1532 ms, FOV = 256 mm, #slices=170, voxel size = $1.0 \times 1.0 \times 1.0$ mm), (iii) a sagittal high-resolution 3D EPI⁸⁸ sequence (TR = 80.2 ms, TE = 35 ms, flip angle = 18° , FOV = 256 mm, #slices = 355, voxel size = $0.67 \times 0.67 \times 0.67$ mm), (iv) quantitative T1map derived from a sagittal 3D MP2RAGE sequence (TR = 1925 ms, TE = 3 ms, TI = 700 and 2500 ms, FOV = 256 mm, #slices=156, voxel size = $1.0 \times 1.0 \times 1.0$ mm), (v) multi-shell diffusion-weighted sequence (TR =4842 ms, TE = 77 ms, Δ = 35.7 ms, δ = 22.9 ms, 64 gradients at b = 1000, 32 at b = 2000, 3000, 5000 and 7 b = 0 s/mm², FOV = 220, #slices = 68, voxel size = $2.0 \times 2.0 \times 2.0$) with reversed phase encoding, and (vi) a post-gadolinium T1-weighted spoiled gradient recalled echo (CESPGR) sequence (TR = 6.9 ms, TE = 2.1 ms, flip angle = 12° , FOV = 240, #slices = 150, voxel size = $0.69 \times 0.69 \times 1.0$ mm).

6.2.3 Imaging data processing

Lesion identification and segmentation

This section presents the different regions of interest (ROI) and the methods to identify them for this analysis. The first identified biomarker is paramagnetic rim lesions (PRL). For their identification, 3D FLAIR and CE-SPGR images were rigidly coregistered using mutual information to the high-resolution 3D EPI scan using ANTs.⁷⁸ Chronic non-enhancing MS lesions were classified as PRL if they showed no enhancement on post-gadolinium CE-SPGR images and demonstrated a paramagnetic rim on unwrapped filtered phase images,¹⁰⁷ in accordance with the latest NAIMS criteria.²⁸ PRL identification required the following features, as previously described:⁵

(i) colocalization with the edge of an MS lesion on FLAIR, (ii) visibility in at least two planes, and (iii) a paramagnetic rim covering at least two-thirds of the lesion's white matter border on the slice of maximum visibility. PRL assessment was performed by consensus of two trained raters (AS and PM).

Whole-brain lesion segmentation was performed using FLAMeS^{128,142} applied to the FLAIR image for semantic binary segmentation, combined with an in-house algorithm to track longitudinal lesion volume changes as previously described³ and detailed in supplementary materials C.1. Lesion volume trajectories were quantified by fitting a Huber^{129,130} linear regression model to normalized annual lesion volume changes. Lesions were classified as expanding if the slope exceeded +9%, shrinking if below -9%, or stable if between -4% and +4%, based on the distribution of our data. Lesions not meeting the $\pm 9\%$ criteria were defined as having a tendency to expand (or shrink).

Finally, whole-brain anatomical segmentation was performed with FreeSurfer⁹¹ and NAWM mask was automatically computed based on the FreeSurfer brain segmentation⁹¹ and corrected by subtracting a lesion mask segmented using FLAMeS¹²⁸ and a ventricle mask computed using an *in-house* algorithm based on Otsu thresholding.¹⁰⁸ To explore the microstructural evolution in different histopathologically relevant lesion area, all lesions were subsequently divided into three areas: lesion rim — corresponding to the 1mm border of the lesion —, the lesion core — corresponding to the inner two third area excluding the lesion rim —, and the lesion periplaque — corresponding to a 1mm border outside of the lesion in the NAWM and corrected by the presence of periplaque of other lesions as previously described.²

Microstructural investigation

Microstructural tissue integrity was investigated using two quantitative imaging methods: quantitative longitudinal relaxation time (T1) derived from the MP2RAGE sequence (MP2RAGE-derived T1map), used as an established marker of MS tissue integrity¹⁰² and multi-shell diffusion MRI, in order to further investigate microstructural alterations. The multi-shell diffusion data were first preprocessed with denoising,¹⁰⁹ top-up,¹¹⁰ motion and eddy-currents correction,¹¹¹ and skull-stripping using FSL BET¹¹² using the *elikopy* pipeline.¹⁴³ Finally, the resolution of the diffusion data was increased to 1mm isotropic by employing a novel model-free "SUPER-Resolution" pipeline using self-similarity^{144,145} as previously described in MS.¹⁴⁶ The generated high-resolution diffusion data were upsampled to the

same resolution as the 3D T1-weighted MPRAGE anatomical volumes, i.e. 1mm^3 isotropic. Finally, the diffusion-weighted data was processed using three multi-compartment diffusion models, as they are the most informative about MS complex pathophysiology² (see Chap. 4):

- Neurite Orientation Dispersion and Density Imaging (NODDI)⁴⁶ is a multi-compartment model aiming at characterizing neurite orientation dispersion. NODDI computes a set of biologically interpretable metrics as the fraction of intra-axonal volume (f_{intra}), the fraction of extra-axonal cell volume (f_{extra}), the fraction of isotropic diffusivity (f_{iso}), and the orientation dispersion index of the fibers (odi). NODDI's limitations come from its two assumptions: (i) one fiber population per voxel and (ii) fixed diffusivity for all the voxels in the brain tissue.
- For fitting of the Soma And Neurite Density Imaging (SANDI) model, we used the AMICO software,¹⁴⁷ applying the default diffusivity parameters and a λ_2 regularization term of 0.005, as previously described.¹⁴⁶ Estimates of the intra-neurite ($f_{neurite}$), extracellular (f_{extra}), and intra-soma (f_{soma}) signal fractions and apparent soma radius (R_{soma}) were used for the microstructural analysis.
- The last diffusion model was implemented using the DIstribution of Anisotropic MicrOstructural eNvironments in DWI (DIAMOND or DMD)¹¹⁵ framework. It was implemented as a three compartments model including: (i) an anisotropic compartment modeled as a tensor representing the fibers (f_{fibers}), (ii) one isotropic compartment with a fixed diffusivity of $3 \cdot 10^{-3} \text{mm}^2/\text{s}$ representing free water (f_{csf}), and (iii) one final restricted isotropic compartment with a variable diffusivity (d_{ear}) corresponding to the presence of other types of spherical cells (f_{ear}). This model, called *DIAMOND_{fear}*, was implemented based on the work of Taquet et al.¹⁴⁸ which showed association between the restricted isotropic compartment with reactive microglia in an animal mouse model.

These three models were chosen for their capabilities to model extra-axonal compartments, mainly corresponding to the presence of astrocytic, glial and microglial cells, a characteristics highly present, and arguably the most important process to observe in MS lesion pathophysiology.

6.2.4 Statistical Analysis

Statistical analyses were performed in R (version 4.5.2) using the *lme4* package for linear mixed-effects models (LMM).

Baseline Characterization: To investigate phenotypic differences at baseline, cross-sectional LMMs were fitted with microstructural metrics as the dependent variable. Fixed effects included *PRL status* (PRL+ vs. PRL-), *Expansion status* (Expanding [EL], Stable, Shrinking [SL]), and their interaction term. A random intercept for *subject* was included to account for intra-patient clustering, and all models were adjusted for age at baseline as a fixed effect.

Longitudinal Evolution: The longitudinal trajectory of lesion microstructure was assessed using repeated-measures LMMs. The primary fixed effect of interest was the interaction between *Time* (in years) and lesion phenotype (PRL $\times$ Time, Expansion $\times$ Time, and PRL $\times$ Expansion $\times$ Time), allowing for the estimation of distinct rates of change (slopes) for each lesion type. To account for the hierarchical structure of the data, nested random intercepts were included for *subject* and *individual lesion ID*. Consistent with the baseline analysis, models were adjusted for age at baseline as a fixed effect.

Sensitivity Analysis: All analyses were carried out with mean value across the lesion, the mean value in the lesion core, rim, and periplaque. In addition, to ensure findings were not driven by partial volume effects from neighboring lesions, analyses were repeated in a subset of "low-confluence" lesions (defined as lesions sharing $< 15\%$ of their border with another lesion). Finally, the longitudinal analysis was repeated in a subset of lesions that had at least three consecutive timepoints, to ensure robustness of linear regression fitting. The purpose of the analysis was mainly to compare the direction of the different contrast differences.

Clinical Associations: To evaluate clinical relevance, the total lesion volume for each phenotype (stratified by PRL and expansion status) was computed per subject and log-transformed to normalize the distribution. Associations between specific lesion loads and clinical outcomes (Brain Volume, EDSS, MSSS) were assessed using multivariable linear regression models, adjusted for age and sex assigned at birth.

Reporting: Model-based contrasts were estimated using the *emmeans* package with Tukey-adjusted post-hoc comparisons. In figures, results are presented as fixed-effect β estimates (representing mean differences or slopes) with 95% confidence intervals (CI). While the primary significance threshold was set at $p < 0.05$, pathophysiologically meaningful trends are also discussed. To enhance readability, p -values in the text are indicated by asterisks: * ($p < 0.05$), ** ($p < 0.01$), and *** ($p < 0.001$). Where significance was observed within a specific lesion sub-area but not in the lesion average, a regional suffix is added to the asterisk: c (core), r (rim), or p (periplaque). Detailed p -values for all contrasts are provided in the supplementary tables.

6.3 Results

6.3.1 Demographics

122 participants were included in this study. The baseline demographics, clinical and MRI characteristics are reported in Table 6.1. The median EDSS and MSSS at baseline were 2 (0 – 7) and 5.26 (0.78 – 9.46) respectively. 64 out of 122 (42.62%) participants had at least one PRL and the mean total PRL volume was 1118.3 mm³. Participants had a median number of 3 sessions (2 – 5) and a mean time of follow-up of 743.5 days (273 – 1652).

6.3.2 Microstructural characterization of MS lesions

12912 individual lesions were analyzed in the microstructural investigations, of which 809 were PRL+, 12037 were PRL-. Overall, there were 4835 SL, 1735 EL, and 1004 stable lesions, and Table 6.2 shows a contingency table of the interaction of lesion PRL and expansion status.

Table 6.1 Demographics, clinical and MRI characteristics of the participants included in the SEL detection experiment. RMS: Relapsing-remitting MS; PMS: Primary progressive MS; EDSS: Expanded Disability Status Scale; MSSS: Multiple Sclerosis Severity Scale; PRL: paramagnetic rim lesions; Baseline Treatment: Glatiramer acetate, Interferon; High efficacy treatment: Dimethyl Fumarate, Sp2-Inhibitors, Teriflunomide, Cladribine; Very high efficacy treatment: Anti-CD20, Natalizumab, Alemtuzumab.

Demographics and clinical metrics	Values
Number of patients (RMS, PMS)	122 (95, 27)
Age, mean (range) [years]	41.7 (29.5 – 72.7)
Female, N (%)	78 (65.5%)
Baseline disease duration, mean (range) [years]	4.87 (0 – 37.24)
EDSS at baseline, median (range)	2 (0 – 7)
MSSS at baseline, median (range)	5.26 (0.78 – 9.46)
Number of sessions, median (range)	3 (2 – 5)
Time of follow-up (days), mean (range)	743.5 (273 – 1652)
Normalized brain volume, mean ± std	0.74 ± 0.03
Normalized gray matter volume, mean ± std	0.41 ± 0.024
Normalized white matter volume, mean ± std	0.29 ± 0.023
Number of PRL, mean (range)	6.6 (0 – 142)
PRL volume, mean ± std [mm ³]	1118.3 ± 2666.9
≥ 1 PRL, N (%)	64 (42.62%)

Treatments:	
untreated, N (%)	67 (54.92%)
baseline treatment, N (%)	8 (6.56%)
high efficacy treatment, N (%)	32 (26.23%)
very high efficacy treatment, N (%)	15 (12.3%)

Table 6.2 Contingency table of the number of lesions based on their paramagnetic rim status (PRL+, PRL-) and their expansion status (shrinking — SL, expanding — EL, stable).

	SL	stable	EL
PRL-	4392	924	1623
PRL+	401	78	110

Baseline lesion characteristics

The cross-sectional β estimates of the most explicit metrics are represented in Figure 6.1, while the β estimates of the rest of the metrics can be found in Figure D.1 in the Annex D, alongside Table D.1 containing all contrast p -values. At baseline, PRL+ lesions displayed a distinctly more severe pathological phenotype compared to PRL- lesions, independent of lesion size. PRL+ lesions were significantly larger^{***} and exhibited higher T1 values^{***}, reflecting greater tissue destruction. Advanced diffusion metrics revealed that this damage was driven by a convergent pattern of: (1) neuro-axonal loss, indicated by lower fiber density (lower DMD f_{fibers} ^{***}, NODDI f_{intra} ^{***}, SANDI $f_{neurite}$ ^{***}); (2) increased interstitial clearance, shown by higher unrestricted or extra-cellular diffusion (higher DMD f_{csf} ^{***}, NODDI f_{extra} ^{***}); and (3) pronounced hypocellularity, particularly within the lesion core (lower DMD f_{ear}^{*c} and SANDI f_{soma}^{**}). Interestingly, PRL+ lesions exhibited larger average soma size (R_{soma}^{*}) and lower diffusivity within the extra-axonal restricted space (d_{ear}^{***}), potentially reflecting the presence of foamy macrophages and the accumulation of extracellular debris.

When stratified by expansion status alone, SL presented a paradoxical profile: they were larger^{***} than EL but appeared microstructurally "healthier" with lower T1^{*} and greater preservation of fiber-related metrics^{***}, particularly within the lesion core. SL also exhibited lower extracellular diffusion compared to EL, most consistently reflected by reduced NODDI^{*c} and SANDI^{*} extracellular fractions. Interestingly, EL showed higher DMD f_{ear}^{*c} but lower f_{soma}^{*} , suggesting a relative dissociation between extra-axonal restricted space and cellular compartment metrics, with EL showing a tendency to have higher DMD $d_{ear}^{\sim}$ in the core.

However, this difference was largely driven by the high prevalence of PRL- lesions in the SL group. While PRL- SL lesions showed signs of tissue preservation, expanding and shrinking PRL+ lesions showed largely overlapping baseline characteristics, with similar lesion size and T1 values. Only non-significant trends suggested slightly higher fiber fractions[~] in shrinking PRL+ lesions, while expanding PRL+ showed a non-significant tendency towards higher cellularity[~], associated with a trend of higher $R_{soma}^{\sim}$ and decreased DMD $d_{ear}^{\sim r}$, predominantly at the lesion rim.

