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In search of lost disease

*Clinical, qualitative and imaging studies to investigate
long-term effects of autologous haematopoietic stem
cell transplantation for multiple sclerosis*

ANDREAS TOLF



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Abstract

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With the introduction of autologous haematopoietic stem cell transplantation (AH SCT) for the treatment of multiple sclerosis (MS), there are now individuals living without experiencing disease activity of MS. Are they cured?

There is a need for terminology to describe the state of these individuals. To address this and to report the ten-year outcomes of the first ten persons treated with AH SCT for MS at our centre, we conducted tests involving six different outcome measures to encompass the inflammatory (new clinical relapses, new MRI lesions, intrathecal antibody production) and degenerative (clinical progression, elevated intrathecal levels of neurofilament light protein, presence of callosal atrophy) aspects of the disease. Half of the participants achieved 'sustained complete remission' (normal values in all parameters, excluding intrathecal measurements), and three 'resolved disease' by displaying standard values across all measures. This terminology can be used when communicating successful treatment outcomes, and 'resolved disease' might serve as a working definition of cure.

How did one perceive oneself, one's health, and one's diagnosis ten years after AH SCT? In this qualitative interview study, the lived experience of the same persons was explored and analysed with qualitative content analysis. The treatment symbolised a transition from a life dominated by uncertainty to a state of health and normality. The participants shared their experience of now feeling healthy, and some even reported not having MS anymore. They expressed a desire for objective criteria to affirm their health status.

Impaired cerebral blood flow has been linked to progressive MS. The third study was a case-control study examining CBF and cerebrovascular reactivity (CVR) in individuals with secondary progressive MS, healthy controls, and persons treated with AH SCT from the previous studies. CBF was measured using positron emission tomography with ¹⁵O-water. CBF was diminished in individuals with progressive MS compared to the healthy controls. In contrast, CBF in the AH SCT group did not differ significantly from healthy controls. CVR was not impaired in progressive MS patients, suggesting an opportunity for therapeutic intervention with a vasodilating agent.

The fourth study was a case-control study assessing factors associated with a favourable response to COVID-19 vaccination in persons with rituximab and MS. B cells emerged as the sole factor influencing antibody production. Consequently, pre-vaccination B-cell measurement was advised to enhance the likelihood of an effective humoral immune response.

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Till mamma och pappa

List of papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.

- I. **Tolf, A.**, Fagius, J., Carlson, K., Åkerfeldt, T., Granberg, T., Larsson, E.-M., Burman, J. (2019) Sustained remission in multiple sclerosis after hematopoietic stem cell transplantation. *Acta Neurologica Scandinavica*, 140(5):320-7
- II. **Tolf, A.**, Gauffin, H., Burman, J., Landtblom, A.-M., Flensner, G. Experiences of being treated with autologous haematopoietic stem cell transplantation for aggressive multiple sclerosis: A qualitative interview study. (Submitted)
- III. **Tolf, A.**, Hedman, K., Appel, L., Larsson, E.-M., Danfors, T., Landtblom, A.-M., Granberg, T., Fagius, J., Lubberink, M., Burman, J. Cerebral blood flow in multiple sclerosis: an ¹⁵O-water PET study. (Manuscript)
- IV. **Tolf, A.***, Wiberg, A.*, Müller, M., Nazir, F.H., Pavlovic, I., Laurén, I., Mangsbo, S., Burman, J. (2022) Factors Associated With Serological Response to SARS-CoV-2 Vaccination in Patients With Multiple Sclerosis Treated With Rituximab. *JAMA Network Open*, 5(5):e2211497

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Related papers

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Abbreviations

ACE	Angiotensin-converting enzyme 2
ACZ	Acetazolamide
AH SCT	Autologous haematopoietic stem cell transplantation
ANOVA	Analysis of variance
ARR	Annual relapse rate
ASL	Arterial spin labelling
ATG	Anti-thymocyte globulin
BBB	Blood-brain barrier
BEAM	Carmustine, etoposide, cytarabine and melphalan
BMI	Body mass index
CBF	Cerebral blood flow
(n)CCA	(Normalized) corpus callosum area
CD	Cluster of differentiation
CIS	Clinically isolated syndrome
CNS	Central nervous system
COREQ	Consolidated criteria for reporting qualitative research
COVID-19	Coronavirus disease 2019
CSF	Cerebrospinal fluid
CVR	Cerebrovascular reactivity
DSC	Dynamic susceptibility contrast
EBV	Epstein-Barr virus
EDSS	Expanded disability status scale
FLAIR	Fluid-attenuated inversion recovery
FS	Functional system
HLA	Human leucocyte antigen
IFN- γ	Interferon gamma
IL-2	Interleukine 2
MRI	Magnetic resonance imaging
mRNA	Messenger ribonucleic acid
MS	Multiple sclerosis
NAWM	Normal-appearing white matter
NEDA	No evidence of disease activity
NFL	Neurofilament light
OCBs	Oligoclonal bands
PBMC	Peripheral blood mononuclear cell

PET	Positron emission tomography
PPMS	Primary progressive multiple sclerosis
RBD	Receptor-binding domain
RRMS	Relapsing remitting multiple sclerosis
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
SFU	Spot forming units
SPMS	Secondary progressive multiple sclerosis
SRQR	Standards for reporting qualitative research
STROBE	Strengthening the reporting of observational studies in epidemiology
TSPO	Translocator protein
VOI	Volume of interest

Introduction

‘I usually don’t complain; I usually accept a situation for what it is. But when you don’t know from one day to another if you’re going to get out of bed, or if you’re going to see anything, or hear anything...’ – *Daniel* (Study II)

Living with multiple sclerosis

Multiple sclerosis (MS) is a disease that causes uncertainty in most aspects of life.¹

Will the disease eventually deprive me of my autonomy and independence?² What will happen if I develop a progressive disease course?³ Will I be able to pay my bills?⁴ Will my child with MS be able to live an independent life?⁵ How will my life change if my partner with MS deteriorates and becomes more dependent on help – will it impact my own freedom and independence?⁶

The diagnosis, the disease, and its consequences change oneself.⁷

Despite major medical advancements in the treatment of MS over the past decades,⁸ consequences like fatigue, cognitive impairment, depression, disability and unemployment have been linked to a decreased quality of life in MS, as reported in a recent comprehensive review.⁹ MS continues to be a disease that inflicts suffering and compromises health.

This thesis encompasses three studies that investigate ten individuals who underwent autologous haematopoietic stem cell transplantation (AHSCT) for aggressive, highly inflammatory MS. In his 2014 dissertation titled ‘Curing Multiple Sclerosis: How to Do It and How to Prove It’, Neurologist Joachim Burman discussed this group and outlined strategies for achieving and defining a cure for MS.

The first three studies in the present thesis revisit these ten individuals, a decade after their treatment with AHSCT. Study I delves into the clinical, radiological, and biomarker outcomes, many of which are frequently used in clinical practice. Study II explores the lived experience of the participants after treatment with AHSCT for MS. Study III assessed them alongside a cohort with secondary progressive MS and a healthy control group, using positron emission tomography to compare cerebral blood flow and cerebrovascular

reactivity. Collectively, these three studies form a search of a disease that might have been lost.

In the 21st century, living with MS typically involves continuous medication with a significant impact on the immune system. This not only increases the susceptibility to infections but can also reduce the efficacy of vaccinations. Conducted during the Coronavirus disease 2019 (COVID-19) pandemic, Study IV addressed the urgent clinical question: How should individuals with MS undergoing rituximab treatment be vaccinated?

Epidemiological and genetic insights

MS is characterised by inflammation and progressive degeneration of the central nervous system (CNS).¹⁰ It predominantly manifests during early adulthood and represents a significant cause of neurological disability; the prevalence in Sweden stands at 190,¹¹ with an annual incidence of 10 per 100,000 inhabitants.¹² Beyond the palpable distress experienced by patients and their families,^{9,13} MS imposes a tangible burden on society, encompassing not only the utilisation of healthcare resources, but also a notable diminution of the workforce.¹⁴

Numerous risk factors have been delineated in relation to MS, including Epstein-Barr virus (EBV) infection as an indispensable prerequisite for the development of the disease,¹⁵ clinically manifest mononucleosis,¹⁶ smoking,¹⁷ obesity during adolescence,¹⁸ and low levels of vitamin D,¹⁹ possibly due to reduced sun exposure. An increasing distance from the equator also heighten the risk of developing MS.²⁰

There also exists a hereditary component. More than 200 genes have been associated with MS, which collectively seem to account for approximately half of the hereditary component.²¹ The risk genes are predominantly those coding for the human leucocyte antigen (HLA) class II, especially *DRB1*15:01*, which stands as the most strongly predisposing genetic variant and was among the first to be identified.²² However, MS-associated genes have been observed across the entire spectrum of immune cells, both from the adaptive and the innate immune systems, and especially in microglia. Notably, neurons and astrocytes do not appear to be targets of genetic vulnerability associated with MS.²¹ Recently, an association between the progression of disability and homozygosity for the risk allele of the *DYSF-ZNF638* variant (rs10191329) has also been demonstrated; it thus appears that the propensity to develop progressive disease may also have genetic components.²³

Risk genes interact with other risk factors; for instance, genetic susceptibility operate synergistically with environmental factors, such as smoking and air pollution.^{24,25} Additionally, HLA-DRB1*15:01 has recently been demonstrated to act as a co-receptor for EBV.²⁶ Risk factors like smoking, high body mass index (BMI), EBV infection and low sun exposure have also been

associated with epigenetic changes,²⁷ which in turn are believed to interact with various disease-driving processes, such as pro-inflammatory activation associated with smoking.^{28,29}

Pathological and clinical characteristics

In parallel to the clinical difficulties estimating the start of the progressive stage in MS, the pathological demarcation between the traditional clinical subtypes of MS is not firmly established. The traditional classification of MS into relapsing remitting MS (RRMS) and the following secondary progressive stage (SPMS) is increasingly questioned.³⁰

In the early stages of the disease, new inflammatory active lesions in the CNS typically occur.³¹ While these occasionally result in acute clinical symptoms ('relapse'), they are pathologically distinguished by what is believed to be activated resident microglia,³² and subsequent infiltration of cells from both the innate adaptive immune systems through a compromised blood-brain barrier, including monocyte-derived macrophages,³³ CD8+ T cells and CD20+ B cells.³⁴ That being said, it is often difficult to separate monocyte-derived macrophages, migrating into the brain from the periphery, from activated CNS-resident microglia, hence they are often collectively named: microglia/macrophages.³⁵ The acute, active and demyelinating perivascular lesions are discernible from gadolinium-enhancement on magnetic resonance imaging (MRI), and usually persists for days to weeks, sometimes resulting in clinical symptoms.

The symptoms of a relapse are as diverse as the CNS itself, manifesting as blurred vision, double vision, vertigo, muscle strength loss, numbness and coordination dysfunction, to name a few.³⁶ Interestingly, a subset of the MS patients does not exhibit the typical initial relapsing remitting trajectory; instead, they display a progressive course from the onset, referred to as primary progressive MS (PPMS).¹⁰ People with MS can also be troubled by severe fatigue,³⁷ which is often perceived as the worst symptom of the disease when present.³⁸

After the influx of peripheral inflammatory cells into the CNS during the acute lesion phase, a significant proportion of the inflammatory cells do not exit but remains as tissue-resident CD8+ memory T cells and plasma cells.³⁹ Although mainly inactive, these cells have the potential to become reactivated when encountering their respective antigens.⁴⁰ The microglia/macrophages produce critical agents, including oxygens radicals, which are believed to cause mitochondrial damage, oxidative tissue injury and induce a hypoxic state potentially leading to demyelination and neurodegeneration.⁴¹

The specific characteristics of the immunological attacks can differ from patient to patient, but seem to be surprisingly consistent in each individual.^{42,43}

Following the acute phase of a newly developed MS-lesion, its further pathological development can take various paths:³¹

Shadow plaque. Lesion indicative of previous damage but showing signs of remyelination.

Inactive lesion. Consists of astroglial scar, is relatively stable and shows no signs of expansion or activity.

Mixed active/inactive lesion. Also known as *smouldering* or *chronic active* lesion. Display an outer layer of activated microglia/macrophages, suggesting a gradual expansion.

While the shadow plaques and inactive lesions remain stable, the mixed active/inactive lesions can be slowly expanding over the course of several years,⁴⁴ causing gradual tissue damage and progressive disability.⁴⁵ The soluble toxic substances subsequently produced by the activated microglia/macrophages are believed to induce demyelination and neurodegeneration in adjacent tissue, as outlined by Airas and Young.⁴⁶ The mixed active/inactive lesions can present with a paramagnetic rim possible due to iron in the phagocytosing cells and leakage of iron over a slightly impaired blood-brain barrier,⁴⁷ detectable on MRI,⁴⁸ and corresponds to the activation of microglia/macrophages as measured by translocator protein (TSPO) positron emission tomography (PET).⁴⁹ Moreover, TSPO PET can be utilised to categorise individual lesions in patients *in vivo*; in SPMS patients, the proportion of rim-active lesions is greater than those with RRMS.⁵⁰ The classical active lesions also manifest in other CNS inflammatory diseases, but the slowly expanding seem to be restricted to MS. In summary, it appears probable that halting or preventing the development of mixed active/inactive lesions could significantly benefit people with MS. However, there are reservations about whether conventional treatments for RRMS can accomplish this.⁴⁵

In many MS patients, disability eventually progresses despite absence of new focal inflammatory lesions, corresponding relapses and inflammatory activity in MRI. In this secondary progressive phase, SPMS, relapses are generally fewer and milder.⁵¹ Axonal damage is present already in the relapsing-remitting phase, but is more severe in the progressive phase and seems to be driven by innate immunity, where microglia/macrophages play a major role.⁵²⁻⁵⁴ This chronic diffuse inflammation is present both in grey and white matter as well as in perivascular areas and in the meninges.⁵⁵⁻⁵⁸ Even in normal-appearing white matter (NAWM) of the SPMS brain, activated microglia have been demonstrated *in vivo* with PET.^{59,60} This notion is further corroborated in biomarker studies, where biomarkers of microglia and astrocyte activation are prominent in the cerebrospinal fluid (CSF) from SPMS patients.^{61,62}

As described above, the mixed active/inactive lesions are believed to play a role in the disease progression. Although the cause of neurodegeneration in SPMS still is not fully understood, it seems that also inflammation in SPMS

to be compartmentalized in the CNS behind an intact blood-brain barrier (BBB).⁵⁷ In elderly patients with an extended disease history, the slow progression may arise or be exaggerated from age-related factors in a previously damaged CNS.

Diagnosing multiple sclerosis

‘I thought I was doomed. At first it was like... a sentence. Yes, that’s it. So I had... it was a long time before I even articulated the diagnosis.’ – *Melissa* (Study II)

Receiving an MS diagnosis is for many incredibly emotionally tumultuous, accompanied by shock, denial, anger, and fear; often, the diagnostic process has also been prolonged.⁶³

The characteristic clinical feature of the disease, to cause recurring periods of focal neurological deficit symptoms from various parts of the central nervous system, caused by multiple and separate inflammatory lesions, are principles referred to as ‘dissemination in time and space’ and have historically been used, and are still used today, to make the diagnosis.⁶⁴

The first major effort to establish consistency in diagnosis is usually attributed to the American group of neurologists chaired by Schumacher in the 1960s, which aimed to create validity and reliability in future therapeutic intervention studies, relying solely on clinical criteria for the definite diagnosis.⁶⁵ With the advancement of diagnostic technologies, new diagnostic criteria for possible, probable and definite MS were suggested two decades later in the early 1982 by Poser *et al.*⁶⁶

It was not until the McDonald criteria another two decades later, published in 2001, that MRI was incorporated in the diagnostic criteria, alongside CSF biomarkers and neurophysiological examination of visual evoked potentials, to support the clinical findings in the making of the diagnosis.⁶⁷ With this revision, Poser's differentiation based on the degree of probability, which had previously been prevalent, also disappeared; now, one either had MS or did not.

Since 2001, the ‘McDonald criteria’ have been revised in 2005,⁶⁸ 2010⁶⁹ and most recently in 2017.⁶⁴ With the 2017 diagnostic criteria, the demonstration of oligoclonal bands (OCBs) in CSF was reintroduced as evidence of dissemination in time, presently being the only included biochemical biomarker.

While the diagnostic criteria continue to be grounded in the presence of clinical relapses, i.e., symptomatic inflammatory lesions in the CNS, the aim of the revised criteria was to enable a more timely diagnosis of MS, promoting appropriate commencement of immunomodulatory therapy.⁶⁴ Compared to the 2010 version, the 2017 updates to the criteria enhance their ability to anticipate a second relapse following the initial clinical episode (clinically

isolated syndrome, CIS), consequently shortening the median time to an MS diagnosis by nearly 10 months.⁷⁰

Modifications to diagnostic criteria may pose challenges in research studies that aim to chart the historical evolution of a disease, such as its age of onset. Moreover, these alterations could raise concerns in broader scientific discussions: can one reliably extrapolate findings from studies on individuals diagnosed with MS under previous criteria? Nonetheless, given that recent amendments to the criteria are comparatively modest and aiming to facilitate an earlier diagnosis, it is likely that the number of individuals who meet the criteria for MS according to an older version but not a newer one, is exceedingly small.

The individuals with MS included in this thesis have been diagnosed based on various diagnostic criteria, but were also been retrospectively assessed and found to meet the criteria for MS according to the 2017 revision.

Defining progression

Given that the transition from RRMS to SPMS typically occurs gradually, the diagnosis of SPMS is frequently made in hindsight. Historically, the elusive character of the character of the clinical phenomenon that we call secondary progression has been reflected in the absence of a precise and universally accepted definition, complicating comparisons between studies on SPMS. Nevertheless, in 2016, Lorscheider *et al.* proposed a definition for SPMS research, centred on readily available clinical parameters:⁷¹

- Disability progression of ≥ 1 EDSS (Expanded disability status scale)⁷² step in patients with EDSS ≤ 5.5 or 0.5 in patients with EDSS ≥ 6.0 in the absence of a relapse;
- An EDSS score of ≥ 4 and a pyramidal FS (functional system) score of ≥ 2
- Confirmed progression over ≥ 3 months including confirmation within the leading FS.

In the 2013 revision of the definition of the clinical courses of MS, both PPMS and SPMS have been subdivided into four categories: ‘active and with progression’ (previously termed relapsing progressive MS), ‘active but without progression’, ‘not active but with progression’ and ‘not active and without progression’. Activity is determined by clinical relapse and new MRI findings, while progression is based fully on clinical evaluation of progressing disability.⁵¹

Cerebrovascular aspects

In recent years, there has been an active discourse that has explored the connection between MS and the vascular system. The risk of suffering from ischemic stroke seems to be slightly increased in people with MS, as evidenced in epidemiological studies.^{73,74} Endothelial dysfunction and atherosclerotic changes resulting from active inflammation in the brain parenchyma, oxidative stress, and elevated homocysteine have been suggested as possible explanations for these observations.⁷⁵ Small, local changes in blood flow also seem to precede the onset of gadolinium contrast-enhancing lesions.⁷⁶

Measurements of cerebral blood flow (CBF) in people with MS have also been of interest for a long time. The first *in vivo* quantification of CBF in humans was performed by Kety and Schmidt in the 1940s with inhalation of the inert gas nitrous oxide, a pioneer work on which the current CBF quantification paradigm still rests upon.⁷⁷ In the 1970s, the PET technology developed considerably, allowing *in vivo* measurement of CBF with radiotracers such as krypton-85.⁷⁸ CBF in people with MS was conducted in beginning of the 1980s by Swank *et al.*, using PET and inhaled xenon-133 gas; an age-increasingly impaired CBF could be demonstrated, much more pronounced than in healthy controls, which correlated with the 'rate of progress of the disease.'⁷⁹ The following year, Brooks *et al.* reported reduced regional CBF in both white and grey matter in a group of people with MS 'in remission', with an average disease duration of 12 years, compared to healthy controls.⁸⁰ They measured CBF using a steady-state PET method with inhalation of C¹⁵O₂ and ¹⁵O₂. Lycke *et al.* also examined CBF in MS patients in the early 1990s using single-photon emission computed tomography and technetium-99m hexamethylpropyleneamine oxime.⁸¹ They were able to demonstrate a relationship between clinical measures such as impaired visual function and reduced occipital blood flow and between reduced frontal blood flow and impaired cognition.

