UPPSALA
UNIVERSITET

Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine 1005

Curing Multiple Sclerosis

How to do it and how to prove it

JOACHIM BURMAN

ACTA
UNIVERSITATIS
UPSALIENSIS
UPPSALA
2014

ISSN 1651-6206
ISBN 978-91-554-8964-9
urn:nbn:se:uu:diva-221888

Dissertation presented at Uppsala University to be publicly examined in Rudbecksalen, Rudbecklaboratoriet, Dag Hammarskjölds väg 20, Uppsala, Friday, 13 June 2014 at 09:15 for the degree of Doctor of Philosophy (Faculty of Medicine). The examination will be conducted in English. Faculty examiner: Clinical Reader Paulo Muraro (Division of Brain Sciences, Department of Medicine, Imperial College London, London, United Kingdom).

Abstract

Burman, J. 2014. Curing Multiple Sclerosis. How to do it and how to prove it. *Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine* 1005. 74 pp. Uppsala: Acta Universitatis Upsaliensis. ISBN 978-91-554-8964-9.

Hematopoietic stem cell transplantation (HSCT) is a potentially curative treatment for multiple sclerosis (MS) with now more than 600 documented cases in the medical literature. Long-term remission can be achieved with this therapy, but when is it justified to claim that a patient is cured from MS? In attempt to answer this question, the outcome of the Swedish patients is described, mechanisms behind the therapeutic effect are discussed and new tools for demonstration of absence of disease have been developed.

In Swedish patients treated with HSCT for aggressive MS, disease free survival was 68 % at five years, and no patient progressed after three years of stable disease. Presence of gadolinium enhancing lesions prior to HSCT was associated with a favorable outcome (disease free survival 79 % *vs* 46 %, p=0.028). There was no mortality and no patient required intensive care.

The immune system of twelve of these patients was investigated further. In most respects HSCT-treated patients were similar to healthy controls, demonstrating normalization. In the presence of a potential antigen, leukocytes from HSCT-treated patients ceased producing pro-inflammatory IL-17 and increased production of the inhibitory cytokine TGF-β1 suggesting restoration of tolerance.

Cytokine levels and biomarkers of tissue damage were investigated in cerebrospinal fluid from a cohort of MS patients. The levels were related to clinical and imaging findings. A cytokine signature of patients with relapsing-remitting MS could be identified, characterized by increased levels of CCL22, CXCL10, sCD40L, CXCL1 and CCL5 as well as down-regulation of CCL2. Further, we could demonstrate that active inflammation in relapsing-remitting MS is a tissue damaging process, with increased levels of myelin basic protein and neurofilament light. Importantly, relapsing-remitting MS patients in remission displayed no tissue damage. In secondary progressive MS, moderate tissue damage was present without signs of active inflammation.

From a clinical vantage point, it seems that we confidently can claim cure of relapsing-remitting MS patients after five years absence of disease activity. The new tools for evaluation of disease can strengthen this assertion and may enable earlier prediction of outcome.

Keywords: biomarkers, cerebrospinal fluid, cytokines, hematopoietic stem cell transplantation, immunology, magnetic resonance imaging, multiple sclerosis, neuroimmunology, neurology

Joachim Burman, Department of Neuroscience, Neurology, Akademiska sjukhuset, Uppsala University, SE-75185 Uppsala, Sweden.

ISSN 1651-6206
ISBN 978-91-554-8964-9
urn:nbn:se:uu:diva-221888 (http://urn.kb.se/resolve?urn=urn:nbn:se:uu:diva-221888)

List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals in superscript. Reprints of the actual papers are located in the second part of this book.

I Burman J, Iacobaeus E, Svenningsson A, Lycke J, Gunnarsson M, Nilsson P, Vrethem M, Fredrikson S, Martin C, Sandstedt A, Uggla B, Lenhoff S, Johansson JE, Isaksson C, Hägglund H, Carlson K, Fagius J. Autologous hematopoietic stem cell transplantation for aggressive multiple sclerosis: the Swedish experience. J Neurol Neurosurg Psychiatry. Epub ahead of print 2014 Feb 19.

II Burman J, Fransson M, Tötterman TH, Fagius J, Mangsbo SM, Loskog ASI. T cell responses after hematopoietic stem cell transplantation for aggressive relapsing-remitting multiple sclerosis. *Immunology*. 2013 Oct;140(2):211-9.

III Burman J, Svensson E, Fransson M, Loskog ASI, Zetterberg H, Raininko R, Svenningsson A, Fagius J, Mangsbo, SM. The cerebrospinal fluid cytokine signature of multiple sclerosis: a homogenous response that does not conform to the Th1/Th2/Th17 convention. Manuscript.

IV Burman J, Zetterberg H, Fransson M, Loskog ASI, Raininko R, Fagius J. Assessing tissue damage in multiple sclerosis: a biomarker approach. Acta Neurologica Scandinavica. Epub ahead of print 2014 Feb 24.

Contents

Abbreviations

AAR	annualized relapse rate
ATG	anti-thymocyte globuline
BEAM	a combination of four drugs: BCNU, etoposide, cytosine-arabinoside and melphalan
CD	cluster of differentiation
CNS	central nervous system
EAE	experimental autoimmune encephalomyelitis
EBMT	European Group for Blood and Marrow Transplantation
EDSS	expanded disability status scale
EMA	European Medicines Agency
FDA	Food and Drug Administration
FoxP3	forkhead box protein 3
G-CSF	granulocyte colony-stimulating factor
Gd+	gadolinium enhancing
GFAp	glial acidic fibrillary protein
HR	hazard ratio
HSCT	hematopoietic stem cell transplantation
HLA	human leukocyte antigen (synonymous to MHC)
IL	interleukin
MBP	myelin basic protein
MHC	major histocompatibility complex (synonymous to HLA)
MOG	myelin oligodendrocyte glycoprotein
MRI	magnetic resonance imaging
MS	multiple sclerosis
NFL	neurofilament light
NK cell	natural killer cell
PFS	progression free survival
PBMC	peripheral blood mononucleated cells
PML	progressive multifocal leucoencephalopathy
PPMS	primary progressive multiple sclerosis
RRMS	relapsing-remitting multiple sclerosis
SPMS	secondary progressive multiple sclerosis
TBI	total body irradiation
Tc	cytotoxic T cell
Th	T helper cell
Treg	T regulatory cell
TRM	treatment related mortality
VCAM-1	vascular cell adhesion protein 1

Prologue

In September 2003 a young woman fell ill. It began with a facial palsy, soon followed by weakness in a leg, pronounced fatigue and loss of vision. A diagnosis of multiple sclerosis was made. Although these symptoms got better, others more ominous took their place. She became paralyzed from the waist down and her bladder stopped working. She got treatment and again she got better for a while. However, this was just a short respite and by spring she was completely paralyzed.

She was transferred to the University Hospital. At the darkest hour, she was offered a novel treatment. Hematopoietic stem cell transplantation. With little to lose and everything to gain, she accepted. None could have guessed at the outcome.

A few days into procedure, she was able move her toes again, for the first time in two months. Rapid improvement followed. Some weeks after discharge, she could walk with a stroller. After three months she could walk unaided. After one year she was working part-time.

Ten years later, she is living a normal life. She works full-time. She is the mother of two healthy children. She has no treatment. She has not had any relapses.

Is she cured from multiple sclerosis?

Chapter 1 - Introduction

> Disseminated sclerosis, as I have informed you, gentlemen, is not an exclusively spinal affection. It invades the cerebrum, the pons Varolii, the cerebellum, the bulbus rachidicus, as well as the spinal cord.
>
> Lectures on the diseases of the nervous system (1877). Lecture VI Disseminated sclerosis. Pathological anatomy.
>
> J M Charcot

Multiple sclerosis (MS) is a debilitating disease affecting mainly young individuals, with a peak incidence around 30 years of age. In Sweden more than 17 000 persons suffer from MS,[1] and worldwide an estimated 2.5 million.[2] Untreated, it often leads to severe disability and premature death.[3-6] It is considered to be an inflammatory and autoimmune disease of the central nervous system (CNS).

At onset, 85% of patients will have a relapsing remitting form (RRMS), with periods of worsening followed by periods of recovery and stable disease.[7] Eventually most of the patients will develop a secondary progressive form of the disease (SPMS), characterized by fewer and milder relapses,[7] less MRI activity[8, 9] but relentless deterioration of neurologic function.[10] After 40 years of disease, more than 80% of RRMS patients will have developed SPMS.[10] A minority of patients will have progressive disease from onset and no relapses; such disease is denominated primary progressive MS (PPMS).[7]

Basic concepts

What is inflammation?

According to the Encyclopædia Britannica, inflammation is “a response triggered by damage to living tissues. The inflammatory response is a defense mechanism that evolved in higher organisms to protect them from infection and injury. Its purpose is to localize and eliminate the injurious agent and to remove damaged tissue components so that the body can begin to heal. The response consists of changes in blood flow, an increase in permeability of blood vessels, and the migration of fluid, proteins, and white blood cells (leukocytes) from the circulation to the site of tissue damage. An inflammatory response that lasts only a few days is called acute inflammation, while a response of longer duration is referred to as chronic inflammation.”[11]

What is autoimmunity?

Autoimmunity is less straightforward than inflammation and considerable thought has been put into the definitions of this concept. In its simplest form it can be viewed as “the state in which the immune system reacts against the body’s own normal components, producing disease or functional changes.”[12] But how do you know if a disease is autoimmune?

In 1957 Witebsky *et al* postulated criteria for autoimmune diseases, the "Witebsky's postulates".[13]

1. An autoimmune response must be recognized with an autoantibody or cell-mediated immunity.
2. A corresponding antigen must be identified.
3. An analogous autoimmune response can be induced in an experimental animal model.
4. The immunized animal must develop a similar disease.

The definition of autoimmunity was later modified by Rose and Bona.[14]

1. Direct evidence by transfer of pathogenic antibody or pathogenic T cells.
2. Indirect evidence based on reproduction of the autoimmune disease in experimental models.
3. Circumstantial evidence
    a. lymphocytic infiltration of target organ.
    b. statistical association with MHC haplotype.
    c. favorable response to immunosuppression.

Polly Matzinger turned the table and suggested that the immune system is more concerned with damage than with foreignness, and is called into action by alarm signals from injured tissues, rather than by the recognition of non-self.[15] According to Matzinger, the tissue shapes the immune response in the normal situation (Figure 1.1).[16] The brain is considered an immune-privileged site, but Matzinger argues that CNS tissue shapes an immune response into a preferable form rather than inhibiting it. In this sense, an autoimmune disease such as MS is a condition where the immune system does not listen to the tissue.

What is a relapse?

One of the most common definitions of a relapse is: *a period of acute worsening of function lasting ≥24 hours*. The word "inflammation" is not mentioned in this definition, although most would agree that a relapse is caused by an inflammatory event in the brain or spinal cord. In clinical practice, diagnosis of a relapse is usually made on clinical grounds only. There are several pitfalls in the diagnosis of a relapse, however. The well-known Uhthoff* phenomenon is one,[17, 18] and even for experienced clinicians it can be difficult to ascertain if the symptoms presented by a patient are caused by inflammatory activity in the CNS. Magnetic resonance imaging (MRI) has been employed to evaluate relapses. The presence of gadolinium enhancing lesions is usually taken as support for an ongoing relapse. The reverse is not true, however. With administration of higher doses of gadolinium, more and larger lesions are seen;[19] with the employment of other contrast agents such as ultra-small iron oxide particles (USPIO) other lesions can be revealed.[20, 21]

Degradation products of myelin, such as myelin basic protein can be measured in the cerebrospinal fluid (CSF) to demonstrate destruction of oligodendrocytes, and high levels of myelin basic protein can indeed be found early on in a relapse.[22, IV]

---

* *In 1890 Wilhelm Uhthoff described a condition of temporary vision loss linked to physical exercise, in patients with previous optic neuritis. This condition was to become known as Uhthoff's phenomenon, and was later found to be caused by a decrease in nerve conduction velocity due to a rise in body temperature.*

Figure 1.1. Overview of Matzinger's danger model.