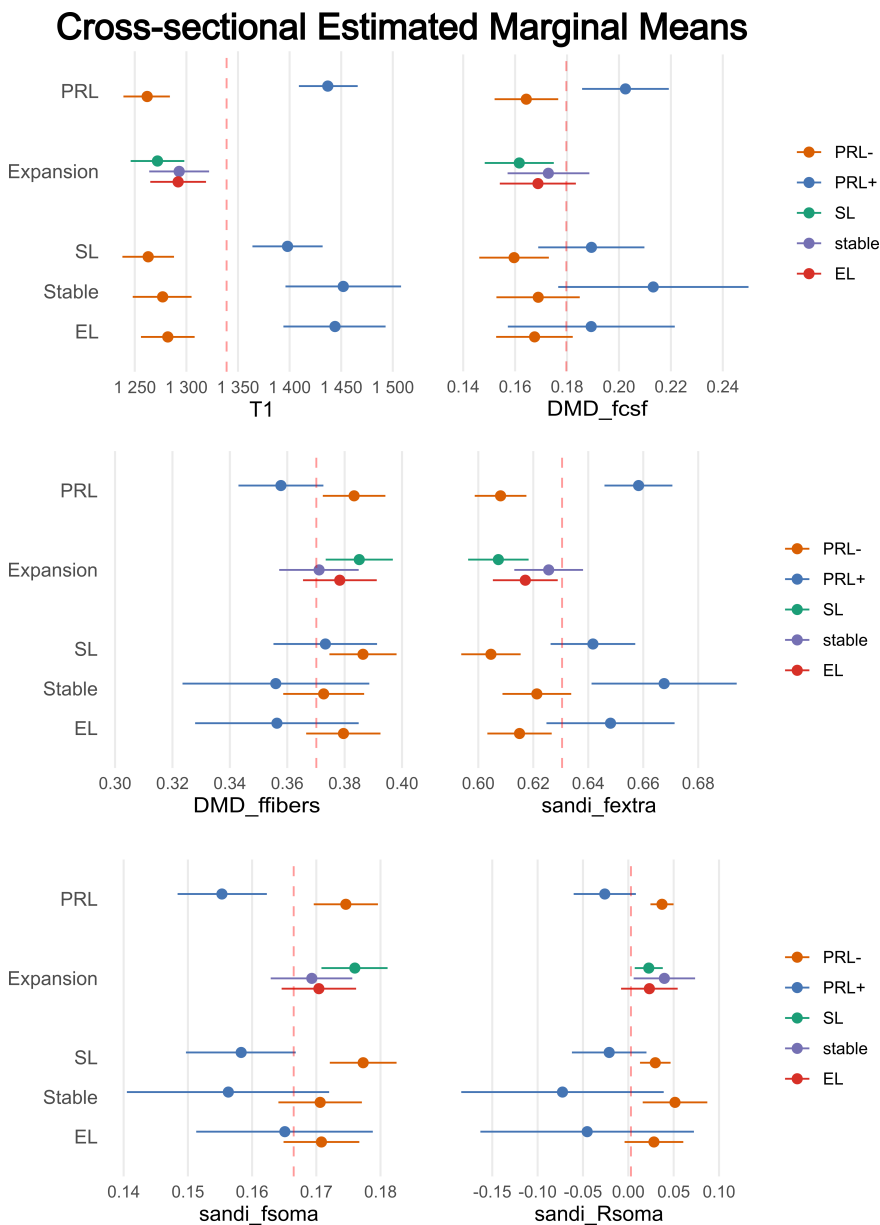


Fig. 6.1 Cross-sectional estimated marginal means at baseline of the different microstructural metrics in different lesion types based on their PRL and expansion status. The first row of the graph describes PRL status alone, the second row expansion status alone, and the third row the interaction of the two. The red dashed line shows the mean value across all lesions. Abbreviations: (non) paramagnetic rim lesion (PRL-/PRL+); shrinking lesion (SL); stable lesion (stable); expanding lesion (EL)

Longitudinal microstructural lesional evolution

The longitudinal slope β estimates of the most explicit metrics are represented in Figure 6.2, while the slope β estimates of the rest of the metrics can be found in Figure D.2 in the Annex D, alongside Table D.2 containing all contrast p -values. Across the cohort, lesions tended to shrink*** over time regardless of PRL status. Despite this overall volume reduction, PRL+ lesions exhibited a distinctly more pathological longitudinal evolution.

Longitudinally, when compared to PRL-, PRL+ lesions showed increasing T1 values***, progressive loss of fiber-related metrics***, and more accumulation of free-water content (DMD f_{csf} *** and NODDI f_{iso} ***) but not in the extra-axonal compartment, indicating ongoing tissue degeneration. PRL+ lesions showed a steep decline in cellularity metrics (f_{ear} **, f_{soma} ***), particularly in the lesion core, suggesting a "burnout" of cellular elements over time. In contrast, PRL- lesions showed relative preservation or even slight increases in these markers. These changes in PRL+ were accompanied by reductions in SANDI R_{soma} *** and greater decreases in extra-axonal restricted diffusivity** compared to PRL-, suggesting progressive cellular loss and extracellular matrix remodeling.

Expansion status alone was a misleading predictor of tissue fate. Indeed, SL generally exhibited signs of tissue repair (decreasing T1***, increasing fibers***, decreasing SANDI f_{extra} ***) compared to EL who showed more signs of increased inflammation (increasing f_{extra} ***). However, Shrinking PRL+ lesions defied this trend: despite a reduction in volume***, they continued to accumulate microstructural damage (increasing T1***, decreasing fibers**, and increasing unrestricted isotropic diffusion***) at a rate significantly faster than shrinking PRL- lesions. Furthermore, shrinking PRL+ had a tendency of steeper decrease in DMD f_{fibers} ~^c and steeper increase in T1~ and DMD f_{csf} ~^c compared to expanding PRL+ (especially in the core), a trend reversed for the SANDI $f_{neurite}$ ~^c metric and for the extra-axonal diffusion compartment~. Shrinking PRL+ also showed a trend towards steeper decrease in cellularity~ and in extra-axonal restricted diffusivity~ compared to expanding PRL+. This would suggest that in PRL+ lesions, volumetric shrinkage may represent tissue collapse driven by tissue destruction rather than resolution of inflammation or remyelination.

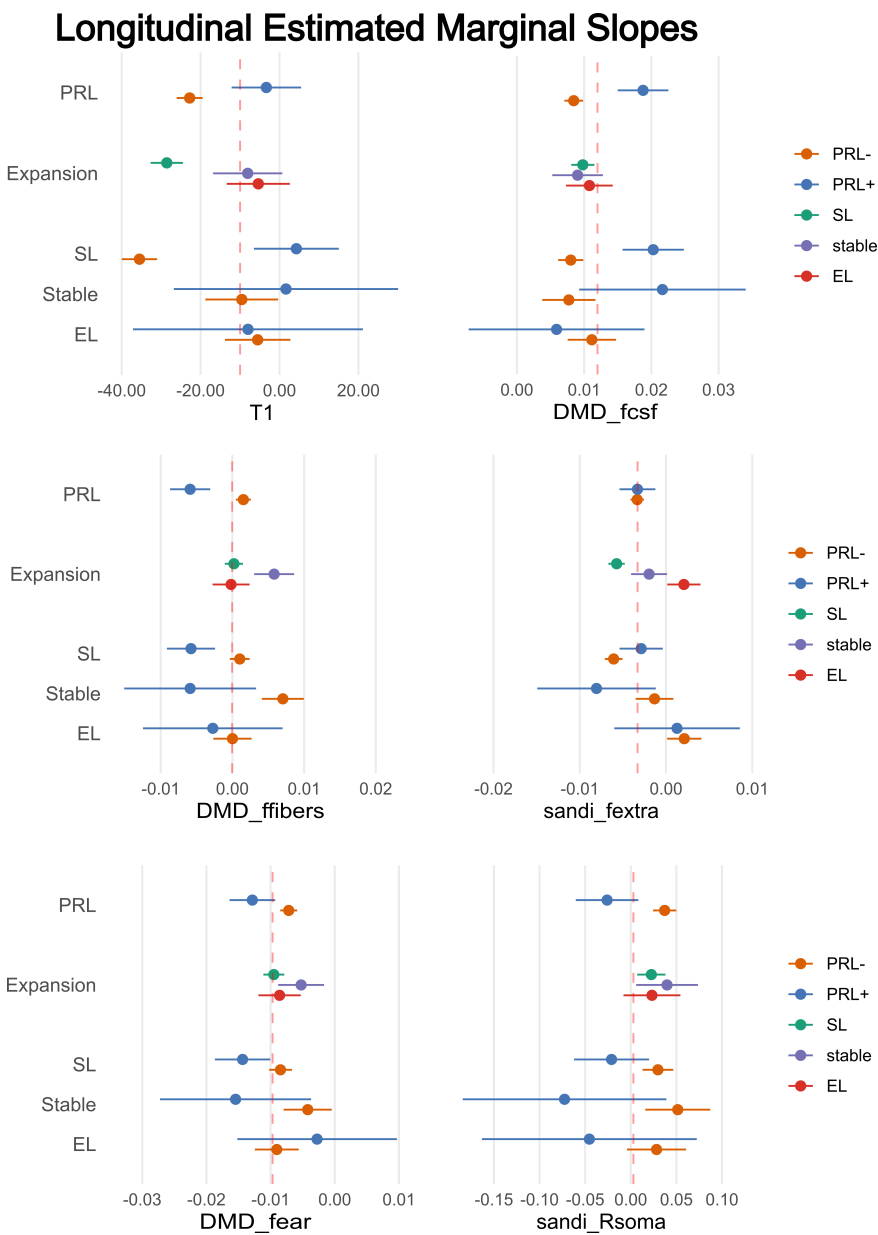


Fig. 6.2 Longitudinal estimated marginal slopes of the different microstructural metrics in different lesion types based on their PRL and expansion status. The first row of the graph describes PRL status alone, the second row expansion status alone, and the third row the interaction of the two. The red dashed line shows the mean value across all lesions. Abbreviations: (non) paramagnetic rim lesion (PRL-/PRL+); shrinking lesion (SL); stable lesion (stable); expanding lesion (EL)

Sensitivity Analysis

Sensitivity analyses were performed using two restricted lesion datasets: (i) a low-confluence dataset, defined as lesions sharing less than 15% of their border with neighboring lesions, and (ii) a longitudinally stable dataset, defined as lesions with at least three available time-points. These analyses were conducted to assess the robustness of the primary findings, with particular emphasis on the consistency of contrast directionality.

Detailed results from both sensitivity analyses are reported in Appendix Tables D.3 and D.4. Overall, both baseline and longitudinal sensitivity analyses revealed a small number of divergent trends compared with the full lesion dataset. Importantly, with one exception, these discrepancies were not statistically significant and are therefore likely attributable to reduced statistical accuracy resulting from the smaller sample sizes in the sensitivity datasets. The only statistically significant discrepancy was observed in the low-confluence dataset, where PRL- lesions exhibited higher baseline NODDI f_{iso} values compared with PRL+ lesions. However, no significant difference in baseline f_{iso} between PRL- and PRL+ lesions was observed in the full lesion dataset, indicating that this finding does not influence the primary results. Furthermore, the low-confluence dataset inherently excludes highly confluent lesions, which tend to represent more advanced pathological states and are disproportionately represented among PRL+ lesions. This selective exclusion likely contributed partly to the observed differences in this sensitivity analysis. Collectively, these findings support the overall robustness of the primary analyses.

6.3.3 Clinical associations

The clinical relevance of lesion subtypes was assessed by examining associations between lesion volumes and measures of disability and neurodegeneration (see Table 6.3). Notably, within the context of this study, all T2 lesions were evaluated for PRL status; therefore, the PRL- subtype serves as a proxy for the overall T2 lesion burden, as reflected by the predominance of PRL- lesions in the contingency table (Table 6.2). Global brain atrophy was broadly associated with total lesion burden, driven largely by the volume of PRL- and shrinking lesions. In contrast, EDSS was associated with both PRL- and PRL+ lesion volumes, including shrinking PRL+ and PRL- lesions, suggesting that disability reflects the combined effects of lesion burden and lesion chronic activity. Finally, MSSS — which accounts for disease duration

and therefore serves an indicator of disease severity — was significantly associated only with the volume of PRL+ lesions and specifically shrinking PRL+ lesions. Interestingly, no associations with expanding lesions were found in our database. This identifies the shrinking PRL+ lesion as a highly eloquent marker of disease severity, independent of total lesion load.

Table 6.3 Clinical association between different lesion types and normalized brain volume, expanded disability status scale (EDSS) and multiple sclerosis severity score (MSSS). The table report estimates and associated p-values of multi-variate linear regression models with the total lesion volume of different lesion phenotype (stratified by paramagnetic rim — PRL — and expansion status — shrinking [SL], stable, expanding [EL]) adjusted for age and sex assigned at birth.

Outcome	Lesion type (Volume)	estimate	p-value
Brain Volume	PRL-	-1.00e-02	p<0.001
Brain Volume	SL PRL-	-3.82e-03	p<0.01
Brain Volume	SL	-3.04e-03	p<0.01
Brain Volume	stable PRL-	-2.26e-03	p<0.05
EDSS	PRL-	3.63e-01	p<0.001
EDSS	SL PRL+	1.66e-01	p<0.01
EDSS	SL	2.24e-01	p<0.01
EDSS	SL PRL-	2.02e-01	p<0.01
EDSS	PRL+	1.15e-01	p<0.05
MSSS	PRL+	2.46e-01	p<0.01
MSSS	SL PRL+	1.99e-01	p<0.5

6.4 Discussion

This study provides a comprehensive *in vivo* microstructural characterization of PRL and their interplay with lesion expansion or shrinkage, all showing a certain degree of chronic activity and tissue remodeling. By leveraging advanced multi-compartment diffusion modeling (DIAMOND, NODDI, SANDI) alongside longitudinal volumetric analysis, we demonstrate that the *chronic active* phenotype is not strictly defined by volumetric expansion and that shrinkage is not always synonymous with tissue repair. Our results reveal three major insights: (1) PRL+ lesions exhibit a severe, distinctive microstructural phenotype at baseline characterized by extensive neuro-axonal loss, increased unrestricted extra-axonal diffusion and hypocellularity, independent of their expansion status; (2) Longitudinal tissue degeneration in PRL+ lesions is decoupled from volumetric evolution,

with *shrinking* PRL+ lesions exhibiting accelerated microstructural decay rather than repair; and (3) The burden of these *shrinking but destructive* PRL+ lesions is a specific, independent predictor of disease severity (MSSS), challenging the assumption that lesion shrinkage necessarily implies tissue regeneration.

The distinct destructive signature of the Paramagnetic Rim lesion

Our baseline analysis confirms that PRL+ lesions represent a particularly nasty lesion phenotype in terms of tissue integrity. While conventional T1 mapping indicated greater tissue destruction, granular diffusion metrics offered a more specific biological narrative. The convergent finding of reduced intra-axonal fiber fractions across multiple models aligns with histopathological evidence of irreversible axonal transection in smoldering lesions.^{21,87} Crucially, we observed a distinct *hypocellular core* phenotype in PRLs associated with an increased free water and extra-axonal diffusion. This likely reflects the *burned-out* center of chronic active lesions, where inflammatory cellularity has retreated to the rim, leaving behind a hypocellular, fluid-filled cavity.²¹ The paradoxical finding of larger apparent soma size and decreased restricted extra-axonal diffusivity may suggest the presence of specific cellular populations, although it should be confirmed in future dedicated MRI-histopathological studies.