In recent years, advancements in MRI techniques, especially arterial spin labelling (ASL) MRI, have provided valuable insights into CBF in individuals with MS. Multiple studies have consistently reported reduced cerebral blood flow in MS patients.⁸²⁻⁸⁴ This widespread cerebral hypoperfusion not only aligns with pathological findings observed in MS but also hints at a possible hypoxic component in the progression of the disease.⁸⁵

There's a growing body of evidence connecting reduced CBF with clinical manifestations in MS; specifically, diminished CBF has been associated with both physical and cognitive impairments in MS patients.⁸⁶ Notably, hypoperfusion in the thalami has been correlated with increased disability in MS patients.⁸⁷ Furthermore, there's an intriguing link between elevated serum NFL levels and reduced thalamic perfusion⁸⁸ as well as decreased cerebral arterial blood flow, the latter being determined using Doppler sonography.⁸⁹

A study by Sowa *et al.* further highlighted reduced perfusion within white matter MRI lesions.⁹⁰

Adding depth to these findings, a study by Mascali *et al.* involving 91 patients with RRMS and 26 healthy controls, unveiled an association between grey matter hypoperfusion and irreversible white matter damage, as determined through ASL MRI. This observation led the authors to hypothesize that cerebral hypoperfusion may not just play a role in neurodegeneration, but could potentially precede and exacerbate it by hampering the tissue repair mechanisms of the CNS.⁹¹

While drawing certain conclusions from historical studies is precautionous, in which MS has been classified and diagnosed differently than today, and where a variety of different methods have been used, the combined literature strongly suggests that there is an association between reduced CBF and MS. Why the blood flow is reduced, and what role a reduced CBF plays in the pathogenesis of progressive disease is not yet clear. Restoring reduced CBF in people with MS has been suggested as a therapeutic intervention, but a trial with the vasodilating drug bosentan in a group of 27 people with RRMS for a time period of four weeks could not demonstrate neither increased CBF nor improvement in symptoms.⁹²

Cerebrovascular reactivity (CVR), also known as perfusion reserve, is the ability of cerebral blood vessels to change their diameter, and thus blood flow, in response to certain stimuli and the neurons' fluctuating metabolic requirements.⁹³ It is considered to be a measure of endothelial function and the integrity of the blood-brain barrier.^{94,95} A reduced CVR has been observed in several neurological conditions such as mild cognitive impairment,⁹⁶ Alzheimer's disease and vascular dementia,⁹⁷ Parkinson's disease⁹⁸ as well as in MS – although the results, primarily reliant on ASL MRI, have been inconsistent.⁹⁹

Treatments

‘The prognosis is extremely severe. We have shown that despite remissions, lasting sometimes for very long period, the disease progresses by aggravation, in the end preventing any movements. We do not know of a single case of healing and one should be aware of complications that may occur during the course of the disease and increase its severity’. – *Jean-Martin Charcot*¹⁰⁰

The treatment era

The advent of the ‘treatment era’ for MS began in the early 1990s, marking a pivotal moment in the management of the disease.¹⁰¹ By 1995, Sweden had sanctioned the inaugural immunomodulatory treatment with the introduction of interferons.¹⁰² Since then, a plethora of treatments has been approved,⁸ albeit accompanied by significant expenses and, in most cases, the necessity of repeated administration.¹⁰³ With judicious treatment, one can ambitiously target the prevention of several aspects of disease activity, including prevention of new relapses, MRI lesions and arresting development of disability.¹⁰⁴

With the initiation of a treatment, people with MS aspire to navigate this uncertainty and reclaim control over their lives and a sense of normalcy.^{105,106} If a treatment does not effectively manage the disease, the typical approach is to escalate to more effective treatments.⁸ Therefore, recognizing the signs that warrant escalation and being informed of alternative treatments are crucial. Unfortunately, a significant number of neurologists feel ill-prepared to identify treatment shortcomings and to stay updated on the latest therapeutic advancements.¹⁰⁷

Almost 60% of patients in Sweden who are on ongoing immunomodulatory treatment for multiple sclerosis are treated with rituximab, see Figure 1. A historical background of the Swedish treatment landscape is outlined in the chapter ‘Approaches to an ethical founding’ further below.

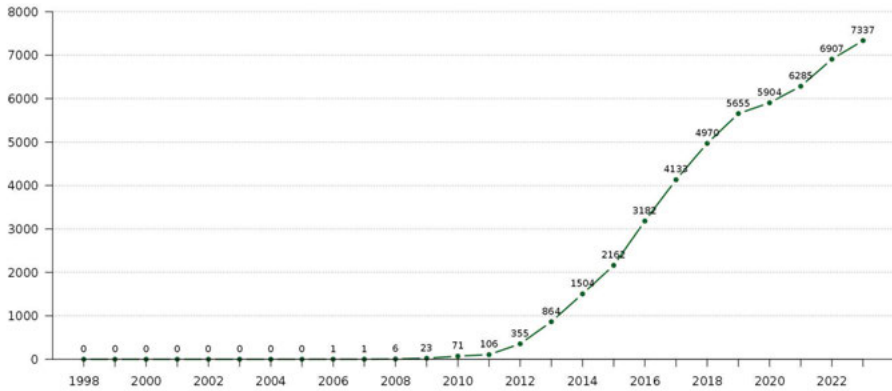


Figure 1. Number of patients with ongoing treatment with rituximab for MS in Sweden, 1998-2023.

Data from the Swedish multiple sclerosis registry, October 2023.

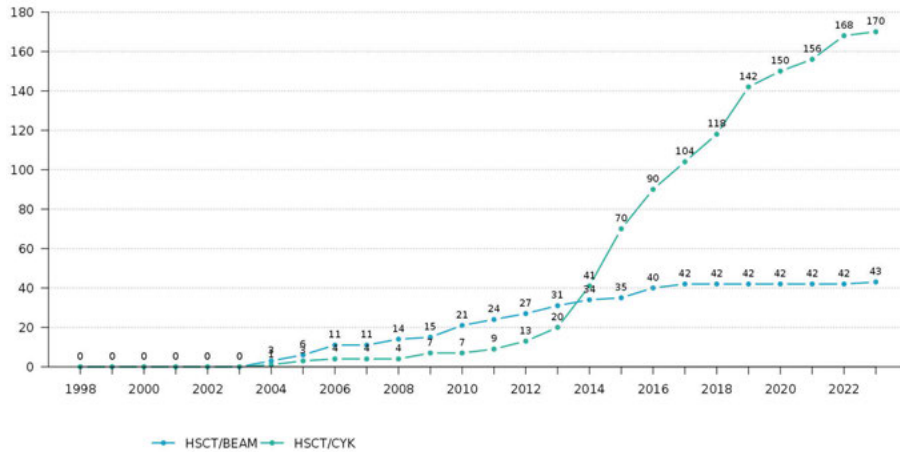


Figure 2. Accumulated number of treatments with AHSCT for MS in Sweden, 1998-2023.

Data from the Swedish multiple sclerosis registry, October 2023. HSCT/BEAM = autologous haematopoietic stem cell transplantation with conditioning regimen consisting of carmustine, etoposide, cytarabine, melphalan and anti-thymocyte globulin. HSCT/CYK = autologous haematopoietic stem cell transplantation with conditioning regimen consisting of cyclophosphamide and anti-thymocyte globulin.

Autologous haematopoietic stem cell transplantation

An alternative strategy to continuous immunomodulation is immune reconstitution therapy. One approach is chemotherapy with stem cell support, often called AHSCT. This procedure was developed for in haematological malignancies¹⁰⁸, but today used to treat various auto-immune diseases including neurological conditions.^{109,110}

Among the available treatments, AHSCT stands out as the most potent against RRMS.¹¹¹ Following AHSCT, NEDA-3 (no evidence of disease activity, see further below) is maintained in approximately half the patients up to a decade after the treatment intervention.¹¹² Moreover, the long-term adverse events are less severe than with other potent therapeutic strategies, such as alemtuzumab.¹¹³ Importantly, treatment-related mortality after AHSCT for MS has seen a significant decline, with a current estimate of 0.2-0.3%.^{111,114}

The first treatment with AHSCT for MS was executed in 1995 by Fassas' team. Findings, reported in 1997, from their initial cohort of 15 patients – all of whom had progressive MS – were moderately encouraging.¹¹⁵ However, it was subsequently discerned that the effectiveness for progressive MS was limited and associated with considerable treatment-related mortality.^{116,117}

Conversely, AHSCT appeared to be safe and much more effective among younger patients presenting with severe and rapidly deteriorating MS. A notable reduction in inflammatory activity such as gadolinium-enhancing MRI lesions and clinical relapses was observed. It was also reported that the safety of the treatment was linked to the expertise of the treating centres.¹¹⁸

Those findings gave rise to the recommendation that individuals with early-stage, highly aggressive RRMS would benefit most from this procedure. Following this, the first Swedish MS patient was treated with AHSCT on a compassionate-care basis, in 2004 at Uppsala University Hospital. It was a young patient with an uncommonly aggressive form of RRMS with short disease duration and a high degree of disability. The outcome was highly favourable and sustained.^{119,120} This pioneering experience paved the way for an early adoption and escalated use of AHSCT for MS within Sweden, see Figure 2. By 2014, data from 48 Swedish patients, who had undergone AHSCT for MS between 2004 and 2013, indicated clinical and radiological remission in approximately two-thirds of the participants, with only a few experiencing serious adverse events.¹²⁰

In 2019, Burt *et al.* reported on the outcomes of the only completed randomized clinical trial, also known as the Multiple sclerosis international stem cell transplant trial, which encompassed 110 RRMS patients with failure of a first-line treatment. Participants were randomized between AHSCT and an alternative or more efficient treatment than previously administered. The primary endpoint was time to disease progression, and AHSCT demonstrated a significant delay in the time to disease progression in comparison to alternative treatment.¹²¹

The AHSCT procedure has four parts: (1) mobilization and (2) collection of haematopoietic stem cells, (3) an immunoablative conditioning regimen, and (4) re-infusion of the cryopreserved stem cells. Since its inception in 1995, several protocols for this procedure have been devised, striking a balance between the therapeutic impact of the lymphoablative or myeloablative regimen and the risk for associated adverse effects. These conditioning regimens have been categorised into low-, intermediate-, or high-intensity strata.¹²² However, the overall intensity of the complete procedure is also contingent upon variables such as the incorporation of chemotherapy during mobilization and the potential administration of lymphodepleting serotherapy.¹¹¹

The BEAM-ATG conditioning regimen (carmustine 300 mg/m², etoposide 800 mg/m², cytarabine 800 mg/m², melphalan 140 mg/m² and antithymocyte globulin (ATG) 10 mg/kg) was predominant in Sweden prior to 2013.¹²⁰ At a Swedish national consensus meeting in 2013, it was decided that a low-intensity cyclophosphamide-based conditioning regimen with ATG should be used instead.¹²¹

Measuring and evaluating disease

Clinical assessment

The basis for the diagnosis of MS continues to be the patient's medical history and the clinical neurological examination.⁶⁴ Typically, during a follow-up, a clinical neurological examination is conducted to systematically map neurological functions, supplement the historical medical data, and identify any functional disturbances that the patient may not have noticed themselves. Based on this thorough examination and the patient's history, scores are determined using the Expanded disability status scale (EDSS), a scale developed by John F. Kurtzke in the early 1980s,⁷² though its inception began as early as the 1950s.¹²³ The scale ranges from 0 to 10 and is divided into 0.5 point increments starting from 1.0; a higher score indicates a higher degree of neurological disability, with 10 representing death due to MS.

The significant advantage of EDSS is its long-standing use across many years and almost all MS research studies. This facilitates, for example, the use of historical cohorts in research studies. It is also based on tests and information usually gathered during a routine follow-up with a neurologist. The drawbacks include its heavy reliance on mobility, which largely reflects lower limbs functions and pathology of the spinal cord, less on cognitive measures, and the fact that it typically can only be performed by a neurologically trained physician, and its moderate reproducibility between examiners.¹²⁴⁻¹²⁶

Several other scales have been developed to compensate for the limitations of the EDSS, such as the Multiple Sclerosis Functional Composite,¹²⁷ and in addition, various tests have been developed to more specifically assess different aspects of neurological disability.

Magnetic resonance imaging

Even if MRI is not formally required for the diagnosis, it has become crucial for diagnosis as well as follow-up.^{128,129} For diagnostic purposes, MRI can support demonstration of dissemination in space by inflammatory lesions in different regions of the CNS, and in time by demonstrating the simultaneous presence of contrast-enhancing and non-contrast-enhancing lesions, or by the appearance of lesions at repeated examinations.⁶⁷ Since it has been known since the 1980s that only a minority of newly developed MS lesions are

symptomatic,¹³⁰ MRI is excellently suited for providing additional information about treatment outcomes.

T₂-weighted sequences, where fluid appears brighter, have high sensitivity for pathologies like inflammatory and demyelinated lesions, which usually have a higher fluid content than surrounding tissue and therefore appear in the form of bright, 'hyperintense', changes. T₂ FLAIR (fluid-attenuated inversion recovery) is a sequence where CSF is suppressed, making MS lesions even more prominent, making it ideal for the detection of new MS lesions. In T₁-weighted sequences, on the other hand, fat is bright and fluid is dark; such sequences are used, among other things, for anatomical sharpness and for detecting contrast-enhancing lesions after the administration of gadolinium contrast. A newly developed MS lesion is usually contrast-enhancing due to a disrupted blood-brain barrier for the first four to six weeks, but rarely for more than two months.¹³¹ Over time, a lesion can become hypointense on T₁-weighted images, which has been interpreted as tissue damage due to irreversible gliosis and lower fat content, i.e., demyelination.¹³² However, the degree of hypointensity depends on the scanner's sensitivity, and the significance of hypointense T₁ lesions in routine healthcare is unclear;¹³³ it is worth noting, however, that the presence of such is not considered to prove dissemination in time according to the present diagnostic criteria.⁶⁷ The presence of new or enlarged lesions on T₂-weighted sequences ('T₂ lesions') or the presence of gadolinium-enhancing lesions on T₁-weighted sequences is often used as radiological evidence of ongoing inflammatory disease activity ('MRI activity').¹³⁴

Atrophy

Already in the late 19th century, Charcot described the demyelination and axonal loss characteristic of MS.¹³⁵ With the help of prospective MRI studies, a gradual brain atrophy in individuals with MS has since been demonstrated,¹³⁶ which is believed to result from the loss of axons and myelin.¹³⁷ The atrophy is primarily considered a marker for neurodegeneration in progressive side of MS, but it is also evident in individuals early in the disease course already after the first relapse.¹³⁸ Of note, the brains of healthy individuals also decrease in size with increasing age, but not to the same extent as in individuals with MS.

A common measure to estimate atrophy in MS, and other neurodegenerative diseases, is to measure the total volume of the brain.¹³⁹ This volume, sometimes referred to as brain parenchymal volume, can be related to the skull volume, resulting in a normalized measure called the brain parenchymal fraction.¹⁴⁰ Several different software programs can be used to measure brain volume, but comparing examinations from different scanners can be problematic.¹⁴¹

Given that an individual's brain volume can be affected by numerous factors — including time of day, fluid intake, medication, pseudo-atrophy post-initiation of immunomodulatory treatment, and other considerations like cardiovascular comorbidities and genetic makeup — it can be challenging to rely on yearly atrophy measurements to monitor treatment effects.¹⁴² Based on what we know today about the methodological difficulties, it seems unlikely that MRI data suggestive of atrophy in an individual patient would justify escalating an immunomodulatory treatment targeting neuroinflammation.

Callosal atrophy

Another early MRI measure of atrophy was based on the area of the corpus callosum in a midsagittal image, which is clearly visible in MRI and is easily measurable.¹⁴³ The demyelination and atrophy of the corpus callosum are considered typical pathological and radiological features, arising from Wallerian degeneration and impact on white matter.^{143,144} In healthy individuals, there is on the other hand virtually no atrophy in the corpus callosum.¹⁴⁵⁻¹⁴⁷ A study of 23 MS patients tracked over more than 17 years revealed an annual callosal atrophy rate of 1.2%; notably, atrophy was more pronounced during the first half of the study period at 1.6%, compared to 0.9% in the latter half.¹⁴⁸ A separate cohort of 37 patients, representing various MS subtypes and monitored over 9 years, reported an annual callosal atrophy rate of 1.8% (95% CI 1.4–2.2).¹⁴⁹

The use of the corpus callosum area (CCA) as a measure for neurodegeneration is considered to have the advantage of being reliably calculated even if MRI examinations are conducted using different scanners and protocols; the correlation with common clinical outcome measures also appears to be better than other atrophy measures.^{148,150,151} This makes the CCA suitable for retrospective studies.

A limitation is that measuring CCA in clinical settings often need manual execution, introducing variability based on the evaluator. Nonetheless, this method boasts high consistency and alignment across different assessors, and it correlates more favourably with key physical and cognitive clinical indicators, such as the EDSS and symbol digits modalities test, compared to other brain atrophy measurements.^{148,151,152} As a result, the CCA can rapidly be determined during a standard MRI evaluation without the necessity for specific software. By normalizing the CCA to a consistent intracranial metric, potential scaling differences can be reduced, ensuring comparability.

Atrophy following AHST

Directly following AHST for MS, there are reports of continuous and even accelerated brain atrophy.¹⁵³⁻¹⁵⁵ This could result from ongoing Wallerian degeneration, stemming from intense brain inflammation in axons already marked for degeneration, even in the absence of inflammatory activity. Certain drugs, such as busulfan, might have neurotoxic effects, further

contributing to this atrophic process. Interestingly, one study highlighted that the atrophy rate returned to normal levels around 2.5 years after AHSCT, lending credence to the aforementioned hypothesis.¹⁵⁴

No evidence of disease activity

Historically, the annualised relapse rate has served as the predominant primary endpoint in clinical trials. However, as treatments became more efficacious, more ambitious goals for treatment were set.^{104,156} The term ‘NEDA’ for MS was introduced in the editorial of Multiple Sclerosis and Related Disorders as an aggregate measure of treatment effect, characterised as a ‘working definition of cure’, drawing inspiration from oncological paradigms. The authors advocated for the employment of the term NEDA, postulating a 15-year duration of no evident disease activity as a ‘starting point for defining a cure.’¹⁵⁷ The inaugural definition of NEDA included absence of relapse, EDSS progression and MRI activity, denoted as NEDA-3.^{157,158} By 2016, the absence of brain volume loss was incorporated into the NEDA definition, referred to as NEDA-4,¹⁵⁹ often defined as an occurrence of ‘whole brain atrophy’ exceeding 0.4% per year.¹⁶⁰ Of presently ongoing treatment studies against RRMS, fewer than 10% however include a brain atrophy measure as one of the outcomes, and of those almost always a measure of global brain volume.¹⁶¹ In recently published studies detailing the treatment effects of immunomodulatory drugs, the older and more established NEDA-3 is still used as an outcome measure.¹⁶²⁻¹⁶⁵

Biomarkers in cerebrospinal fluid

Normally, antibodies are present in the CSF in low concentration. These antibodies diffuse over the blood-brain barrier and originate from plasma cells in the periphery. However, in over 95% of European patients with MS, there are indications of intrathecal antibody production within the CNS.¹⁶⁶ This can be shown by the demonstration of CSF specific OCBs on isoelectric focusing¹⁶⁷ or by increased levels of IgG antibodies in the CSF relative to the blood.¹⁶⁶ These intrathecal antibodies are thought to stem from expanded clones of CNS resident plasma cells and plasmablasts.¹⁶⁸ OCBs are not entirely specific to MS, and can be seen following various inflammatory, infectious and rheumatological diseases.¹⁶⁹ Once formed, the bands typically remain consistent over time in MS.¹⁶⁹

Neurofilament light (NFL) is a marker of axonal damage in the CNS, elevated in many neurological degenerative conditions,¹⁷⁰ and used in clinical praxis as a biomarker of ongoing disease activity in MS. A high level of NFL in the CSF at onset, is associated with a worse prognosis of MS.^{171,172}

Lived experience

The historical privilege and duty of healthcare and medical science have been to independently formulate both the problems, questions, and seek the answers. Especially in a data-driven age, in a commendable quest for outcome measures to define, measure, improve, and report, it can be easy to lose sight of the patient-centred perspective,¹⁷³ even though the goals of healthcare often align with those of the patient. The problem with a perspective that primarily starts from the provider's side is that the patient's objectives may risk being overlooked, which contradicts the fundamental role of a physician: to treat human beings, not just diseases.¹⁷⁴ This is stating the obvious for skilled, experienced clinicians who have always aspired to place the individual at the centre – first and foremost, by listening to them.¹⁷⁵

Although many neurologists possess extensive first-hand knowledge of various treatment strategies and the ensuing patient outcomes, the scientific literature on patients experiences with different MS therapies is remarkably limited. At best, there are quantitative data on self-reported 'quality of life', but a deeper understanding of the lived experience, which cannot be achieved through survey studies, is mainly lacking.