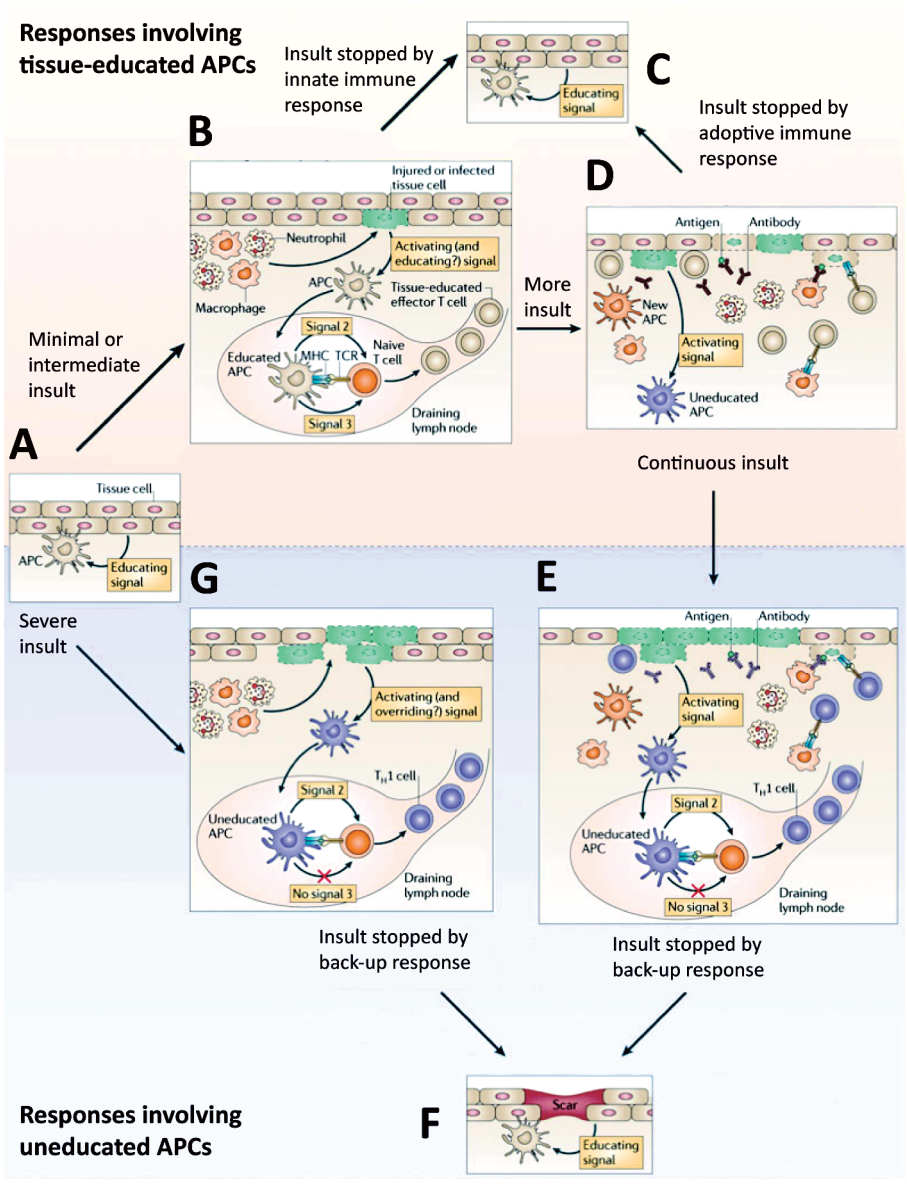


Figure 1.1 Overview of Matzinger's danger model (continued).

(A) Resting tissues educate local antigen-presenting cells (APCs). **(B)** Following an insult (such as an injury or infection), the APCs leave the tissue to stimulate naive T cells to make tissue-educated responses. **(C)** If the innate immune response clears the infection (or injured tissue), the tissue heals and educates newly arriving APCs. An adaptive immune response is not needed and ceases. **(D)** If the innate immune response does not stop the infection, then tissue-educated adaptive immune responses are initiated. If these clear the pathogen, then the tissue heals. **(E)** If the tissue-educated adaptive immune response cannot resolve the infection, then a second wave of newly entering APCs will be activated in a local tissue environment that now contains more extensive damage. The new APCs may be properly educated or they may not be (because the high level of damage would result in fewer signals from the tissue). If not, they will leave the tissue and stimulate the emergency backup response. **(F)** If the backup response clears the pathogen, then the tissue heals, but with some scarring or fibrosis occurring. **(G)** If the initial insult is severe, the local APCs leave the tissue without receiving a complete education. This could be because the severely damaged tissues cannot provide the right signals or because the tissue provides signals that override the original education. These APCs launch the immediate backup response. Reprinted with permission (see Appendix).

How is the clinical diagnosis of multiple sclerosis made?

The cornerstone of MS diagnosis is the demonstration of dissemination of disease in space and time. In the Schumacher criteria from 1965, a diagnosis of MS could be made after two attacks with symptoms characteristic of multiple sclerosis separated in time by no less than one month and from two anatomically distinct sites of the central nervous system.[23] Today, diagnosis can be made after one attack if supportive MRI data are available.[24]

What is secondary progressive disease?

From a clinical point of view SPMS is a loss of function that occurs slowly and gradually over years or even decades. Relapses tend to be absent or mild. SPMS cannot be treated successfully,[25] and therapy can at best achieve slowing of deterioration.[26] From an imaging standpoint, SPMS is characterized by axonal damage and increasing atrophy of the brain and spinal cord.[27] This axonal damage can be measured in the CSF of MS patients as increased levels of neurofilament.[28, IV]

Pathogenesis

Ultimately, symptoms in MS can be related to neuronal dysfunction or destruction. The main target of the inflammation seen in MS is the oligodendrocyte,[29] one of the four main cell types in the CNS. The oligodendrocyte contains myelin, and together several oligodendrocytes will form a myelin sheath, providing insulation for axons traversing the brain and spinal cord. The attack on the oligodendrocyte leaves the axon denuded; hence, MS has been considered a demyelinating disease. Demyelination occurs in discrete areas of the brain and spinal cord, typically located around the lateral ventricles, juxtacortically or in subtentorial white matter of the brain and spinal cord. In these demyelinated *plaques*, axonal transmission is impaired, leading to conduction block of neuronal signaling. However, the concept of demyelination is in many ways misleading, since it has been established that axonal damage can be present from onset, increases with time and correlates with disability.[27]

The preceding events leading to the inflammatory attack is not fully known. Several risk genes and environmental risk factors have been discovered. More than 100 genes affecting the risk of developing MS have been described and a majority of them are coding proteins important for the immune system.[30] The strongest association with MS is seen in the HLA-DR locus; carriers of the HLA-DRB1*1501 allele have an odds ratio of 5.80 for development of MS. Polymorphisms in other loci increase the risk of MS to a lesser degree, *e g* single-nucleotide polymorphisms in the IL-2 receptor alpha and IL-7 receptor alpha genes confers an odds ratio of 1.25-1.18.[31] Some alleles have proven to be protective, such as the HLA-A*02, which confers an odds ratio of 0.4 for development of MS. The relative contribution of the genetic background to the risk of developing MS can be estimated from twin studies. The concordance rate among monozygotic twins is 15-24 % and in dizygotic twins 1.7-3 %.[32, 33]

Epstein-Barr virus infection is the strongest environmental risk factor, and the hazard of developing MS is approximately 15-fold higher among individuals infected with Epstein-Barr virus in childhood and about 30-fold higher among those infected with mononucleosis.[34] Other risk factors associated with MS are smoking, childhood obesity and vitamin D insufficiency, whereas sun exposure seems to be protective.[34]

A simplified model of MS pathogenesis

For the purposes of this thesis a modified version of Matzinger's danger model will be used. In the special case of MS, I shall also make the following assumptions:

1. Some lymphocytes of MS patients have acquired the ability to recognize oligodendrocytes as something dangerous that must be destroyed.
2. MS relapses are caused by damage to the CNS orchestrated and mediated by these lymphocytes.
3. Progressive disease is a consequence of previous inflammatory mediated damage to the CNS.

Recognition

It has been demonstrated that auto-reactive T cells recognizing oligodendrocyte epitopes are present in healthy individuals as well as in MS patients.[35] From this we can conclude that the ability to recognize self is not enough to generate an autoimmune response in this context. The auto-reactive lymphocytes are probably fewer in healthy individuals, under influence of powerful regulatory mechanisms and still listening attentively to tissue signals. What causes the transition from this state to MS is presently unknown, but it is reasonable to believe that the above-mentioned risk factors increase the likelihood that this event will occur.

Infiltration

From early histopathological studies, it was clear that MS lesions contained cells that would later be recognized as essential parts of the immune system. Today, there is abundant evidence that these cells are disrupting nerve cell function and destroying tissue in the CNS.[36, 37] The central questions are: "Why are those cells there?" and "Why at this particular time?" Even if a fair amount of dysregulated auto-reactive T cells are in circulation, they are not necessarily gaining access to the brain or spinal cord where they can interact with their molecular targets. It is well known that new plaques may appear anywhere within the white matter, but hitherto there has been no satisfactory explanation as to why the lymphocytes are lured to a specific area of the CNS. Until this issue has been resolved, this must be viewed as an inherent randomness of the disease.

Progression

There is currently an on-going debate whether unfavorable inflammation is absent in SPMS or if it still plays an important role. It is clear that continuous degeneration of axons as a consequence of prior damage to the CNS can be seen in the absence of inflammation, at least in animal models.[38, 39] On the other hand the evolution of SPMS is paralleled by the emergence of lymphoid like structures in the meninges[40] and studies have revealed a shift of immunity towards the innate system.[41, 42] Maybe, inflammation is not absent in SPMS, but rather of a different kind: low grade and compartmentalized behind an intact blood-brain barrier. Under any circumstance, it is widely believed that secondary progression is a consequence of previous inflammatory disease. Although rigorously hard to prove, there is some evidence that early treatment might delay the onset of secondary progressive disease.[43]

The usual suspects

The immune system contains several key components, which contributes to the pathogenesis of MS in different ways. Below, you will find a summary of what is known about how these contribute to MS.

Antibodies

More than fifty years ago, it was observed that MS patients had an increased level of antibodies in CSF.[44] Later on it became clear that this increased production of antibodies were oligoclonal in distribution, *i e* only a limited number of plasma cell clones are contributing to the increased levels of antibodies.[45] A further development was the development of the IgG-index, which is an estimate of intrathecal IgG production,[46] and today the demonstration of intrathecal IgG production is part of the clinical routine in establishing a diagnosis of MS. However, the specificity of these antibodies has not been established. Most of the oligoclonal antibodies present in the CSF are not directed to the major myelin components,[47] and some controversy exists as to the importance of those that do exist.[36] Additionally, intrathecal antibody production can be seen in a variety of conditions.[48] At present it is unclear whether these antibodies are harmful, protective, neither or both. It has been demonstrated that patients with RRMS and SPMS have antibodies directed towards oligodendrocyte precursor cell lines, but only the SPMS patients had antibodies directed towards a neuronal cell line.[49] This supports the idea that the concept of epitope spreading is important in MS.