The *Shrinking Rim* paradox: Tissue Collapse vs. Repair

A key contribution of this work is the dissociation between volumetric shrinkage and microstructural repair. In the general MS lesion population, shrinkage is typically associated with resolution of edema/inflammation and remyelination, as evidenced by the decreased T1 and increased fiber density we observed in shrinking PRL- lesions. However, shrinking PRL+ lesions defied this rule. Despite reducing in volume, these lesions continued to accumulate severe microstructural damage, including rising T1 values and progressive loss of fiber integrity. This observation challenges the conventional radiological interpretation of lesion shrinkage. In the context of the paramagnetic rim, we propose that shrinkage likely represents the physical collapse of the supporting extra-cellular matrix following severe axonal loss rather than a return to healthy tissue density. This *destructive shrinkage* suggests that once a lesion has established a chronic inflammatory

rim, the cessation of expansion does not necessarily signal the cessation of damage.

On the other side, while lesion expansion is widely recognized as a marker of chronic activity, our results suggest that the paramagnetic rim may be the dominant driver of tissue fate. Indeed, expansion is associated with worse tissue integrity and longitudinal pathological accumulation, however, the presence of a rim effectively erased the microstructural benefits seen in stable or shrinking lesions; a shrinking PRL+ lesion looked microstructurally similar to an expanding one. Furthermore, shrinking PRL+ displayed a potential trend of accumulating more axonal loss, free-water, and hypo-cellularity, while expanding PRL+ showed a tendency to a steeper accumulation of extra-axonal fractions. The latter trends might suggest a different phenotypic microstructural signature evolution characterizing the difference in pathological processes present in expanding and shrinking PRL. While the accumulation of more extra-axonal fractions in expanding lesions might represent ongoing inflammatory cell infiltration — effectively driving lesion expansion —, shrinking PRL+ seems to be characterized by increased axonal loss and accumulation of free-water, potentially reflecting an irreversible tissue collapse.

Clinical relevance of shrinking PRL

While total lesion load (here represented as PRL- volume) correlated with general brain atrophy and EDSS — likely reflecting cumulative disease burden — the MSSS (which adjusts for disease duration) was specifically driven by PRL+ burden, as previously described,⁹⁵ and most notably by shrinking PRL+ lesions. This identifies the *destructive shrinking PRL+* phenotype as a highly eloquent marker of aggressive disease, independent of total lesion load. The lack of association with expanding lesions in our cohort may reflect the relatively short observation window or suggest that expansion is a transient phase of ongoing persistent inflammation and reversible damage, whereas the microstructural destruction captured by shrinking PRL+ might be cumulative and irreversible.

A hypothesized life-cycle of chronic active lesions

Our findings offer valuable insights into the pathological evolution of chronic active lesions (defined as PRL). While some specific microstructural distinctions between shrinking and expanding PRL+ lesions remained trend-level — likely due to the low signal-to-noise ratio (SNR) inherent to high *b*-value diffusion MRI and the complexity of the statistical modeling — these patterns allow us to propose a hypothetical *in vivo* life cycle of the chronic active lesion. We caution that this framework remains speculative and requires validation in future dedicated prospective studies.

Following the resolution of the acute inflammatory phase, a lesion may evolve into a chronic inactive or chronic active phenotype. The determinants of this trajectory are not yet fully understood but would likely involve the severity (intensity and magnitude of the demyelinating process) of the initial blood-brain barrier disruption and the efficacy of debris clearance.¹⁴⁹ For chronic inactive lesions, subsequent evolution is most likely characterized by partial remyelination and gliosis, manifesting radiologically as T2/FLAIR lesion shrinkage associated with tissue repair.

In contrast, we hypothesize that chronic active lesions undergo a distinct biphasic evolution. The initial post-acute phase would likely involve minor shrinkage due to edema resolution. However, we propose that this is followed by a *smoldering expansion* phase, driven by the infiltration of iron-laden macrophages and microglia at the rim, resulting in the centrifugal accumulation of myelin and axonal damage,¹⁵⁰ ultimately driving lesion expansion. Crucially, we propose a subsequent *tissue collapse* phase. Once axonal loss would surpass a critical structural threshold — potentially exacerbated by inflammation-induced cellular senescence¹⁰ — the extra-cellular matrix may mechanically collapse. This phase would manifest as volumetric shrinkage but would represent irreversible tissue loss rather than repair, contributing directly to the accumulation of permanent clinical disability and overall neurodegeneration. Crucially, this model suggests that the optimal therapeutic window lies within the *smoldering expansion* phase, where intervention could potentially halt the accumulation of damage before the tissue reaches the point of mechanical collapse, where a certain degree of repair could still be achieved. Significant open questions remain, including whether this cycle of expansion and collapse is a singular or recurrent phenomenon, and what biological determinants dictate why certain expanding lesions manifest the characteristic paramagnetic rim while others do not.

Limitations

Our first study limitation arise from the use of advanced multi-compartments like SANDI and DIAMOND. While these advanced diffusion models offer "histology-like" specificity, they remain mathematical approximations, reliant on assumptions about tissue compartments and optimization process. Hardware constraints, including limited gradient strength, low SNR at high *b*-value, and long acquisition times, inevitably introduce variance in microstructural estimations. While state-of-the-art preprocessing algorithms mitigated these effects, this likely reduced statistical power, particularly for longitudinal interactions, compared to more robust metrics like T1 mapping. Nevertheless, the consistency of the observed trends across multiple diffusion models supports the biological coherence of our phenotypes.

Secondly, the classification of "shrinking" vs. "expanding" relies on linear regression of automated lesion masks and empirical thresholding, which can be sensitive to segmentation errors or partial volume effects. While our sensitivity analysis in low-confluence lesions and lesions with 3 observation time-points confirmed the trends of the main findings, a more refined description of volume dynamics is needed for the definition of a robust biomarker. Finally, histological validation using ultra-high field *ex vivo* MRI is required to confirm the cellular origin of the specific signatures observed here, particularly the increased somatic size and restricted extra-axonal diffusivity at the lesion rim.

Future perspectives

The MS field should recognize the heterogeneity underlying lesion shrinkage, which can represent either beneficial tissue repair or detrimental tissue collapse depending on the lesion's microstructural activity. Future research should leverage multi-modal MRI to refine these phenotypes. Specifically, studies utilizing high-performance gradient hardware (at high- or ultra-high field MRI) are needed to acquire high-resolution diffusion sequences with sufficient longitudinal follow-up to confirm the proposed life cycle of chronic active lesions. Furthermore, the development of diffusion models specifically tailored to MS pathology — accounting for demyelination, inflammatory interplay, and gliosis — should be prioritized over standard models designed for healthy white matter. Validation of these hypotheses will ultimately require *post mortem* MRI-histology correlations

or potentially the observation of neuroinflammatory dynamics in bioengineered organoids.

Conclusion

In conclusion, this study demonstrates that PRLs are characterized by a relentlessly destructive microstructural trajectory that persists even when the lesion volume decreases. We identified a distinct *destructive shrinking* phenotype in PRL+ lesions, characterized by the decoupling of volumetric reduction from microstructural repair. This finding challenges the current reliance on volumetric endpoints in clinical trials, suggesting that lesion shrinkage is not a universal proxy for therapeutic efficacy. Instead, the tissue collapse of chronic active lesions may display ongoing and irreversible neurodegeneration. These results advocate for the integration of PRL status and advanced diffusion metrics as essential biomarkers in MS precision medicine, enabling the identification of patients at risk of progressive disability who may benefit from therapies targeting compartmentalized inflammation.

7

Reflexive synthesis and discussion of my PhD journey

7.1 Purpose and scope of this reflective discussion

At the end of this four-and-a-half-year doctoral journey, this chapter proposes a reflexive synthesis of the work carried out throughout the thesis. Rather than reiterating the individual conclusions already presented at the end of each chapter, the aim here is to step back and reflect on the intellectual, methodological, and conceptual trajectory that structured this research.

This discussion therefore adopts a deliberately different tone from the preceding chapters. This final synthesis seeks to contextualize the work within the broader process of scientific inquiry, acknowledging that a PhD is not only a cumulative scientific endeavor, but also a formative research journey. The reflections presented here are grounded in the concrete analyses developed throughout the thesis and are intended to clarify the motivations, assumptions, and methodological choices that shaped the work.

Two overarching themes emerged as particularly structuring throughout this doctoral research: (i) the central role of biomedical engineering in neuroimaging research, and (ii) the importance of creativity as an essential component of meaningful scientific investigation. These two themes provide the conceptual framework for the present synthesis.

7.2 Biomedical engineering as a driver of neuroimaging research

One of the most striking lessons of this PhD was the inherently multidisciplinary nature of neuroimaging research. This aspect was extensively developed in the *Theoretical Background* chapter (Chapter 2), which highlighted the fact that MRI spans a wide spectrum of domains, ranging from physics and engineering to biology and clinical neuroscience. The production of clinically interpretable images relies on sophisticated engineering solutions grounded in fundamental physical principles, while the resulting contrasts are ultimately shaped by microscopic molecular and cellular interactions.

This complexity makes MRI one of the most powerful but also one of the most challenging medical imaging modalities to interpret. Approaching MRI data as if they were natural images, without an understanding of the underlying physical and biological mechanisms, severely limits the ability

to extract meaningful information or to develop innovative neuroimaging methods. In this context, biomedical engineering plays a critical role by providing the conceptual and technical tools required to bridge these domains. A strong biomedical engineering background enables the necessary flexibility to navigate between technical implementation and biological interpretation, a prerequisite for robust neuroimaging research.

Despite the frequent emphasis on interdisciplinarity in contemporary research discourse, the central role of biomedical engineering in neuroimaging is still often underestimated. Structural limitations, such as the lack of genuinely interdisciplinary evaluation panels for funding applications (as observed in the Belgian context), illustrate the practical challenges of conducting research that genuinely sits at the interface between engineering and clinical sciences. This structural context directly shaped the trajectory of this PhD and reinforced the necessity of explicitly addressing the engineering–clinical gap throughout the thesis.

Lowering technical barriers: from algorithms to tools

The development of the BMAT software (Chapter 3) emerged directly from this interdisciplinary perspective. A simple but critical observation motivated this work: most neuroimaging algorithms rely on command-line interfaces and require a level of computational expertise that can constitute a significant barrier for clinical researchers. This limitation restricts the dissemination and practical use of advanced neuroimaging methodologies.

To address this issue, a graphical user interface was developed as a wrapper around commonly used neuroimaging tools, with the aim of improving accessibility without compromising methodological rigor. Although initially conceived as an internal laboratory tool, BMAT progressively evolved into a more mature and transferable software solution. Its development ultimately led to open-source dissemination and publication, enabling its adoption by other research groups.

Beyond its practical utility, BMAT provided a foundation for the transparent implementation and sharing of subsequent processing pipelines developed during this PhD. More broadly, this work illustrates the essential role of biomedical engineering in translating methodological advances into tools that can be effectively used by the clinical and scientific community.

Methodological transparency and interpretability in diffusion MRI and lesion biomarkers

The importance of bridging engineering and clinical perspectives was particularly evident in the context of diffusion MRI. The development of meaningful diffusion models must be guided by biologically plausible assumptions, while their interpretation requires a clear understanding of their theoretical hypotheses and optimization. This issue formed the core of the work presented in Chapter 4, which compared multiple diffusion models in the specific context of multiple sclerosis.

This study was motivated by the observation that many diffusion MRI studies in MS relied on a single model, making cross-study comparisons difficult and potentially limiting the robustness of the conclusions. By systematically comparing different types of diffusion models, this work highlighted the respective strengths and weaknesses of each approach and identified multi-compartment models grounded in strong biological assumptions, especially those modeling the extra-axonal compartment, as particularly informative for disentangling MS-related microstructural damage. A final contribution of this work was the creative integration of complementary diffusion metrics to provide a more comprehensive representation of pathological differences between active, chronic active and inactive lesions.

A similar concern for methodological transparency motivated the critical examination of the Slowly Expanding Lesions (SELs) algorithm presented in Chapter 5. While the conceptual hypothesis underlying SELs — namely that lesion expansion reflects a form of chronic inflammatory activity — is biologically compelling, discussions with clinical collaborators revealed limited understanding of the algorithmic implementation itself. A detailed analysis showed that certain methodological limitations constrained the extent to which the algorithm could faithfully represent the underlying biological process.

This work does not diminish the importance of SELs as a significant advance for the MS field. On the contrary, SELs contributed to shifting attention toward the longitudinal dynamics of MS lesions, or what I conceptualize as "MRI-evolving lesions", a previously underexplored aspect of the disease. However, this analysis underscores the necessity of transparent algorithmic design and interpretation, emphasizing that clinically meaningful biomarkers require a close alignment between biological hypotheses and engineering implementation.

7.3 The critical role of Creativity in scientific inquiry

A second, equally central theme of this PhD was the role of creativity in scientific research. Although science is often portrayed as a purely rational and objective endeavor, creativity plays a fundamental role in hypothesis generation, methodological design, and the interpretation of unexpected results. Technical expertise alone is insufficient if it is not coupled with the ability to formulate novel questions and to adapt when results challenge initial assumptions.

A recurring methodological lesson throughout this PhD can be summarized as follows: initial hypotheses should not constrain interpretation, but rather be revised in light of empirical findings. This principle became particularly salient during an exploratory study comparing microstructural damage in expanding versus shrinking lesions (Annex E). Contrary to expectations, shrinking lesions exhibited greater baseline damage than expanding lesions. At first glance, this observation may appear inconsistent with the results presented in Chapter 6. However, this discrepancy can be explained by key methodological differences. In the exploratory study, lesion expansion was characterized using a Jacobian-based approach within manually segmented masks, inherently introducing selection bias and limiting the analysis to a restricted subset of lesions. In contrast, Chapter 6 relies on volumetric trajectories and an unbiased lesion-splitting framework, enabling the investigation of the full lesion spectrum. After extensive methodological verification, this result prompted a re-examination of the literature, ultimately revealing prior work that supported this observation.¹³⁷

Rather than being dismissed as anomalous, this finding provided the conceptual groundwork for the final contribution of this thesis: the longitudinal characterization of the interplay between chronic inflammatory activity and lesion volume dynamics, presented in Chapter 6. By adopting a data-driven approach and minimizing selection bias, this work aimed to develop a more comprehensive understanding of "MRI-evolving lesions" in MS. This approach enabled the characterization of distinct microstructural patterns associated with lesion expansion and shrinkage in chronic active lesions, and led to the identification of *shrinking PRLs* as a particularly eloquent biomarker of irreversible tissue collapse, clinical disability, and overall disease severity. Finally, although the proposed conceptual model of CALs life-cycle dynamics remains hypothetical and should be interpreted

with caution, its value lies in its ability to provide a coherent framework for interpreting these observations and to stimulate further investigation and refinement.