Miller *et al.* is the exception, as they have published articles employing a phenomenological method to examine the lived experience of drug treatments with interferon beta, glatiramer acetate, and natalizumab. Experiences with interferon beta revolve around the medication's injection-related side effects, although it also instilled hope for the future.¹⁷⁶ Glatiramer acetate served as a tool to attempt to retain control over one's body.¹⁷⁷ Natalizumab was well-tolerated but involved a delicate balance between the risk of the dreaded side effect, progressive multifocal leukoencephalopathy, and the treatment's effectiveness.¹⁷⁸ As for AHSCT, there are only reports on quantitative data related to quality of life published, suggesting an decrease in the self-reported disability of life post-treatment.^{179,180}

As nursing research has gained greater prominence, methods for systematically gathering, organizing, and incorporating patient experiences have become more sophisticated. These methods often stem from a humanistic scientific tradition rather than a medical one, and because their results cannot be quantified through statistical analysis, they are often labelled as 'qualitative', as opposed to 'quantitative'. Insights gained from qualitative research can enhance the understanding of patients' motivations, particularly in their choice of treatments; by leveraging these insights, healthcare professionals can offer more personalized support in guiding patients' treatment decisions.¹⁸¹

The concept of 'lived experience' is a central idea within phenomenology, which has its roots in the teachings of Edmund Husserl, and was subsequently developed and expanded by, among others, Martin Heidegger and Maurice Merleau-Ponty.¹⁸² As phenomenology is understood in nursing research today, it focuses on the subjective experience ('noesis', 'Vorhandenheit' /

'appearance') of a phenomenon ('noema', 'Zuhandenheit' / 'essence') and the idea that consciousness, and therefore experiences, always have intentionality, meaning they are directed towards something. The individual experience of people also exhibits intersubjectivity (comparable to the Heidegger's concept of 'In-der-Welt-Sein'); they are connected by a shared cultural preunderstanding, and meaning is created together with others. The researcher is obligated to set aside their own assumptions in order to approach the subject of study with an open mind ('epoché' / 'bracketing'), being in search of the essence of their experience.¹⁸³ With this perspective, within a phenomenological context, lived experience is seen as the subjective encounter of an individual, rendering it a suitable approach when investigating experiences related to a particular medical treatment.

Cerebral blood flow and cerebrovascular reactivity

CBF, as previously described, can be examined in various ways. Different methods have distinct strengths and weaknesses, and the choice of method depends not only on the purpose and research question but also on availability and resources.

Transcranial Doppler sonography can be performed quickly, inexpensively, non-invasively, and provides immediate results.¹⁸⁴ However, it primarily measures blood flow in larger arteries, not the perfusion of brain parenchyma. MRI methods for measuring CBF differ, but two common approaches are ASL¹⁸⁵ and dynamic susceptibility contrast (DSC) MRI.¹⁸⁶ DSC MRI uses gadolinium contrast to measure perfusion, while ASL MRI employs radiofrequency pulses to magnetically label water protons in arterial blood, which can then be imaged and compared to an image without magnetic labelling. ASL is believed to yield more absolute values than DSC, though the latter has a well-established clinical application, notably in the diagnosis and follow-up of brain tumours.¹⁸⁷

¹⁵O-water PET is considered the gold standard for measuring CBF, since water is freely diffusible, metabolically inert and has a very high extraction. Hence, it provides absolute quantification of tissue perfusion.^{188,189} This is done by measuring the radioactivity in arterial blood, which serves as an input factor in kinetic modelling, described in more detail below. The method requires establishing arterial access, often performed by an anaesthesiologist. Although this fairly straight-forward, it does represent a logistical drawback compared to methods like xenon-computed tomography, Doppler sonography, and MRI. Efforts have been made to measure the input factor without arterial blood sampling, but no method is in routine use yet. Unlike Doppler sonography and MRI, ¹⁵O-water PET involves exposure to ionizing radiation, albeit in small doses due to the isotope's short half-life. This short half-life also

necessitates that cyclotron production occurs at the same location as the PET camera, demanding substantial logistical and resource considerations.

The measurement of CVR is based on two consecutive CBF measurements, between which a vasodilatory intervention is introduced, believed to reveal cerebrovascular reserve or reactivity. Vasodilation can be achieved through various methods, such as the administration of acetazolamide (ACZ), a breath-holding test, or the inhalation of CO₂-enriched gas. An advantage of ACZ is that its dosage can be very precise and standardized.

¹⁵O-water PET

After production with an on-site cyclotron and quality controls, the tracer ¹⁵O-water is injected intravenously as an automated controlled rapid bolus dose, which can be dosed based on the body weight or with a fixed dose. The PET scanning begins concurrently with the intravenous bolus injection and continues for 4-10 minutes. The ¹⁵O-water mixes with the venous blood, reaches the heart's right atrium and ventricle, and then proceeds via the pulmonary circulation back to the heart's left atrium and subsequently into the systemic arterial circulation, including the brain. Only a few seconds after the injection, the radiolabelled water reaches the brain, as illustrated by the time-activity curve in Figure 3.

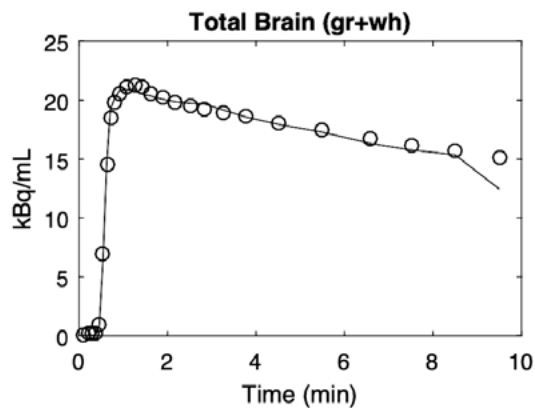


Figure 3. Example of a time-activity curve and single-tissue compartment model fit

¹⁵O-water behaves in the body just like regular water, moving freely from the blood across the blood-brain barrier into the brain tissue, with a rate constant K_1 , without being metabolised or trapped intracellularly. The same molecules can also move in reverse from the brain tissue back into the blood, just like non-labelled water, with a rate constant k_2 (Figure 4).

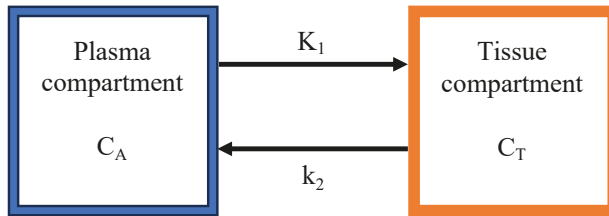


Figure 4. The principle of a single-tissue kinetic model.

The commonly used term ‘CBF’, is often used to describe what more accurately could be termed ‘cerebral perfusion’. In this dissertation, the term ‘CBF’ is used in accordance with the accepted nomenclature, synonymously with ‘cerebral perfusion’.

Roughly at the same time as the PET scanner can detect radioactivity in the brain, constituting the ‘tissue compartment’ C_T , radioactivity can also begin to be measured in the blood, which represents the ‘blood compartment’ C_P and the ‘input function’ (Figure 4). The radioactivity in the arterial blood is measured thanks to continuous arterial sampling at 3 mL/min throughout the 10-minute PET scan with a special sampling system, visible in blue in the lower right corner of Figure 5. Thereby, a curve of the arterial blood radioactivity in C_A over time can be created. For calibration of the individual on-line blood curves, two extra blood samples are taken at 5 and 10 minutes and analysed in well counters.



Figure 5. A PET scan being performed with on-line arterial blood sampling for determination of the arterial input function.

From the moment of injection until the radiotracer reaches the brain and the machine measuring radioactivity in the arterial blood, the tracer has time to mix with the blood, become diluted, and experience turbulence in a process called ‘dispersion’. Furthermore, it undergoes a delay while traveling through the tubing of the blood system, where additional dispersion occurs. Both dispersion and delay must be incorporated into the kinetic model, and appropriate corrections need to be made.

PET offers limited anatomical visualisation of the brain. To investigate the blood flow in specific brain regions, such as in the grey and white matter, the high resolution provided by MRI is essential. This can be achieved by co-registering the PET images with T₁-weighted images, allowing the uptake in distinct structures to be studied separately by segmentation of the brain tissue into various volumes of interest (VOI) with a special software. Time-activity curves are constructed, representing the mean radioactivity concentration (kBq/mL) over time in the different VOIs, as shown in Figure 4.

For the calculation of the cerebral blood flow, a kinetic modelling of the PET data is necessary. For ¹⁵O-water PET, a single-tissue compartment kinetic model is usually applied, utilizing the TAC from a specific VOI, C_T(t), and the time-activity curve from the arterial blood C_A(t), as outlined in Figure 4.

The relationship between C_A(t) and C_T(t) can be represented by a convolution integral, which mathematically describes how, over time, the radioactivity concentration in the arterial blood C_A(t) influences the radioactivity concentration in the tissue C_T(t):^{190,191}

$$C_T(t) = K_1 \times \int_0^t C_P(\tau) \otimes e^{-k_2(t-\tau)} d\tau$$

The CBF can then be calculated as:

$$CBF = K_1$$

Vaccination and multiple sclerosis

Individuals undergoing immunomodulatory treatment for MS face a heightened risk of severe infections compared to the general populace; this risk is most pronounced with continuous high-efficacy treatments, such as rituximab.¹⁹² As early initiation of long-term high-efficacy treatments is becoming more prevalent, (Figure 1 and 2) and expected to last many years, the issue of vaccinations and MS has never been more pertinent, especially given the urgent need for vaccination in light of the COVID-19 pandemic. A recent European consensus document on the subject of MS and vaccination has been published by European Committee for Treatment and Research in Multiple Sclerosis and European Academy of Neurology.¹⁹³ Today, various vaccines are available to prevent infectious diseases. Over the years, concerns arose about the immune response triggered by vaccines potentially impacting the disease progression adversely. However, extensive studies with inactivated vaccines have reassuringly not found evidence of such a risk,^{194,195} including with the COVID-19 vaccine.¹⁹⁶

With the outbreak of the COVID-19 pandemic in early 2020,¹⁹⁷ physicians and patients on immunomodulating therapies worldwide were faced with difficult decisions: should treatments continue or be paused, and how should the risk of, for example, MS disease breakthrough be balanced against the risk of contracting severe COVID-19? It soon became apparent that the risk of developing severe COVID-19 was 2-3 times higher with B-cell depleting anti-CD20 therapy, although, fortunately, not the mortality.^{198,199} In Sweden, where half of all ongoing drug treatments for MS consist of rituximab, a common strategy emerged to significantly extend the infusion interval of rituximab in patients with stable disease.²⁰⁰ Furthermore, the initiation of treatments that might elevate the risk for severe COVID-19, such as alemtuzumab, anti-CD20 treatment or AHSCT, was temporarily avoided.²⁰¹

As vaccines started becoming available in early 2021,²⁰² clinicians faced additional questions: how best to ensure that patients treated with anti-CD20 therapy, who were most at risk of a severe COVID-19, were protected by the vaccine?²⁰³ Knowledge about the response to other vaccines administered during rituximab treatment indicated a weak or absent immune response.²⁰⁴⁻²⁰⁹ Consequently, guidelines typically recommended administering vaccines prior to starting the treatment or after a specific time interval following the previous anti-CD20 infusion.^{210,211}

SARS-CoV-2 and basic vaccine immunology

Severe acute respiratory syndrome corona virus 2 (SARS-CoV-2) is the virus responsible for COVID-19. The spike protein on the virus's surface contains a region known as the receptor-binding domain (RBD), which allows the virus to bind to the ACE-2 (angiotensin-converting enzyme 2) receptor on human cells, facilitating entry into the human cells. The nucleocapsid protein in the core of the virus plays a crucial role in viral replication and packaging.

mRNA (messenger ribonucleic acid) vaccines, such as the Pfizer-BioNTech vaccine (tozinameran), function by introducing a small piece of mRNA that encodes the spike protein of SARS-CoV-2. This mRNA enters cells at the injection site, often muscle cells, and is processed by ribosomes, just like other mRNA molecules. This processing leads to the production of the SARS-CoV-2 spike protein. Subsequently, antigen-presenting cells, such as dendritic cells and macrophages, engulf the spike proteins and present the antigens to various cells, including T cells, initiating an immune response.

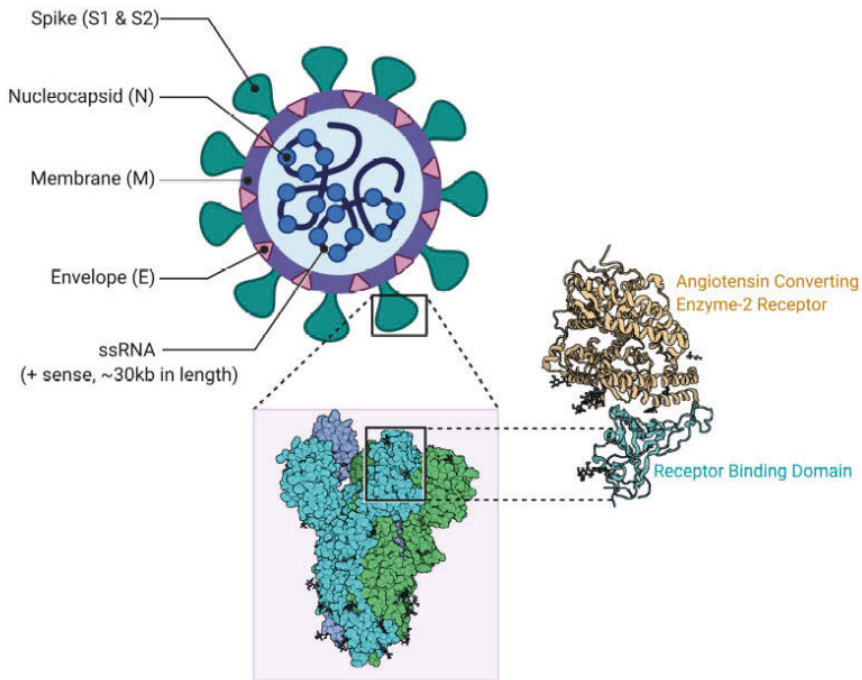


Figure 6. SARS-CoV-2

SARS-CoV-2 structure with molecular architecture of the Spike S protein and ACE2-Spike S protein complex. Illustration by Rohan Bir Singh, MD; made with Biorender. Licenced under the Creative Commons Attribution 4.0 International.²¹²

The immune response to both infection and vaccination involves two primary components: humoral and cellular immunity.²¹³

Humoral immunity is primarily mediated by B cells, specialized white blood cells, and results in the formation of antibody-producing plasma cells and memory B cells.²¹³ Humoral immunity can be assessed by serological measures through determining the concentration of antibodies (quantification) or by measuring the binding capacity of the antibodies to an antigen, e.g., the ACE-2 receptor or spike protein (qualitative measurement).

Cellular immunity focuses on the role of T cells and can be assessed by measuring the release of cytokines from peripheral blood mononuclear cells (PBMCs) when they are exposed to viral antigens. PBMCs, which are isolated from whole blood samples, include all cells with a round nucleus, including B cells, T cells, monocytes, natural killer cells and dendritic cells.²¹⁴ Upon exposure to viral antigens, such as peptide pools from SARS-CoV-2, activated T cells release cytokines like interleukine 2 (IL-2) and interferon gamma (IFN- γ). These cytokines can be quantified using various techniques, including the Fluorescent Spot Assay (FluoroSpot).²¹⁵

Background and aims of the studies

Study I

With the introduction of highly effective immune reconstitution treatments for MS, such as AHSCT, there are now individuals who have been living for many years without signs of ongoing disease activity, even in the absence of ongoing immunomodulatory treatment. There is therefore a need for a terminology with clear definitions to describe the state of these individuals. To address this, and to report the ten-years outcome of the first ten persons treated with AHSCT for MS at our hospital, we conducted tests involving six different outcome measures to encompass the inflammatory and neurodegenerative aspects of the disease. The primary endpoint was ‘sustained complete remission of MS’. This was considered to be present if the all the following criteria were fulfilled for a period of at least five years and if no disease-modifying treatment was started:

- No clinical relapses occurred.
- No EDSS progression was present.
- No MRI event was detected.
- No ongoing atrophy was demonstrated.

Further, MS was considered ‘resolved’ if ‘sustained complete remission of MS’ was present *and*:

- Intrathecal IgG production was absent.
- No evidence of axonal damage was present.

Study I thus aimed to investigate whether AHSCT could lead to ‘sustained complete remission of MS’ and to determine whether the disease could be considered ‘resolved’ in any of these patients.

Study II

The father of neurology, Jean-Martin Charcot (1825-1893), noted that no one had recovered from MS, and that the disease inexorably progresses. This bleak picture of the disease has continued to prevail for most of the 20th century and perhaps still does today for many. However, with AHSCT, long-lasting freedom from what we call 'disease activity' has been achieved without ongoing immunomodulatory treatment, and for many, neurological functioning has improved after treatment, as extensively described in Study I. But how does this affect the person who has been diagnosed with MS, experienced a dramatic course of illness, received treatment, and then improved? How does one perceive oneself, one's health, and one's diagnosis today, ten years later? Questions of this kind require a qualitative method to be answered.

Study II thus aimed to explore the long-term lived experiences of people with MS treated with AHSCT.

Study III

Concerning the long-term outcomes after AHSCT for RRMS, there is a concern that patients may still progress to SPMS. The cohort of ten RRMS patients treated with AHSCT (Study I-III) in this dissertation showed no clinical progression over ten years, but subclinical neurodegeneration might emerge over time. PET offers promise for studying ongoing pathological processes in vivo, and various tracers are under investigation.²¹⁶ To explore PET's potential in imaging neurodegeneration, we examined ten SPMS patients and ten healthy controls using three PET methods: ¹⁵O-water with ACZ challenge, ¹¹C-PK11195, and ¹¹C-L-deprenyl. The rationale for examining CBF and CVR was the emerging interest in the connection between impaired CBF and MS, and that ¹⁵O-water PET, despite being gold standard for measuring CBF, never had been done in MS patients before. Prior ¹⁵O-water PET studies in other neurodegenerative conditions, such as Alzheimer's disease, have also revealed a compromised CBF; these studies demonstrated that CBF alterations could be identified several years before the clinical diagnosis of Alzheimer's disease and can serve as a predictive marker for the transition from mild cognitive impairment to Alzheimer's disease.^{217,218}

Study III thus aimed to investigate whether CBF and CVR were impaired in patients with SPMS compared to healthy controls. We then wanted to explore whether similar changes could be observed in patients treated with AHSCT ten years prior.

Study IV

During the COVID-19 pandemic, the urgent question arose regarding how to effectively vaccinate individuals undergoing rituximab treatment. Since SARS-CoV-2 was a novel virus, there was no established evidence to guide this. Existing guidelines recommended waiting for a specific time after rituximab treatment before vaccinating. However, we hypothesized that the level of B cells, rather than the time since the last rituximab infusion, determined the vaccine response.

Study IV thus aimed to investigate factors associated with a favourable vaccine response to tozinameran in rituximab-treated individuals with MS.

Approaches to an ethical founding

Navigating as a medical researcher in the 21st century requires, among many other necessary tools, both an ethical map and a compass. The eccentric antique philosopher Heraclitus claimed that ‘all things come about by strife’,²¹⁹ and when it comes to the historic development of clinical research ethics, this is indeed an appropriate aphorism. Few areas are likely to have been shaped to such an extent by war, scandals and maltreatment.

Medical research ethics in a historical context

One of the earliest examples of an elaborated strict ethical code for medical research is the 1931 Weimar Republic ‘Richtlinien für die neuartige Heilbehandlung und für die Vornahme wissenschaftlicher Versuche an Menschen’,²²⁰ developed in response to an appalling medical scandal of the time, involving the death of 75 children in experiments with tuberculosis vaccine.²²¹ This remarkable document was however not sufficient to prevent the atrocities of the Nazi experiments on humans in the following two decades. In the following post-war Nuremberg trials of 1947, a ten-point statement on fundamental principles on ethics in medical research was presented by the prosecutors, now commonly known as the Nuremberg Code.²²²

Striving to elaborate and adapt the ethical principles in the Nuremberg Code to clinical research, World Medical Association developed their own ethical recommendations ‘guiding doctors in clinical research’, presented in the Declaration of Helsinki 1964 and subsequently repeatedly revised.²²³

Studies clearly violating the Nuremberg Code were however pursued even after 1947. One infamous example is the Swedish Vipeholm Study between 1945 and 1954, where hundreds of institutionalized psychiatric patients were subject to extensive, irreversible and involuntary caries experiments.²²⁴ Half a century later, in 2001, one of the main authors defended the study and argued that, although an ethics committee today would not have accepted such a study design, the Vipeholm Study was conducted before the Declaration of Helsinki and that the results made a significant impact on dental health for coming generations; ‘the end sometimes justifies the means’.²²⁵

Today, most clinical studies involving humans include a semi-liturgical statement assuring editor, reviewers and the readership that the present study

was conducted in accordance to the Declaration of Helsinki; the studies included in this dissertation are no exception to that rule. The principles of the declaration are now often incorporated in the national legislation, e.g., in the Swedish Ethical Review Act of Research Involving Humans. However, just as health is not merely the absence of disease, an ethically justified study is more than a study protocol within the boundaries of the Declaration of Helsinki and approved by an ethics committee. Ethical considerations should permeate the scientific method from the very definition of a problem to storing the data many years after publication, and in a wider perspective make us ready to abandon our beliefs when we are proven wrong.