Cytokines

Cytokines are molecules that play a pivotal role in the regulation of inflammatory responses and tissue repair. The term cytokine has replaced the older terminology of interleukins and chemokines. Cytokines orchestrate all phases of immune responses and act in highly complex, dynamic networks in a paracrine and/or autocrine fashion and play a central role in the recruitment of leukocytes to sites of inflammation. They are secreted by cells of the immune system, but also by tissues such as the CNS. Immune responses have been characterized by the pattern of cytokines produced; the distinction between Th1 and Th2 type responses is the classical example.[50] MS has been associated with the Th1 response and the more recently discovered Th17 type response.[36, 51] Of late the stereotyped cytokine responses have come into question and it has been theorized the tissue shapes the immune response locally and Th1 and Th2 responses are only crude simplifications.[16]

B cells

For a long time, B cells were thought to be important in MS in their capacity to differentiate into plasma cells and produce antibodies.[36] The advent of B cell depleting therapies has challenged this view. It was noted that clinical improvement in patients treated with rituximab often preceded reduction in autoantibody levels.[52] Further, in a phase II trial of atacicept, a fusion protein that blocks plasma cell function and the late stages of B cell development, treatment was found to aggravate MS.[53] This is in contrast to rituximab, which in clinical trials has been proven to be effective in MS.[54, 55] The above suggests that B cells are important in some other capacity than antibody production. Rituximab treatment results in a noticeable decline of T cell numbers in CNS of treated patients, suggesting that B cells sustain pathogenic T cell responses. Recently, it was demonstrated that IL-6 production is the major mechanism of B cell contribution to the pathogenesis of EAE (experimental autoimmune encephalomyelitis, an experimental model of MS), and also that this inflammatory pathway was increased in RRMS patients.[52] Another possibility is that the B cells act as antigen presenting cells.[56]

B cells may also be important for the evolution of SPMS. Ectopic lymphoid follicles, enriched with B cells and plasma cells, have been found in the meninges of a subset of patients with SPMS.[40] The preferential localization of these ectopic follicles is the subarachnoid space in the cerebral sulci and their presence is correlated to severe cortical pathology and an aggressive clinical course.[57] Interestingly, rituximab is the only drug that has been shown to exert some effect in primary progressive MS.[55]

T helper cells

CD4+ T cells or T helper cells (Th cells) are the most implicated culprits in MS. One of the first observations suggesting an important role for Th cells came from studies of EAE. It was demonstrated that EAE could be transferred from diseased to naïve animals by *in vitro* reactivated myelin specific Th cells.[58] Since then, several studies have focused on Th cells in EAE and MS and today there is very compelling evidence that Th cells are key players in the inflammatory process of MS:

1. Th cells are part of the CNS infiltrating cells in MS.[59]
2. Genetic risk is to a substantial degree conferred by HLA-DR and -DQ molecules.[31]
3. Mice expressing both MS-associated HLA-DR molecules and MS patient derived myelin basic protein specific T cell receptor develop spontaneous EAE.[60, 61]
4. A therapeutic trial with an altered peptide ligand of myelin basic protein induced cross reactive Th cells that led to disease exacerbations of MS patients.[62]
5. Antibody production, maturation of cytotoxic T cells and many other steps of adaptive and innate immunity are at least in part controlled by Th cells.[36]

On the other hand, in a clinical trial with a monoclonal CD4 depleting antibody, no effect was seen on MRI parameters.[63] This suggests that the human situation is much more complicated than what can be gathered from the EAE model.

There are several types of Th cells and for a long time it was believed that MS was a Th1 mediated disease.[36] During the last years, attention has been shifted to Th17 cells, which are induced by IL-23. The role of Th17 cells in host defense against pathogens has been characterized extensively in mouse models, with the general consensus that IL-17 is necessary for protective immunity against bacteria and fungi at mucosal barriers. In humans, the role of Th17 cells in anti-bacterial responses is largely unexplored.[64]

Increased numbers of IL-17A producing Th17 cells have been demonstrated in MS patients, particularly during relapses. Further, with a microarray approach, IL-17A was found to be elevated in MS plaques in comparison to brain tissues from control subjects.[50] Quite recently it was reported that secukinumab, a monoclonal antibody directed against IL-17A decreased the number of gadolinium enhancing lesions of MS patients in a phase I trial.[65]

Cytotoxic T cells

CD8+ cytotoxic T cells (Tc cells) have been much less investigated in MS than Th cells. Nevertheless, there are several reasons to believe that Tc cells are important in the pathogenesis of MS:

1. The MHC class I allele HLA-A*0301 has been associated with increased risk of developing MS, whereas the HLA-A*0201 allele is protective.[66]
2. Depending upon the severity of the disease and the activity of the lesions, astrocytes, oligodendrocytes and neurons express MHC class I molecules, making them potential targets for Tc cells.[67]
3. Prominent oligoclonal expansion of Tc cells can be seen in MS brain tissue and within parenchymal lesions Tc cells can be detected with their cytolytic granules polarized towards demyelinated axons indicative of imminent Tc cell mediated killing.[68]
4. Distinct T-cell clones are present in lesions in anatomically disparate regions from MS patients confirming that infiltration of CD8 T cells in the lesions is selective, rather than stochastic.[69]

A subset of CD8+ T cells, $CD161^{high}$CD8+ T cells, sometimes denominated mucosal-associated invariant T cells (MAIT) are found in increased frequency of the blood from MS-patients. These MAITs produce pro-inflammatory cytokines such as IL-17, IFN-γ and TNF-α and have also been demonstrated in MS plaques.[70, 71]

T regulatory cells

T regulatory cells (Tregs) are capable of restricting the proliferation and cytokine production of a wide range of immune cells. They are characterized by the production of the transcription factor forkhead box protein 3 (FoxP3), which has been called a master regulator of Treg function. Since transcription factors are located intracellularly, FoxP3 cannot be used as a surface marker for Tregs. In order to measure it, cells have to be permeabilized, making functional studies impossible. For studies on live cells other markers have been used, such as CD25, CD127 and CD62L.

One difficulty when studying Tregs is that their phenotype is not stable. It has been demonstrated that stimulation of white blood cells with bacterial exotoxin results in dose-dependent increase of FoxP3 expression in exotoxin specific T cells.[72] These Tregs are sometimes referred to as peripherally induced Tregs (pTreg) in opposition to the naturally occurring Tregs (nTreg). The pTregs are very unstable and can convert back to effector cells.[73] To complicate matters further, FoxP3 is expressed in activated effector T cells, albeit transiently and at low levels.[74, 75]

Studies on Tregs have generated much conflicting data, which is not surprising, given the plethora of definitions for Tregs that have been used and their unstable phenotype. For example, Tregs from MS patients have been demonstrated to be defective in function, but not in number.[76, 77] Others have demonstrated normal function and number, decreased frequency during remission or increased frequency during relapse.[78, 79]

Helios is a transcription factor of the Ikaros family. It has been proposed as a marker of nTregs, which are believed to be educated in the thymus and are more stable than pTregs.[73, 80] The combined analysis of FoxP3 and Helios may improve the precision in analyses of Tregs.

Natural killer cells

Natural killer cells (NK cells) are large granular lymphocytes, important in the defense against intracellular infections and tumors. In contrast to B and T lymphocyte receptors, NK cell receptors do not undergo somatic rearrangement, enabling NK cells to mediate host defenses without any prior sensitization by antigen.[81] NK cells are important inhibitors of inflammation in MS. They were thought to exert their suppressive function through secretion of cytokines, but in the clinical trials with the anti-CD25 antibody daclizumab it was discovered that daclizumab expanded the CD56high subset of NK cells by up to 500 %.[82] It has thereafter been demonstrated that this cell population can act as a regulatory NK cell population, exerting its action by killing activated autologous T cells.[82] This supports the notion that NK cells exert their suppressive function through cell-to-cell interactions. It has also been shown that NK cells must be present in the CNS to regulate the development of autoimmune responses in EAE. The CX3CL1 (fractalkine) receptor is critical for CNS NK cell recruitment, but not for B or T cells. CX3CL1 knockout mice have fewer NK cells infiltrating the CNS, but normal numbers in the periphery and develop more severe EAE.[83]

Prognosis

It is notoriously hard to predict the outcome of MS; even so, some predictors have been identified. Symptoms from efferent systems, high frequency of relapses and incomplete remissions of relapses during the first five years after diagnosis are associated with development of SPMS and a worse prognosis.[84] MRI has also been used to establish prognosis: high lesion load, whole brain atrophy and large ventricle size are associated with worse prognosis.[85-87] High levels of neurofilament light in the CSF measured at the time for diagnosis is associated with a five-fold risk of development of severe MS.[88]

MS therapy

The first drug to be approved for MS by the FDA and EMA was interferon beta.[89] Today, several therapies are available, with different risk/benefit profiles. Generally speaking, increased benefit comes at the prize of greater risk, and the most effective treatments are associated with severe adverse events, and even death. Therefore, it is paramount that patients can be properly assessed, and that treatment benefit is constantly reevaluated. What these therapies all have in common is that they act on the immune system, need to be administered continuously and are not very effective against progressive forms of MS.

Currently, many treatments are tried in an experimental setting. One of the most interesting is autologous hematopoietic stem cell transplantation (HSCT), which differs from conventional therapy in that it aims to remove the inattentive listeners of the immune system in a one-time treatment which could be curative. But what is meant by cure? This will be discussed in the next chapter.

Chapter 2 – Curing multiple sclerosis

Pilate saith unto him, What is truth?

King James Bible. John 18:38

In this chapter I will explore the concept of "cure". In order to do so, it is necessary to begin with defining more fundamental concepts such as disease, illness and health. Clinical medicine is a hybrid of art and science and to fully appreciate the meaning of these concepts, both must be accounted for.

Health, illness and disease

Sydenham, "the English Hippocrates", believed that diseases exist "by convention" and suggested that their effects on patients could be documented by charting the course of symptoms and signs observed at the bedside. "Nature, in the production of disease, is uniform and consistent; so much so that for the same disease in different persons the symptoms are for the most part the same; and the self–same phenomena that you would observe in the sickness of a Socrates you would observe in the sickness of a simpleton."[90] Sydenham knew little of the ultimate nature of his conventional disease however, largely due to the undeveloped state of the natural sciences in the 17th century.

Further progress was made in the early 19th century, when clinicians began to turn their attention to the physical examination of the patient. New instruments such as the Laennec stethoscope revealed a new range of clinical information. At the same time, clinicians began to examine the internal organs after death and to correlate physical signs with postmortem appearances. The result was a radically new classification of disease based on morbid anatomy, far more able to exclude organic disease.[91] In 1963 Foucault coined the term "medical gaze" to denote the dehumanizing medical separation of the patient's body from the patient's person. "It meant that the relation between the visible and the invisible… …changed its structure, revealing through gaze and language what had previously been

below and beyond their domain." This change involved "a reorganization of the elements that make up the pathological phenomenon (a grammar of signs has replaced a botany of symptoms), a definition of a linear series of morbid events (as opposed to the table of nosological species), a welding of the disease on to the organism".[92]

This dichotomy was further expounded upon in the following years when the distinction between disease and illness was made.[93] Disease is used for pathologic bodily change, while illness is reserved for experienced suffering. Patients are concerned primarily with their illness (*i e* their suffering), while physicians are more concerned with their disease.

Although the concept of disease as departure from natural functions of the human body may seem fairly straightforward, controversy still exists between objectivists and constructivists. Constructivists strive to uncover the role that moral and social values have always played in medical diagnosis and argue that the categories of disease can never be objective. Szasz took an extreme position and maintained that mental illness was a myth rather than a disease, "If you talk to God, you are praying; if God talks to you, you have schizophrenia."[94] While many of his arguments have been invalidated, we must still acknowledge that the categorization of clusters of symptoms into separate diseases is biased by our previous experiences.

Health is usually viewed as the opposite of illness, rather than disease. One of the most used definitions of health comes from the constitution of the World Health Organization; according to this, health is "a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity".[95] In this sense, treatment or even cure of a disease does not necessarily lead to improved health, as McWhinney points out "Healing in its deepest sense - the restoration of wholeness... …is not the same as treating or curing. It is something that happens to a whole person; that is why we can be cured without being healed and vice versa. A person who remains in spiritual anguish even after physical recovery cannot be said to be healed."[91]

Defining cure

Some diseases are considered curable; most are not. A few examples of curable diseases are bacterial infections, kidney stone and some cancers. Others are today considered incurable, notably many neurological diseases such as migraine, Duchenne muscular dystrophy and amyotrophic lateral sclerosis. That is not to say that they will be forever incurable - in principle most if not all diseases are theoretically curable.

In the past, a diagnosis of multiple sclerosis was made on clinical grounds only.[23] MS has been called a great mimic, and in all likelihood some of these early diagnoses were wrong. Clinical diagnosis is an educated guess at the

underlying pathologic disorder based on a patient's self-reports, behavior and any observed signs and so is necessarily provisional.[96] Great effort has been put into improving this state, and with greater knowledge and evolving medical technology, diagnostic accuracy has improved considerably. Today, diagnosis is made with a combination of clinical and paraclinical observations,[24] and most clinicians would agree that diagnosis of MS is straightforward in but a few cases.

Demonstrating absence of disease is much harder, and has previously not been studied in the context of MS. The reason is fairly obvious: MS is a chronic disease with no spontaneous cure and available treatments have aimed at slowing down the accumulation of disability. Since cure of MS have been nowhere in sight, it has been pointless to discuss this concept in detail. However, as we shall see, this concept contains more than meets the eye.

At this point, it is important to again bring up the distinction between RRMS and SPMS. It has been established that RRMS is primarily an inflammatory disease, while the pathogenesis of SPMS is still largely unknown. Let us for the sake of argument make the assumption that RRMS is an utterly inflammatory disease and that SPMS is an entirely degenerative disease. If this holds true (or some part of it), a cure of RRMS does not necessarily imply cure of SPMS and *vice versa*. Consequently, we shall have to remind ourselves of this particular relationship. As mentioned previously, it is believed that if RRMS is successfully treated, onset of SPMS will be delayed or even averted. As a special case we must therefore consider that formation of SPMS may be prevented by the cure of the relapsing-remitting part of the disease.