7.4 Concluding words

This thesis contributes to the field of MS neuroimaging through three main avenues:

- The development of user-friendly neuroimaging software that lowers technical barriers for clinical researchers and facilitates the transparent sharing of processing pipelines within a standardized and open framework. This includes the implementation and dissemination of two main processing pipelines: SEL detection and MS lesion splitting algorithms, enabling reproducible and large-scale lesion analyses.
- The construction of explanatory bridges between engineering and clinical domains, through comparative diffusion MRI modeling and critical methodological analysis of lesion biomarkers.
- The advancement of our understanding of longitudinal lesion dynamics, particularly the relationship between apparent lesion shrinkage, tissue collapse, chronic inflammation, and clinical disability.

Looking forward, there are reasons for optimism regarding the continued integration of advanced MRI-based biomarkers into both clinical practice and research in multiple sclerosis. Recent work from our laboratory highlights the increasing role of multi-contrast MRI for earlier and more specific disease diagnosis.^{4,9} The ongoing longitudinal follow-up of study participants using an advanced multimodal MRI protocol, combined with strong international collaborations in neuropathology, ultra-high-field MRI, and biological biomarker research, provides a promising framework for validating and extending the hypotheses proposed in this thesis. Together, these efforts may contribute to a more comprehensive understanding of MS pathology and, ultimately, to improved care for individuals affected by the disease.

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Supplementary materials for
BMAT: an open-source
BIDS Managing and Analysis
Tool

This section presents additional information about the utilization of the software. Firstly, the renaming strategy for the MRI files is presented in detail. Secondly, the process of manually adding new pipelines to the software is explained and finally, the results of the utilization experiment of the software by 5 independent raters are shown.

A.1 Renaming strategy

An example of the sequences.csv file is presented in Table 1. During the renaming process, the software recognizes keywords in the file name coming from the conversion from DICOM to NIfTI (conversion realized using dcm2niix), and maps them to the corresponding BIDS name. The renaming scheme works in 3 steps:

1. The software identifies the modality of a sequence by trying one by one all the possible keyword(s) (one or several separated by '+') in the modality column of the sequences.csv file and maps these keywords to the corresponding BIDS modality in the 'modality_bids' column.
2. Once the modality has been found, the BMAT naming scheme continues by searching other possible keywords eventually present in the file name (e.g. the field column 'acq' in the sequence.csv file) and uses the corresponding BIDS label (the 'acq_bids' column) for the renaming. The user can add other columns to the sequence.csv file corresponding to other field of the BIDS specification. This is done by adding one column, named with the BIDS field, containing the specific keyword in the file name, and a second column with the '_bids' extension containing the corresponding label to use when renaming the file. An empty cell is not taken into account while a '_' cell is always considered.
3. Finally, according to the BIDS specification, the MRI_type column contains the name of the subfolder in which the BIDS renamed file needs to be stored. The "IGNORED" keyword is used to discard the image.

Table A.1 Mapping file to rename the NIfTI files according to the BIDS standard. The renaming scheme is based on the recognition of keywords and mapping to their corresponding BIDS modality/field label (column with ‘_bids’ extension). The MRI_type provides the folder where the MRI sequence is stored in the BIDS dataset.

modality	modality_bids	acq	acq_bids	run	run_bids	MRI_type
3Plane_Loc	3Plane_Loc	i0001	ax			IGNORED
3Plane_Loc	3Plane_Loc	i0013	sag			IGNORED
3Plane_Loc	3Plane_Loc	i0025	cor			IGNORED
Ax_T2_Propeller	T2w	Ax	ax			anat
Opt_DTI+Eq1	sbref					dwi
Opt_DTI	dwi	_	multishell	_4_	4	dwi
Opt_DTI	dwi	_	multishell	_5_	5	dwi

The software always search for a given keywords by screening from the top to the bottom of the file and stops as soon as it gets a match. Importantly, when completing the sequence.csv file from top to bottom, the user should always remember to first list files containing multiple keywords separated by “+” and then files containing only keyword (e.g. first “Opt_DTI+Eq1” and then “Opt_DTI” in Table A.1). In general, in the overall BMAT renaming scheme, it is up to the user to follow the BIDS specification. The software only proposes a BIDS validation tool (BIDS validator) to detect if the dataset is BIDS compliant or not.

A.2 Local Pipelines

The conception of the “LocalPipelines” drop-down menu allows the implementation of new local pipelines to run specific image processing tasks. To add a new local pipeline to the software, the user must add its source code in python containing a dedicated graphical interface written in PyQt5 and the computation code, as well as an associated JSON file containing meta-information about the pipeline, in the “LocalPipelines” folder of the software source code. The computation part of the pipeline can use docker containers or can be implemented locally, allowing adaptability of the software to a wide range of Neuroimaging applications.

The JSON file must be written in a dictionary-like structure and should contain the following information:

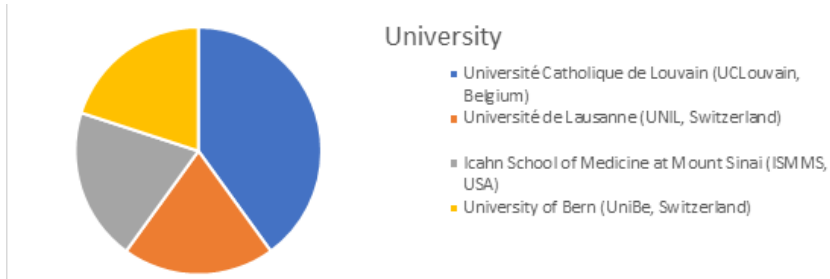
- “name”: the name of the Pipeline that will be displayed in the ‘Pipelines’ drop-down menu in the software.

- “source_code”: the name of the python file containing the source code for the graphical interface of the pipeline. This file needs to be implemented using PyQt5 python module and must contain a launch function, taking the Main Window of the software as only argument, and that launches the graphical interface of the Pipeline. Users can take the Template pipeline as an example.
- “import_name”: the name of the corresponding module that needs to be imported in python. It corresponds to the name of the python source code file without the ‘.py’ extension.
- “attr”: the name of the attribute of the imported ‘Pipelines’ python module corresponding to the python source code of the pipeline. Again, this corresponds to the name of the python source code file without its ‘.py’ extension.
- “add_info” (optional): a dictionary structure that can easily be modified by the user to provide the pipeline with additional information for its successful running. For example, it can contain the name of the sequence that the pipeline must use, this allowing the use of the same pipeline on different datasets with different sequence names.

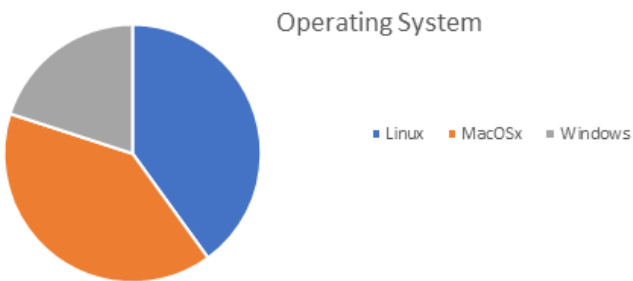
A.3 BMAT utilization survey

This section presents the raw results of a survey on the installation and use of BMAT that was taken by 5 independent researchers from 4 different universities. Researchers were asked to rate their experience using a scale from 0 (bad) to 5 (excellent) for questions 3-9) of the survey, see below.

1. From which University are you coming from?

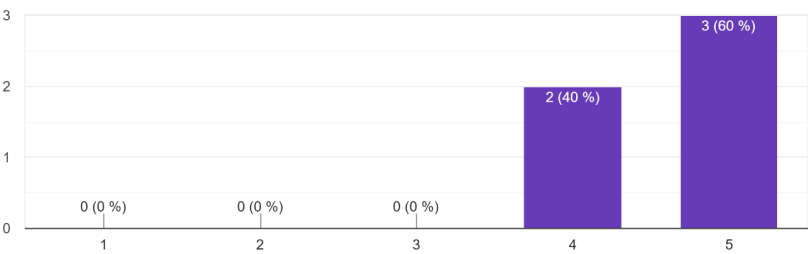


2. On which Operating System did you use BMAT?



3. Rate your experience of installing the dependencies of BMAT

Rate your experience of installing the dependencies of BMAT (python, docker, git, itk-snap)
5 réponses

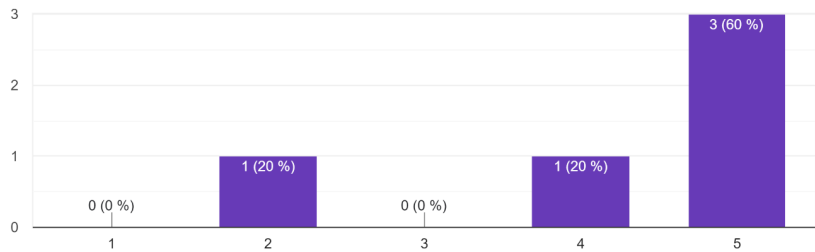


Comments

- Got an error with the following command that I solved with a google search. `docker pull colinvdb/bmat-ext:0.0.1`
Erreur:
Got permission denied while trying to connect to the Docker daemon socket at unix:///var/run/docker.sock:Post"http://%2Fvar%2Frun%2Fdocker.sock/v1.24/images/create?fromImage=colinvdb%2Fbmat-ext&tag=0.0.1": dial unix /var/run/docker.sock: connect: permission denied
- I think that the tutorial of docker was a bit hard to understand, but I also worked with it on another computer and had negative a-priori about it so it might not help
- I had them already, the installation is not difficult at all.

4. Rate your experience of installing BMAT

Rate your experience of installing BMAT; - Download the GitHub repository - Download the python required packages - First launch BMAT software
5 réponses

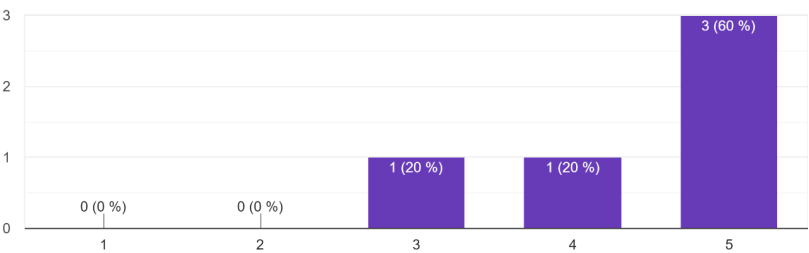


Comments

- I had to look to the video to know how to launch the program but once I checked it it was ok
- I was using MacBook Air Apple chip M1. I got several errors when installing PyQt5 and PyQtWebEngine requirements, probably because of the M1 chip. What I did in the end was to install those requirements through brew (directly in the device, not in a virtual environment) and then copy the PyQt5 folder from the brew path (/opt/homebrew/Cellar/pyqt@5/5.15.7_1/lib/python3.9/site-packages/PyQt5) into the virtual environment site packages path (/Users/jaimebarranco/miniconda3/lib/python3.9/site-packages/PyQt5)

5. Rate your overall experience of installing BMAT

Rate your overall experience of installing BMAT
5 réponses



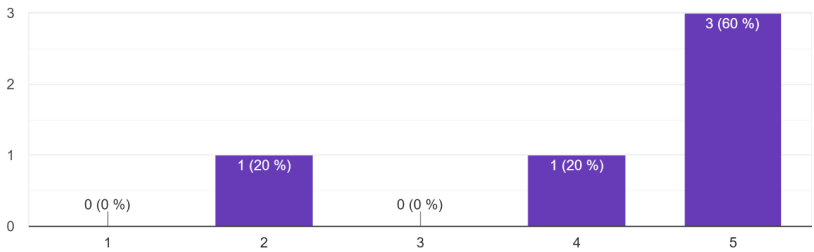
Comments

- The experience is not the ideal WHEN INSTALLING ON MAC, SPECIALLY WITH THE M1 VERSION. I also installed BMAT on Linux and everything went well when installing the requirements. Also, I think is good to have the above comment if anyone wants to install it on M1 mac :)
- Lots of things to do, but very well explained in the documentation.

6. Rate your experience of DICOM to BIDS conversion using BMAT

Rate your experience of the DICOM to BIDS conversion in BMAT (with the completion of sequences.csv file)

5 réponses



Comments

- Very convinient tool
- I think my experience is not that good because of the not so well labeled dataset that I have, it comes from ophthalmologists from Germany and I aslo tried to put it in BIDS format and it was a headache, using dcm2bids and dcm2niix and in the end it didn't pass the bids validator.

First, I think it's important to know how the dataset I'm using is organized. There are 35 subjects, each one contains 3 different folders (1 T1-w DICOM images and 2 T2-w DICOM images) as well as an itknap workspace. Inside the T1-w folder there is one .nii.gz file which contains the labels of that T1-w images.

| - 0000815176

| | - I_Kopf_t1_mpr_tra_iso_p2_FIL (176 .dcm + 1 .nii.gz)

| | - I_Kopf_t2_spc_irprep_tra_dark-fluid_p2_FIL (48 .dcm)

| | - I_Kopf_t2_tse_sag_FIL (11 .dcm)

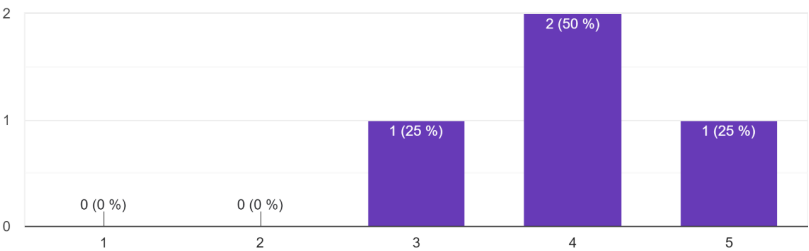
My final intention is only to convert the files into the T1-w folder from each subject.

Having said that, I have different results depending on the input I fed to the BMAT program. If I feed the whole folder (both T1-w and T2-w folders), I have as output /sub-001/ses-01/unrecognized_sequences/ and inside all the images (from t1 and t2) converted to .nii.gz along with their .json files. If I feed the whole folder, but removing this time the T2-w images, the itksnap workspace, and the .nii.gz inside the T1-w folder, I get as output /sub-001/ses-01/unrecognized_sequences/ with the .nii.gz and their .json and also sub-002/ses-01/ empty. If I feed the T1-w folders one by one, I get correctly the different subjects. In any case, I didn't pass the BIDS validator.

- Very intuitive and useful way to use of the program !