In the last few years, medical research ethics has once again caught the attention of the general public with the grave scandal of the surgeon Paolo Macchiarini who, without successful preclinical animal studies and in conflict with the prevailing biomedical paradigm, repeatedly transplanted synthetic tracheae in humans with disastrous results.^{226,227} It is striking how those experiments in all its aspects violate not only the Declaration of Helsinki, but also the Nuremberg Code and even the Reichsrichtlinien. Without doubt, such scandals damage the trust in medical research – a trust that is crucial for people who consider participating in clinical trials. On the other hand, there is a risk that the establishment of insurmountable regulations in response to obviously unethical and presumably illegal acts will quell provocative and unconventional ideas, resulting in a more homogenic research environment.²²⁸

Ethical incentive to perform research when others do not

In court, Macchiarini argued that the tracheal transplantations were performed under ‘compassionate care standards’, implying that the procedures, although unconventional, were made in good faith, with no other better and less dangerous treatment available.²²⁹ This thesis includes two groups of MS patients with therapeutic strategies that likewise can be considered unconventional: in Studies I-III, treatment with AHSCT, and in Study IV rituximab.

At the time when the first Swedish AHSCT for the purpose of treating MS was performed in May 2004, this had never been done in any Nordic country before, and the decision was motivated as being an ‘acute rescue treatment’ under compassionate care.¹¹⁹ Unlike the case of the Macchiarini’s tracheae, AHSCT had however been performed for many years for treating haematological conditions, it had successfully been carried out in animal models for MS and other auto-immune diseases,²³⁰ and AHSCT for MS had been performed abroad, e.g., in a multi-centre study including 85 MS patients.¹¹⁶ The decision to proceed with AHSCT in the patients included in Studies I-III, although being an unconventional treatment, was thus in legal and ethical accordance with ‘science and proven experience’;²³¹ ²³² the selection of patients was restricted to very severe, ‘malignant’ cases and was monitored through a

thorough follow-up.²³³ Most importantly, the patients (and for the two children, their parents) were provided extensive information about the risks of AHSCT, and the choice was clearly their own, as reported in Study II.

The cohort in Study IV includes patients treated with rituximab, or patients planned to start this medication. In Sweden, rituximab is the most common disease-modifying treatment for MS, despite lacking formal indication for treating MS, starting with a few patients from 2006 and onwards, and now constituting almost 60% of all MS-patients with ongoing disease-modifying treatment.²³⁴ The first systematic use of rituximab for MS in Sweden was launched following a promising phase II trial,²³⁵ and given within the frames of a national multicentre open-label phase II-study.²³⁶ Encouraged by the favourable results and clinical experience of both high tolerance and efficiency, patients failing on other first-line treatment gradually accumulated in the rituximab group. A parallel development in the understanding of multiple sclerosis in the last decade, facilitating the use of rituximab, is a gradually lower toleration towards ongoing inflammatory activity. With increasing access to highly efficient immunomodulatory treatment and reports of beneficial long-term outcome with early high-efficacy treatment strategy,²³⁷ the general consensus among neurologists in Sweden today is that relapses and new lesions on MRI with an ongoing immunomodulatory treatment motivates a switch to a more effective treatment.²³⁸

One could argue that the possibility of Swedish physicians to prescribe medication off-label and without rigid treatment guidelines from the authorities, thus has paved the way to a rapidly evolving, diverse and cost-effective treatment landscape, where scientific results can be put into practice to maximum benefit of the patient. When prescribing medication off-label, the treating physician has the responsibility.²³⁹ Thus, with an extensive off-label prescription follows an ethical demand for a rigorous clinical follow-up and monitoring, and in wider sense a responsibility to conduct research for deepening our understanding of a certain therapeutic strategy, when such studies are not expected to be performed by the medical-industrial complex. Shouldering this duty, an academic randomised controlled phase III-trial was finally conducted comparing rituximab to dimethyl fumarate, where clinical and neuroradiological superiority of rituximab could be demonstrated.²⁴⁰ In this spirit, Study IV contributes to a deepened knowledge about the safe use of rituximab, aiming to establish a practical strategy to reach a protective vaccine response in patients with MS.

In parallel to the case of rituximab treatment for MS, the research about AHSCT for MS has not been performed by the medical-industrial complex, but rather by the academic institutions world-wide. Although AHSCT for MS cannot be classified as off-label prescription, since the treatment is a procedure and not one single medication, the same ethical appeal to conduct studies can be applied, constituting a major motivation for our research group.

Informed consent in the 21st century

One of the main critics against the Vipeholm Study was the lack of informed consent from the participants,²⁴¹ despite the very first paragraph of the Nuremberg Code stating that '[t]he voluntary consent of the human subject is absolutely essential'.²²² Such a rigid formulation however, formulated by lawyers in the context of a juridical process, risks creating a significant scientific gap in how to treat patients, from whom a consent is not possible to obtain, e.g., patients with acutely impaired consciousness, aphasia or infants. Also, individual consent in large retrospective cohort studies is generally not possible to obtain, and the permission to proceed with a study is then given by an ethics committee.

Information to potential study participants should ideally be both easily comprehensive and sufficiently exhaustive, and never be persuasive. Inclusion of participants in the present Studies I-IV were made in different manners, although all were provided oral and written information and given time to decide upon their participation. All gave their written consent and were repeatedly informed that they could withdraw from the study at any time.

The ten patients in the AHSCT group of Study I-III were asked for participation by their treating physician through personal communication, followed by written information. In Study II, where the participants in this group speak for themselves, it is easy to sense a strong and loyal engagement in the research conducted and an almost infinite trust in the treating physician. We cannot know if they would have declined participation in Study I-III if the offer would have come from another researcher with no previous relationship to them. It is likely to presume that the personal trust is the foundation on which their participation rests. Such a relationship between researcher and participant, based on mutual respect and trust, may be the quintessence of the ethical guidelines for medical research of the past decade.

Potential participants in the SPMS group of Study III were identified through the Swedish MS registry.²⁴² The selection of potential participants was based on age and sex, to match both the AHSCT group and the healthy controls. As the mean age in the AHSCT group was relatively low in comparison to most SPMS patients, the number of potential participants in the SPMS group was limited. Since our impression is that most persons with MS with follow-up at our clinic are happy to participate in research, we chose to contact the potential participants directly. Thus, the identified potential participants were phoned by a researcher with no previous relationship to the patient and first provided oral information about the study, followed by written information by mail. An alternative, less direct strategy, would have been to try reaching those patients through general advertisement, with a significant risk of not reaching out, but with a lower risk of the potential participants feeling persuaded to participate.

To conduct almost every kind of research in the natural sciences, it is necessary to tabulate information and often, one needs to link this information in some way to the source, i.e., the participant. Performing ethical medical research in a digital and globalized era is therefore not possible without carefully considering how to handle sensitive information about the participants. In the last decade, there has been a major shift in the view on personal data, following the implementation of the European General Data Protection Regulation in 2018.²⁴³ Thus, this is not only a question of ethics, but also of law. In Studies I-IV, the participants were throughout pseudonymised to hide the link to the individual participant, and the keys were stored in separate, encrypted documents. For example, in Study III, the pseudonymisation was performed by a researcher not directly involved in the later data processing and interpretation, and in the measurements of corpus callosum area in Study I, pseudonymisation of all MRI scans were made in a random order. Apart from protecting the direct link between the participant and the data, pseudonymisation also aims to increase validity, as our preunderstanding and hypotheses might influence how we process and interpret the data. However, also pseudonymised information can be sensitive, and must be handled with great care. This was especially apparent in Study II, where printed versions of the transcripts of the full interviews together with USB-sticks with the digital files were posted to co-authors and not e-mailed, following the sensitive nature of the data. Also, data were not stored in cloud-based services, since many servers are abroad and protection of sensitive data cannot be assured.

However, once published, the data are open for everyone to read, everywhere and infinitely – one of the advantages of performing and taking part of research in the 21st century. Many journals encourage or even demand the publishing of raw data, to support the results and conclusions. While this is an attempt to ensure validity, support data sharing and facilitate replication, it is not a strategy without ethical concerns. Although the data are pseudonymised or even anonymised, individuals may identify data describing themselves, or others, especially in small case-series or cohort studies where individual data on the participants are published. In this thesis, such data are published in Studies I and II, and once published, the data cannot be withdrawn. Therefore, to avoid unexpected or unpleasant surprises, the manuscript of Study I was shared with the participants and consent was reconfirmed before submission. For methodological reasons, the manuscript of Study II was not shared in the same way, as discussed below. However, the data in Study II do not consist of external measurements but of the participants' own verbal communication, well aware of the interview's purpose, the aims and the fact that the interviews were recorded.

Conducting research in parallel with a clinical routine

As the COVID-19 pandemic entered Sweden in 2020, there were great concerns about severe disease courses in patients with ongoing immunosuppressive treatment. Therefore, several strategies were applied to minimize the risk, including individual assessment of extending treatment intervals in MS patients with ongoing rituximab treatment.^{200,201,244} Soon, international and national data confirmed a two to three times higher risk of a severe COVID-19 when treated with rituximab, although the absolute risk fortunately was rather low.^{198,245} At the beginning of 2021, vaccines became available at our hospital for selected groups, and we identified patients with rituximab treatment as a prioritized group; besides having a higher risk of a severe COVID-19, we also postulated a high risk of achieving a poor humoral immune response after immunisation, based on previous knowledge about vaccination and rituximab treatment.^{204,205,207} Some guidelines for vaccination during immunomodulating treatment proposed a minimum of three but preferably six months from the last infusion of rituximab before vaccination,^{210,211} while national guidelines by the Swedish MS Association suggested a B-cell count of at least 20/ μ L.²⁴⁶ Based on the vital role of B cells in the development of a humoral immune response after vaccination, and our clinical experience that B-cell repopulation after rituximab infusion has a substantial inter-individual variation, we decided to develop a clinical praxis with vaccination for COVID-19, as further described below. In short, patients with ongoing rituximab treatment were offered to perform flow cytometry six months after the last infusion of rituximab for the determination of B-cell count; if 20/ μ L or above, we scheduled vaccination with two doses of tozinameran at our clinic, followed by infusion of rituximab six weeks after the second dose. If under 20/ μ L, we proposed waiting another two months, and then repeat the flow cytometry. We suggested proceeding with vaccination after three flow cytometries even if the B-cell count was under 20/ μ L.

Our vaccination strategy was neither intended, nor designed to constitute a clinical trial, but was solely striving for a maximal protection against severe COVID-19 for our patients, based on the best of our knowledge in January 2021. Nonetheless, we identified a unique opportunity to study the vaccination effect in what was a previously remarkably unexplored field. Our hypothesis was that the humoral immune response to vaccination would increase with higher B-cell count at time of the vaccination. Patients with ongoing or planned rituximab treatment were invited to participate in the study through giving blood samples after the vaccination to check the immune response. Although the clinical routine and the study ran in parallel, the participation (and the non-participation) in the study did not affect the clinical practice. Those who wanted to proceed with vaccination despite having low B-cell counts were also informed about the study and invited to participate. In this way, the

study design not only allowed the diverse cohort of the real world, but was strengthened by it.

Although we had few ethical concerns regarding the study itself, the clinical procedure however raised important ethical questions. Was it morally justified to propose a delayed vaccination and prolonged interval to the next rituximab treatment for those with a low B-cell count? Were we withholding effective protection against a dangerous, pandemic disease while increasing the risk of a deterioration in MS? The decision to propose the clinical routine to our patients was based on the following facts and hypotheses: (1) there is a higher risk for patients with rituximab to have a severe COVID-19 disease course, (2) humoral immune response after vaccination for other diseases is impaired when having rituximab treatment, (3) humoral and not cell-mediated immunity constitutes the major protection against COVID-19, (4) humoral immune response after vaccination is related to the level of circulating B cells and (5) the rituximab infusion interval in clinically and radiological stable MS patients can be extended to at least one year without a high risk of disease recurrence. On the contrary, we argued that proceeding with a general vaccination procedure without believing in a following favourable protection would be immoral; firstly, the patients could be given a false sense of security and thus risk getting a severe COVID-19, and secondly, we had an obligation to use the few available vaccine doses to the best of our knowledge.

As we did not have any definite answers, our strategy was to be as transparent as possible about the present knowledge. The patients were informed about our proposed vaccination procedure in an information letter, in which we stressed the current lack of evidence for the best vaccination strategy, and that they could choose to proceed with vaccination at any time, also with a low number of B cells or without previous flow cytometry. We spent a lot of time guiding our well-informed patients on this matter, discussing both immunology and arguments for or against applying our suggested strategy. However, for patients with an active MS or neuromyelitis optica spectrum disorder, we recommended proceeding with the scheduled rituximab treatment without postponement, and adjusting the vaccination accordingly, without consideration of B-cell count.

In the end, it turned out that our main hypotheses were correct, although some patients developed a strong humoral immune response despite a low number of circulating B cells. Also, the optimal B-cell count for humoral vaccine response was higher than previously known. Three participants (5%) had signs of disease activity; although a low proportion, one can never know for sure if they would not have had disease activity if the rituximab infusion would not have been postponed due to our vaccination routines. Was it worth it? What if 30% of the participants would have suffered relapses? Or if someone would have died in COVID-19, unvaccinated, waiting for the B cells to recover? Though not immediately transferable, since the vaccination routine described in Study IV was not part of the study protocol, such questions

expose the very core of the ethical dilemma of clinical trials, especially when comparing an agent to a placebo group. For example, few today would consider it ethical justified to compare a treatment for RRMS against a placebo.

Under different circumstances, Study IV could have been planned as a drug trial, and other questions could have been answered. However, since the primary purpose of the vaccination strategy was not research, this was never considered. Such a setup would have taken longer to implement and likely required a more rigorous ethical review with more extensive regulations. Thus, there is a temptation to adapt clinical routines to serve as a good basis for research. An example of such a setup comes from stroke medicine, where two different treatment protocols were used every other week as the clinical standard of care, effectively creating a randomised intervention group and a control group. This kind of arrangement not only sidesteps ethical review but also undermines opportunities for patients to provide informed consent. Although not illegal, it risks undermining the important trust that medical researchers rely on to continue driving medical advancements forward.

Conducting academic studies today is a major challenge for clinicians, who are often overwhelmed by the demands of daily patient care. With increasing legal requirements for drug trials, there are concerns that only large pharmaceutical companies will be able to conduct such trials in the future. This development risks leading to a narrower, more expensive and poorer range of medications, to the detriment of patients and society.

Treating history with respect

Like medical science in its broadest sense, ethics is not a set of static truths but a constantly evolving field. The ethical principles we uphold in our culture and time may not necessarily apply in the next century or even in the upcoming decade. With the same humility that we hope future generations do not unjustly judge us, we must approach the past with respectful curiosity. Paraphrasing the words of Sir Isaac Newton and other great past thinkers: if we see further, it is by standing on the shoulders of the successes and failures of previous generations.

Statistical considerations

In search of cause and effect

The birth of natural science is often attributed to 585 BC when the Greek philosopher Thales is said to have predicted a solar eclipse. Such an achievement would not have been possible without precise and systematic observations of celestial body movements and accurate timekeeping. Human endeavours to systematically seek relationships in nature have hardly diminished since then. It is through identifying associations that both humans and animals can survive in their environments. However, our strong drive to perceive associations and believe in cause and effect can also lead us astray, as history and contemporary times offer numerous examples.

Even when an association exists, it does not necessarily imply causality. For instance, Study IV, which is a type of case-control study, does not provide sufficient evidence to claim a causal relationship. In this case, we can conclude that we have found an association between B-cell levels and humoral vaccination response. Furthermore, causality, meaning that B follows A, may not be as we envision it within our paradigm but could be due to other mechanisms. An example of this is the development of the first immunomodulating treatment for RRMS, interferon-beta, which mimics the body's antiviral defence and was developed within a paradigm positing that MS was caused by an unidentified virus.²⁴⁷ The medication had a clear inhibitory effect on MS,²⁴⁸ but the virus paradigm was later abandoned, and the exact mechanism of action of this drug class remains incompletely understood.²⁴⁹ As described above, the virus paradigm has however seen some resurgence with the rediscovery of the central importance of the Epstein-Barr virus in the development of MS.¹⁵

Hypothesis testing

Medical research is often hypothesis-driven, and the studies in this thesis follow suit. Based on assumptions, experience, and experiments, we believe we have reason to assume the existence of a relationship or difference. Here are three examples of hypotheses from this thesis for illustration:

H_1 = Atrophy progression stops after 2.5 years in RRMS patients treated with AHSCT. (Study I)

H_1 = The higher the EDSS score in SPMS patients, the lower the cerebral blood flow. (Study III)

H_1 = Patients undergoing rituximab treatment who do not achieve protective antibody levels after vaccination have lower B-cell levels than those who do. (Study IV)

In the scientific method, one starts with an absence of a relationship or difference by formulating a null hypothesis, which is maintained until it can be rejected through statistical analysis.

H_0 = Atrophy progression does not stop after 2.5 years in MS patients treated with AHSCT.

H_0 = There is no relationship between higher EDSS scores and lower cerebral blood flow in SPMS patients.

H_0 = There is no difference in B-cell levels between rituximab-treated patients who achieve a protective antibody response after vaccination and those who do not.

Through hypothesis testing, our goal is to derive conclusions that apply universally, rather than only to our study's participants. Since it is not feasible to examine all individuals in the group we wish to study, we must take samples. Nonetheless, our goal is to make statements about the entire population, such as all rituximab-treated MS patients who are vaccinated against COVID-19.

In classical hypothesis testing, there are two basic errors that can be made. First, one can incorrectly reject the null hypothesis, even though what we believe we have demonstrated would not exist if we had examined the entire population (Type I error). Second, we can fail to reject the null hypothesis, even though it is false, and there is actually a relationship or difference in the population (Type II error). In medical science, a risk of Type I error is often accepted at 5% ($\alpha = 0.05$) by rejecting the null hypothesis at a p value < 0.05 . The typical approach to avoiding Type II errors is to design the study

with sufficient power, which requires a power calculation to determine the minimum number of participants needed to detect a difference between two groups and, consequently, reject the null hypothesis. In this calculation, one must also consider the level of risk accepted for a Type II error, often set at 20% ($\beta = 0.2$). Additionally, assumptions about expected means and standard deviations are necessary, often requiring pilot studies.

It becomes more problematic to perform power calculations when preliminary studies are lacking. Examples of such cases include the investigation of cerebral blood flow in patients with RRMS who have undergone AHSCT in Study III or the level of antibodies against SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) after vaccination in rituximab-treated MS patients in Study IV. Often, researchers rely on available but arbitrary cohorts, such as in Study I, which consisted of the first ten patients treated with AHSCT for RRMS in Sweden, or in Study IV, which included participants during a specific inclusion period. In the case of Study I, the examinations were conducted as part of a larger research project that also included investigations with ^{11}C -PK11195 on the same groups. Based on published data,⁵⁹ a difference of 15% between healthy individuals and MS patients was assumed. With $\alpha = 0.05$ and $\beta = 0.1$, it was estimated that ten participants in each group would be sufficient for the study to achieve adequate power.