A chronic disease such as MS can be viewed as the sum of current symptoms and the threat of future disability. A cure could affect either of these parts and be defined from the patient's, the clinician's or the scientist's perspective. With this in mind, cure could be defined as either of:

1. Reduction of the disease to a non-threat.
2. Freedom from clinical disease activity.
3. Cessation of biological disease activity.

In addition to the above, cure could also entail:

4. Reversal of accumulated disability.

Let us examine these in greater detail and see how they relate to the special case of MS.

Reduction of the disease to a non-threat

If we can we eliminate the risk of future disability, the disease is reduced to a non-threat. This means that the patient could experience new symptoms, develop new signs of disease discernable at a physical examination or new MRI lesions; and still be cured. This may seem counter-intuitive but there exist many conditions that are considered abnormal or unphysiological but impede function very little (if at all) and are most often not labeled as disease. A few examples are color-blindness; mild nearsightedness; everyday headache, vitiligo, and so on. It can be argued that an MS relapse with mild numbness in a limb for a few weeks followed by full recovery is more a nuisance than a sign of disease.

This definition has a few problems. First, from a biological standpoint it is really hard to argue against the possibility of emergence of a secondary progressive course. Second, from a clinical point of view it is well known that MS has a highly variable disease course and how can we be sure that what we observe is really an effect of treatment and not just a random fluctuation. From natural history studies it is clear that a seemingly innocuous course may be followed by unexpected flares or secondary progression.[6] Additionally, since new symptoms could appear anytime, many patients would probably still experience MS as an illness.

Freedom from clinical disease activity

This definition of cure includes absence of clinical relapses, development of new neurological signs as well as secondary progression. This can be viewed as a somewhat stronger version of definition 1, with similar problems. At this point, it is not clear how long such a period of disease absence must be in order to accurately predict cure. Nevertheless, it should be possible to perform such an analysis, which should be valid on a group level at least.

Cessation of biological disease activity

If disease is defined as a pathological process leading to disruption of normal physiology with or without tissue damage, then cure can be defined as the termination of such a process. If the pathogenesis has been sufficiently described, cure can be demonstrated by showing normalization. An obvious problem with this definition in the special case of MS, is that our understanding of the pathophysiological processes leading to and maintaining MS is incomplete. Nevertheless, in some key areas, we are likely to find pathology, and those areas are good candidates for demonstrating absence of disease, *i e* cure. This will be discussed further in Chapter 4.

Reversal of accumulated disability.

Lastly, cure could encompass reversal of accumulated disability. A true reversal would require restoration of damaged tissue to a normal cytoarchitecture and in most cases neurogenesis. With few exceptions this is not possible in mammals, and certainly not in any disease of the human central nervous system.[97] For patients with a large amount of accumulated disability this aspect of cure will be very important, typically patients with long-standing disease or progressive forms of MS.

In other diseases we often use the word cure even although disability or impairment persists. *E g* in patients with breast cancer it is sometimes necessary to remove the breast to save the woman's life. Even if all tumor cells can be removed, she will still be stigmatized by the loss of a breast. Nevertheless, most of us would agree that she is cured from cancer. In necrotizing fasciitis, tissue is rapidly destroyed by aggressive bacteria. Even if the patients can be cured from the infection, they are still left with severe damage to the limbs for the rest of their lives. Again, most of us conceptualize these patients as being cured.

Additional criteria

In addition to the above, I shall maintain that a curative therapy also requires:

A. A reasonable mode of action.
B. Absence of on-going therapy.
C. Persistence of therapeutic effect for some time.

These criteria can be illustrated with the following examples:

a. Herbal tea is not a cure of cancer, because we have no reason to believe that herbal tea can affect the biological processes of cancer.
b. Carbamazepine is not a cure of epilepsy, since seizures are likely to reappear after cessation of therapy.
c. A patient who is treated with antibiotics for pneumonia who immediately gets worse after a treatment course was never cured - the antibiotics were just suppressing the infection.

Concluding remarks

In this chapter, I have discussed different aspects of the concepts of health, illness, disease and cure. The word “cure” is very powerful, evoking images from folklore and religion. Nevertheless, it is possible to arrive at a rather precise definition of this word. For the purposes of this thesis, I shall adopt the view of clinician and scientist and define cure of MS as freedom from clinical and biological disease activity (definition 2 & 3) with this proviso: the cure only applies to the RRMS part of the disease. In the next chapter, I will discuss hematopoietic stem cell transplantation, which could be a cure for RRMS.

Chapter 3 - How to do it

You will burn and you will burn out;
you will be healed and come back again.

The Brothers Karamazov, Chapter 4.

F Dostojevskij

In chapter two, we investigated the concept of cure in a broad sense. In this chapter I will discuss hematopoietic stem transplantation as a treatment for MS.

Hematopoietic stem cell transplantation

Hematopoietic stem cell transplantation has been used for malignant disease since the 1950s[98, 99] and since then more than one million transplants have been performed world-wide.[100] In 1990, Edward Donnall Thomas was awarded the Nobel Prize in Physiology or Medicine for the development of HSCT as a treatment for leukemia. Today, it is mainstay in the treatment of acute myeloid leukemia and plasma cell disorders.[100]

Already in 1995, Burt *et al* suggested that HSCT should be tried for malignant RRMS, based on experiences from animal studies.[101, 102] However, when Fassas *et al* performed the first autologous HSCT for multiple sclerosis in April 1995, it was tried for progressive MS. Their experiences with fifteen patients were summarized in a seminal paper published in 1997, which set the stage for the coming years. The treatment could "be used with relative safety" and some evidence was found that "this kind of therapy can suppress disease progression and reduce disability".[103]

Despite the suggestions from Burt *et al*, the procedure was initially reserved for patients with treatment resistant progressive forms of MS. It soon became evident that this therapy was not able to stop worsening in patients with progressive disease, eloquently demonstrated by Burt *et al* in

the 2003 paper *Hematopoietic stem cell transplantation for progressive multiple sclerosis: failure of a total body irradiation-based conditioning regimen to prevent disease progression in patients with high disability scores.*[25] Since then, this has been one of the strongest arguments against a prominent role of adoptive immunity in progressive disease.

In the following years it became clear that HSCT could be a very effective treatment for RRMS and in particular highly aggressive RRMS.[104, 105] It seems that long-term remission, and maybe even cure, can be achieved with this form of therapy.[106-108, I] During the last 20 years, reports of more than 600 patients treated with HSCT for MS have appeared in the medical literature.[109]

The goal of this therapy is to achieve long-term remission through short-lasting ablation of the immune system. In this setting it is important to recognize that the terminology autologous hematopoietic stem cell transplantation is a misnomer. There is no transplant in the real sense of the word, and the hematopoietic stem cells are mainly viewed as a supportive blood product.[110] On the other hand the stem cells could be important for establishing immunological tolerance after HSCT. Their significance in this regard was investigated in an EAE model and it was reported that stem cell transplantation was necessary for complete and long-time remission, and that those beneficial effects probably were related to induction of Tregs.[111]

Procedure

In contrast to treatment of malignant disease, HSCT performed for autoimmune disease has almost always been autologous due to a significantly higher risk of treatment related mortality with allogenic procedures. Nevertheless, anecdotal reports of patients treated for hematological disease with allogenic transplantation describes stabilization of disease.[112-114]

In the past, hematopoietic stem cells were usually collected from the bone marrow by multiple aspirations from the iliac crest. This procedure has been superseded by pharmacological mobilization of stem cells. Through administration of a combination of cyclophosphamide and granulocyte colony-stimulating factor (G-CSF), stem cells are released into the blood. Stem cells can also be mobilized with G-CSF alone, but this has been associated with MS flare, possibly due to release of auto-reactive cells.[115]

Hematopoietic stem cells can reliably be identified through the surface marker CD34 and when sufficient amounts of stem cells are in circulation, they are collected by leukapheresis. A minimum of 2×10^6 CD34+ cells/kg bodyweight are usually collected and stem cells are thereafter cryopreserved, and stored until reinfusion. The graft can be manipulated to remove possibly auto-reactive T cells through positive selection of CD34+ cells or depletion of T cells (*ex-vivo* T cell depletion), which is rarely used nowadays.[116] Auto-

reactive lymphocytes that survive the conditioning regimen or are re-infused with the graft can be eliminated through infusion of anti-thymocyte globulin (ATG) or monoclonal antibodies directed towards lymphocytic antigens (*in-vivo* T cell depletion).

After a variable number of days, often 2-8 weeks after the stem cell harvest, a conditioning regimen (with chemotherapy, biologics, and/or radiation) is performed. The conditioning can be done in several ways and can be divided into:

1. High intensity regimens, including any busulfan or total body irradiation (TBI) containing regimens.
2. Low intensity regimens restricted to cyclophosphamide alone, melphalan alone or fludarabine-based regimens.
3. Intermediate regimens, encompassing all the other combinations.[117]

The two most commonly used conditioning regimens for MS have been a low intensity cyclophosphamide/ATG protocol (cyclophosphamide 200 mg/kg; ATG 5-10 mg/kg) and an intermediate regimen usually denominated BEAM/ATG (BCNU 300 mg/m^2; etoposide 800 mg/m^2; cytosine-arabinoside 800 mg/m^2; melphalan 140 mg/m^2; ATG 7.5-10 mg/kg).

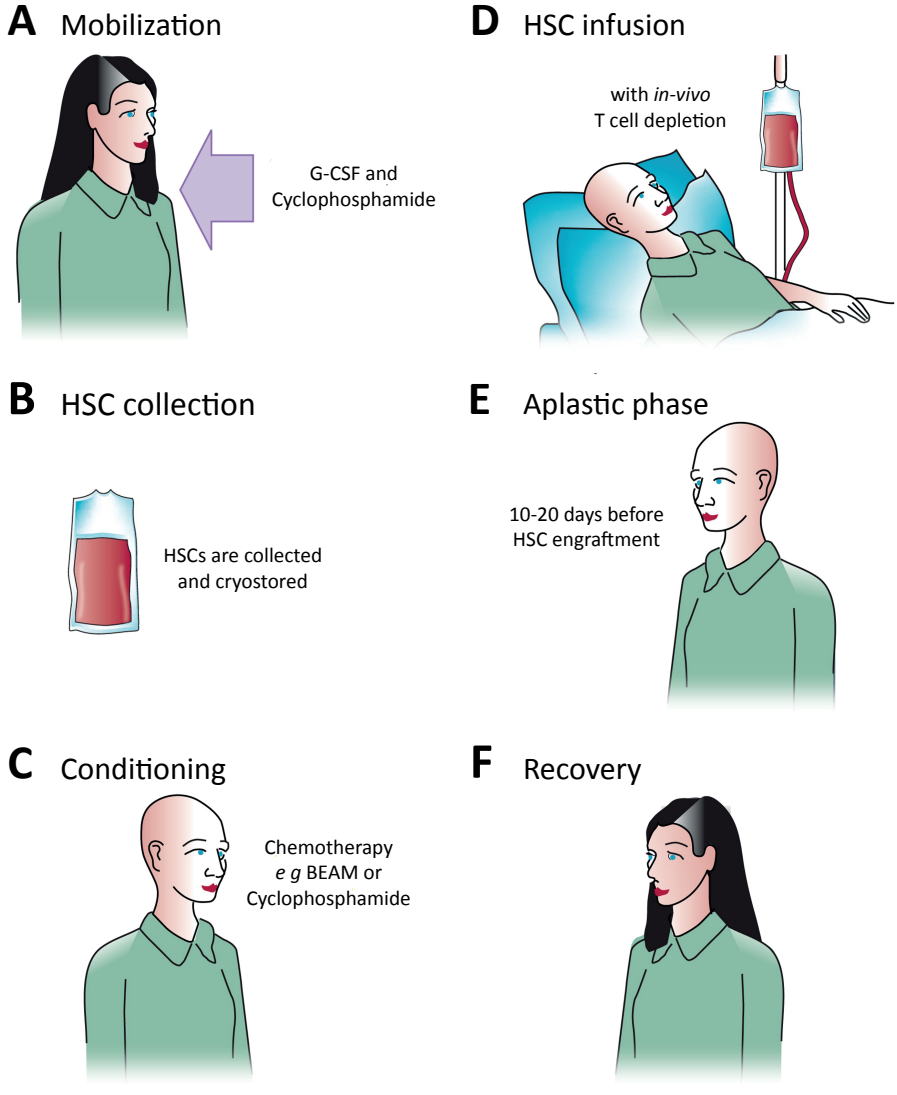
After the completion of the conditioning regimen, the thawed stem cells are re-infused through a central venous catheter. A critical aplastic phase follows, which is characterized by low counts of white and red blood cells as well as platelets. During this period infections and culture negative fever are common and most patients are treated with antibiotics. With low and intermediate intensity regimens, recovery of cell counts usually occurs at 10–15 days after infusion of stem cells.