7. Rate your experience of using pipelines with BMAT

Rate your experience of using Pipelines with BMAT
4 réponses

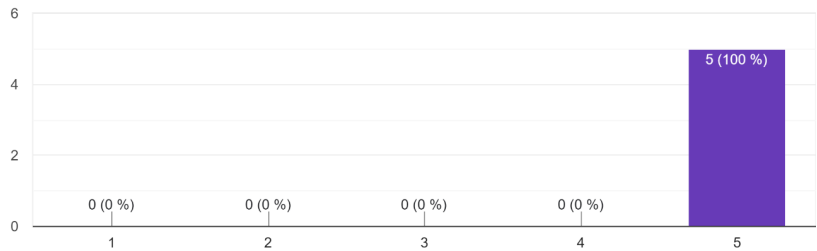


Comments

- I haven't used any pipeline contained in BMAT, so I voted neutral
- Allows a really good management of the files and to already start working on them in a single program

8. Rate the overall Graphical User Interface

Rate the overall Graphical User Interface
5 réponses

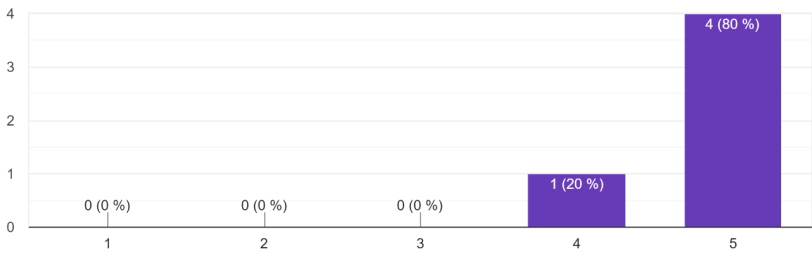


Comments

- I was lazy to read the user manual, and it was clear enough without it
- sometimes the app crashed unexpectedly and also I tried to change subject name, sequence and session but it didn't seem to change anything in participants.tsv nor the name of the corresponding folders nor the .nii.gz or .json filenames. Anyway, the GUI is easy to use and easy to understand what to do.
- Very good !

9. Rate your overall experience of using BMAT

Rate your overall experience of using BMAT
5 réponses



Comments

- The overall experience was good despite the dataset that I'm using which I suspect is not correctly annotated.
- It will be very useful for studies involving lots of data / subjects

10. Would you recommend BMAT to fellow researchers? Why?

- Yes, because it saves a lot of time
- Yes, especially because it is user-friendly and quite easy to learn to work with.
- Yes, I know many people having always trouble when transforming a DICOM set into BIDS format, trying to do it through specific codes for each dataset using dcm2bids and dcm2niix but not always having good results (as my case). So I think having a program with Graphical User Interface would be easier to use and would encourage people to use it.
- Yes, it facilitates the whole organization of several datasets, requiring little time and experience
- Yes, very useful and intuitive use, centralization of all data, possibility of working with the data in the same program and therefore keeping the same structure

11. Have you ever used another BIDS managing software? Which one? Would you say that BMAT is worse, equivalent or better than other BIDS managing software?

- Yes, BMAT is equivalent to “dcm2bids” and “dcm2niix”

B

Supplementary materials for
Comparative overview of
multi-shell diffusion MRI
models to characterize the
microstructure of multiple
sclerosis lesions and
periplaques

B | Comparison of multi-shell diffusion MRI models
supplementary materials

Table B.1 contains a detailed microstructural analysis of the difference between paramagnetic and non-paramagnetic rim lesions (PRL+, PRL-) in relapsing-remitting (RRMS) vs progressive (PMS) multiple sclerosis phenotypes. Figure B.1 contains additional graphs of the evolution of additional diffusion parameters coming from DTI, NODDI and DIAMOND models across the different regions of interest (ROI) investigated in this study. A detailed summary of all statistical analyses performed is displayed in Table B.2. Table B.3 displays a supplementary ROC analysis comparing NODDI's parameters in the different ROI investigated in this study. Figure B.2 shows an axial view of the free-water compartment of NODDI (fiso), DIAMOND (fscfDMD) and MF (fscfMF), suggesting that the numerical description from MF is more suited to describe the free-water compartment in vivo.

Table B.1 Mapping file to rename the NIfTI files according to the BIDS standard. The renaming scheme is based on the recognition of keywords and mapping to their corresponding BIDS modality/field label (column with '_bids' extension). The MRI_type provides the folder where the MRI sequence is stored in the BIDS dataset.

	RRMS	PMS	Statistical analysis
Participants, n	56	27	n/a
PRL+, n	239	78	n/a
PRL-, n	157	75	n/a
PRL+, volume [mm3]	163.08±179.73	292.64±377.51	p=0.00129
PRL-, volume [mm3]	72.89±66.22	110.26±142.37	1
PRL+, T1	1615.96±280.23	1743.73±224.23	p=0.000108
PRL-, T1	1321.15±190.36	1320.52±206.62	p=0.64844
PRL+, AD_DTI	0.001069±0.000200	0.001121±0.000223	p=0.0726842
PRL-, AD_DTI	0.000955±0.000173	0.000965±0.00021	p=0.81155775
PRL+, FA_DTI	0.3715±0.1213	0.3613±0.1053	p=0.62705820
PRL-, FA_DTI	0.432±0.1244	0.4531±0.1271	p=0.2777337
PRL+, MD_DTI	0.000759±0.000159	0.000793±0.000133	p=0.07133407
PRL-, MD_DTI	0.000637±0.000119	0.000635±0.000140	p=0.34450464
PRL+, RD_DTI	0.000604±0.000166	0.000629±0.000124	p=0.22735145
PRL-, RD_DTI	0.000479±0.000126	0.000469±0.000140	p=0.39699097
PRL+, AD_DMD	0.001871±0.000279	0.001888±0.000323	p=0.49850188
PRL-, AD_DMD	0.00178±0.000276	0.001746±0.000282	p=0.42677462
PRL+, FA_DMD	0.5597±0.1287	0.5416±0.1235	p=0.49032250
PRL-, FA_DMD	0.604±0.1402	0.6447±0.1279	p=0.07649490
PRL+, MD_DMD	0.00109±0.000169	0.001109±0.000134	p=0.56743515
PRL-, MD_DMD	0.000994±0.000184	0.000927±0.000162	p=0.00548044
PRL+, RD_DMD	0.000699±0.000196	0.000719±0.000149	p=0.72396115
PRL-, RD_DMD	0.000601±0.000224	0.000517±0.000188	p=0.00851461
PRL+, fcsf_DMD	0.2914±0.194	0.3278±0.18	p=0.03864478
PRL-, fcsf_DMD	0.2479±0.1159	0.30964±0.1299	p=0.00013237
PRL+, ecvf	0.5999±0.1115	0.5923±0.1098	p=0.52433077
PRL-, ecvf	0.515±0.12	0.4726±0.0986	p=0.00226256
PRL+, icvf	0.2671±0.0964	0.245±0.0669	p=0.16215540
PRL-, icvf	0.3240±0.0951	0.3344±0.0984	p=0.26140813
PRL+, fiso	0.1330±0.1094	0.1627±0.1217	p=0.04709107
PRL-, fiso	0.161±0.103	0.193±0.0897	p=0.00293991
PRL+, odi	0.233±0.0801	0.2356±0.0968	p=0.95065047

PRL-, odi	0.2372±0.0771	0.2325±0.0854	p=0.76808243
PRL+, fcsf_MF	0.2583±0.1782	0.2582±0.1414	p=0.32589598
PRL-, fcsf_MF	0.1804±0.0829	0.191±0.0975	p=0.59093515
PRL+, fvf	0.1477±0.0721	0.1376±0.0478	p=0.50323570
PRL-, fvf	0.197±0.0714	0.2143±0.0757	p=0.07860340

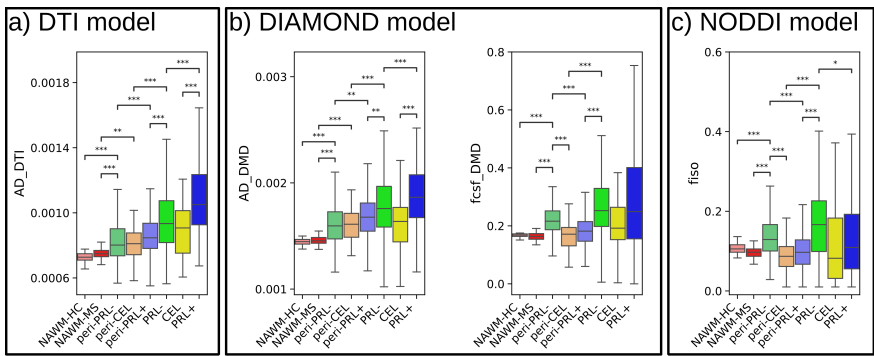


Fig. B.1 Evolution of microstructure into different region of interest (ROI) according to axial diffusivity (AD) from DTI (a) and DIAMOND (b), and to the free-water compartment from DIAMOND's fcsfDMD (b) and NODDI's fiso (c). On each graph, the different ROI are; NAWM-HC (desaturated red), NAWM-MS (red), peri-PRL- (desaturated green), peri-CEL (desaturated orange), peri-PRL+ (desaturated blue), PRL- (green), CEL (yellow), PRL+ (blue). The ROI are ordered by order of microstructural damage according to histopathological analysis. Statistical differences are only noted between ROI with a range of 2, with * ($p < 0.05$), ** ($p < 0.01$), *** ($p < 0.001$).

Table B.2 Microstructural differences between paramagnetic and non-paramagnetic rim lesions (PRL+, PRL-) between relapsing-remitting (RRMS) vs progressive (PMIS) multiple sclerosis phenotypes. Mann-Whitney U test was used for statistical analysis and significant results are highlighted in bold.

ROI1	ROI2	T1	AD DTI	FA DTI	MD DTI	RD DTI	AD DMD	FA DMD	MD DMD	RD DMD	fcst DMD	ecst	icst	fsso	odt	fcst MF	fsf
NAWM-HC	NAWM-MS	n/a	0.213	0.281	0.044	0.033	0.89	0.463	0.025	0.059	0.977	0.009	0.063	0.214	0.863	0.993	0.998
NAWM-HC	post-PRL-	n/a	.001	0.996	.001	.001	.001	0.778	.001	.001	.001	.001	.001	.001	.001	.001	0.997
NAWM-HC	post-PRL+	n/a	.001	0.993	.001	.001	.001	0.961	.001	.001	0.992	.001	.001	0.622	0.415	0.068	0.01
NAWM-HC	PRL-	n/a	.001	0.026	.001	.001	.001	0.002	.001	.001	0.004	.001	.001	1	.001	.001	.001
NAWM-HC	PRL+	n/a	.001	.001	.001	.001	0.012	.001	.001	.001	0.28	.001	.001	0.992	0.444	.001	.001
NAWM-HC	CEL	n/a	.001	.001	.001	.001	.001	.001	.001	.001	.001	.001	.001	.001	.001	.001	.001
NAWM-MS	post-PRL-	0.949	.001	0.303	.001	0.004	.001	0.059	.001	0.025	.001	.001	.001	.001	.001	.001	1
NAWM-MS	post-PRL+	0.297	0.005	0.291	.001	0.003	.001	0.291	.001	0.006	1	.001	.001	0.999	0.565	0.105	0.009
NAWM-MS	PRL-	.001	.001	0.772	.001	.001	.001	.001	.001	.001	.001	.001	.001	0.16	.001	.001	.001
NAWM-MS	PRL+	.001	.001	.001	.001	.001	0.022	.001	.001	.001	0.218	.001	.001	0.574	0.348	.001	.001
NAWM-MS	CEL	.001	.001	.001	.001	.001	.001	.001	.001	.001	.001	.001	.001	0.823	.001	0.666	0.999
NAWM-MS	post-CEL	0.508	1	0.288	0.594	0.235	1	0.158	0.159	0.091	.001	.001	.001	.001	0.666	0.999	0.015
NAWM-MS	post-PRL-	.001	.001	0.034	.001	.001	.001	.001	.001	.001	.001	.001	.001	.001	1	0.714	.001
NAWM-MS	post-PRL+	.001	.001	.001	.001	.001	.001	.001	.001	.001	1	.001	.001	.001	0.668	.001	.001
NAWM-MS	CEL	.001	.001	.001	.001	.001	.001	.001	.001	.001	1	.001	.001	0.998	.001	0.001	.001
NAWM-MS	post-CEL	.001	.001	.001	.001	.001	.001	.001	.001	.001	.001	.001	.001	0.998	0.363	.001	.001
NAWM-MS	post-PRL+	.001	.001	0.986	0.228	0.986	0.244	0.987	0.992	1	0.15	1	0.848	0.54	0.63	1	0.993
NAWM-MS	PRL-	0.733	0.089	0.858	.001	.001	.001	.001	.001	0.182	.001	0.499	.001	.001	0.171	0.006	0.046
NAWM-MS	post-PRL-	.001	0.222	.001	.001	.001	1	.001	0.005	.001	0.207	.001	.001	0.889	0.079	0.003	.001
NAWM-MS	post-PRL+	.001	.001	.001	.001	.001	.001	.001	.001	.001	.001	.001	.001	.001	0.107	.001	.001
NAWM-MS	CEL	.001	.001	.001	.001	.001	.001	.001	.001	.001	.001	.001	.001	.001	0.674	.001	.001
NAWM-MS	post-CEL	.001	.001	.001	.001	.001	.001	.001	.001	.001	.001	.001	.001	.001	0.995	.001	.001
NAWM-MS	post-PRL-	.001	0.979	.001	.001	.001	0.77	.001	0.005	.001	0.758	.001	.001	.001	0.365	.001	.001
NAWM-MS	post-PRL+	.001	.001	.001	.001	.001	.001	.001	.001	0.027	.001	.001	.001	.001	0.365	.001	.001
NAWM-MS	CEL	0.032	0.326	.001	0.913	0.073	0.058	.001	0.93	0.027	0.819	0.014	0.998	0.222	0.365	0.076	0.004
NAWM-MS	post-CEL	.001	.001	.001	.001	.001	.001	.001	.001	.001	0.243	.001	.001	0.177	.001	.001	.001
NAWM-MS	PRL-	0.015	.001	0.262	0.006	0.455	.001	0.219	0.259	1	0.179	0.315	0.048	0.988	.001	0.946	1
NAWM-MS	PRL+	.001	.001	.001	.001	.001	.001	.001	.001	.001	.001	.001	.001	.001	.001	.001	.001

Table B.3 Receiver operating characteristic (ROC) curve comparing the different parameters from the NODDI diffusion model and their ability to differentiate between different regions of interest (ROI); *ecvf*, *icvf*, *fiso*, *odi*. The table under displays a summary of the full performance across all applications. Abbreviations: area under the curve (*auc*); of each parameter for differentiating ROIs between one another and their mean performance across all applications. Periphaque of paramagnetic rim lesion (pPRL+); periphaque of non-paramagnetic rim lesion (pPRL-); periphaque of contrast-enhancing lesion (pCEL); Neurite Orientation Dispersion and Density Imaging (NODDI); extra-neuronal cell volume fraction (*ecvf*); intra-axonal cellular volume fraction (*icvf*); fraction of isotropic diffusion (*fiso*); orientation dispersion index (*odi*).