Science beyond p values

There is an ongoing critique from the statistical community against the dichotomization of scientific results as either ‘true’ or ‘false’ based on whether the p value falls below or above 0.05. The pursuit of ‘significant results’ and increasingly inventive and complex statistical methods to achieve significance, risks obscuring a deeper understanding of the data and may lead to overlooking important but non-significant differences. Furthermore, a low p value tells us nothing about whether this ‘statistically significant difference’ is relevant or not. If possible, a clear graphical presentation of the data, including individual data points, can often provide researchers and readers with a visual and intuitive sense of the data, with statistics then used to quantify and clarify the findings.^{250,251}

Understanding measurement values

The foundation of our statistical analyses often consists of observations and measurement values with different characteristics. Values are often represented on scales, reflecting our efforts to describe, categorize, and rank our environment to interpret and understand it. To properly compile and interpret our data, we also need to understand its nature, which can pose varying

degrees of intellectual challenge. Many measurements in this dissertation are easily understood intuitively as ratio scales: age, weight, relapse frequency, normalized corpus callosum area. Measurement values like EDSS scores and oligoclonal bands can be more deceptive; EDSS is an example of an ordinal scale with quantitative, discrete variables, making the use of the mean as a central tendency measure inappropriate, and the presence of oligoclonal IgG bands in cerebrospinal fluid is more appropriately considered a nominal scale with qualitative variables (presence or absence of oligoclonal bands). Additionally, assumptions often need to be made about the distribution of measurement values and whether they can be assumed to be normally distributed.

The precision of measurements also has consequences for statistics; the smallest number of significant figures in our measurement values on a ratio scale will limit the number of significant figures in the final result; for example, it would be incorrect in Study I to state the normalized corpus callosum area as 2.92361% if it is based on measurement values of 421 and 144. Too many significant figures would inaccurately indicate a level of precision not supported by the measurement values.

Rank-based statistics

The majority of the statistics in this dissertation are based on rank-based methods. These methods were chosen for several reasons. First, the data under study often cannot be assumed to be normally distributed. Second, the sample sizes are relatively small in most cases. This could mean that extreme but randomly measured data points, known as outliers, could have a disproportionately large impact and risk leading to false correlations, i.e., Type I errors. Furthermore, rank-based statistics are less sensitive to measurement errors than parametric tests, and with a small number of observations, even minor measurement errors can have a significant impact on the results. However, the disadvantages of non-parametric, rank-based tests are that they operate on ranks instead of actual data values, which can sometimes result in overlooking smaller differences between groups.

For example, when it comes to the relationship between two variables, Karl Pearson developed a method in the early 1900s to measure the strength of a linear relationship in a normally distributed population using a correlation coefficient that bears his name.²⁵² A Pearson correlation coefficient of 0 indicates a complete absence of a linear relationship, while 1 or -1 indicates a perfect linear relationship. Pearson's correlation relies on normally distributed data. Therefore, in some cases, we chose to use 'Spearman's rank correlation coefficient' instead,²⁵³ such as in the calculation of the correlation between blood flow and EDSS in Study III or between the level of B cells and the level of antibodies against SARS-CoV-2 in Study IV.

In comparisons between two groups, Student's *t* test is often used when normal distribution can be assumed. In this dissertation, where non-parametric tests were mostly used, Mann-Whitney *U* test was preferred, for example, in comparing blood flow in different volumes of interests between SPMS patients and healthy controls in Study IV or to compare the level of antibodies against SARS-CoV-2 between individuals who were under rituximab treatment and those who had not yet started this treatment in Study III. In these two examples, where the groups are independent of each other and the null hypothesis is that there is no difference between the groups, the Mann-Whitney *U* test is applicable. The *U* value represents the difference in the ranking of the different groups. However, when comparing the level of antibodies before and after vaccination in Study IV, and the two groups being compared consisted of the same individuals, who were sampled before and after the intervention, the null hypothesis is not an absence of difference between the groups but rather the absence of difference between pairs of observations. The groups being compared were dependent, and therefore, the Wilcoxon matched-pairs signed rank test is used; instead of ranking individual observations in each group, as in the Mann-Whitney *U* test, here the differences between pairs of observations are ranked.

Regression analyses

To discern relationships between different phenomena or variables, various regression models are often employed. These variables are often composed of measurable and quantifiable data, such as time, corpus callosum area, cerebral blood flow, or antibody levels. However, they can also include non-measurable categories, such as sex or type of MS. In regression models, one needs to decide which variable is considered the dependent variable and which is the independent one, based on the hypothesis about causality or at least association that one intends to demonstrate. Often, this decision is obvious and intuitive, such as the relationship between time (independent) and normalised corpus callosum area (dependent) in Study I or between B-cell count at vaccination (independent) and subsequent antibody levels (dependent) in Study IV. In some cases, making this distinction requires more deliberation and introduces a greater degree of uncertainty. For instance, in Study III, we designated the EDSS scale steps as the independent variable and treated blood flow as the dependent variable.

Simple linear regression is used when analysing the relationship between two variables, one dependent and one independent, and the model assumes that the relationship is linear. This method is used multiple times in this dissertation. The slope of the regression line represents the change in the dependent variable for each unit change in the independent variable; for example, an increase of 18 B cells/ μ L per month for 'fast mobilizers' (Study VI) or a

decrease of 3.3 mL/minute in total cerebral blood flow for each increase in whole EDSS scale step (Study III). In this way, predictions can be made, such as the approximate time period when a certain B-cell count can be expected when planning vaccinations. The variation between the model and the actual values of the dependent variables can be quantified by the R-squared value, where 1 indicates perfect linear correlation, i.e., complete agreement between the model and data points, and 0 represents the total absence of correlation, which is used as the null hypothesis in significance testing. The calculated p value complements the R-squared value but does not provide information about the strength of the linear relationship. Even a weak linear relationship with a low R-squared value can be highly significant.

In Study I, we wanted to examine the atrophy development in the ten study participants before and after AHSCT. The mid-sagittal normalized corpus callosum area (nCCA) was used as a measure of atrophy, which can be measured in an MRI scan. The data points consisted of 81 nCCA calculations from an equal number of MRI scans of the ten participants conducted before and after AHSCT. If these 81 data points had come from 81 different participants, a simple linear regression model could have been used. However, when repeated measurements over time are made on the same participants, the measurements are often correlated over time within the same individual. Individuals can also have different starting points (i.e., intercepts), which become evident in Study I when examining the individual nCCA measurements. To handle this data, a linear mixed-effects model with a random intercept was chosen, where the ‘subject’ is treated as a random effect to account for individual differences, while the nCCA value is still treated as a dependent fixed variable. The simple linear regression model also assumes a constant variance in the error effect over time, which a linear mixed-effects model handles by including random effects, epsilon. Within this model, the slope of the regression line could also be allowed to change at specified time points. Based on previous studies showing a decrease in nCCA in MS patients and continued brain atrophy 2.5 years after AHSCT, we postulated that the rate of atrophy, and hence the slope of the regression line, would change at the time of AHSCT ($t = 0$) but plateau within 2.5 years after treatment ($t = 2.5$).

When investigating how more than one variable may influence a dependent continuous variable, a multiple linear regression model can be used. In this dissertation, such a model was used in Study IV to examine which factor or factors had the strongest association with antibody levels and T-cell reactivity after vaccination. We identified nine parameters as potential predictors, which were included in the model. The choice of these independent variables in a multiple linear regression analysis needs to be made carefully, and variables that are believed to be highly correlated should be avoided. If two variables are highly correlated, i.e., have high multicollinearity, it can be difficult to distinguish the effects of both predictors, and the model can become unstable. In the current case, there might be concerns that the predictors ‘No of previous

anti-CD20 infusions' and 'Accumulated dosage' were highly correlated, and also that 'Time since anti-CD20-mAb' and 'Pre-vaccination CD19+ B-cell count' were. In the latter example, the choice to include both variables was motivated by clinical experience that there is a large variation in B-cell recovery among different individuals, and it was therefore crucial to include these two variables in the model. To investigate if variables have high collinearity, a correlation matrix can be generated, and then one can choose to exclude one of the predictors that is highly correlated with another variable. In Study IV, we did not perform a correlation matrix, though it would have been advisable. The results of a multiple linear regression are often presented in the form of an estimate (beta coefficient), which shows the expected change in the dependent variable for each unit change in the independent variable while keeping all other variables in the model constant. Along with the confidence interval of the beta coefficient, the uncertainty in the estimation can be assessed. The standard error is a measure of the uncertainty in the estimate of the beta coefficient, and based on the estimate and standard error, a *t*-value can be calculated, and thus the *p* value.

In cases where the dependent variable is binary, a multiple logistic regression model is used. Such a model was applied in Study IV to investigate which factors were associated with the presence of low B-cell count (<10 cells/ μ L) six months after the last rituximab dose and exhibited a slower recovery rate of B cells ('slow mobilizers') compared to those with a B-cell count \geq 10 cells/ μ L and had a faster recovery rate ('fast mobilizers'). The binary outcome was either belonging to the 'slow mobilizers' group or the "fast mobilizers" group, and five covariates that we postulated could affect the outcome were chosen. The beta coefficient in a multiple logistic regression model represents the change in the logarithmic odds ratio for the binary outcome for each change in the independent variable by one unit while keeping the other variables constant, and is often converted into odds ratios for easier understanding. Similarly to the multiple linear regression model, the uncertainty in the estimation can be assessed with a confidence interval of the beta coefficient, after which one can evaluate which predictor or predictors appear to be associated with the dependent variable.

Conclusions from a non-rejected null hypothesis?

Classical hypothesis testing is based on the assumption that the researcher's goal is to reject the null hypothesis, and the significance level is a way to manage the risk of incorrectly rejecting this null hypothesis, i.e., committing a Type I error. Therefore, the burden of proof lies with the researcher to prove the alternative hypothesis, and until the alternative hypothesis is proven, the null hypothesis prevails. Concluding that the null hypothesis is true solely because it wasn't rejected is fallacious. Concluding that the absence of a

significant difference is evidence of equivalence is a logical mistake. Before Study III was initiated, we formulated the research hypothesis that we would find reduced blood flow and impaired cerebrovascular response in individuals with SPMS compared to healthy controls, based on previously published data. Additionally, we wanted to investigate whether the same potential difference could be observed in individuals who had undergone AHSCT for RRMS compared to healthy controls.

An alternative research question could have been whether the blood flow is the same in individuals who underwent AHSCT for RRMS and healthy controls. However, this would likely require a different study design and statistical method. To demonstrate equivalence, equivalence testing is required, which is typically used when comparing, for example, a generic drug to an original drug; it aims to prove equivalence or at least ‘non-inferiority.’ The most intuitive method for this is to define a confidence interval that represents a clinically or scientifically irrelevant difference (‘minimally clinically important difference’ or ‘least relevant difference’), and this pre-defined accepted difference is denominated delta.^{254,255} Another equivalent approach is to formulate a null hypothesis that two outcomes are not equivalent, i.e., outside the pre-defined delta value. Attempting to apply equivalence testing in the comparison of blood flow between the AHSCT group and healthy controls in Study III would face several challenges. First, the conventional equivalence testing assumes a normally distributed population, even though non-parametric methods are available.²⁵⁶ Second, there is currently no scientific basis for determining delta, i.e., what constitutes a clinically relevant (or irrelevant) decrease in blood flow in MS, rendering an equivalence test impossible.

Sometimes, researchers use a post hoc power analysis to support the null hypothesis when statistical significance is absent. In this case, an ‘observed power’ is calculated. However, this approach is heavily criticized as a logical fallacy and cannot contribute to an assessment of the risk of a Type II error.²⁵⁷

So, what do the results in Study III tell us when comparing the AHSCT group in comparison to the healthy controls, and what conclusions can be drawn? Can we make any assertions beyond a non-rejected null hypothesis? Even if equivalence cannot be demonstrated, it can still be concluded that the reduction in blood flow observed in the AHSCT group compared to healthy controls could not be detected, while this was the case when comparing the SPMS group to the same healthy controls.

Why was no statistical analysis performed including all three groups, and why was no direct comparison made between the SPMS and AHSCT groups? A comparison between all three groups could have been conducted using the Kruskal-Wallis test, the non-parametric equivalent of ANOVA (analysis of variance), which can be used when comparing three or more independent groups. However, if the Kruskal-Wallis test had been performed, there would have been a considerable risk that the data collected in Study III would not have been sufficient to detect smaller but real differences. A direct comparison

between the SPMS and AHSCT groups was not conducted because it did not address the research question we posed, and therefore, such a comparison was not performed. Performing a multitude of hypothesis tests on the same data increases the risk of multiple testing errors, i.e., false positive results. Such an ‘exploratory’ data analysis procedure usually requires a reduced alpha level to minimize the risk of Type I errors, but this comes with the risk of masking real differences, i.e., increasing the risk of Type II errors.

Since PET scans are resource-intensive, time-consuming, and involve radiation exposure for participants, research questions, the study, and statistical analyses need to be planned as efficiently as possible, especially when the study is designed as an experimental pilot study as in Study III. It is likely that existing data could have been processed differently to potentially yield different levels of significance, or alternative statistical tests could have been used. However, there is value in using conventional, reliable, and relatively simple statistical methods that are reproducible and understandable by researchers who are not statisticians. There is no perfect statistical method to uncover the ‘truth’, and pursuing such perfection places unreasonable trust in statistical methods. The need for careful statistical planning also relates to ethical considerations, where researchers must balance a desire to include enough participants to detect real differences between groups without excessive resource utilization and a commitment to minimize factors such as radiation exposure or participant discomfort.

Methods

Reporting guidelines

Many medical journals require articles to be written based on commonly used reporting guidelines,²⁵⁸ and adherence to these must usually be documented in a specific form upon submission. All four papers in this thesis have been written, and to some extent also planned, with consideration to the established guidelines; for study I, III, and IV, the ‘Strengthening the reporting of observational studies in epidemiology’ (STROBE) reporting guidelines were used,²⁵⁹ and for study II, the ‘Standards for reporting qualitative research’ (SRQR),²⁶⁰ and ‘Consolidated criteria for reporting qualitative research’ (COREQ) guidelines.²⁶¹ The use of such checklists aims not only to increase uniformity in reporting, which is beneficial when comparing studies or conducting meta-analyses, but also to enhance quality, transparency and reproducibility, which collectively makes it easier for the reader to assess and possibly implement the conclusions.²⁵⁸

Study I-III

Study population

Clinical and disease-related data of the participants in Study I-III are presented in Table 1.

People with RRMS treated with AHSCT (Study I-III)

At the centre of the thesis lies the group of ten individuals with aggressive RRMS who were the first to be treated with AHSCT for this indication at Uppsala University Hospital, between 2004 and 2007. These ten participants constituted the entire cohort in Study I and II, as well as one of the three examined groups in Study III. They have been described in various publications preceding the studies in this thesis,^{119,120} and have also been included in larger cohorts subsequently.²⁶²

At the time of the mobilisation before AHSCT, the participants had a clinically and radiologically highly inflammatory disease activity, characterized by frequent relapses and often pronounced functional impairment; the median number of gadolinium-enhancing lesions on T₁-weighted images was 11

(range 0-40), the median number of relapses in the year preceding the treatment (annual relapse rate, ARR) was 8 (range 3-12), and the median EDSS score was 6.5 (range 2.0-8.5). The median disease duration was 28 months (range 4-113). In the two cases where the disease duration was less than one year, the ARR was calculated based on the relapse frequency during the disease duration. All participants except one had immunomodulatory treatment prior to AHSCT (median 2, range 0-3). A complete overview of clinical data for this group, both before AHSCT and during follow-up, is presented in Supplementary Table I, Study I.

AHSCT procedure

For the mobilization of blood stem cells from the bone marrow, a single dose of cyclophosphamide (2 g/m²) and filgrastim (5-10 µg/kg/day) for 6-7 days were administered. The stem cells, now mobilized to the peripheral blood, were collected through apheresis 10-11 days after the start of the mobilization regimen, and were then cryopreserved. No *ex vivo* graft manipulation was performed. In nine out of the ten participants, a myeloablative intermediate-intensity BEAM-ATG conditioning regimen was used, which consisted of carmustine (300 mg/m²), etoposide (800 mg/m²), cytarabine (800 mg/m²), and anti-thymocyte globulin (10 mg/kg). For the youngest participant, who was nine years old at the time of AHSCT, a lymphoablative low-intensity conditioning regimen with cyclophosphamide (200 mg/kg) and anti-thymocyte globulin (6 mg/kg) was used.

People with SPMS (Study III)

People with diagnosed SPMS who were followed at the Department of Neurology at Uppsala University Hospital, and who had agreed to be contacted about participation in research studies, were identified through the Swedish MS registry.²⁴² As we aimed for a group of SPMS patients that, in terms of age and gender distribution, did not differ too much from the AHSCT group at the time of the ten-year follow-up, relatively young individuals with SPMS were identified. These individuals were then contacted by phone, followed by written information about the study sent by mail. We carefully reviewed the SPMS diagnosis and only included patients that we were convinced had a true progressive disease. The recruitment procedure continued until ten individuals were included in the study.

Healthy controls (Study III)

The healthy volunteers were recruited through digital advertising on social media and through the researchers' own networks, via adverts on the university campus and at Uppsala University Hospital. Interested individuals were then provided with a link to an electronic form containing health-related questions. Those with ongoing daily medication, current or previous regular use of tobacco, ongoing or past cardiovascular, cerebrovascular, systemic

inflammatory, hypertensive, or metabolic disease, or a first-degree relative with MS or another autoimmune or inflammatory disease were excluded at this stage. The remaining participants were selected to as closely as possible match the age and gender of the other two groups. Routine blood tests, along with neurological and somatic exams, were conducted and found to be normal in all cases. However, the PET examination for Study III could not be completed for two of the healthy controls due to a technical failure for one and difficulties in obtaining intra-arterial access for the other. As a result, an additional two healthy controls were included, following approved supplementary application from the local Radiation Ethics Committee and the Regional Board of Medical Ethics, to bring the total number to 10. One of these two was recruited with the assistance of Clinical Trials Consultant AB, Uppsala, Sweden.

Table 1. Demographic and disease-related data of participants in Study I-III.

	Study I-III AHSCT n = 10	Study III SPMS n = 10	Study III Healthy controls n = 10
Demographic data			
Age, years, mean (SD)	34.0 (8.5)	43.5 (5.4)	37.1 (9.5)
Female, <i>n</i> (%)	7 (70)	6 (60)	6 (60)
Body mass index, kg/m ² , mean (SD)	25.6 (4.9)	24.4 (4.0)	24.0 (3.6)
Cardiovascular risk factors*, <i>n</i> (%)	0 (0)	4 (40)	0 (0)
Multiple sclerosis related data			
Multiple sclerosis duration, years, mean (SD)	13.1 (2.9)	13.8 (7.3)	NA
SPMS duration, years, mean (SD)	NA	5.2 (4.1)	NA
EDSS score, median (range)	2.0 (0–6.0)	5.75(3.0–9.0)	NA
SPMS phenotype ⁵¹			
Active and with progression, <i>n</i> (%)	NA	2 (20)	NA
Active but without progression, <i>n</i> (%)	NA	0 (0)	NA
Not active but with progression, <i>n</i> (%)	NA	4 (40)	NA
Not active and without progression, <i>n</i> (%)	NA	4 (40)	NA
Ongoing immunomodulating treatment			
Glatiramer acetate, <i>n</i> (%)	2 (20)	0 (0)	NA
Rituximab, <i>n</i> (%)	0 (0)	3 (30)	NA
Natalizumab, <i>n</i> (%)	0 (0)	3 (30)	NA
None, <i>n</i> (%)	8 (80)	4 (40)	NA

*Cardiovascular risk factors identified in the study groups were body mass index >35 kg/m², essential hypertension, diabetes mellitus type II, hyperlipidaemia and smoking.

Investigations

Clinical assessment

Each individual underwent a standard somatic and neurological assessment, and for those with MS, the EDSS score was established. Routine blood tests were taken, and body weight, height and blood pressure measured.

Magnetic resonance imaging

Participants were scanned including 3D T₂-weighted fluid-attenuated inversion recovery (FLAIR) and 3D T₁-weighted MRI sequences with and without intravenous gadolinium contrast. For the AHSCT group, past images from the clinical follow-up after AHSCT were reassessed.

Determination of callosal atrophy

The normalized midsagittal corpus callosum area was determined in the AHSCT group, in order to assess callosal atrophy. While T₁-weighted images without gadolinium contrast were the primary choice for measurements, for two participants only a limited number of sequences of this type were available; consequently, T₂-weighted sequences were consistently used for these two individuals to ensure consistent longitudinal comparisons. The images were pseudonymized and randomized ahead of measurements to eliminate potential bias. To normalize the CCA, the midline internal skull surface (MISS) area was determined in the same view. The coefficient of variation was derived as the ratio of the standard deviation of the measurements to the mean to validate inter-rater reliability. For the CCA, the average coefficient of variation was 1.9% (range 0.37-5.4), and for the MISS area 0.26% (range 0.11-0.60). For the SPMS group participants and the healthy controls, nCCA was measured in the same way, but only in the most recent image. For details on the measurements, see Supplementary Methods, Study I, and for an example of CCA and MISS, see Supplementary Figure 1 and 2, Study I.