Efficacy

As of yet, no randomized control trial of HSCT for MS has been completed. The treatment effect of the procedure must therefore be estimated from case series reports. Patient selection, treatment regimens and outcome measures have been very dissimilar between studies making comparisons difficult. Nevertheless, some conclusions can be drawn.

Early on, it was evident that HSCT is not an effective treatment of progressive forms of MS. In a well done study of 21 MS patients (20 with progressive MS and 1 with RRMS) treated with a high intensity conditioning, only 2/12 patients with an expanded disability status scale (EDSS, see Appendix) score > 6 remained stable during the follow-up period (mean follow-up time 2.6 years).[25] Patients with EDSS ≤ 6 fared a little better and 3/9 remained stable. Similar results have been found in many

Figure 3.1 HSCT procedure.



(A) Peripheral blood stem cells (HSCs) are mobilized with granulocyte colony-stimulating factor, usually in combination with cyclophosphamide. **(B)** HSCs are then collected through leukapheresis. **(C)** Subsequently, the patient is treated with high-dose chemotherapeutic agents. **(D)** The cryopreserved graft is subsequently re-infused into the patient with ATG (in-vivo T-cell depletion) to remove autoreactive T cells that survived the conditioning regimen or that might have been reinfused with the graft. **(E)** After a period of 1–3 weeks, hematological engraftment occurs. Reprinted with permission (see Appendix).

other studies leading investigators to conclude that "…this extremely protocol did not prevent clinical progression" and "The lack of efficacy […] does not favor the use of similar rigorous protocols in the future."[118] Further deterioration has also been demonstrated at long-term follow-up. Fassas *et al* described the outcome of their first 35 patients (of which all but one were diagnosed with progressive MS) fifteen years after HSCT.[119] Only 25 % remained stable in EDSS and if patients with gadolinium enhancing lesions on MRI were excluded this figure decreased to 10 %.

During the first ten years, only sporadic cases of RRMS were treated; but when they were, outcome was often good. Burt *et al* reported an improvement in EDSS score of 2.5 sustained at two years of follow-up[25] and in another study 2/5 RRMS patients with high EDSS scores improved.[120] This led to the insight that the procedure probably should be reserved for patients with RRMS.

In 2004 a young woman with an unusually aggressive RRMS, with rapid deterioration of neurologic function into complete tetraparesis within six months, became the first to be treated at our hospital and throughout Scandinavia. She made an astounding recovery, and within a year after HSCT she was almost completely recovered. This success prompted us to treat other patients and the experiences of the first nine patients were summarized some years ago.[104] In short, after a mean follow-up time of 32 months, 8/9 patients were free from all signs of disease activity and the median patient had improved by 3.5 in EDSS score. Other reports have followed and today descriptions of about 150 cases of RRMS exist in the medical literature (Table 3.1 and 3.2). Unfortunately, in most instances data from combined cohorts of RRMS and progressive forms of MS have been presented, making it even harder to sort out efficacy and risks associated with HSCT.

One of the most commonly used outcome measures have been progression free survival (PFS). PFS has varied considerably between studies. In one of the few studies containing solely RRMS patients, PFS was 100 % at three years.[105] In a long-term follow-up study of a mixed cohort, PFS was 84.4 % at three years for RRMS patients, and although few patients were followed longer, no progression was seen among them.[107] In a slightly larger study with shorter follow-up, PFS in RRMS patients at five years was 71 %.[108] These results are similar to our own, where PFS in the entire cohort was 77 % and no real difference between RRMS and SPMS patients could be demonstrated.[I] A stronger outcome measure is freedom from disease (*i e* freedom from new relapses, freedom from deterioration in neurological function, and freedom from new or enlarging MRI lesions). In practice, this equates to absence of all forms of measurable disease activity using all available methods employed in clinical routine of modern state-of-the-art health care. Only two studies have reported disease free survival and both

reach similar figures, with disease free survival of 62 % at three years[105] and 68 % at five years.[I]

Prognostic factors of efficacy

The most consistent prognostic factor has been presence of gadolinium enhancing lesions at baseline, which improved PFS at five years from 46 % (no gadolinium enhancing lesions) to 87 % in one study,[108] and improved disease free survival at five years from 46 % to 79 % in Swedish patients.[I] Interestingly, in neither study, presence of progressive disease was a negative prognostic factor. This is unexpected in view of previous experiences, but may reflect residual inflammation in transient cases of SPMS or too short follow-up time. In a study with longer follow-up time it was evident that patients with RRMS had a better prognosis, with an odds ratio of 39 to remain free from progression at six years.[107]

Safety

Safety of the procedure has been a major concern. In a 2006 report from the register of the European Group for Blood and Marrow Transplantation (EBMT), a transplant related mortality (TRM) of 5.3 % in 183 examined patients was reported.[121] The authors noted that heavy intensity conditioning protocols with busulphan were associated with a higher TRM and also that no deaths had occurred after the end of 2000. In a later (2010) follow-up of EBMT data from 345 MS patients, TRM was 3.8 %. In the pooled analysis of patients with different autoimmune diseases, age < 35 years and centers' experience were associated with lower risk of death (HR 1.7 for age and 2.5 for center experience).[117]

Avoiding heavy intensity conditioning can improve TRM, and from the Italian study a TRM of 2.7 % was reported.[108] However, in this study 35 % of patients were transplanted at centers with experience of less than five patients. Restricting treatment to experienced centers can reduce mortality even further, and absence of mortality has been reported in four studies with in total 180 patients.[105, 107, 122, I]

Long-term side effects have been less studied. In one study varicella zoster or urinary tract infections were seen in about 5% of patients.[108] In our experience, the most common long-term side effects are herpes zoster reactivation (15 %) and thyroid disease (8.4 %).[I]

Table 3.1 Number and type of patients treated with HSCT.

	n	Age	MS sub-type (n)				Disease duration (years)	EDSS	AAR in RRMS patients	Conditioning		
			RRMS	SPMS	PPMS	PRMS				BEAM	Cy	other
Burman et al	(41‡) 48	31*	34	5	2		5.5†	6†	4.8*	34	6	
Mancardi et al	74	36*	33	41			11.2*	6.3*	2.8*	74		
Shevchenko et al	(90‡) 95	35*	42	35	15	3		3.5†				95
Krasulova et al	26	33†	11	15			7†	6†	2†	25		
Burt et al	21	33†	21				5†	3.1*			21	

*Reports that contained ≥10 RRMS patients were included in this table. ‡ The number in parenthesis are patients analyzed for outcome (in Table 3.2). * Mean. † Median.*

Table 3.2 Outcome of HSCT.

	n	Follow up time (months)	PFS		Disease free survival		Mortality		Prognostic factors
			Timepoint	Percentage	Timepoint	Percentage	n	Percentage	
Burman et al	41	47*	60	77 %	60	68 %	0	0	Gd+ at baseline
Mancardi et al	74	51*	60	66 %			2	2.7 %	Gd+ at baseline
Shevchenko et al	90	46*	60	82 %			0	0	
Krasulova et al	26	66†	60	32 %			0	0	Disease duration <5 years RRMS vs SPMS
Burt et al	21	37*	36	100 %	36	62 %	0	0	

*Reports that contained ≥10 RRMS patients were included in this table. * Mean. † Median.*

How does HSCT compare to approved treatment?

Natalizumab and alemtuzumab are considered to be the most efficacious over-the-counter therapy available today. Both have been compared to placebo and an active comparator (interferon beta-1a) in phase III studies.

Natalizumab is a monoclonal antibody directed against the cell adhesion molecule α4β1 integrin, present on lymphocytes and monocytes. Natalizumab prevents the interaction between α4β1 integrin and its cognate ligand VCAM-1 on brain endothelial cells,[123] thus preventing extravasation of lymphocytes.

Alemtuzumab binds to CD52, a cell surface protein of unknown function that is expressed at high levels on T cells and B cells and at lower levels on monocytes, macrophages, and eosinophils. In effect, peripheral lymphocytes are depleted, probably by an antibody-dependent, cell-mediated cytotoxicity, leaving cells of the innate immune system unaffected.[124] Thus, the biological effect is somewhat similar to HSCT, but cell depletion occurs to a lesser degree.

Since HSCT mainly have been used as a rescue or third line therapy, the most relevant phase III studies of natalizumab and alemtuzumab are those made with patients failing first line therapy. After two years of follow-up, natalizumab treatment in addition to interferon beta-1a, led to freedom from relapses in 54 % of patients (Table 3.3); freedom from new MRI lesions in 67 % of patients; and halved annualized relapse rate (AAR) in comparison to treatment with interferon beta-1a alone (AAR 0.34 *vs* 0.75).[125] After two years of follow-up, alemtuzumab treatment led to freedom of relapses in 65 % of patients (Table 3.3); a reduction in AAR from 1.7 to 0.26; and a disease free survival of 32 %.[126] The risk of progressive multifocal encephalopathy associated with natalizumab treatment is now well appreciated; the risk is 0.2 % *per annum* and about 20 % of those affected died.[127] Otherwise, this substance is well tolerated. In the pivotal studies of alemtuzumab, thyroid disorders were seen in 18-23 % of patients; immune thrombocytopenic purpura in 1-2.8 %; agranulocytosis in 1 %; and mortality was 0.27-1.9 % (in total seven deaths in 1188 patients, equating to a TRM of 0.59 %, Table 3.3).[126, 128, 129]

Table 3.3 Comparisons of natalizumab, alemtuzumab and HSCT.

	Natalizumab	Alemtuzumab	HSCT
Follow-up time (months)	24	24	47
AAR baseline	1.44	1.7	4.1
AAR on study	0.34	0.26	0.03
Freedom from relapses	54 %	65 %	87 %
Freedom from disease	no data	32 %	68 %
Mortality	0 %	0.59 % (pooled data)	0 % (pooled data 0.76 %)
PML	0.2 % per annum (post-marketing data)	n/a	n/a

This table compares data from the SENTINEL[125] and CareMS-2[126] trials with data from the Swedish experiences of HSCT for MS[I]. Pooled alemtuzumab data are from the CAMMS223,[128] CareMS-1[129] and CareMS-2[126] studies, whereas pooled HSCT data are derived from the studies in table 3.1 and 3.2.

Concluding remarks

We have come a long way of understanding how HSCT should be performed and which patients should be treated to reach optimum results. Preferably, patients should be younger, diagnosed with RRMS and inflammatory active with presence of gadolinium enhancing lesions. Heavy intensity conditioning regimens should be avoided and treatment should only be performed at experienced centers. So far, too few patients have been treated to accurately estimate the TRM. Nevertheless, treatment related mortality can never be reduced to zero. Before more data on safety can be provided, HSCT cannot be recommended as a second-line treatment. Rather, available data support HSCT as a third line treatment, which is in accordance with the guidelines from the Autoimmune Disease Working Party of the EBMT.[130]

Meanwhile, it is paramount to improve safety as much as possible. Although no proper controlled study of an intermediate intensity conditioning regimen, such as BEAM, *vs* a low intensity conditioning regimen has been performed; a low intensity regimen should be better tolerated and associated with lower mortality, a supposition that is also backed by some data.[131] Since there are no convincing data that either regimen has superior effect, there are currently very few arguments for continuous use of intermediate intensity regimens for the treatment of MS.

It is safe to say that HSCT is more effective than any other available treatment. Two-thirds of patients are free from all forms of disease activity. But are they really cured? In the next chapter, I will expound on how we can strengthen the position that this is indeed the case.