	PRL+-PRL-	pPRL+-pPRL-	PRL+-CEL	pPRL+-pCEL	CEL-PRL-	pCEL-pPRL-	mean	variance
icvf								
auc	0.6785	0.6311	0.6498	0.4176	0.4095	0.4473	0.539	0.013356
accuracy	0.6393	0.6083	0.7014	0.6331	0.5815	0.5986	0.627	-
sensitivity	0.6246	0.6139	0.7192	0.6551	0.4737	0.439	0.5876	-
specificity	0.6595	0.6008	0.5526	0.4634	0.5991	0.6261	0.5836	-
ecvf	0.7287	0.669	0.6585	0.3486	0.6622	0.7217	0.6315	0.016782
auc	0.6776	0.6372	0.4845	0.5602	0.5963	0.5663	0.587	-
accuracy	0.6467	0.5506	0.4511	0.5443	0.6316	0.8049	0.6049	-
sensitivity	0.7198	0.7521	0.7632	0.6829	0.5952	0.5252	0.6731	-
fiso	0.6551	0.6892	0.4014	0.4198	0.7452	0.7433	0.609	0.020673
auc	0.5975	0.6552	0.6	0.4454	0.7185	0.6738	0.6151	-
accuracy	0.5741	0.6487	0.5994	0.4082	0.6053	0.7317	0.5946	-
sensitivity	0.6293	0.6638	0.6053	0.7317	0.7371	0.6639	0.6719	-
odi	0.4434	0.4497	0.7543	0.5898	0.7519	0.4181	0.5679	0.020165
auc	0.5337	0.4765	0.7408	0.6751	0.7259	0.638	0.6317	-
accuracy	0.4953	0.1519	0.7571	0.6994	0.6053	0.5122	0.5369	-
sensitivity	0.5862	0.9076	0.6053	0.4878	0.7457	0.6597	0.6654	-

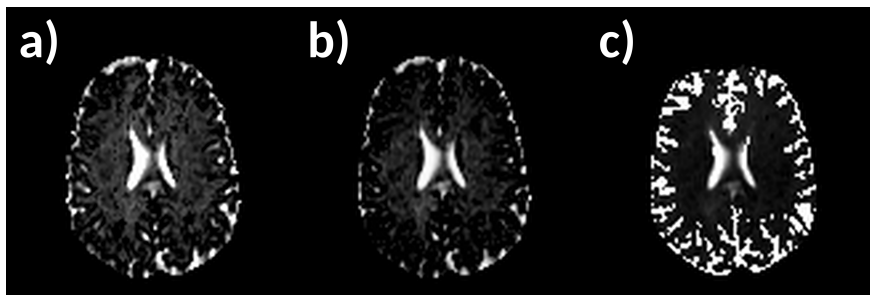


Fig. B.2 Comparison of different diffusion models water content modelling; NODDI's fiso (a), DIAMOND's fcsfDMD (b), and MF's fcsfMF (c). We can observe a somewhat suboptimal NAWM's water modelization when using analytical models (NODDI, DIAMOND), suggesting that a numerical approach (MF) may improve water modelling. Abbreviations: Neurite Orientation Dispersion and Density Imaging (NODDI); DIstribution of Anisotropic MicrOstructural eNvironments in DWI (DIAMOND); Microstructure Fingerprinting (MF).



Supplementary materials for
Challenges in identifying
multiple sclerosis chronic
active lesions as slowly
expanding lesions

C.1 Automatic lesion splitting

Lesion splitting is computed utilizing the fluid-attenuated inversion recovery (FLAIR) and magnetization prepared rapid gradient echo (MPRAGE) contrast to localize different clusters of tissue inside lesional area with different apparent T1w and T2w values, likely corresponding to different lesion instances with different ongoing pathological processes (representation of the lesion splitting in Figure C.1).

First, preprocessing was applied on both FLAIR and MPRAGE images independently and included non-local means adaptive denoising filter,¹³¹ skull-stripping using Synthstrip,⁹⁰ rigid registration of the MPRAGE image onto the FLAIR space using the mutual information metric (ANTS),⁷⁸ and contrast enhancement using a voxel-wise squaring intensity transformation. Finally, we applied a frequency-domain sharpening step to both the MPRAGE and FLAIR images. The magnitude of their Fourier (F) transform was modulated via a power-law operator ($|F|^{0.75}$), and subsequently recombined with the unaltered phase for reconstruction. The inverse Fourier transform yielded images with enhanced high-frequency content, resulting in sharper lesion boundaries and facilitating subsequent separation of lesions.

Within the binary lesion mask obtained from FLAMeS on the FLAIR images,¹²⁸ the sharpened FLAIR image was divided by the sharpened MPRAGE image to enhance contrast between regions with differing T2/T1 characteristics. The resulting map was further processed with a multi-scale Laplacian-of-Gaussian (LoG) filter to accentuate clusters with distinct T2/T1 ratios. Candidate lesion centers were identified as voxels where the gradient magnitude of the first derivative was near zero ($\epsilon < 10^{-3}$) and where the Hessian's eigenvalues were all negative, indicating a local maximum. These centers were subsequently used as markers to initialize a watershed segmentation of the lesions, yielding the final lesion delineations.

To quantify the performance of the automated lesion-splitting approach, we carried out a correlation analysis comparing automated and manual lesion volumes. The evaluation was performed on a total of 1578 lesions manually delineated in the cross-sectional dataset. For each lesion, volumes derived from the automated algorithm were matched to their corresponding manual reference labels. Pearson's correlation was computed to quantify the

performance of the automated lesion-splitting approach to truly represent the volume of individual lesions.

This evaluation revealed a robust correlation between automated and manual lesion volumes ($r = 0.8029$, 95% CI: [0.7845; 0.8197], $p < 0.00001$), indicating that the automated procedure reliably approximates expert-derived measurements (Figure C.2).

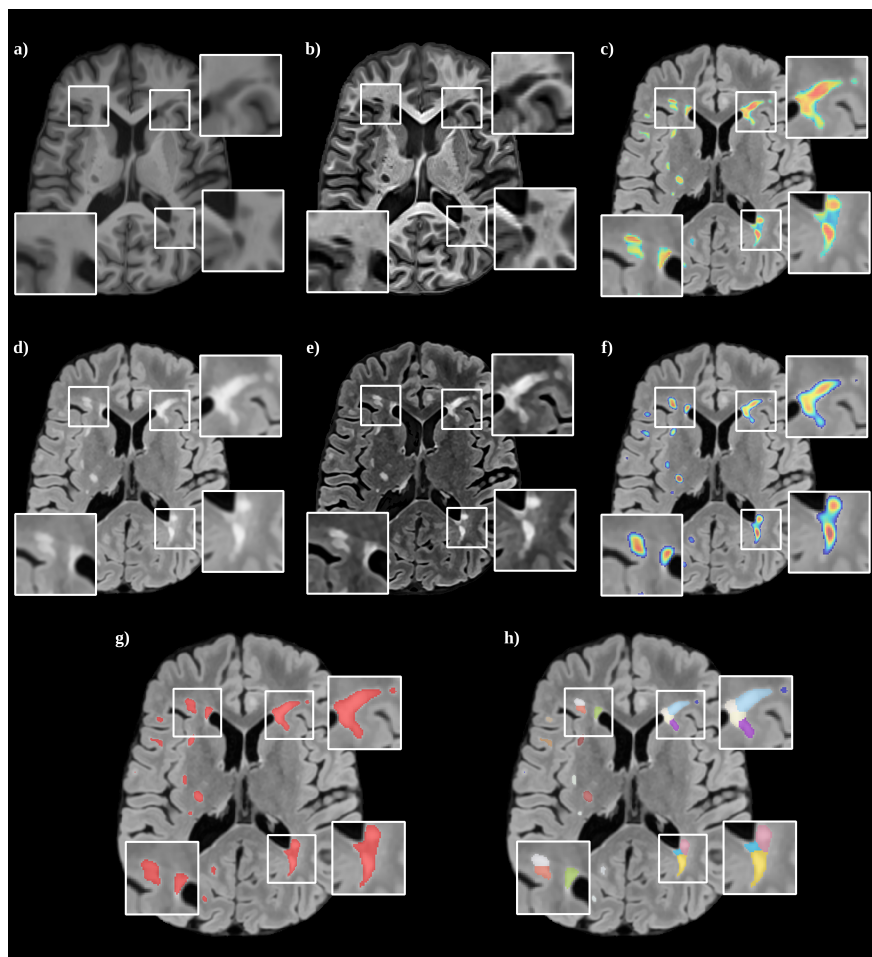


Fig. C.1 Representative figure of the lesion-splitting algorithm in axial images of an individual in their 20's with relapsing-remitting multiple sclerosis (RRMS). (a) magnetization prepared rapid gradient echo (MPRAGE) image; (b) preprocessed and sharpened MPRAGE image; (c) fluid-attenuated inversion recovery (FLAIR) image with the FLAIR/MPRAGE ratio overlay; (d) FLAIR image; (e) preprocessed and sharpened FLAIR image; (f) FLAIR image with the multi-scales Laplacian of gaussian (LoG) filter applied on the FLAIR/MPRAGE ratio overlay; (g) FLAIR image with automatic binary lesion segmentation; (h) FLAIR image with the resulting lesion-split segmentation.

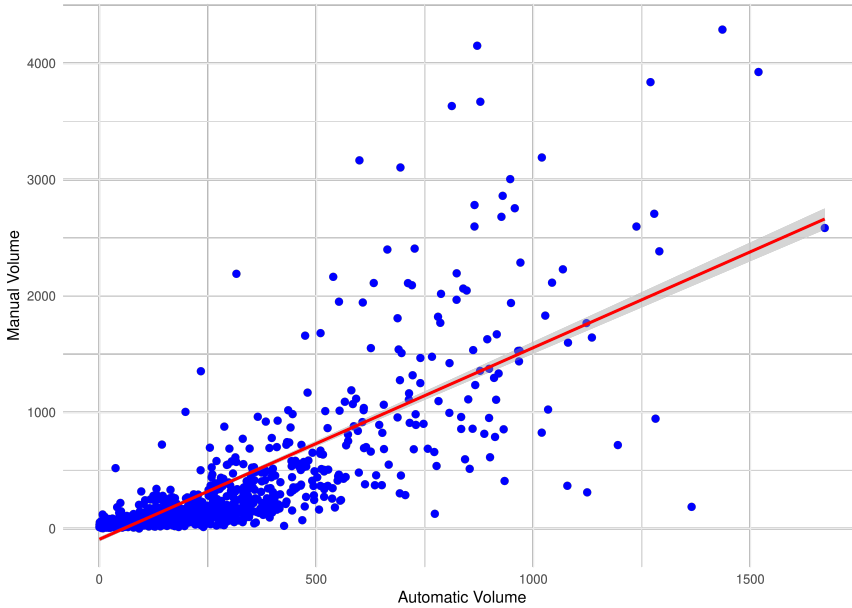


Fig. C.2 Scatterplot illustrating the relationship between automated and manual lesion volumes. The line of best fit and corresponding correlation coefficient ($r = 0.8029$, 95% CI: [0.7845; 0.8197], $p < 0.00001$) indicates strong agreement between the two methods.

C.2 Longitudinal lesion tracking

To enable longitudinal tracking of individual lesions across sessions, a lesion correspondence algorithm was developed to map lesion labels from the current session mask to those of a reference (prior) session mask registered to the current session using ANTs.⁷⁸ For each labelled lesion region in the current session, the algorithm determines the most appropriate matching reference label using spatial overlap criteria, supplemented by local neighbourhood analysis when required.

Overlap computation For each lesion l in the current session mask, containing N voxels, the algorithm extracts the reference mask values at the same spatial coordinates. Two overlap profiles are computed: (i) the fractional overlap with each distinct labelled region r in the reference labelled mask, defined as the proportion of the N voxels of l coinciding with region r ; and (ii) the fractional overlap with the reference binary lesion mask (all

lesion labels collapsed to 1). Both profiles are sorted in descending order of fractional overlap. A minimum overlap threshold τ (here $\tau = 15\%$) is then applied to the labelled overlap profile to retain only candidate reference regions whose overlap with the current lesion exceeds this threshold.

Case 1 — Unique match If exactly one reference region exceeds τ :

- If that region corresponds to a labelled lesion (label $\neq 0$), the current lesion is assigned the corresponding reference label, indicating a stable lesion present in both sessions.
- If the dominant region is background (label = 0), the lesion is considered a candidate new lesion. This is confirmed only if the binary mask overlap analysis also shows a majority background overlap (i.e., the voxels of the current lesion predominantly fall outside any reference lesion region) with a binary background fraction exceeding 85%. In this case, the lesion is assigned a new unique label not previously used. If either condition is not met, the lesion is not classified as a new lesion and is excluded from the output mask because it would correspond to a region that was manually excluded from the prior reference session.

Case 2 — Multiple matches If more than one reference region exceeds τ , background (label = 0) is excluded from further consideration:

- If a single non-background reference region remains, the current lesion is matched to that region (interpreted as a lesion that has slightly expanded into background territory but remains unambiguously associated with one reference lesion).
- If multiple non-background reference regions remain, the current lesion is interpreted as spatially overlapping with two or more distinct reference lesions — corresponding to either a merged lesion or ambiguous boundaries. In this case, label assignment is performed at the voxel level: for each voxel of the current lesion, the reference mask is sampled within a local neighbourhood of ± 2 voxels in each spatial dimension, and the voxel is assigned to the most frequent non-background reference label within that neighbourhood. If no non-background reference label is found in the neighbourhood, the voxel is assigned to the nearest reference lesion based on Euclidean distance to lesion centroids.

Results The resulting corrected mask preserves the spatial extent of all current-session lesions, with labels aligned to the reference session where a match exists, and novel labels assigned to confirmed new lesions. In case of oversplitting, multiple current lesions mapped to the same reference label are automatically reassigned to the same common reference id, effectively merging them and enabling consistent correction of lesion splitting across time points.