Construction of T₂ lesion masks

For ¹⁵O-water PET measurements of blood flow and cerebrovascular reaction in MS lesions in Study III, masks were created based on T₂-weighted FLAIR images. Initially, masks were generated from the FLAIR images using automatic segmentation in the Freesurfer software. To enhance specificity, we utilized co-registered T₁ images within the same software to exclude T₂ lesions mistakenly identified in the ventricles, CSF, choroid plexus, thalami, and caudate nucleus. Subsequently, these masks were meticulously refined on a voxel-by-voxel basis. For details on the construction of the masks, see Study III.

Biomarkers in cerebrospinal fluid

For assessment of CNS-specific oligoclonal bands, IgG-index and NFL, the patients treated with AHSCT underwent a lumbar puncture. For comparison, all previously made agarose gels for the detection of OCBs from the participants were retrieved and re-examined, with the exception of two that could not be located.

Exploring lived experience

The purpose of Study II was to delve into participants' personal perspectives, leading us to adopt a phenomenological viewpoint. In the context of nursing research, this approach emphasizes the significance of individuals and their unique experiences and views.²⁶³ We aimed to probe the 'lived experience' to better comprehend the 'essence' or intrinsic structure they convey. As researchers and interviewers, we focus on understanding the phenomenon through the eyes of the participants.

To achieve a highly inductive and adaptable interview, no predetermined interview template was used, as is common with structured or semi-structured interview techniques.²⁶³ At the outset of each interview, interviewers reintroduced themselves, reiterated the study's aim, and reconfirmed participants' consent. The participants were then prompted to recount their memories from disease onset. The discussion evolved organically with open-ended questions, and topics brought up by participants were delved into deeper. These interviews were digitally recorded, transcribed verbatim, pseudonymized, and shared with the group of researchers.

As analysis method, a qualitative content analysis was chosen, as described by Graneheim and Lundman in a frequently cited article from 2004.²⁶⁴ The choice of this analysis method is not in conflict with a phenomenological approach but works well with such an entry point. The method has its roots in a quantitative text analysis,²⁶⁵ and has the advantages of being structured, systematic, and well-described.^{264,266,267} Three of the authors were also well familiar with the analysis method from before, which was a strength. The method is also suitable for an inductive approach, which does not seek to fit the results into already existing theories or models (deductive approach), but on the contrary, tries to create abstraction and categorization from the text, or the unit of analysis one intends to explore.²⁶⁶

The content analysis involved identifying individual meaning units, condensing them for clarity, and interpreting their deeper meaning. This interpretative phase, especially regarding latent content, was conducted by three of the researchers independently. We then met in digital meetings to discuss, compare, and reach a consensus on our findings, aiming to ensure trustworthiness. The findings were organized into themes and subthemes, connecting latent content across various units and codes. No categories were formulated;

in content analysis as described by Graneheim and Lundman, this is not always a necessary step, ‘when data is rich and codes are expressive’.²⁶⁶

Using the MAXQDA 2020 software, these codes were categorized under their respective themes and subthemes. Selected quotations, illustrative of the themes, were then translated from Swedish to English by a professional translator. The entire team then achieved consensus on the finalized themes, subthemes, and chosen quotations.

¹⁵O-water PET

To assess cerebral blood flow and cerebrovascular reactivity, participants underwent two 10-minute ¹⁵O-water PET scans, both before and after the injection of the vasodilating agent acetazolamide. An arterial catheter was inserted into the radial artery before the scans, for continuous blood sampling.

A 10-minute baseline dynamic PET scan commenced concurrently with the intravenous injection of ¹⁵O-labelled water. The dosage was determined based on body weight. After this initial scan, participants were administered an intravenous bolus dose of acetazolamide, calculated at 9 mg/kg of body weight but capped at 1,000 mg, to induce cerebral vasodilation. Following this, a subsequent ¹⁵O-water PET scan was made.

Before processing, the PET data were pseudonymized. The images then underwent various stages of processing. First, a realignment was performed to correct for minor movements during each examination. This was followed by a co-registration of the two scans made pre- and post-administration of acetazolamide, facilitating mutual comparison. The specific VOI selected for this analysis encompassed the total brain, grey matter, white matter, and thalamus. Using the constructed T₂ lesion masks, which were derived from each MS patient’s T₂ FLAIR images as described above, the blood flow within the lesions was also calculated.

Additionally, we aimed to study the cerebral blood flow in the white matter areas that appeared unaffected by inflammatory lesions on T₂ FLAIR, termed the normal-appearing white matter (NAWM). These masks were generated by subtracting the T₂ lesion masks from the white matter VOI. To prevent falsely elevated values due to the spill-in of radioactivity from the surrounding grey matter, which has a higher blood flow, the NAWM VOI was then reduced to represent an area of approximately 20 cm³ in the centrum semiovale. Since the grey matter of the cortex is very thin, we also performed a partial volume correction when calculating the CBF in the grey matter.

Study IV

As outlined in ‘Approaches to an ethical founding’, the study was conducted in parallel to the clinical routine for vaccination against COVID-19.

Clinical routine

Individuals with ongoing treatment with rituximab for MS at the neurological department of Uppsala University Hospital were offered the Pfizer-BioNTech COVID-19 vaccine, tozinameran. Vaccination was recommended if six months had passed since their last rituximab infusion and if their B-cell counts were 20/ μ L or above. If below this threshold, vaccination and treatment could be delayed, with check-ups every second months for up to six months. After this, vaccination was advised regardless of B-cell count. Some patients chose to receive immediate vaccination irrespective of their counts. Two doses of the vaccine were given three weeks apart. Rituximab treatment was resumed six weeks after the second vaccine dose and patients were urged to report any side effects or MS relapse symptoms.

Study population and clinical outcome

Blood samples were drawn six weeks after the second vaccine dose. After participants were enrolled in this observational study, blood samples taken routinely before the administration of the first vaccine dose were incorporated into the analysis. Of the 75 individuals assessed for eligibility in the study, 69 were enrolled, and data from 67 were analysed. Among these, 60 were already receiving rituximab treatment, while seven were set to start the treatment. The latter group acted as an internal control, consisting of participants not undergoing any ongoing B-cell depleting therapy. Clinical and disease-related data for the participants can be found in Table 1 of Study IV. Upon concluding the study, the medical records of all participants were reviewed to detect COVID-19 before or after vaccination, or any clinical or radiological signs of MS activity.

Humoral immune response

The presence of antibodies targeting the nucleocapsid, RBD and spike proteins of the SARS-CoV-2 virus was assessed using the V-PLEX SARS-CoV-2 Panel 2 (IgG) kit from Meso Scale Diagnostics. Additionally, to evaluate the ability of these antibodies to neutralize the virus, the V-plex SARS-CoV-2 Panel 2 (ACE2) kit was employed. This kit specifically measures the antigen neutralization capacity through an angiotensin-converting enzyme 2 competitive assay.

Cellular immune response

After incubating T cells with one of two distinct peptide pools, the SARS-CoV-2 specific T-cell reactivity was determined using a human IFN- γ and IL-2 FluoroSpot assay. The first peptide pool was a commercially available product from Mabtech, consisting of 100 peptides all sourced from the human SARS-CoV-2 spike protein. The cells were also exposed to an inhouse-developed peptide pool that contained eight peptides. Once the stimulation was completed, the subsequent number of 'spot forming units' (SFUs) was measured using a Mabtech IRIS FluoroSpot reader. To further analyse the data from the spots, the Mabtech Apex software, version 1.1, was employed.

Results

Study I

Clinical assessment

Two patients experienced clinical relapses and subsequently started disease-modifying drug treatment. One of these patients had another relapse three years later. However, none of the patients showed progression in EDSS.

MRI assessment

For three participants, at least one MRI event was detected. One patient had a total of three MRI events throughout the follow-up period, with two occurring in connection to a clinical relapse. Another patient had one MRI event detected in connection to a clinical relapse, and the third patient had one gadolinium-enhancing lesion in the spinal cord during the 6-month follow-up scan. Upon comparing the last set of images with the images from the first follow-up scan, three new T₂ lesions were found in one of these patients, while no new T₂ lesions were observed in the remaining patients.

On a group level, the estimated annual change in nCCA remained relatively stable before and immediately after AHSCT, but approached zero after 2.5 years. The individual-level analysis revealed ongoing brain atrophy in two patients, where the normalized CCA decreased according to the pre-specified definition.

Composite scores

Five out of the ten patients reached the primary endpoint of ‘sustained complete remission of MS’, and in three of these patients, MS was ‘resolved’. Additionally, seven out of ten patients maintained NEDA-3 throughout the ten-year follow-up period. All patients showed an improvement in EDSS scores, with the median score decreasing from 6.5 at HSCT to 1.75 at the end of the study, reflecting a median improvement of 3.0 in EDSS scores. Importantly, all patients were alive at the end of follow-up.

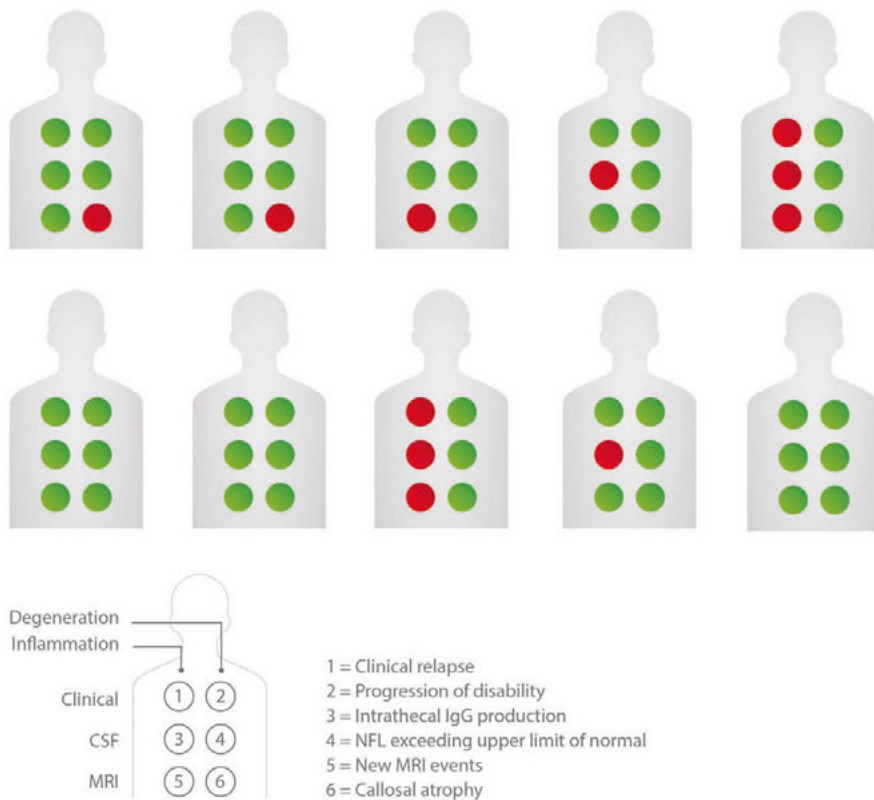


Figure 7. 'Resolved disease' in individual patients.

The clinical course after haematopoietic stem cell transplantation for MS in individual patients. Red represents pathological findings and green represents normal findings. In patients with normal findings only, MS was considered to be 'resolved'.

Adverse events

There were sixteen grade 3 and three grade 4 adverse events occurring within 100 days of AHSCT, while three grade 3 and one grade 4 adverse event occurred during the remainder of the follow-up period. There was no mortality.

Study II

Five main themes emerged from the interviews: (1) being diagnosed with MS – an unpredictable existence, (2) a new treatment – a possibility for a new life, (3) AHSCT – a transition, (4) reclaiming life, and (5) a bright future accompanied by insecurity.

Theme 1: Being diagnosed with MS – an unpredictable existence

Under this theme, two sub-themes were identified: (1) abnormal bodily experiences, and (2) affecting the participants' whole life and living in uncertainty.

Subtheme 1: Abnormal bodily experiences

The theme explored the phenomenon where individuals experienced unfamiliar and unsettling sensations within their bodies, which resulted in feelings of fear and the emergence of unusual symptoms. This peculiar sense of alienation was frequently articulated through phrases like 'this is not me' and 'what is happening?' The underlying suggestion of this theme was that it thrust individuals into a state of uncertainty, exerting a significant influence on various aspects of their lives.

Subtheme 2: Affecting the participants' whole life and living in uncertainty

This theme delved into the profound impact that the described experiences had on individuals' lives. It was characterized by a pervasive sense of uncertainty, as individuals lived day-to-day without knowing how their bodies would behave, resulting in a lack of trust in their own bodies. There was a dramatic transformation in their lives due to overlapping relapses, where no treatment seemed effective, leading to a diminished sense of life's value and a relentless struggle for existence. These individuals felt vulnerable, exposed, helpless, and powerless, often dependent on others. The dramatic changes rippled through various dimensions of life, affecting relationships, work or school, freedom, autonomy, and equality. This process was accompanied by grief and sorrow over the losses incurred. Overall, the theme highlighted the extensive and multifaceted challenges that individuals faced when living with these experiences.

Theme 2: A new treatment – a possibility for a new life

This theme centred on the introduction of a new treatment option, which presented both a possibility for a fresh start and a looming threat. It represented a pivotal moment in the lives of these individuals, where the prospect of regaining their lives was offered, albeit with the shadow of potential risks. The theme explored several aspects:

Information and decision-making Individuals affected by MS and AHSCT were confronted with life-threatening conditions. They became active participants in the decision-making process, recognizing that life with MS may not have been sustainable. Questions like ‘What kind of life do you have now?’ underscored the gravity of the situation.

Grasping hope There was a sense of grasping the last straw, where individuals held onto hope, viewing the new treatment as a chance for a better life. It embodied their aspirations for improvement.

Surrender and trust In their pursuit of a potential cure or betterment, individuals found themselves in a state of surrender. This surrender was marked by trust, confidence, and a willingness to place their hopes in the hands of medical interventions.

Theme 3: AHSCT – a transition

This theme delved into the experience of undergoing AHSCT, representing a significant transition in individuals’ lives. It comprised two central aspects:

Life on pause This phase encapsulated the period during the stem cell transplantation process, marked by the challenges of rigorous treatment. Individuals faced a tough regimen that left them with hazy memories, as if in a fog. They grappled with a sense of being out of control, vulnerability, and dependence. This phase represented a time when their lives were put on hold due to the demanding treatment.

Transformation This phase signifies the profound changes individuals underwent as they transitioned from the world of illness to the world of wellness. It involved a shift from the realm of disease and its challenges to the realm of health and recovery. While this transition was disruptive and challenging to navigate, it held the promise of regaining bodily functions and a healthier existence.

Theme 4: Reclaiming life

This theme explored the process of individuals recovering and reasserting themselves after undergoing AHSCT, revolving around the following key elements:

Regaining ability In this phase, individuals underwent a challenging journey to regain their physical and cognitive abilities. Their recovery was gradual and could be likened to a form of rebirth or even a miracle. They worked diligently to reclaim skills and activities they once enjoyed, such as running.

Reclaiming life and work During this phase, individuals actively took back control over their lives and careers. They saw it as an opportunity for redemption and a chance to regain a sense of agency.

Feelings of recurring difficulties Emotions ran high as individuals navigated the ups and downs of recovery. They oscillated between moments of happiness and periods of sadness. Some struggled with not recognizing themselves anymore, and past emotions, including disappointment over unmet expectations, resurfaced.

Time and assistance for healing and wholeness Recovery was recognized as a gradual process that required both time and support. This phase underscored the importance of patience and the role of assistance in the journey toward ‘becoming whole again’.

Theme 5: A bright future accompanied by insecurity

This theme delved into the complex emotions and experiences of individuals who had undergone stem cell transplantation and were facing an uncertain yet hopeful future. It captured the paradox of a bright and hopeful future tinged with feelings of insecurity and the desire for objective validation of their health status. This theme comprised two central aspects:

A unique experience Highlighted the individuals’ recognition that their journey was unique. Despite the presence of symptoms and lasting effects, they found stability in their circumstances.

Experience of being healthy and desiring objectivity The participants shared their experience of feeling healthy and not having MS anymore, yet they expressed a desire for objective criteria to affirm their health status. They hesitated to declare themselves as cured but found joy in their regained physical well-being and everyday life.

Study III

Ten healthy controls, 10 patients with SPMS and 9 MS patients treated with AHSCT completed all scans.

Magnetic resonance imaging

Among the SPMS patients, 8 had no gadolinium-enhancing lesions and no new T₂ lesions when comparing to an MRI scan made >12 months before. Two of the SPMS patients had an increased number of T₂ lesions, and one of them also had 1 gadolinium-enhancing lesion.

The median T₂ lesion volume in the SPMS group was 17 mL and in the AHSCT group 12 mL; there was no significant difference in T₂ lesion volume in those groups.

The median nCCA in the healthy controls was 4.0%, in the SPMS group 3.1% and in the AHSCT group 2.8%. In comparison to the healthy controls, the normalized corpus callosum area was lower in both the SPMS group ($p < 0.05$) and in the AHSCT group ($p < 0.0001$); there was however no difference between the SPMS and AHSCT groups.

Cerebral blood flow

SPMS versus healthy controls

In the healthy controls, the median total brain CBF at baseline was 55 mL/100 g/min and in the SPMS patients 42 mL/100 g/min ($p < 0.01$).

In the grey matter, the difference was more pronounced, with a median of 58 mL/100 g/min in healthy controls and 42 mL/100 g/min in the SPMS patients ($p < 0.001$). After partial-volume correction was made, the median of healthy controls was 98 mL/100 g/min and 76 mL/100 g/min in the SPMS patients ($p < 0.05$).

In the white matter there was a trend towards lower CBF in the SPMS group. Also, in the thalami, CBF was lower in the SPMS patients compared to the healthy controls ($p < 0.01$).

The CBF in NAWM in the centrum semiovale did not differ between healthy controls and SPMS patients.

There was an inverse correlation between the EDSS score and total brain CBF of the SPMS participants ($r_s = -0.75$, $p < 0.05$); in the whole brain, the CBF was 3.3 mL/100 g/min lower per EDSS unit ($p < 0.05$). When comparing regional CBF in the T₂ lesions to the NAWM in the centrum semiovale within the SPMS group, the CBF in the T₂ lesions was lower ($p < 0.05$).

AHSCT versus healthy controls

In the AHSCT group, the median total brain CBF at baseline was 54 mL/100 g/min; there were no significant differences in CBF between patients who underwent AHSCT and healthy controls for total brain.

In NAWM in the centrum semiovale at baseline, CBF was higher in AHSCT patients than in healthy controls ($p < 0.05$).

Cerebrovascular reactivity

ACZ challenge resulted in a higher total and regional median CBF in all VOIs. The median increase in total brain CBF in healthy controls was 9.0 mL/100 g/min and in SPMS 16 mL/100 g/min. In all VOIs, there was a trend towards higher CVR in SPMS group than in the healthy controls, reaching statistical significance in the thalami ($p < 0.05$); the median total brain CVR in healthy controls was 18% and in the SPMS group 34%.