Chapter 4 – How to prove it

> If we are uncritical we shall always find what we want: we shall look for, and find, confirmations, and we shall look away from, and not see, whatever might be dangerous to our pet theories.
>
> The Poverty of Historicism (1957) Chapter 29: The Unity of Method
>
> Karl Popper

In the previous chapter the effects of hematopoietic stem cell transplantation for multiple sclerosis were discussed, and it could be demonstrated that a majority of patients have no signs of disease activity five years after the procedure.[1] For all purposes they seem to be cured from their MS, but is this really true? Let us assume for a moment that this claim is true. How would you prove it? Strictly speaking, you couldn't. According to Popper, scientific truths can never be proven, only disproven.[132] Applied to the current scenario, we must therefore disprove that the cured patient still has MS in a sufficient number of ways to convince ourselves that the disease is actually gone. In addition, we must also apply our criteria for a curative treatment (*cf* Chapter 2) and see if they are met.

Clinical cure

Disproof by clinical follow-up

One position is that it is necessary to do follow-up of a patient until death, in order to disprove MS. It is well known that MS is a capricious disease,

where relapses or conversion to SPMS may occur after many years of innocuous disease course. Even though this may happen, most clinicians would agree that it is a rare phenomenon. Looking at the data from available studies, we see that re-emergence of MS is most common early on (Figure 4.1). After a variable amount of time (6 months – 4 years) the survival curves flattens out and no further progression can be detected.

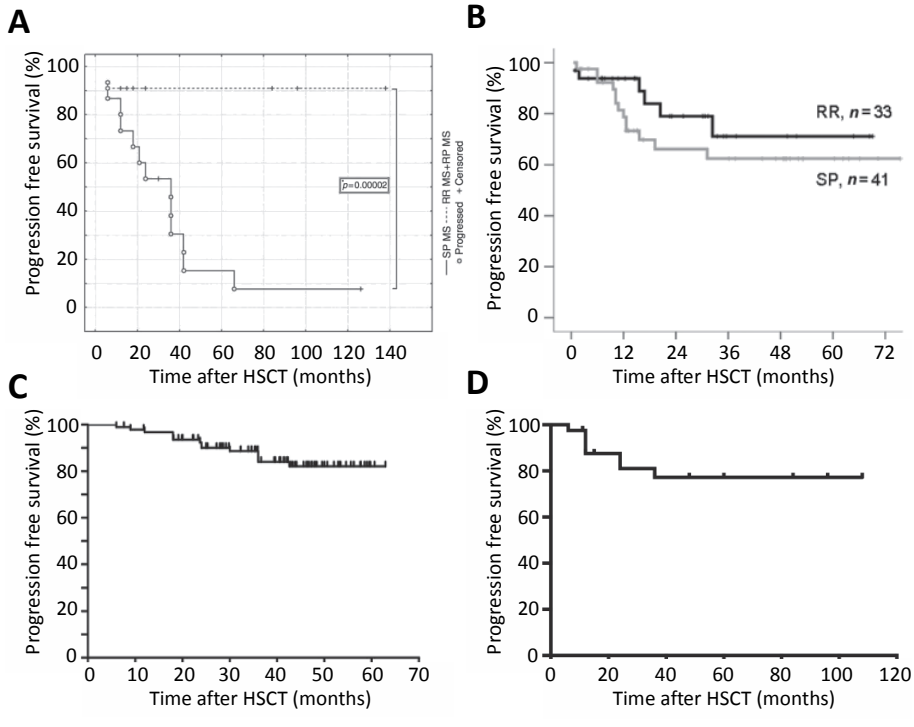
In Chapter 2, it was stipulated that the disease must be undetectable for some time, for us to be able to use the word cure. From the available data, it seems that this time point, at least from a practical point of view, could be set to five years after the procedure. What if someone has a relapse six years after the procedure? First, for all we know this is an unlikely event. Second, if we have access to no other data it is equally valid to claim that the disease has been there all along or that it is a question of reoccurrence of disease. A patient that is successfully treated for pneumonia who again becomes ill some years later cannot be said to have had the pneumonia the entire time.

Disproof by radiological follow-up

Although MS lesions cannot directly be investigated *in vivo*, MRI provides an indirect method of visualization of tissue integrity. Presence of gadolinium enhancing (Gd+) lesions is usually taken as evidence of on-going inflammation[59] although enhancement is really a sign of a permeability disturbance in the vessel wall, which *per se* is unspecific. The enhancement is only present for weeks-months, but as a rule the inflammation creates a persisting demyelinated plaque that can be visualized as a new T2 lesion. It has been demonstrated that presence of Gd+ and formation of new or enlarging T2 lesions are equally valid end-points for clinical trials.[133] It is well known that the number of evolving lesions is far greater than the number of clinical relapses, and that lesions may appear in a clinically stable patient.[37] Absence of new MRI lesions should provide a stronger argument for cure than clinical parameters only.

Fassas *et al*[119] described the long-term outcome of 35 patients with mainly progressive disease with a median follow-up time of 11.3 years. Although not described in great detail, Gd+ lesions were seen in 24/110 (22 %) of MRI scans performed within the first year after transplant (Figure 4.2). Thereafter only one new Gd+ lesion was seen in 84 scans (1.2 %). MRI data on patients who underwent HSCT for RRMS are scarce. Burt *et al*[105] reported that in 3/5 patients (60 %) with clinical relapses at least one new T2 lesion could be demonstrated, and in 2/16 patients (13 %) without clinical relapses. Again, most of the patients with new lesions (4/5) developed these within the first year after transplant, and only one clinically stable patient acquired a new lesion in the interval 1-2 years after transplant. In the Swedish patients, we found that MRI event free survival at five years was 85 % with no new lesions appearing after three years of follow-up (Figure 4.3).[I]

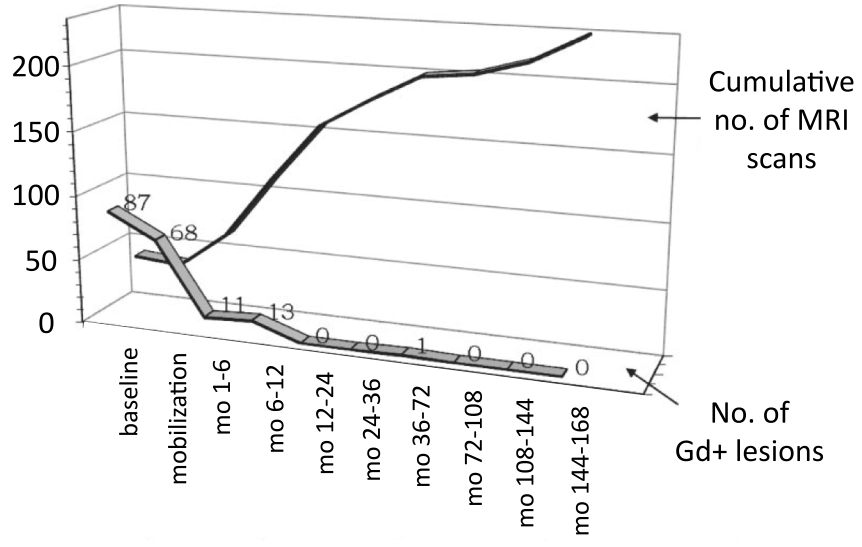
Figure 4.1 Progression free survival after HSCT for MS.



Progression free survival in patients treated with hematopoietic stem cell transplantation for MS in **(A)** a Czech cohort of 35 patients using a BEAM/ATG conditioning regimen, **(B)** an Italian cohort of 74 patients using a BEAM/ATG conditioning regimen, **(C)** 90 patients treated in Russia with a reduced intensity conditioning regimen based on BEAM and **(D)** the Swedish cohort of 41 patients treated with a BEAM/ATG or a Cyclophosphamide/ATG conditioning regimen. Reprinted with permission (see Appendix).

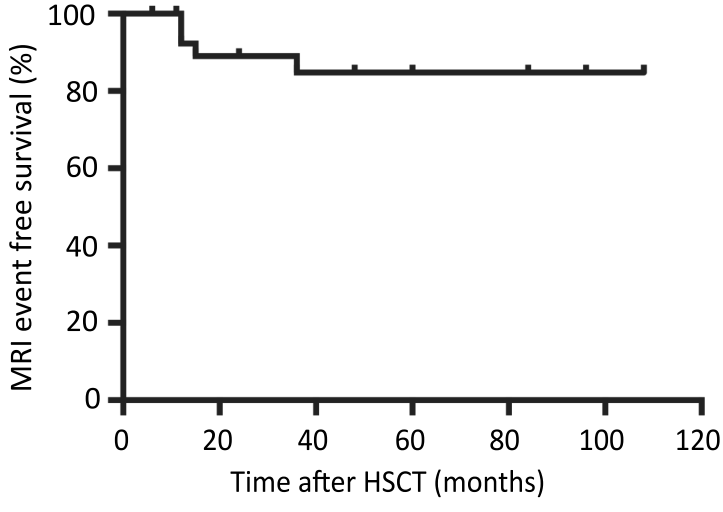
Imagers operating at 1.5 T are the clinical standard today. If field strength is increased to 3 T and the dose of gadolinium is increased, more lesions can be visualized.[134, 135] It is very reasonable to suppose that even more lesions can be detected if field strength and amount of contrast agent are increased further. In addition, other contrast agents such as ultra-small iron particles reveal lesions not visualized with gadolinium containing contrast agents.[20, 21] Thus, it could be argued that routine MRI is not sensitive enough to provide arguments for a cure of RRMS in the biological sense.

Figure 4.2 Impact of HSCT on gadolinium enhancing lesions at brain MRI.



Number of gadolinium enhancing lesions on MRI scans performed before and after HSCT in 35 Greek patients. Reprinted with permission (see Appendix).

Figure 4.3 MRI event free survival after HSCT.



Survival curve of MRI event free survival after HSCT in 41 Swedish MS patients. Reprinted with permission (see Appendix).

Caveats of clinical cure

Should we be satisfied with this evidence for clinical cure? HSCT is a one-time treatment, which in general requires no on-going treatment after the procedure. We have also seen that the therapeutic effect persists over time. Thus criterion B and C have been fulfilled. We have yet to demonstrate a reasonable mode of action, however. In addition, subclinical disease, undetectable by standard MRI, could still be brooding somewhere. Can we also disprove this?

Biological cure

When trying to build support for a clinical cure, we are chiefly concerned with outcome. If the objective is to demonstrate that the biological processes leading to disease has ceased, we must open the black box and examine its contents. However, doing that, we will find that the content is a mess, since the pathogenesis of MS is not fully known, and in the case of SPMS it can be argued that it is not known at all. In Chapter 1, it was postulated that MS is caused by lymphocytes recognizing oligodendrocytes as something dangerous that must be destroyed. Assuming that this supposition is true, shouldn't we demand a disproof of these lymphocytes existence after HSCT? If that is not possible, what are the alternative approaches?

Disproof by demonstrating absence of pathogenic lymphocytes

In practice it has proven to be very difficult to demonstrate absence of pathogenic lymphocytes. The major obstacle is that it is not known which these lymphocytes are. Nevertheless some efforts have been made. In 2005 Muraro *et al*[136] investigated the T cell repertoire pre- and post HSCT. When the pool of circulating lymphocytes was examined at six months after HSCT, a majority of T cells were phenotypically memory cells, even though a high intensity conditioning regimen was used. This can be interpreted as a homeostatic proliferation of surviving T cells, *i e* the procedure does not reliably eliminate all T cells. During follow-up, the share of memory cells successively decreased during due to an expansion of naïve T cells educated in the thymus. Evidence for increasing clonal diversity after the procedure in some patients was also provided (Figure 4.4).