**Supplementary materials for
In vivo *characterization of
multiple sclerosis "MRI
evolving lesions"***

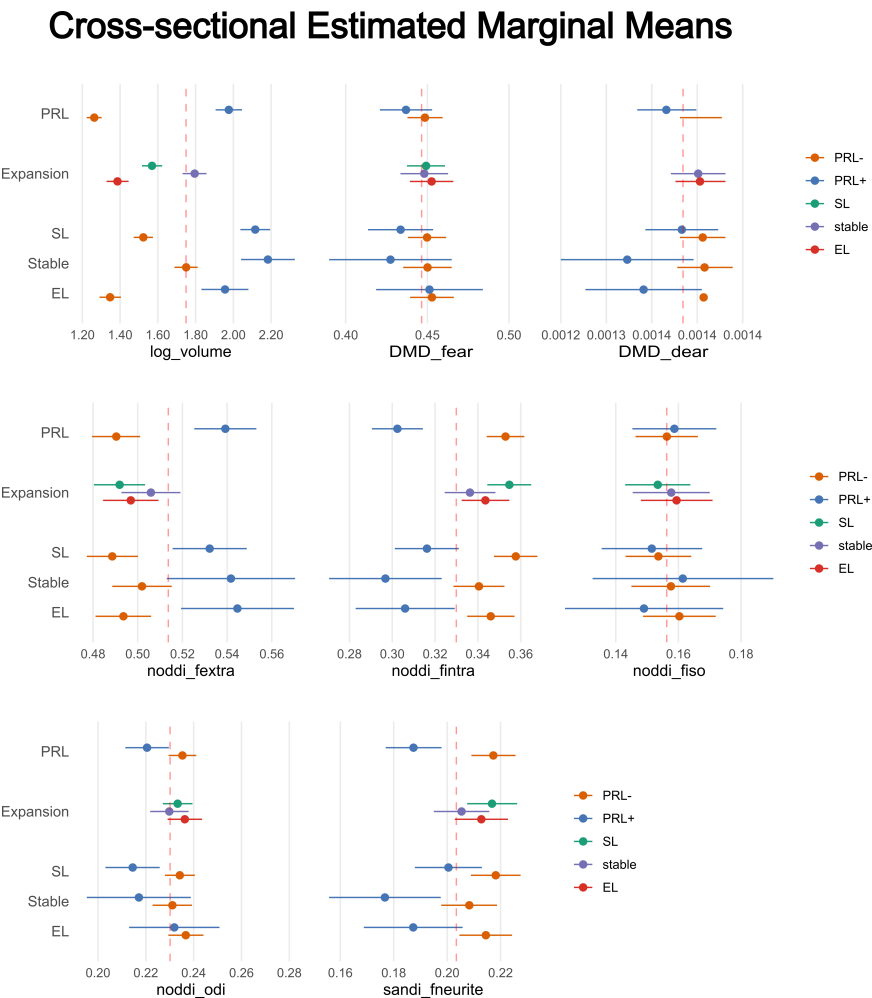


Fig. D.1 Cross-sectional estimated marginal means at baseline of the different microstructural metrics in different lesion types based on their PRL and expansion status. The first row of the graph describes PRL status alone, the second row expansion status alone, and the third row the interaction of the two. The red dashed line shows the mean value across all lesions. Abbreviations: (non) paramagnetic rim lesion (PRL-/PRL+); shrinking lesion (SL); stable lesion (stable); expanding lesion (EL)

Table D.1 Cross-section contrast analysis of the different microstructural metrics at baseline in different lesion types. Abbreviations: (non) paramagnetic rim lesion (PRL-/PRL+); shrinking lesion (SL); stable lesion (stable); expanding lesion (EL)

	PRL-/+	SL/stable	SL/EL	stable/EL	SL PRL-/+	stable PRL-/+	EL PRL-/+	PRL-/SL/stable	PRL-/SL/EL	PRL-/stable/EL	PRL+/SL/stable	PRL+/SL/EL	PRL+/stable/EL
log_volume	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.949	0.16	0.112
T1	<0.001	<0.05	<0.05	0.996	<0.001	<0.001	<0.001	0.617	0.129	0.993	0.417	0.48	1
DMD f_{fibers}	<0.001	<0.05	0.295	0.394	0.503	0.911	0.549	0.0884	0.678	0.839	0.918	0.883	1
DMD f_{csf}	<0.001	0.115	0.334	0.794	<0.01	0.148	0.722	0.604	0.651	1	0.822	1	0.905
DMD d_{cor}	<0.01	0.966	0.999	0.98	0.771	0.191	0.282	1	1	1	0.644	0.836	0.999
DMD d_{par}	0.072	0.982	0.796	0.774	0.444	0.844	1	1	0.994	0.999	1	0.923	0.92
NODDI f_{extra}	<0.001	<0.01	0.409	0.15	<0.001	0.0544	<0.001	<0.05	0.822	0.555	0.988	0.938	1
NODDI f_{intra}	<0.001	<0.001	<0.01	0.242	<0.001	<0.05	<0.01	<0.001	<0.05	0.836	0.724	0.963	0.993
NODDI f_{iso}	0.627	0.596	0.286	0.934	1	1	0.942	0.955	0.576	0.995	0.987	1	0.984
NODDI d_{di}	<0.001	0.573	0.6	0.213	<0.01	0.811	0.996	0.948	0.97	0.709	1	0.561	0.899
SANDI f_{extra}	<0.001	<0.001	<0.05	0.136	<0.001	<0.01	<0.05	<0.001	0.0514	0.742	0.404	0.995	0.835
SANDI f_{intra}	<0.001	<0.001	0.325	0.0777	<0.01	<0.05	<0.05	<0.05	0.784	0.498	0.219	0.731	0.96
SANDI d_{parite}	<0.001	<0.05	<0.05	0.912	<0.001	0.465	0.958	0.0924	<0.05	1	1	0.945	0.952
SANDI d_{rema}	<0.05	0.619	0.931	0.493	0.349	1	0.282	0.884	0.997	0.745	0.982	0.971	0.852

Longitudinal Estimated Marginal Slopes

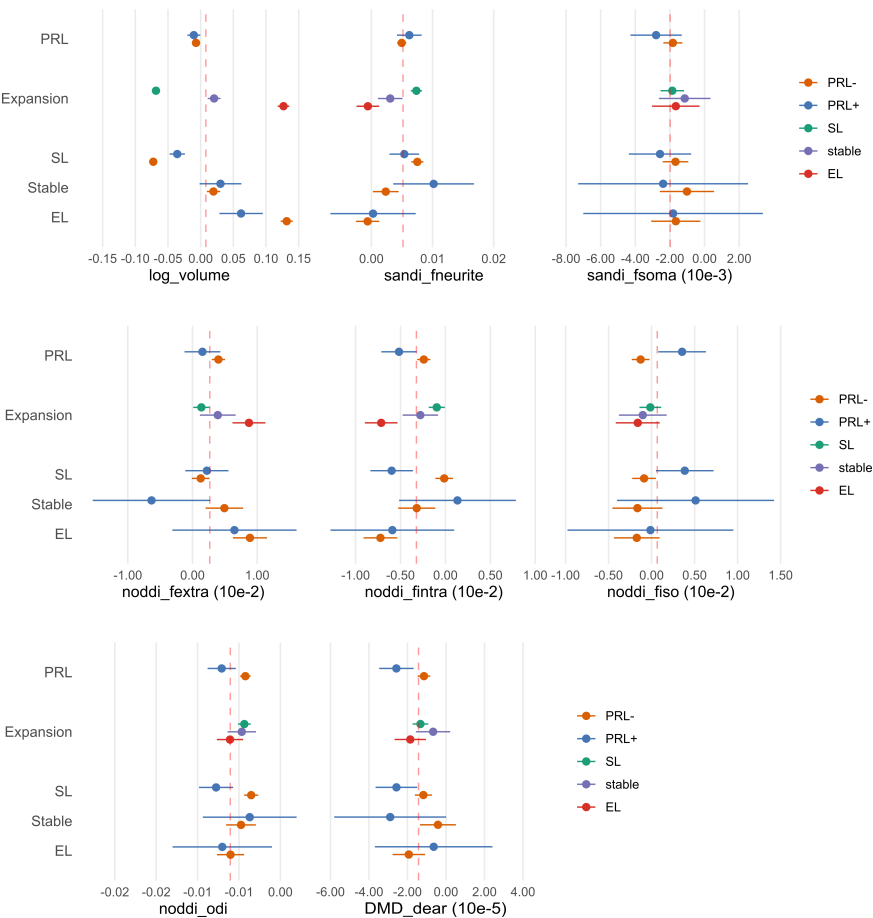


Fig. D.2 Longitudinal estimated marginal slopes of the different microstructural metrics in different lesion types based on their PRL and expansion status. The first row of the graph describes PRL status alone, the second row expansion status alone, and the third row the interaction of the two. The red dashed line shows the mean value across all lesions. Abbreviations: (non) paramagnetic rim lesion (PRL-/PRL+); shrinking lesion (SL); stable lesion (stable); expanding lesion (EL)

Table D.2 longitudinal contrast analysis of the different microstructural metrics slope in different lesion types. Abbreviations: (non) paramagnetic rim lesion (PRL-/PRL+); shrinking lesion (SL); stable lesion (stable); expanding lesion (EL)

	PRL-/+	SL/stable	SL/EL	stable/EL	SL PRL-/+	stable PRL-/+	EL PRL-/+	PRL-/SL/stable	PRL-/SL/EL	PRL-/stable/EL	PRL+/SL/stable	PRL+/SL/EL	PRL+/stable/EL
log_volume	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.949	0.16	0.1112
T1	<0.001	<0.05	<0.05	0.996	<0.001	<0.001	<0.001	0.617	0.129	0.993	0.417	0.48	1
DMD f_{fibers}	<0.001	<0.05	0.295	0.394	0.303	0.911	0.549	0.0884	0.678	0.839	0.918	0.883	1
DMD f_{csf}	<0.001	0.115	0.334	0.794	<0.01	0.148	0.722	0.604	0.651	1	0.822	1	0.905
DMD d_{car}	<0.01	0.966	0.999	0.98	0.771	0.191	0.282	1	1	1	0.644	0.836	0.999
DMD f_{car}	0.072	0.982	0.796	0.774	0.444	0.844	1	1	0.994	0.999	1	0.923	0.92
NODDI f_{extra}	<0.001	<0.01	0.409	0.15	<0.001	0.0544	<0.001	<0.05	0.822	0.555	0.988	0.938	1
NODDI f_{intra}	<0.001	<0.001	<0.01	0.242	<0.001	<0.05	<0.01	<0.001	<0.05	0.836	0.724	0.963	0.993
NODDI f_{iso}	0.627	0.596	0.286	0.934	1	1	0.942	0.955	0.576	0.995	0.987	1	0.984
NODDI d_{ad}	<0.001	0.573	0.6	0.213	<0.01	0.811	0.996	0.948	0.97	0.709	1	0.561	0.899
SANDI f_{extra}	<0.001	<0.001	<0.05	0.136	<0.001	<0.01	<0.05	<0.001	0.0514	0.742	0.404	0.995	0.835
SANDI f_{intra}	<0.001	<0.001	0.325	0.0777	<0.01	<0.05	<0.05	<0.05	0.784	0.498	0.219	0.731	0.96
SANDI d_{ad}	<0.001	<0.05	<0.05	0.912	<0.001	0.465	0.958	0.0924	<0.05	1	1	0.945	0.952
SANDI R_{suma}	<0.05	0.619	0.931	0.493	0.349	1	0.282	0.884	0.997	0.745	0.982	0.971	0.852

Table D.3 Baseline contrast sensitivity study of the different microstructural metrics means in different lesion types compared to the full lesion dataset. The top part of the table displays the sensitivity analysis in the low confluence (<15% shared lesion border) lesion dataset, while the bottom part displays the sensitivity analysis in the subset of lesions having ≥ 3 observations. Red color shows metrics where the direction of the contrast difference diverges from the full lesion dataset.

Abbreviations: (non) paramagnetic rim lesion (PRL-/PRL+); striking lesion (SL); stable lesion (stable); expanding lesion (EL)

outcome	PRL-/+	SL/stable	SL/EL	stable/EL	SL-PRL-/+	stable PRL-/+	EL-PRL-/+	PRL-/SL/stable	PRL-/SL/EL	PRL-/stable/EL	PRL+/SL/stable	PRL+/SL/EL	PRL+/stable/EL
low confluence													
log. volume	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.999	0.215	0.257
T1	<0.001	0.324	<0.05	0.579	<0.001	<0.05	<0.01	0.928	0.0894	0.819	0.669	0.978	0.987
DMD <i>fibers</i>	0.916	0.408	0.429	0.987	0.86	1	1	0.808	0.799	1	0.986	0.997	1
DMD <i>csf</i>	0.0788	0.991	<0.05	0.109	0.863	0.755	0.997	1	0.0949	0.248	0.992	0.996	1
DMD <i>dear</i>	0.177	0.89	0.792	0.618	0.957	0.772	0.866	0.985	0.992	0.886	0.993	0.985	1
DMD <i>far</i>	0.156	0.994	0.847	0.936	0.616	0.863	0.86	1	0.997	0.998	1	1	1
NODDI <i>extra</i>	<0.001	<0.05	0.075	0.0712	<0.01	0.514	0.06	<0.05	0.925	0.338	0.999	0.985	0.999
NODDI <i>intra</i>	<0.001	<0.01	0.949	0.712	0.145	0.513	0.578	0.202	<0.05	0.992	0.957	0.998	0.999
NODDI <i>iso</i>	<0.05	0.551	0.085	<0.05	0.525	1	0.362	0.895	0.253	0.0724	0.997	0.997	0.987
NODDI <i>ed</i>	<0.001	0.551	1	0.588	0.0601	0.774	0.968	0.953	1	0.969	0.997	0.974	0.992
SANDI <i>extra</i>	<0.001	0.058	<0.05	1	0.713	0.285	0.996	0.418	0.0643	0.998	0.661	0.937	0.886
SANDI <i>neutric</i>	<0.01	0.102	0.394	0.517	0.567	0.478	0.815	0.801	0.828	0.997	0.61	0.937	0.935
SANDI <i>Reyna</i>	0.0536	0.659	0.0538	0.936	0.861	0.962	0.948	0.975	0.117	0.775	1	0.924	0.933
SANDI <i>Reyna</i>	<0.001	<0.05	0.936	<0.05	<0.05	0.999	0.451	<0.05	1	0.0569	0.869	1	0.94
≥ 3 ses													
log. volume	<0.001	<0.01	<0.001	<0.001	<0.001	<0.001	<0.001	<0.05	<0.001	<0.001	0.969	0.799	0.593
T1	<0.001	0.868	0.969	0.996	<0.001	<0.001	<0.001	0.993	1	0.597	0.909	0.963	1
DMD <i>fibers</i>	<0.01	0.165	0.997	0.189	0.589	0.643	0.579	0.654	0.761	0.896	0.98	0.998	0.954
DMD <i>dear</i>	<0.01	0.957	0.505	0.73	0.276	0.586	1	0.92	0.804	0.878	0.894	1	0.984
DMD <i>far</i>	0.101	0.995	0.563	0.676	0.986	0.676	1	0.92	0.92	0.925	0.999	0.982	0.981
NODDI <i>extra</i>	<0.001	0.986	0.727	0.679	0.972	1	<0.05	0.136	0.908	0.925	0.999	0.982	0.989
NODDI <i>intra</i>	<0.001	<0.05	0.998	0.0589	<0.001	0.726	0.212	0.19	0.957	0.158	0.995	0.981	0.999
NODDI <i>iso</i>	0.874	0.864	0.666	0.249	<0.001	0.167	0.829	0.998	0.986	0.92	0.993	0.971	0.852
NODDI <i>ed</i>	0.0739	0.728	0.847	0.978	0.976	1	0.451	0.49	0.968	1	0.673	0.971	1
SANDI <i>extra</i>	<0.001	0.0763	0.988	0.146	<0.001	<0.05	0.414	0.843	0.986	0.286	0.995	0.985	0.985
SANDI <i>neutric</i>	<0.001	0.193	0.621	<0.05	0.369	0.0904	0.986	0.828	0.995	1	0.995	0.999	0.999
SANDI <i>Reyna</i>	0.628	0.319	0.966	0.513	0.999	0.918	0.24	0.554	1	0.608	0.995	0.995	0.993

Table D.4 Longitudinal contrast sensitivity study of the different microstructural metrics slopes in different lesion types compared to the full lesion dataset. The top part of the table displays the sensitivity analysis in the low confluence (<15% shared lesion border) lesion dataset, while the bottom part displays the sensitivity analysis in the subset of lesions having ≥ 3 observations. Red color shows metrics where the direction of the contrast difference diverges from the full lesion dataset.