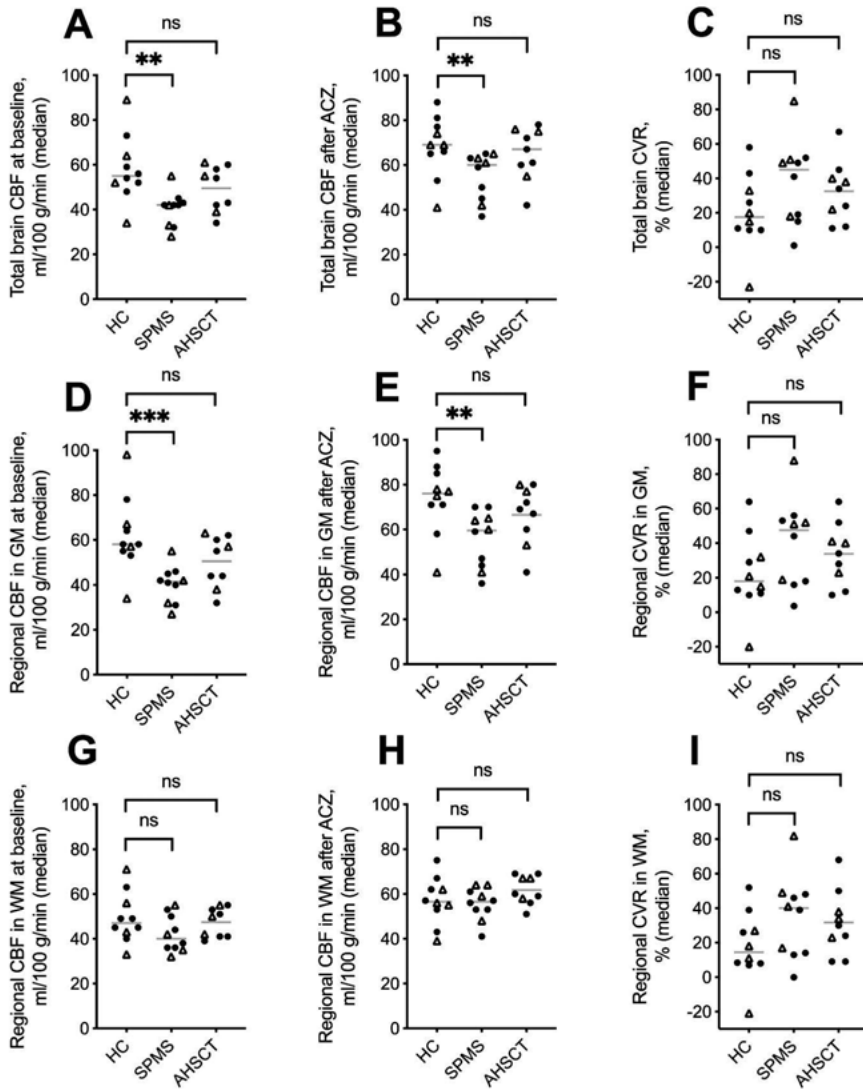


Figure 8. CBF in total brain, grey matter and white matter before and after ACZ in healthy controls, SPMS and AHSCT.

(A) Total brain CBF at baseline. (B) Total brain CBF after ACZ. (C) Total brain CVR. (D) CBF in grey matter at baseline. (E) CBF in grey matter after ACZ. (F) CVR in grey matter. (G) CBF in white matter at baseline. (H) CBF in white matter after ACZ. (I) CVR in white matter. For statistical analysis, Mann-Whitney test was used and statistical significance was determined by two-tailed p value. $**P < 0.01$; $***P < 0.001$; ● = female; △ = male; ACZ = acetazolamide; AHSCT = autologous haematopoietic stem cell transplantation; CBF = cerebral blood flow; CVR = cerebrovascular reactivity; HC = healthy control; GM = grey matter; ns = not significant; SPMS = secondary progressive multiple sclerosis; WM = white matter.

Study IV

Humoral immune response

Serum samples from 48 rituximab-treated patients before vaccination and 60 post-vaccination were analysed, along with seven post-vaccination samples from MS patients never exposed to anti-CD20 treatment ('CD20-naïve').

After vaccination, 72% of rituximab-treated patients had protective anti-S IgG antibodies, and 57% had protective anti-RBD antibodies. Yet, 28% did not achieve protective levels for either. The antibody response was higher in CD20-naïve participants. Antibody levels correlated with B-cell counts. Rituximab-treated patients with non-protective antibody levels had a median B-cell count of 22/ μ L, while patients with protective antibody levels had 51/ μ L. 75% of rituximab-treated patients achieved over 90% inhibition in S-ACE-2 and RBD-ACE-2 binding. All anti-CD20-naïve patients reached over 90% inhibition in both. Inhibition levels correlated with B-cell counts and respective antibody levels.

Cell-mediated immune response

Peripheral blood mononuclear cells from 40 rituximab-treated MS patients before vaccination and 52 after vaccination, along with 7 post-vaccination samples from CD20-naïve participants were analysed. Stimulation with two spike protein peptide pools led to an increase in IFN- γ SFUs post-vaccination in both rituximab-treated and CD20-naïve patients. Similar results were observed for cells secreting IL-2 and those co-secreting IFN- γ and IL-2. After vaccination, 91% of rituximab-treated MS patients showed an IFN- γ response with the Mabtech peptide pool and 83% with the in-house pool. Eighty percent had positive responses with both pools, while 7% showed no response with either pool.

Factors associated with favourable outcome

In analyses examining factors linked to humoral and cellular responses, only the pre-vaccination B-cell count was significantly associated with post-vaccination anti-spike IgG levels. No factors were significantly tied to IFN- γ SFUs.

Safety

The vaccination approach led to an average delay of 11 weeks in rituximab treatment, with a range of -3 to 37 weeks. Out of all participants, 5% experienced MS-related symptoms between their last rituximab infusion pre-vaccination and study conclusion. This included one individual with a brief leg

numbness episode and two with MS activity detected via MRI. Post-vaccination, there were no reports of unexpected side effects, severe MS symptoms, or COVID-19 infections.

B-cell recovery

Approximately 6 months after the last rituximab infusion before vaccination, B-cell count data were available for 48 patients, ranging widely from 0/ μ L to 163/ μ L with a median of 17/ μ L. Patients with B-cell counts below 10/ μ L experienced a slower increase in B-cell counts (7.5 B cells/ μ L/month) compared to those with at least 10/ μ L (18 B cells/ μ L/month); there was a significant difference in the regression line slopes ($p = 0.007$), see eFigure 2, Study IV.

A logistic regression model was used to investigate factors associated with slow B-cell mobilization (<10/ μ L at 6 months). Among covariates such as age, sex, accumulated dose, treatment duration, and treatment interval, treatment duration was the only variable with a nonoverlapping 95% confidence interval in the model. Slow mobilizers had a median treatment duration of 4.0 years, while fast mobilizers had a median duration of 2.1 years ($p = 0.002$).

Discussion

Study I

Background and aim of the study

‘If [my treating physician], for example, would say... keeping in mind that he is very hopeful, but still, if he were to say: “But now [slams their hand in the table]... now I consider you cured!” Then I would probably buy it.’ – *Jennifer* (Study II)

‘No cure for multiple sclerosis is known’,²⁶⁸ states the English Wikipedia page on multiple sclerosis. However, as a result of highly effective immune reconstitution treatment for MS, there are now individuals diagnosed with MS who, without ongoing immunomodulatory treatment, no longer meet the criteria for the classical understanding of MS - dissemination in time. They have neither relapses nor new MRI changes, they have no detectable secondary progression, and in some, even the oligoclonal bands have disappeared. Do they still have MS? Are they cured? These questions are at least raised by the patients, evident in Study II. The medical community is hesitating to use the big word ‘Cure’, perhaps out of concern not to raise false hopes. Fact remains that there is a desire for terminology with clear definitions to describe the condition that they, and neurologists who follow up individuals who have undergone AHST for MS, experience. Against this background, we tested a combination of six different outcome measures to reflect the inflammatory and neurodegenerative nature of the disease and proposed based on these terms ‘sustained complete remission’ and ‘resolved disease’, which can be understood as a proxy for ‘cure’. The aim of the Study I was to test these outcome measures, alongside the more traditional ones like NEDA-3, in the group of individuals who underwent AHST for aggressive RRMS ten years earlier.

Results in a broader context

Half of the participants met our primary endpoint termed ‘sustained complete remission of MS’; this was defined by an absence of disease activity across four of the six specified measures (excluding CSF data) for a minimum duration of five years and the lack of initiation of other MS immunomodulatory

treatments. Among these five, three also achieved the secondary endpoint, ‘resolved disease’, marked by the normalization of CSF measures as well.

In the selection of markers for on-going disease, we were keen to use methods that were highly accessible, robust, and well-established, and which are or could be implemented in clinical routine.

The use of a more comprehensive measure to assess the overall treatment outcome of an intervention against MS, as suggested in this study, has not gained much traction, although similar ambitions have been sporadically discerned. For instance, in a case report from 2020 with an approach akin to Study I, Lycke and Axelsson described a patient with highly active RRMS treated with AHSCT 13 years prior, where measures reflecting disease activity are included, encompassing a normalised NFL in CSF and decreased IgG index, but lacking radiological indicators of degeneration.²⁶⁹

High inflammatory disease activity clearly has the potential to cause atrophy, as was evident when the change in nCCA just before AHSCT was studied; the annual rate of atrophy at the group level was 10%. In line with previous studies, continued atrophy was observed even after treatment.^{270,271} White matter in particular appears to be affected.²⁷² Unlike these studies, however, accelerated atrophy after AHSCT was not observed in Study I; whether this is due to the small study population or the choice of nCCA as a measure of atrophy is uncertain. Both carmustine and cytosine-arabioside, which are included in the BEAM protocol, have shown neurotoxicity in the corpus callosum in animal models both *in vivo* and *in vitro*.²⁷³ Another plausible explanation for the continued atrophy after treatment is that the neurons that have already sustained irreversible damage due to the massive inflammation at the time of treatment continue to degenerate according to the principle of Wallerian degeneration. In the period from 2.5 years after AHSCT to the end of the follow-up time, no continued atrophy of the corpus callosum was detected at the group level, and at the individual level, it was only in two of the ten participants where the measurements indicated continued atrophy from 2.5 years after AHSCT. This indicates that the previously pronounced rate of atrophy observed during the initial high-inflammatory phase subsided after AHSCT.

In six out of the ten participants, the OCBs had disappeared ten years post-treatment, and in the remaining four participants, these bands were described as being ‘fainter’, even if this is not a quantifiable variable. The bands persisted in two of the three who did not meet the criteria for NEDA-3. Only in one participant, who experienced two relapses after the treatment and subsequently had to resume immunomodulatory treatment, was the IgG index still elevated. In the other participants, the IgG index had normalized. Historically, the prevailing view held that OCBs remain substantially unchanged once acquired, emblematic of the compartmentalised inflammation believed to characterise secondary progression.²⁷⁴ However, it has also been reported that OCBs may disappear following treatment with natalizumab^{275,276} and also in half of the patients treated with cladribine a decade prior; this outcome also

correlated with clinical stability.²⁷⁷ Furthermore, in a larger cohort treated with AHSCT, which also includes participants from Study I, it was reported that the bands had disappeared in 50% after 1,500 days.¹⁷² The significance of OCBs remains unclear, but the current view is that they consist of antibodies directed against intracellular targets, suggesting they might be indicative of ongoing CNS damage.²⁷⁸

None of the patients in Study I showed signs of secondary progressive disease. On the contrary, the EDSS had decreased in all of them by the end of the follow-up period, even in those with clinical and radiological evidence of recurrent disease activity. The most significant reduction in EDSS occurred in the first year, but further declining values were observed several years after the treatment. These data, along with the halted corpus callosum atrophy, argue against the presence of subclinical neurodegeneration and thus strengthen the thesis that a neurodegenerative process is the consequence of neuroinflammation, not a separate process distinct from inflammation. Furthermore, recent reports on the outcome after AHSCT for RRMS are consistent with the results in Study I, with decreasing EDSS after treatment, strongly suggesting that the improvement cannot be solely attributed to a methodological ‘regression to the mean’,^{112,121,155,165,262,279,280} but has to be validated in randomized clinical trials. To create the best possible conditions for such neurological improvement, it is crucial that the treatment can be offered before permanent neurological damage has been established. This raises the question to what extent AHSCT, in selected cases, can and should be used as first-line treatment.²⁸¹ When comparing different treatment paradigms, a strategy of starting high-efficacy treatment earlier appears to be long-term beneficial.^{237,282}

The use of AHSCT for RRMS has historically been limited by the perceived high risk associated with the treatment. The compilation of side effects in Study I showed that most severe side effects, defined as at least grade 3 according to the Common Toxicity Criteria 4.03,²⁸³ occurred within the first hundred days after the treatment, directly related to the AHSCT procedure, and were treatable and reversible. In Study I, there was no secondary autoimmunity among the long-term side effects, although it has been described in other follow-up studies after AHSCT for MS; predominantly thyroid disease, even if the risk for secondary autoimmunity is less than with alemtuzumab.^{113,262,284} The side effect profile also depends on the choice of conditioning regimen. Unlike the prevailing practice in Sweden and many other countries today, a medium-intensity protocol with BEAM was primarily used in Study I, except for the youngest participant where a low-intensity protocol was used with cyclophosphamide and ATG. When comparing treatment outcomes between different conditioning protocols, the outcome of treatment with high-intensity myeloablative conditioning regimens unsurprisingly come at the cost of a higher risk for acute and long-term side effects, infertility, and treatment-related mortality.^{111,285} Against this background, it appears to be a

future challenge to selectively assign patients to different conditioning regimens to achieve the best risk-benefit ratio for the individual patient.

Limitations and strengths

The principal weaknesses of Study I are the limited number of participants and the absence of a control group, which is inherent to the nature of a case series. Choosing a comparable control group would have presented significant challenges; however, it might have been possible by identifying matched individuals based on disease duration, gender, age, and EDSS level from a historical cohort, and then offering them participation in the study encompassing follow-ups including clinical assessment, lumbar puncture, and MRI. In such a case-control study, avoiding selection bias in the control group would have been a delicate task. The risk for selection bias in Study I can otherwise be assessed as low since everyone who underwent AH SCT against RRMS at Uppsala University Hospital within the specified timeframe was included in the study. Any potential selection bias would then stem from circumstances beyond the study's scope: were there individuals who were offered treatment but declined, or were there those who desired treatment but could not be offered it – and if so, why?

Predominantly, the material for Study I (and Study IV) are data gathered from routine healthcare, often referred to as 'real-world data'. This approach offers many advantages: it is cost-effective, allows for extended follow-up periods, and the study population typically mirrors the group intended for treatment. Although there are evident strengths in this approach, the data is often sporadic, not as systematically collected, and measurement methods can differ. Such weaknesses were present in this study as well. For instance, MRI scans were largely made using different cameras, occasionally at different hospitals, and with varying protocols; however, all scans were re-examined within the scope of the study, and to enhance comparability, images from the last examination were reformatted to the same slice thickness as earlier examinations. In the clinical assessment, there was a notable consistency, as the entire clinical follow-up was conducted by a singular, highly experienced examiner who was meticulous in identifying and monitoring potential side effects. Naturally, this introduces a risk of bias. Hence, it is valuable not to solely rely on the clinical assessment in the evaluation but also to include biomarkers and patients' own experiences.

The strengths of the study lie in its comprehensive and consistent evaluation of the different aspects of the disease, as well as the relatively long follow-up period in this context.

Summary

The study demonstrates the possibility of achieving a long-lasting and comprehensive freedom from disease activity in MS safely after AHSCT, without continuous immunomodulatory treatment. We propose a terminology to define this condition, ‘resolved disease’, based on a combination of clinical outcomes and biomarkers reflecting the inflammatory and neurodegenerative aspects of the disease. This terminology could be used in clinical practice as a proxy for ‘cure’. Furthermore, the study contributes to the understanding of the favourable treatment outcome after AHSCT by providing detailed 10-year data from a well-described cohort.

Future perspectives

As more individuals with MS undergo AHSCT or other immune reconstitution therapies, there will be an increasing need to establish terminology for the ambitious long-term outcomes that can be achieved. Several randomized trials are currently ongoing with AHSCT, and if the results are positive, it is likely that more people will be offered this treatment. It remains to be seen whether our proposed definitions of ‘sustained complete remission’ and ‘resolution of disease’ have prognostic relevance for the absence of future disease relapse.

Study II

Background and aim of the study

MS has traditionally been considered a chronic and incurable disease, but reports on the treatment outcomes of AHSCT, and other highly effective treatments, challenged these old truths. In newspapers, on radio, in books, and on television, people shared their experiences of the treatment. Despite the extensive medical literature describing the outcomes of AHSCT for MS, patients’ perspectives were missing from the scientific literature. Occasional reports from surveys on ‘quality of life’ indicated some form of improvement but did not provide a deeper understanding of what had occurred. How did patients view their treatment? How did they perceive their health, the MS diagnosis? Was there concern that the disease might return? What was the experience of being treated with AHSCT like? The aim of this qualitative interview study was to explore the lived experiences of patients who had undergone treatment with AHSCT for MS.

Results in a broader context

Many of the topics raised by the participants in relation to their diagnosis and the early stages of the disease bear a striking resemblance to the broader qualitative literature on MS: an initial bodily insecurity and sense of alienation²⁸⁶, followed by the diagnosis itself as a traumatic event described as a 'doom' or a 'sentence' (26, and then a prevailing feeling of fundamental uncertainty that permeates their entire existence and leads to mental distress²⁸⁷⁻²⁸⁹. This uncertainty pertains to the present and the future, involving fears of losing autonomy and facing loneliness^{1,2,4}. In our study, the participants were severely affected by the disease, experiencing frequent and severe relapses. Several described waking up each morning with the apprehension that one of their functions had been compromised overnight: their vision, hearing, sensation, or ability to walk.

For the participants, AHSCT marked a turning point, offering a second chance and an opportunity for a new life. The treatment represented a transition from a state of illness to a state of health, allowing the profound uncertainty they had previously experienced to diminish, ultimately restoring a sense of normalcy. They felt like themselves again.

Although many participants experienced significant improvement after the transplantation, some still had lingering symptoms from previous relapses. However, this did not hinder them from considering themselves as 'no longer having MS,' as the disease was characterized by the risk of future relapses, which contributed to the ongoing uncertainty.

Some expressed disappointment at having received the treatment too late, before irreversible damage to the nervous system had occurred. There was also a sense of shame that their lives had not turned out as they had imagined after being given a 'second chance.'

Limitations and strengths

The low number of participants and their unique, aggressive disease course make them unrepresentative of the typical MS patient. They were all treated by the same neurologist, at the same hospital, and were aware of each other, with some even being friends, potentially influencing their perception of treatment outcomes. This limits the generalizability of the results. Additionally, the ten-year gap since treatment may have led to memory distortions and recall bias. Variability in participants' articulateness and the unstructured interview format could affect data reliability. Despite efforts to minimize response bias, there's still a possibility that participants tailored their stories to align with a 'success story' of AHSCT. Lastly, technical issues resulted in the loss of part of one recording.

Despite the small study group, the findings regarding the initial disease experience, subsequent uncertainty, and identity change align closely with previous qualitative research, suggesting that they resonate with the wider MS community. However, this cohort stands out due to its exceptionally aggressive disease course, previous unsuccessful attempts with conventional treatments, the decision to undergo AHSCT, resulting neurological improvements, and the subsequent reduction in uncertainty and restoration of self-identity. The study included a diverse group of participants, spanning various demographics and treatment outcomes, ensuring a lack of inclusion bias by recruiting all AHSCT-treated patients within a specific timeframe. The research methodology was well-suited for this exploratory study, bolstered by multiple strategies to enhance trustworthiness, such as investigator triangulation, prolonged data engagement, comprehensive contextual descriptions, participant involvement in data interpretation, rigorous analysis, and reliance on a well-established methodology.

Summary

AHSCT was perceived in terms of a second chance and an opportunity for a new life. The treatment became a transition from a state of illness to a state of health, enabling a previous profound uncertainty to vanish and normality be restored. In a wider perspective, the results give a first insight into the experience of not having MS anymore, following a highly effective induction treatment, opening for a paradigm shift in the view of MS as a chronic disease with no possible cure.

From this study, there are several key takeaways:

- Patients are in need of terminology to describe successful treatment outcomes, a proxy for 'cure' based on objective measurements.
- Disappointment can arise if one feels that the treatment was offered too late.
- It is the uncertainty about future functional impairment that appears to create the greatest sense of uncertainty and disease perception, rather than existing and stable functional impairment, which one can develop strategies to manage.

Future perspectives

It has now been many years since the first AHSCT were performed, and the range of treatments for MS has since advanced significantly. It is unusual today to see such a highly inflammatory disease as in this cohort; often, we can control disease activity with other medications. The individuals in this cohort

were severely affected by the disease. They had quickly transitioned from a 'normal' life to a life marked by disability and experienced difficulties in adapting to the rapid deterioration. The turnaround after AHSCT became all the more evident.

Does the result in our study also apply to a group of people with MS who have not become as disabled? To answer these questions, we need to continue our research. With the experiences gained from this study, an interview manual can be developed, and perhaps the analysis can have a deductive approach to interpret the material more quickly and focus on deepening the various themes that emerged in this study. Such a study should also include repeated interviews, preferably starting before AHSCT, where expectations of the treatment can be better mapped out.

Study III

Background and aim of the study

One of the key questions currently confronting us in the field of MS is whether the early and aggressive use of immunomodulatory treatments at the onset of RRMS can potentially halt the subsequent secondary progression that often follows the initial relapsing-remitting phase. The ultimate measure of this progression is, of course, clinical, traditionally characterized by a dominant 'pyramidal syndrome' involving progressive gait difficulties, tetraplegia,²⁹⁰ and an escalating EDSS score.⁵¹ However, employing such an outcome measure for research purposes is hindered by its requirement for an extended observation period. Moreover, it's of limited utility for clinical purposes, as it usually signifies irreversible neurodegeneration. Detecting and predicting progression early on could be profoundly impactful, especially if future therapies demonstrate the capability to arrest or decelerate this progression.