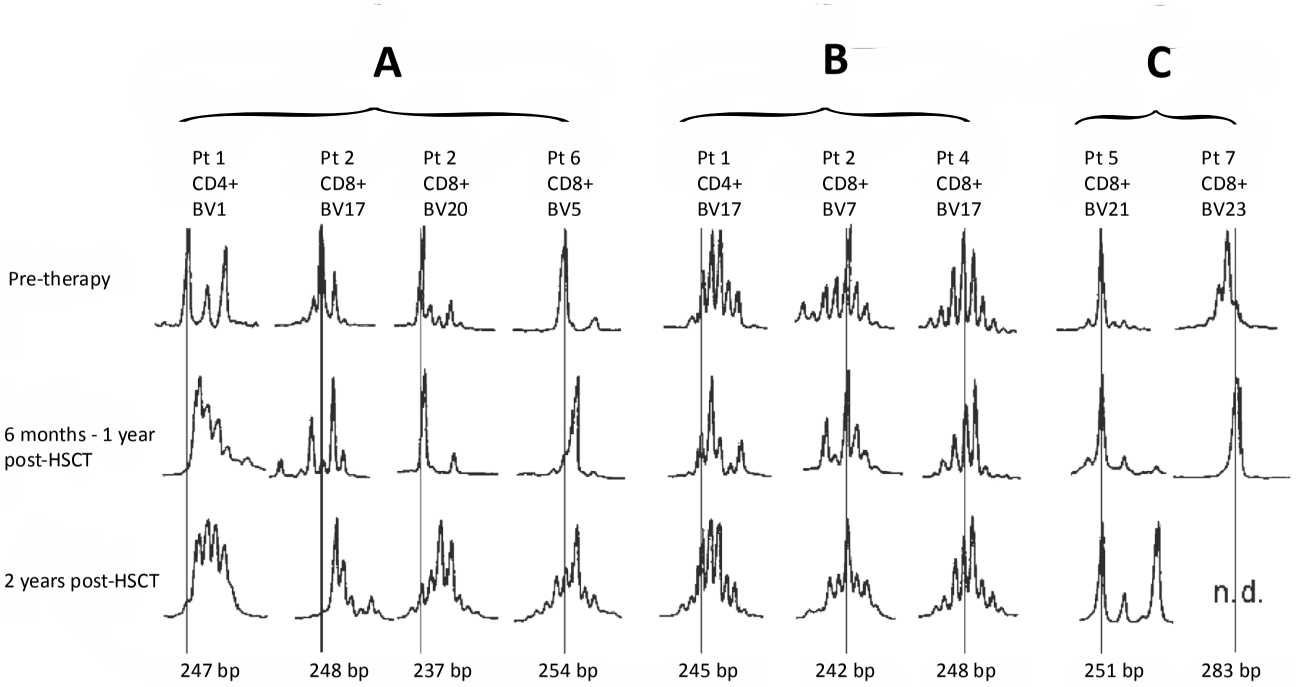
The results from Muraro *et al* are usually interpreted as evidence for a profound renewal of the immune system providing an argument against the view that HSCT only causes immune suppression. In essence this provides solid arguments for a plausible mode of action and thereby fulfilling criterion A. On the other hand, it is possible that the lymphocytes causing MS are still present.

In a later study from the same group, high-throughput deep TCRβ chain sequencing was performed to further characterize the T cell repertoire pre- and post HSCT.[137] While it was shown that a majority of the dominant TCR clones in CD4+ T cells present before treatment were undetectable after HSCT, the dominant CD8+ T cell clones were not effectively removed, and the reconstituted CD8+ T cell repertoire was created by clonal expansion of cells present before treatment. It seems important to get rid of the dominant clones, since a low clonal diversity at two months after the procedure was associated with re-occurrence of disease at two years (Figure 4.5). Again, this study does not contradict the possibility that the lymphocytes causing MS are still present. In addition, B cells were not investigated in either study, which is a significant weakness. An increasing corpus of evidence suggests that they play a crucial part in the pathogenesis of MS.[138]

Some researchers have tried to address this issue by looking closer at certain subpopulations of lymphocytes believed to play particular important roles in MS. One example is the CD161+ subpopulation of CD8+ T cells, which has been implicated in MS.[70] It was present in high frequencies pre-HSCT, but virtually absent after HSCT.[71] Although an interesting find, the role of the CD161+CD8+ T cells has not been sufficiently characterized for us to properly assess the importance of this finding. Another example is the pro-inflammatory Th17 cells which are increased in MS patients but present in frequencies similar to healthy controls in patients who underwent HSCT.[139, II] Since IL-17 blockade provided only modest clinical benefit,[65] these findings can be seen as supportive at best.

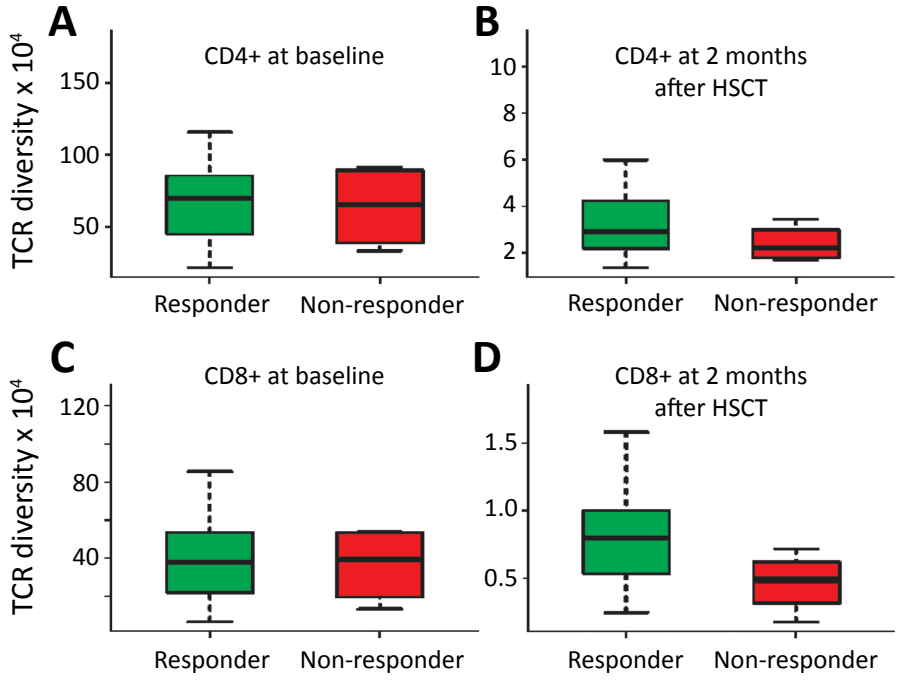
Another approach is to investigate how cells of the adoptive immune system react to tentative antigens and other stimuli. It has been demonstrated that the levels of myelin reactive cells are similar pre- and post-HSCT, even though a significant weakness of these studies is that neither study provided data on healthy controls.[139, 140] Reactivity provides no clue to what they do *in vivo*, however. Darlington *et al*[139] provided conflicting data; on the one hand normalization of the proliferative response and IFN-γ production in cultures of peripheral blood mononucleated cells (PBMC) inoculated with myelin peptides and proteins; and on the other hand a proliferative response after stimulation with myelin basic protein that was similar at baseline and after two years of follow-up. In this study it was also noted that when PBMCs were stimulated polyclonally with an agonistic anti-CD3 antibody they produced less IL-17 than before the procedure. This is in agreement with our findings that PBMCs from HSCT patients that were stimulated with the same anti-CD3 antibody produced much less IL-17 than controls or MS patients.[II] In addition, when PBMCs were cultured with myelin oligodendrocyte glycoprotein (MOG) peptides, IL-

Figure 4.4 Analysis of TCR diversity.



High-resolution TCRBV CDR3 spectratyping analysis identified three basic patterns of evolution of repertoire diversity: **(A)** recovery of diversity from a restricted repertoire; **(B)** reconstitution of diversity from a normally diverse repertoire; and **(C)** total or partial persistence of repertoire skewing. Reprinted with permission (see Appendix).

Figure 4.5 Relationship between TCR diversity and response to HSCT.



(A) TCR diversity at baseline and **(B)** 2 months after HSCT in CD4+ and **(C-D)** CD8+ T cells. Low clonal diversity at two months after HSCT was associated with re-occurrence of disease. Reprinted with permission (see Appendix).

17 production ceased entirely, IFN-γ production was low and CD4+ T cells produced high levels of TGF-β1.[II] Thus, it seems like myelin proteins elicit an inhibitory response rather than a pro-inflammatory response post-HSCT.

Caveats of the disproof by demonstrating absence of pathogenic lymphocytes

All of the studies referred to in this section were performed on lymphocytes acquired through blood samples. This may or may not be representative to the conditions present in the CNS. Further, until we can pinpoint the lymphocytes responsible for MS and actually demonstrate that those lymphocytes are gone after HSCT, we have to rely on functional studies of tentative antigens *ex-vivo*. Those antigens may or may not be relevant to MS. Nevertheless, an inhibitory response elicited from PBMCs in the presence of proteins likely to be found in the local microenvironment of the demyelinated plaque is compelling.

Disproof of inflammation in the CNS

If we can't prove that the lymphocytes attacking the brain and spinal cord are gone and aren't satisfied with the artificial situation of studies on *ex-vivo* lymphocyte cultures, are there other ways to investigate inflammation *in vivo*?

The interstitial fluid of the brain is in direct connection with the CSF through the glymphatic system, a recently defined brain-wide paravascular pathway that facilitates efficient clearance of solutes and waste from the brain.[141] Thus soluble mediators of inflammation in the CSF mirror local inflammation in the brain. A disproof of inflammation in the CNS should require demonstration of normalization in the levels of such soluble factors. Measurement of antibodies and cytokines should be ideal for this purpose.

As discussed in Chapter 1, intrathecal antibody production can be demonstrated in a vast majority of MS patients. It has been claimed that oligoclonal IgG bands persist in the CSF of almost all patients,[118, 142] but in another study with a high intensity regimen 75 % retained oligoclonal bands.[143] In Swedish patients, only 69 % retained oligoclonal bands after a low-intermediate conditioning.[I] This discrepancy may be due to that Swedish patients were on average treated earlier, most of them still in the relapsing-remitting phase. The biological significance of these antibodies is not clear and for all we know they could be an epiphenomenon. It is well known that oligoclonal bands are present to variable degrees in other inflammatory CNS disease,[48] not least in infection with *B burgdorferi*, when antibody production can persist for years after the infection has been cleared.[144-146] The latter illustrates that is not necessary to lose the oligoclonal bands to allow us to speak of cure.

Cytokines have been the subject of many prior studies, but only recently has technology advanced to a point where simultaneous measurement of a large number of cytokines is feasible. Such multiplex assays enables characterization of diseases through their pattern of cytokines, *e g* neuromyelitis optica has been characterized by high CSF levels of IL-17A, IL-6, G-CSF, CXCL8, CXCL10 and CCL4 .[147] In MS, we could demonstrate that RRMS patients were characterized by an increase in the CSF levels of CCL22, CXCL10, sCD40L, CXCL1 and CCL5 and down-regulation of CCL2.[III] Only 7/37 RRMS patients had normal levels of these cytokines, showing that an aberrant cytokine response is the norm and that patients who appear stable nevertheless display an activation of the immune system. Many patients with SPMS also had high levels of predominantly CXCL1 and CCL5. In view of the above, a panel of CCL22, CXCL10, sCD40L CXCL1, CCL5 and CCL2 could be used to rule out on-going inflammatory activity in MS. Some other cytokines that were not investigated in the above study could be added to this panel. CXCL13 in particular comes to attention. It is a B cell chemo-attractant that consistently has been associated with

inflammatory active MS.[148-150] IL-8 and IL-12p40 are two other important cytokines in MS.[148, 151]

If we can demonstrate that inflammation is truly absent in the CNS after HSCT we have another cogent argument for HSCT being a curative therapy. No study demonstrating absence of inflammation in the CSF after HSCT has been made so far – but in order to disprove on-going inflammation after HSCT it should be performed. Patients should preferably be sampled at regular intervals and after two years (when the healing process has reached its end) stable levels should be reached and normalization should be the norm.

Caveats of disproof of inflammation in the CNS

As long as there is on-going inflammation in the CNS, we can probably detect it with the above-mentioned method. Most of the RRMS patients and many of the SPMS patients had elevated values of the cytokines in the suggested panel. However, some of these cytokines may play a role in an inhibitory response and may be beneficial. It is even possible that local production of inhibitory cytokines may be part of a treatment effect. In addition, it is conceivable that inflammation could be absent from the CNS for prolonged durations of time during the course of MS. This can in part be countered by repeated sampling, but the discomfort associated with lumbar punctures makes it impractical to be performed more often than once a year or so.

Disproof of tissue damage in the CNS

Disability in MS originates with tissue damage. Relapses cause acute tissue damage, progressive disease is a low-grade tissue damaging process and permanent disability is a consequence of previous tissue damage to the CNS. If we can demonstrate that no tissue damage is taking place we have reason to believe that the disease process is dormant or absent.

When the brain or spinal cord is damaged, structural proteins are released from the tissue into the CSF where they can be quantified. The amount of such proteins correlates with the amount of cell death and tissue damage.[152] In contrast to MRI, biomarkers of tissue damage provide a direct means to estimate on-going tissue damage.