Abbreviations: (non) paramagnetic rim lesion (PRL-/PRL+); shrinking lesion (SL-); stable lesion (SL-); expanding lesion (EL)

outcome	PRL-/+	SL/stable	SL/EL	stable/EL	SL PRL-/+	stable PRL-/+	EL PRL-/+	PRL-/SL/stable	PRL-/SL/EL	PRL-/stable/EL	PRL-/SL/stable	PRL+/SL/EL	PRL+/stable/EL
low confluence													
log _e volume	0.988	<0.001	<0.001	<0.001	<0.001	1	0.119	<0.001	<0.001	<0.001	0.7	<0.01	0.366
T1	0.143	0.999	0.845	0.918	0.673	1	0.901	0.998	0.966	1	0.99	0.998	0.968
DMD f_{fibers}	<0.01	<0.01	0.564	<0.01	0.372	0.421	0.938	<0.01	0.93	<0.01	1	0.994	1
DMD f_{sf}	0.277	0.948	0.994	0.984	0.996	0.999	1	0.998	1	1	1	1	1
DMD d_{car}	0.86	0.675	0.627	0.341	0.928	0.998	1	0.872	0.98	0.629	0.995	1	1
DMD d_{far}	0.864	0.103	0.417	0.776	0.991	0.99	1	0.365	0.904	0.968	1	1	0.999
NODDI f_{extra}	<0.05	0.371	<0.001	<0.01	0.986	0.771	0.581	0.561	<0.001	<0.05	0.996	1	0.995
NODDI f_{itra}	0.557	0.0604	<0.001	<0.05	0.508	0.72	1	0.605	<0.001	0.311	0.82	0.919	0.545
NODDI f_{iso}	<0.01	0.973	0.12	0.205	0.286	0.951	0.449	0.997	0.591	0.415	0.995	0.998	0.975
NODDI d_{all}	0.801	0.876	0.257	0.693	0.906	0.951	1	0.826	0.289	0.92	0.94	1	1
NODDI f_{extra}	0.147	<0.001	<0.001	0.0524	<0.01	0.529	0.995	<0.01	<0.001	0.434	0.359	0.974	0.314
SANDI $f_{incurite}$	0.498	<0.01	<0.001	<0.05	<0.01	0.443	0.987	<0.01	<0.001	0.2	1.271	0.746	0.93
SANDI f_{oma}	0.417	0.968	0.805	0.955	1	0.629	1	0.987	0.987	0.997	0.99	0.753	0.93
SANDI $f_{oma} - \geq 3$ ses	<0.01	0.538	0.305	0.958	0.347	0.978	0.602	0.913	0.643	0.999	0.998	0.99	0.99
≥ 3 ses													
log _e volume	0.104	<0.001	<0.001	<0.001	<0.01	0.987	<0.001	<0.001	<0.001	<0.001	<0.01	<0.01	1
T1	<0.001	<0.001	<0.001	0.879	<0.001	0.768	1	<0.001	<0.001	0.74	1	0.994	0.987
DMD f_{fibers}	<0.001	<0.001	0.806	<0.05	<0.001	<0.05	0.968	<0.001	1	<0.05	0.999	0.99	0.978
DMD f_{sf}	<0.001	0.969	0.408	0.703	<0.001	<0.05	1	1	1	1	0.869	0.295	0.147
DMD d_{car}	<0.001	0.418	0.588	0.967	<0.05	0.994	1	0.667	0.997	0.958	0.924	0.82	0.557
DMD d_{far}	<0.001	0.111	0.132	0.993	0.0603	0.0792	0.993	0.328	0.893	0.966	0.935	0.82	0.295
NODDI f_{extra}	0.0882	0.836	<0.05	0.183	1	0.0574	0.975	0.813	0.0523	0.836	0.152	1	0.494
NODDI f_{itra}	<0.01	0.574	<0.001	<0.05	<0.001	0.905	0.997	0.327	<0.001	0.222	0.367	0.997	0.906
NODDI f_{iso}	<0.001	1	1	1	<0.05	0.284	0.996	1	0.995	0.999	0.909	1	0.926
NODDI d_{all}	<0.001	0.86	0.962	0.975	<0.05	1	0.999	1	0.992	1	0.794	0.967	1
SANDI f_{extra}	0.294	<0.001	<0.001	<0.05	0.0918	0.903	1	<0.01	<0.001	0.231	0.967	0.902	0.734
SANDI $f_{incurite}$	0.752	<0.001	<0.001	<0.05	0.442	0.543	1	<0.001	<0.001	0.163	0.956	0.726	0.491
SANDI f_{oma}	<0.05	0.587	0.462	0.99	0.834	0.918	1	0.948	0.956	1	1	0.998	0.996
SANDI $f_{oma} - \geq 3$ ses	<0.001	0.992	0.46	0.687	0.0873	0.161	0.804	1	0.734	0.883	0.736	0.972	0.999



Shrinking multiple sclerosis lesions are characterized by a more destructive phenotype than expanding lesions

Preliminary Note:

This annex expands a conference abstract:
Vanden Bulcke, C., Stölting, A., Macq, B., Maggi, P., *Shrinking multiple sclerosis lesions are characterized by a more destructive phenotype than expanding lesions.* in Proc. Intl. Soc. Mag. Reson. Med. vol. 32 (2024).

E.0 Synopsis

Motivation Slowly expanding lesions (SEL) have gained significant attention as a biomarker of chronic active multiple sclerosis (MS) lesions, however, one study¹³⁷ suggests that all MS lesions tend to shrink over a long period of time.

Goal To investigate the microstructure of expanding lesions (EL), shrinking lesions (SL), and stable lesions.

Approach EL and SL were computed using deformation-based volumetric MRI and microstructure was investigated using quantitative T1 and multi-shell diffusion MRI.

Results SL showed a more destructive phenotype at baseline when compared to EL, while stable lesions were considerably less destructive.

Impact This preliminary study underlies the importance of MRI-evolving lesions, whether shrinking or expanding, to better understand the pathophysiology of tissue damage in MS.

E.1 Introduction

Multiple sclerosis (MS) is the most prevalent chronic inflammatory disease of the central nervous system characterized by focal demyelinating lesions.²⁰ Slowly expanding lesions (SEL) are a subset of chronic active MS lesions, found more prominently in patients with progressive MS, and have recently raised attention due to their association with neurological and cognitive disability.¹²⁴ However, SEL showed only a limited correspondence with paramagnetic rim lesions (PRL),¹⁵¹ another novel imaging biomarker for chronic active lesions. In addition, a previous study showed that all MS lesions have a tendency to shrink over a long period of time (about 16 years), hypothesizing that the primary pathological process in chronic lesions, even those described as “slowly expanding,” is likely to be tissue loss.¹³⁷ The inability to directly study MRI-evolving lesions with histology

underscores the necessity for a more comprehensive understanding of their underlying pathology.

The objective of this work is to investigate the microstructural differences at baseline between shrinking and expanding lesions over one year.

E.2 Methods

43 MS patients, with an MPRAGE at baseline and one-year follow-up, were included in this study. First, a deformation field was calculated by non-rigidly registering intra-subject baseline and follow-up MPRAGE images using ANTs.⁷⁸ The Jacobian of this deformation field, normalized over a year, was used to compute local tissue expansion and shrinkage, as previously described.²⁵ More specifically, expanding — resp. shrinking — lesional tissue was described as contiguous regions within lesions exhibiting positive — resp. negative — local volume change of at least 12%, extended by the surrounding area showing positive — resp. negative — volume change of at least 4%. Expanding lesions (EL) — resp. shrinking lesions (SL) — were defined as non-active chronic lesions displaying only or a majority (60%) of expansion — resp. shrinkage. Stable lesions showed neither expansion nor shrinkage, while ambiguous lesions showing both were discarded. The algorithm is represented in Figure E.1. Overall, 1229 lesions (196 EL, 203 SL, 830 stable) were included in the analysis.

Microstructural tissue integrity at baseline was investigated using quantitative MP2RAGE-derived tissue longitudinal relaxation time (T1), a known marker of myelination and axonal density,¹⁰² and multi-shell diffusion MRI ($TR = 4842$ ms, $TE = 77$ ms, $\Delta = 35.7$ ms, $\delta = 22.9$ ms, 64 gradients at $b = 1000$, 32 at $b = 2000, 3000, 5000$ s/mm²). Multi-shell diffusion data were preprocessed with denoising,¹⁰⁹ motion and eddy-currents^{110,111} correction, skull-stripping using FSL BET,¹¹² and then processed by two advanced diffusion models — Neurite Orientation Dispersion and Density Imaging (NODDI)⁴⁶ and Microstructure Fingerprinting (MF)² — to study NODDI's extra-cellular volume fraction (ecvf) and MF's weighted fiber volume fraction (wfvf).

E.3 Results

Results are displayed in Figure E.2. At baseline, SL were bigger ($p=0.013$) than EL and showed longer T1 values ($p=0.020$). In addition, SL were characterized by more prominent extra-axonal cell infiltrate with higher NODDI's ecvf ($p=0.004$) and by lower axonal integrity with lower MF's wfvf ($p=0.010$). In comparison, stable lesions were considerably smaller than evolving lesions and characterized by a less destructive phenotype with shorter T1 values, lower ecvf, and higher wfvf ($p<0.001$).

E.4 Discussion

Our results confirm that MRI-evolving lesions, whether shrinking or expanding, are considerably more destructive than stable lesions, underlying the dynamic nature of MS pathology. Interestingly, SL were characterized by a more destructive phenotype at baseline when compared to EL, with a more prominent accumulation of extra-axonal cells as the most significant difference, hypothesized to reflect a more important presence of astrogliosis in SL. These results are in line with previous findings on chronic shrinking lesions,¹³⁷ but further investigations are needed to confirm that the most prevalent pathological process in chronic lesions is indeed tissue loss.¹³⁷

This preliminary study underlies the importance of considering all MRI-evolving lesions, whether shrinking or expanding, to gain a more comprehensive understanding of the dynamics of tissue damage within MS lesions. To build upon these initial findings, further investigations with a larger sample size are planned to investigate the association of MRI-evolving lesions, lesional age and other biomarkers of smoldering inflammation as well as with disability scores.

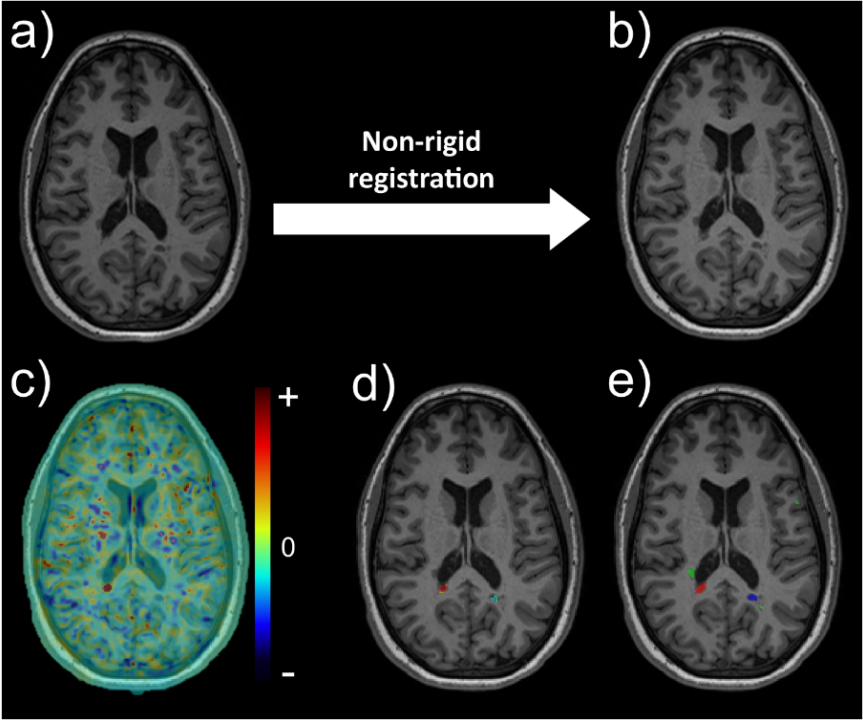


Fig. E.1 Computation of expanding lesions shrinking lesions and stable lesions by non-rigidly registering baseline (a) and follow-up (b) MPRAGE images and computing the Jacobian of the deformation field (c). EL (resp. SL) corresponds to lesion showing minimum local tissue expansion (resp. shrinkage) of 12% over a year (d). (e) shows the final segmentation of EL (red), SL (blue) and stable (green) lesions.

E | Shrinking lesions are more destructive than expanding lesions

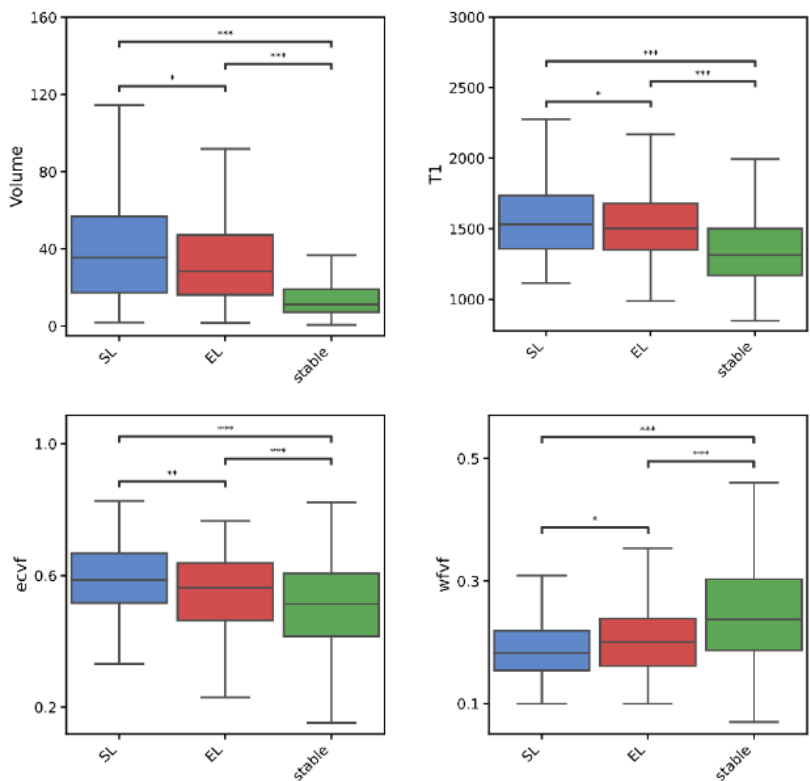


Fig. E.2 Comparison of shrinking (SL – blue), expanding (EL – red) and stable (stable – green) multiple sclerosis lesions in terms of volume, quantitative T1 value, NODDI's ecvf and MF's wfvf. Significance level are indicated as * ($p<0.05$), ** ($p<0.01$) and *** ($p<0.001$).