Hence, there is an urgent need for a method to detect and quantify ongoing pathological processes that underlie progressive disease. Broadly, measurement of these pathological processes can be approached in two ways: either by directly quantifying the pathophysiological process itself or by assessing a phenomenon indirectly associated with it. Both approaches can yield meaningful insights. PET emerges as a promising method in this context, as it uniquely allows in vivo imaging of structures that would otherwise be studied post mortem. Currently, various tracers associated with pathological processes are being explored for this purpose.²¹⁶

Regarding the long-term outcomes after AHSCT for RRMS, there has been a concern that patients might progress into SPMS, even in the absence of detectable inflammatory activity as defined by clinical and standard radiological measures. In the cohort of ten individuals with RRMS treated with AHSCT examined in Studies I-III within this dissertation, we observed no clinical

progression during the ten-year follow-up. Nonetheless, it remains plausible that a subclinical neurodegenerative process may eventually manifest as secondary progression over time.

To investigate the potential of PET for imaging neurodegeneration, as suggested by prior studies,⁵⁹ our plan involved the examination of ten individuals with SPMS and ten healthy controls using three PET methods: ¹⁵O-water with ACZ challenge, ¹¹C-PK11195, and ¹¹C-L-deprenyl. Notably, ¹⁵O-water and ¹¹C-L-deprenyl had not been previously studied in this cohort to the best of our knowledge. Our approach consisted of two steps: firstly, we sought to determine whether there were differences between SPMS patients and healthy controls. Subsequently, in the second step, we aimed to investigate whether such differences could also be observed when comparing the ten individuals from the previously described AHSCT cohort to the healthy control group. In light of these considerations, the results are presented in two steps: first as a comparison between SPMS patients and healthy controls, followed by a comparison between AHSCT patients and healthy controls. Our study design is inherently experimental, driven by a spirit of inquiry, and structured as a pilot study. This dissertation includes the results from the ¹⁵O-water study; the other findings are in the process of manuscript preparation.

Results in a broader context

SPMS versus healthy controls

To the best of our knowledge, this is the first study to investigate cerebral blood flow using ¹⁵O-water, which is considered gold standard for measuring CBF. The study demonstrated that CBF is lower in SPMS patients compared to healthy individuals. The difference is observed both when studying the total brain and is even more pronounced when specifically comparing grey matter. This observation confirms previous findings of reduced blood flow in grey matter, which has also been detected using various methods in individuals with CIS,²⁹¹ RRMS,⁸³ and SPMS.²⁹² However, comparisons with MRI techniques are not entirely accurate, as these provide relative, non-quantitative data and do not align well with ¹⁵O-water PET in subcortical regions. Therefore, they cannot be used interchangeably with ¹⁵O-water PET.²⁹³⁻²⁹⁶

The cause of reduced blood flow in the brains of individuals with MS is not fully understood, but as hinted in the ‘Cerebrovascular aspects’ chapter, there is a growing body of evidence suggesting that blood flow is related to neurodegeneration and may play a role in it. There has been speculation about a dysfunctional neurovascular unit with contributing factors such as impaired axonal activity, reduced astrocyte energy metabolism, decreased levels of N-acetylaspartate, and elevated levels of vasoconstricting agents like endothelin-1.^{75,297-300}

Even in the thalami, blood flow was lower in SPMS patients than in healthy individuals. The thalami are gray matter structures with a significant relay function for both motor and sensory functions, as well as for regulating alertness,⁵ and have previously been of interest from an MS perspective.^{87,292} Studies have shown that hypoperfusion in thalami is associated with disability.⁸⁷

In SPMS patients, we compared CBF between NAWM (Normal-Appearing White Matter) and T₂ lesions and found that CBF was lower in the T₂ lesions, which is reasonable considering the lower metabolic demands in these areas. Nevertheless, CVR was preserved in the lesions, indicating that there might be room to increase perfusion here using vasodilatory medications.

No significant correlation between CBF and age could be seen in our healthy controls. This is in line with a previous study, showing that CBF does not decrease in healthy aging, when corrected for dilution-effect of cerebral atrophy.³⁰¹ In another study, the CBF in grey matter was slightly higher in young women, then slowly decreasing to the same level as men at the age of 65.³⁰²

AHSCT versus healthy controls

No significant difference in CBF in total brain, grey matter or white matter was observed between AHSCT and healthy controls, either in the entire brain, grey matter, or white matter. For a discussion of conclusions when the null hypothesis is not rejected, please see the chapter 'Statistical considerations'. However, it can be noted that the difference that was clear in the comparison between the SPMS group and healthy controls could not be demonstrated between AHSCT and healthy individuals.

Cerebrovascular reaction

In our study, no difference in CVR could be detected. Studies of CVR in MS patients have partly been inconsistent, showing both lower CVR in MS patients,^{303,304} and no difference between MS patients and healthy controls.³⁰⁵ In that sense, our results differed from two previous studies showing an impaired cerebrovascular reactivity in MS.^{303,304} In those studies, by Marshall *et al.*³⁰³ and Krogias *et al.*,³⁰⁴ the majority of the examined MS patients had a relapsing-remitting disease (RRMS). The studies were also performed with different imaging modalities (MRI⁸⁰ and Doppler sonography³⁰⁴) and other methods of examining CVR (induction of hypercapnia by inhaling 5% CO₂-enriched gas⁸⁰ and holding breath for 30 seconds³⁰⁴). Another possibility of course is that our study had too few participants to prove a subtle difference in CVR.

Our results on CVR were however more in line with the study by Metzger *et al.*,³⁰⁵ where blood oxygen level dependent MRI was used to measure CBF, and CVR assessed by inhaling 8% CO₂-enriched gas. No difference in CVR could be seen, neither in global nor in regional CVR, including grey matter regions and thalamus. Metzger *et al.* studied a population consisting of both

RRMS and SPMS patients and baseline CBF is not reported. A significant difference in CVR was however seen when comparing MS patients with cognitive impairment to those without.³⁰⁵

In Alzheimer's disease, also a disease of the CNS with marked brain atrophy, changes in CBF can be detected years before the clinical diagnosis of AD, and also predict conversion from mild cognitive impairment to AD.^{217,218} Comparing CVR in AD to vascular dementia, CVR seems to be more impaired in vascular dementia.³⁰⁶ Both AD and vascular dementia are associated with decreased CBF.³⁰⁶ When comparing our results to CVR studies of different forms of vascular dementia, our results more resemble the pattern seen in multi-infarct dementia rather than lacunar dementia, where a global loss of CVR was seen.³⁰⁷

There was a wide variability in CVR between the participants in both groups of our study. One participant in the HC group even had a marked decrease in CBF after the ACZ challenge. Negative CVR can be seen e.g., in Moyamoya disease patients, explained by a cerebrovascular steal phenomenon increasing regional CBF in some vascular territories at the expense of regional CBF in adjacent territories where increase in regional CBF is limited by angiopathy.³⁰⁸ The negative CBF values in our study was however equally distributed in all vascular territories, not indicating a cerebrovascular steal phenomenon. In our participant with negative CVR, we have scrutinized the PET scans, ACZ challenge, technical factors and the modelling without finding a good explanation. One could however note, that this participant's baseline CBF was relatively high and that the CBF after the ACZ challenge was not particularly low. Maybe the baseline CBF of this participant was higher than normally, and that ACZ challenge did not succeed to increase the perfusion further. Another explanation could be that the dose of ACZ was not sufficient for properly evaluating the CVR, compared to other studies. In some reports, a fixed dose of 1,000 mg ACZ was used³⁰⁹, or a higher dose per kg body weight, e.g. 15-20 mg/kg²⁹⁴ or 22 mg/kg.³⁰⁷ In our study, 9 mg/kg ACZ was used up to a maximum of 1,000 mg.

Limitations and strengths

Is the lower CBF only a function of brain atrophy? The previously dominating view, that reduction of CBF in MS patients and other neurological patient groups with pronounced cerebral atrophy, mainly is a phenomenon secondary to atrophy and diminished metabolic demands, has been challenged in recent years.^{75,297,310} Reduction of cortical CBF has also been seen in the absence of atrophy⁸³ and in different cortical areas than those showing pronounced atrophy.³¹¹

There was a substantial brain atrophy in both the SPMS and the AHSCT group as defined by nCCA, but no significant difference in nCCA when comparing those groups. As described previously, the brain atrophy in the AHSCT

group was ongoing prior to AHSCT and continued unabated 2.5 years after the AHSCT, when the atrophy rate essentially dropped to zero.³¹² It is however still possible that brain atrophy could influence the measurements of CBF by partial-volume effects, which is why partial-volume corrections were performed in grey matter VOI:s with consistent results.

As previously described, the main limitations of the study are the relatively small number of participants and the cross-sectional design, which can raise concerns about the validity of the negative results of this study. It would also have been advantageous to follow patients prospectively with repeated investigations over time to detect individual changes and to evaluate changes that can occur after treatment with AHSCT.

Due to a facility upgrade, two different PET scanners were used. To compensate for this, reconstruction settings were chosen to result in matching image resolution.

Summary

The study provided clear evidence of reduced CBF in individuals with SPMS, with the most significant decrease observed in grey matter and the thalami. Importantly, there was no indication of impaired cerebrovascular reactivity, indicating the potential for therapeutic intervention using vasodilators. Notably, patients who underwent AHSCT exhibited CBF levels similar to those of healthy volunteers, and we found no signs of a subclinical disease process in this group, despite extended disease duration and an initially aggressive disease course.

Future perspectives

In light of the decreased CBF and preserved CVR observed in our study in the group of SPMS patients, there may be potential for the testing of vasodilating medications, especially given the limited availability of other treatment options.

Study IV

Background and aim of the study

During the COVID-19 pandemic,¹⁹⁷ physicians and patients faced the urgent question of how to achieve effective vaccination protection for individuals undergoing treatment with the B-cell depleting drug rituximab. Since the world faced a completely new virus, SARS-CoV-2, there was no established evidence to rely on. Moreover, data indicated that the risk of severe COVID-

19 was higher in individuals with MS who were being treated with rituximab.^{198,199} Previous guidelines often suggested waiting for a certain period after the last dose of rituximab before administering vaccination.^{210,211} However, our hypothesis was that the level of B cells, rather than the time since the last rituximab infusion, determined the humoral vaccine response. But what level of B cells should one aim for? Clinical experience also suggested that the rate of B-cell recovery could vary significantly among different individuals. Previous studies on other vaccines and patient groups supported this hypothesis, but the evidence base was limited.²⁰⁴ Additionally, other factors such as age or the duration of rituximab treatment could potentially influence the vaccine response. Therefore, the objective of this study was to investigate the factors associated with a favourable vaccine response to the vaccine against COVID-19 that was available for us, tozinameran, in individuals receiving rituximab treatment.

Results in a broader context

The main finding of the study was that B-cell levels were related to the humoral immune response. Individuals who had a robust humoral vaccine response had higher levels of B cells before vaccination compared to those who did not. Among those with B-cell levels exceeding 40/ μ L, 90% achieved protection. This observation aligns with a few studies conducted on individuals treated with rituximab before the pandemic. For example, Oren *et al.* studied 25 individuals receiving rituximab treatment for rheumatic diseases who were vaccinated against influenza in 2008. They found that B-cell levels were higher in those who had a humoral immune response. However, a level exceeding 1 per microliter was sufficient for a response, even though the overall vaccine response was low.²⁰⁴ Subsequent studies have also confirmed the relationship between low B-cell levels and an impaired humoral immune response following vaccination against COVID-19.³¹³⁻³¹⁷

The threshold of 40 B cells/ μ L for achieving 90% protection with tozinameran seems surprisingly high and considerably higher than the previous guideline of 20 B cells/ μ L in Sweden.³¹⁸ Can this be explained by tozinameran potentially having relatively low immunogenicity? For example, Wang *et al.*, in a literature review and meta-analysis, found that immunosuppressed individuals vaccinated with tozinameran had a higher risk of COVID-19 and hospitalization compared to those vaccinated with elisasomeran.³¹⁹

We were not the only group faced with the dilemma of vaccinating rituximab patients, as the rich literature published in 2022 and 2023 on this topic attests to. However, comparing these studies is challenging because they often differ in many aspects, such as the type of vaccine, the time between vaccine doses, treatment indications, concurrent use of other immunomodulatory and immunosuppressive drugs, timing of analyses, analysis methods, and outcome measures. Compiling review articles will be a complex task.

As an example, Stefanski *et al.* studied 19 individuals receiving rituximab treatment for rheumatological diseases who were mainly vaccinated with tozinameran ($n = 14$).³²⁰ They found that a level of 10 B cells/ μL was the lowest threshold above which seroconversion to anti-SARS-CoV-2 spike IgG occurred seven days after the second vaccine dose. In our study, we defined serological immune response as anti-SARS-CoV-2 spike IgG > 264 BAU/mL, as this level, at the time of our data analysis, had been shown to provide 80% protection against primary symptomatic COVID-19 infection.³²⁰ In addition, our blood samples were taken six weeks after the first dose, five weeks later than Stefanski *et al.* Furthermore, we administered the second dose three weeks after the first, following the registration study, but early on, for example, the UK chose to extend the interval to 12 weeks to provide more people with the first dose, which turned out to yield a better vaccine response when the second dose was eventually given.³²¹ Information on dosing intervals was not available in Stefanski *et al.*

In a study by Kornek *et al.* involving 82 individuals receiving anti-CD20 treatment for various neurological conditions and an equally large control group, 100% of participants with B-cell levels of $\geq 1/\mu\text{L}$ or more achieved seroconversion, i.e., a level above their cut-off titre of 0.8 BAU/mL anti-SARS-CoV-2 spike IgG.³²² This led the authors to conclude that vaccination should be administered as soon as B cells become detectable.

Due to the clinical routine we applied, only a few individuals in our study had undetectable B cells, and our sample size was too small to draw a definitive conclusion regarding the difference between 0 B cells/ μL and detectable levels. Among the 13 individuals in the group with a total of 0-19 B cells/ μL before vaccination, 5 achieved anti-spike > 264 BAU/mL. Given the current state of knowledge, this observation led us to the conclusion that a first vaccine dose should be offered regardless of the time since the last rituximab infusion, as there is still a chance of developing a protective antibody response despite low B-cell levels, especially considering the severe condition that COVID-19 can cause in this patient group.

Once a humoral vaccine response is established, it appears that the level of B cells is of little or no importance for the efficacy of a booster dose.^{322,323} This is reasonable because plasma cells do not express CD20. These crucial observations open the possibility that booster doses, once basic vaccination has been completed, do not need to be timed with rituximab doses.

However, this conclusion does not imply that a failed or low immune response should be accepted. On the contrary, there is now evidence that lower antibody levels are associated with a higher risk of (symptomatic) breakthrough COVID-19 infection after vaccination and, more importantly, with a need for hospitalization as a result.³²⁴ Therefore, if one wants to be more certain of achieving a protective humoral vaccine response, one should aim for a more substantial recovery of B-cells.

Extending the dosing interval to create a vaccination window seems to be feasible without major risks. In our study, only one participant experienced a mild and transient relapse and two had new lesions on MRI. An increasingly large body of evidence also supports the long-lasting and effective effect of rituximab treatment, even without continuous total depletion.^{315,325} On the contrary, some B-cell recovery between anti-CD20 infusions might be beneficial to avoid side effects such as infections and hypogammaglobulinemia.^{326,327} For example, van Lierop *et al.* successfully tested a strategy during the COVID-19 pandemic where ocrelizumab, another anti-CD20 treatment, was administered first when B-cell repopulation levels after the previous ocrelizumab infusion exceeded 10/ μ L.³²⁸

Our study also confirmed our clinical experience that the rate of B-cell recovery varies significantly among individuals. Based on the B-cell level results six months after the last rituximab infusion, we could categorize participants into 'slow mobilizers' (<10 B cells/ μ L) and 'fast mobilizers' (\geq 10 B cells/ μ L). Slow mobilizers had a slower recovery rate with an increase in B-cell levels of 7.5 B-cells/ μ L/month, while fast mobilizers had an increase of 18 B cells/ μ L/month. This could serve as a rough but straightforward clinical rule of thumb when estimating the timing of the next blood sample if one wants to wait for B-cell recovery to a certain level before vaccination.

Cellular immunity in the form of T-cell reactivity was not associated with B-cell levels, as many other subsequent studies have also confirmed.³²⁹ This suggests that B-cell independent cellular immunity can be developed despite anti-CD20 treatment, but the extent to which cellular immunity can protect against severe COVID-19 and death in the absence of humoral immunity is not clear.

Limitations and strengths

With its observational study design, the study had several limitations. There was no randomization, and a large control group was missing. The internal control group consisted of individuals about to start rituximab treatment, and no pre-vaccination blood samples were available to serve as baseline values. The follow-up period was also short, which means that knowledge about the extent and rate of waning immunity is lacking. It would also be valuable to determine the minimum time interval between vaccination and the next rituximab infusion needed to optimize long-term protection. Furthermore, established cut-off values and definitions for T-cell responses were lacking, which was of lesser importance since no difference between the groups was observed. Moreover, the study relied on laboratory outcome measures; however, for individual patients, the only relevant outcome measure is severe COVID-19. Our study was too short and too small to evaluate this.

The strength of the study was that, despite being an observational study, it was a relatively uniform cohort. All individuals were treated with rituximab

(except for the internal controls) for multiple sclerosis, all received the same vaccine, and most were vaccinated with a three-week interval between the first and second dose. The subsequent blood sample was taken approximately six weeks after the second dose. We also had data on previous COVID-19, and we have reason to believe that this data was reliable; at that time, patients were encouraged by healthcare providers and authorities to get tested for COVID-19 when experiencing symptoms. Another strength of the study was its relatively fast turnover, from the initial study plans in early 2021 to publication in May 2022.

Summary

In summary, Study IV contributed to the understanding of the significance of B-cell levels in generating a robust humoral immune response following vaccination with tozinameran in individuals with MS and rituximab treatment. It is reasonable to assume that this relationship also applies to other anti-CD20 treatments, different diagnoses, and other vaccines. The study has, therefore, been able to contribute to more evidence-based clinical practices.

Future perspectives

As B-cell depleting treatment becomes more common, we are increasingly confronted with the downsides of this treatment. Only by conducting studies can we learn more about the best strategies for optimal drug use. Many questions remain about optimizing vaccination strategies in anti-CD20 treatment. What serological cut-off can be associated with a reduced incidence of severe COVID-19 in this patient group? What protective effect does cellular immunity have? How can we optimize the effectiveness of booster doses? Which vaccine is best for this patient group?

Currently, most research is focused on COVID-19 vaccines, but knowledge about other vaccines is still limited. For example, vaccination against tick-borne encephalitis is becoming more relevant due to the effects of climate change,³³⁰ and fatal outcomes due to this disease have been reported during rituximab treatment.³³¹ Furthermore, mRNA vaccine technology has the potential to rapidly develop a wide range of important vaccines,³³² which patients receiving anti-CD20 treatment are likely to benefit from. Conducting systematic studies to optimize strategies for different vaccines during anti-CD20 treatment is crucial.

Final remarks

This dissertation contributes to the growing body of knowledge regarding successful treatment outcomes with AHSCT for MS. In Study II, it is revealed that prior profound uncertainty vanishes as a result of the treatment. Some individuals also report an experience of no longer having MS, a phenomenon that is not easily refuted when examining the outcomes in Study I. Furthermore, there is a demand for objective criteria for terms like 'cure' or similar concepts.

In Study I, we propose concepts that can be used to describe a maximally successful treatment outcome and demonstrate that it is achievable.

Study III investigates individuals with SPMS using measurements of CBF and CVR, showing that cerebral blood flow is reduced in these individuals compared to healthy subjects, confirmed by the gold-standard method. AHSCT patients did not exhibit the same difference. The role of reduced blood flow remains to be explored, but the preservation of CVR in SPMS individuals suggests some reversibility, potentially opening avenues for therapeutic interventions with vasodilators.

In Study IV, we address the side effects of long-term immunomodulatory treatment by seeking the optimal way to achieve vaccine responses in individuals treated with rituximab. The level of B cells plays a crucial role in the formation of humoral immune responses, emphasizing the need to measure B-cell counts rather than counting days from rituximab infusion to achieve vaccine response.

In a broader perspective, the entire issue of vaccinating individuals undergoing rituximab treatment contributes to the argument favouring immune reconstitution therapy, if it can be conducted safely, given the excellent treatment outcomes. Therefore, it is suggested that AHSCT should be offered to more individuals and at an earlier stage.

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