A comprehensive assessment of tissue damage caused by disease in the central nervous system must at the very least include an analysis of at least one protein corresponding to each of the main cell types: neurons, oligodendrocytes and astrocytes. Cytoskeletal proteins are ideal in this regard, since they are abundantly expressed. The best candidates so far are neurofilament light (NFL), myelin basic protein (MBP) and glial fibrillary acidic protein (GFAp).

1. Neurofilaments are 8-10 nm heteropolymers with three major subunits: the light, intermediate and heavy chain. They constitute the predominant cytoskeletal component in large-diameter myelinated axons, and are scarcely expressed in the neural soma.[153]
2. MBP is positioned at the intracellular surface of myelin membranes, and via interactions with acidic lipid moieties involved in maintaining the structure of compact myelin.[36]
3. GFAp is the principal intermediate filament in mature CNS astrocytes. GFAp is thought to be important in modulating astrocyte motility and shape by providing structural stability to astrocytic processes. Following tissue injury, astrocytes become reactive and respond with rapid synthesis of GFAp.[154]

These biomarkers have been previously studied to some extent in the context of MS, but not together in a single study and rarely in relation to MRI data. The simultaneous measurement of NFL, MBP and GFAp constitutes a new method of evaluation of tissue damage in MS, which we have validated with MRI and clinical data.[IV] No study demonstrating absence of tissue damage after HSCT has been made so far, but such a study would provide very strong arguments for absence of disease activity and against development of future disability. Again, sampling at regular intervals should be done, and after two years normalization should have been reached. Persistent moderately increased values should be interpreted as incipient SPMS.

Caveats of disproof of tissue damage in the CNS

Similar to the analysis of cytokines in the CSF, repeated sampling may not be feasible at intervals short enough to ensure that no tissue damaging event might slip through unnoticed. Additionally, it is possible that a clinically relevant low-grade tissue damaging process may not to be detected with this method.

Concluding remarks

The effect of HSCT is likely related to the destruction of pro-inflammatory auto-reactive leukocytes and restoration of tolerance. Before the procedure, the immune system regarded myelin as something dangerous that must be attacked; after the procedure, it sees myelin as something to protect. Whether we have the improved listening skills of the immune system remain to be proven.

From a clinical vantage point, it seems that we confidently can claim cure in relapsing-remitting MS patients after five-year absence of disease activity. To strengthen this assertion and provide arguments for a cure in the scientific and most strict sense of the word, further studies should be done, utilizing the new tools described herein. In addition, those tools may prove to be useful in predicting outcome of the procedure at earlier time points than five years.

Chapter 5 - Afterword

> The commencement of this final period is marked, as I mentioned to you, by the progressive enfeeblement of the organic functions; inappetency becomes habitual, diarrhœa frequent, and soon a general emaciation supervenes which grows more and more evident. At the same time, there ensues an aggravation of all the symptoms proper to this disease, the obnubilation of the intellect proceeds even to dementia, the difficulty of enunciation is carried to its extreme, and the patient can only utter an unintelligible grunting; then the sphincters become paralysed…
>
> Lectures on the diseases of the nervous system (1877). Lecture VIII Apoplectiform seizures in disseminated sclerosis. Periods and forms. Pathological physiology. Etiology. Treatment.
>
> J M Charcot

Multiple sclerosis is a gruesome disease. On average it takes ten years of life. Even today, the most severely afflicted will die in their middle age. Not only will life be significantly shorter, the final years will be spent in agony. As a neurologist, I have seen too many wither away in the heart of life.

I sincerely believe that MS can be cured, and that we have the means to do it. Every year, some one thousand Swedes are diagnosed with MS. Currently, less than 1 % of them will be treated with hematopoietic stem cell transplantation. Why are so few patients treated?

The difficulty of making an accurate prognosis is one serious obstacle. Even though we know that more than half of patients will end up with secondary progressive MS and severe disability, we cannot with any certainty identify who they are at diagnosis. Neither do we know what an adequate treatment response to conventional treatment is.

The perceived peril of the procedure is another impediment. We are today very far from the close to 10 % treatment related mortality that was reported in early years. In fact, we saw no mortality or life threatening adverse effects among the Swedish patients. We have come to a point when adverse events occur so rarely, that they are hard to measure accurately.

A third hindrance is that patients and neurologists adopt a wait-and-see approach to MS. It usually takes several years before MS start to manifest itself with permanent disability, and many patients and doctors do not take MS seriously until then. In fact, most of the requests for transplantation are self-referrals from patients with progressive disease and EDSS ≥ 6.

These hurdles are not insurmountable. Newly diagnosed patients should be continuously and carefully monitored to evaluate disease activity and tissue damage. Non-trivial disease should be hit hard and hit early. Patients and doctors need to be educated, in order to disperse prejudices against the treatment. Data from a phase III trial would be helpful to convince the skeptics and to establish the therapy as a second line treatment. We are currently participating in one (ClinicalTrials.gov Identifier: NCT00273364).

Meanwhile, every wheelchair is a failure and every premature death is a tragedy.

Epilogue

- Do you still have MS, I asked.
- I want to say no, she said, but I dare not. Some of my friends who have gone through the procedure say that they had MS, but when I'm asked, I usually say that I still have it, but that it is inactive.
- Do you still think a lot about your disease? I continued.
- Not that much. In the beginning I thought about my disease all the time, but now many days can pass without me thinking about it. Then something comes along that reminds me of it, a scent or a tune, and then I am back again. Nevertheless, I consider myself lucky. I don't think I would still be around if I hadn't got the transplant. Ever since I got ill, I have celebrated two birthdays, my original birthday in November and my second birthday in May. That's when I got the transplantation and life began anew. This year, at the 10th anniversary, I will have a real jamboree, to celebrate life. After that, maybe I'll be able to let go. Then I'll stop. No more celebrations…

Ten years have now passed since those dreadful months when she fell ill and she has made an astonishing recovery. Even though we have carefully searched for signs of MS, we have found none. For all we now, she is cured of her multiple sclerosis. With time, perhaps she will be fully healed as well.

Acknowledgements

I could never have done this by myself. First and foremost my thanks goes to all the patients who volunteered to participate in my studies. My involuntary supervisor Jan Fagius has been a tremendous support throughout the years - may he be a happy retiree at last. My co-supervisors, Angelica Loskog - a constant source of reassurance when I am in doubt of myself and my data and Sara Mangsbo who taught me to be on lookout for the story hidden within the data set.

Professor Erik Stålberg and Dr Kristina Carlson were early inspirers. Professor Thomas Tötterman convinced me of the virtues of laboratory science. The head of the Neurology department Karin Rudling believed in me from the start and granted me ample of time to do research. Moa Fransson taught me how to work in the lab. Lisa Christiansson showed me the secrets of Excel and GraphPad. Hannah Karlsson always kept me in a good mood. Lina Liljenfeldt just barely beat me to it. Other former and present PhD student colleagues have been great friends and immensely helpful: Erika Gustafsson, Viktoria Hillerdal, Birgitta Jacobsson, Chuan Jin, Justyna Leja, Camilla Lindqvist, Mohanraj Ramachandran, Alma Rystedt, Linda Sandin, Emma Svensson, Luuk van Hooren, Johan Virhammar and Di Yu. Berith Nilsson, Kenneth Wester and Gabriella Paul Wetterberg were invaluable sources of experience and assistance. Professor Magnus Essand always had an open door.

I am indebted to my co-authors, Professor Henrik Zetterberg - the nicest man I know and Professor Raili Raininko, who always pushed me to do better. Drs Richard Burt, Polly Matzinger, Paulo Muraro and Anders Svenningsson were role models and sources of inspiration.

My colleagues at the Department of Neurology and all the nurses, especially Susanne Eriksson who makes ends meet for me. My colleagues at the Department of Hematology, who are actually doing the things I write about.

Friends and family. Fredrik Weisner, who was born to run. Mikael Huss, I sincerely wanted us to do a research project together, it is not too late. My brother Robert is the Master of figures. My wife Sara and my daughters Filippa, Hanna and Lovisa - you are the sunshine in my life.

Sources of funding

Research is expensive. Numerous sources contributed to the work presented in this thesis. Lars Tenerz made a very generous donation. The Swedish State Support for Clinical Research (ALF), the Swedish Research Council, the MÅH Ländell foundation, the Selander foundation, Uppsala University Hospital, the Swedish Society for Neurologically Disabled and Åke Löwnertz foundation for neurological research all contributed to the research described herein. In addition, Merck Serono and BiogenIDEC provided us with unrestricted research grants.

Appendix

The Uppsala cohort

Study III and IV of this thesis were made with samples and data acquired from a patient cohort of MS patients recruited from 2008 to 2011. MS patients with a diagnosis of RRMS or SPMS were identified in the Swedish multiple sclerosis registry. Patients seen in the MS clinic at Uppsala university hospital (n=390) were invited to participate in the study. The records of those who agreed to participate (n=110) were scrutinized; those who had another diagnosis than RRMS or SPMS were excluded. Patients who were treated with chemotherapy, had a history of psychiatric disease, severe cognitive impairment or otherwise considered not suitable were also excluded. Remaining patients (n=52) were included.

In addition, patients visiting the clinic for evaluation of possible MS or clinically isolated syndrome were offered to join the cohort (n=16). Lastly, patients with an established diagnosis of MS with a recent onset relapse were offered to join (n=7). In total, 73 unique individuals joined the cohort.

Participants underwent a clinical examination, MRI investigation, lumbar puncture and blood sampling. If possible, 10 cc CSF and 10 cc blood were drawn.

Patients with a recent relapse with onset ≤7 days were offered to repeat all investigations three weeks and three months after inclusion. Seven individuals were repeatedly investigated: three patients agreed to repeat all investigations; one patient underwent clinical examination, lumbar puncture and blood sampling only; three patients underwent clinical examination and blood sampling only. In addition, two patients were examined at two different time points, once in remission and once in relapse.

Sixteen individuals were recruited as possible controls. Fourteen were patients with other non-inflammatory neurological disease (*i e* idiopathic intracranial hypertension) or patients investigated for MS where no signs of disease could be demonstrated. Two controls were healthy volunteers.

The expanded disability status scale (EDSS)

0 - Normal neurological exam.

1.0 – 1.5 - No disability.

2.0 – 2.5 - Minimal disability.

3.0 – 3.5 - Moderate disability.

4.0 - Fully ambulatory without aid, self-sufficient, up and about some 12 hours a day; able to walk without aid or rest some 500 meters.

4.5 - Fully ambulatory without aid, up and about much of the day, able to work a full day, may otherwise have some limitation of full activity or require minimal assistance; able to walk without aid or rest some 300 meters.

5.0 - Ambulatory without aid or rest for about 200 meters; disability severe enough to impair full daily activities (e.g., to work a full day without special provisions.

5.5 - Ambulatory without aid for about 100 meters; disability severe enough to preclude full daily activities.

6.0 - Intermittent or unilateral constant assistance (cane, crutch, brace) required to walk about 100 meters with or without resting.

6.5 - Constant bilateral assistance (canes, crutches, braces) required to walk about 20 meters without resting.

7.0 - Unable to walk beyond approximately 5 meters even with aid, essentially restricted to wheelchair; wheels self in standard wheelchair and transfers alone; up and about in wheelchair some 12 hours a day.

7.5 - Unable to take more than a few steps; restricted to wheelchair; may need aid in transfer; wheels self but cannot carry on in standard wheelchair a full day; May require motorized wheelchair.

8.0 - Essentially restricted to bed or chair or perambulated in wheelchair, but may be out of bed itself much of the day; retains many self-care functions; generally has effective use of arms.

8.5 - Essentially restricted to bed much of day; has some effective use of arm(s); retains some self-care functions.

9.0 - Helpless bedridden patient; can communicate and eat.

9.5 - Totally helpless bedridden patient; unable to communicate effectively or eat/swallow.

10.0 - Death due to MS.

From Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS*) by Kurtzke in Neurology. 1983 Nov;33(11):1444-52.*[155]

Origin of figures

Figure 1.1
Adopted from *Tissue based control: the other side of tolerance* by Matzinger P and Kamala T in Nature Reviews Immunology (2011)[16] with permission from Nature Publishing Group.

Figure 3.1
Adopted from *Autologous haematopoietic stem-cell transplantation in multiple sclerosis* by Mancardi G and Saccardi R in The Lancet Neurology (2008)[116] with permission from Elsevier.

Figure 4.1
Reprinted from (A) *High-dose immunoablation with autologous haematopoietic stem cell transplantation in aggressive multiple sclerosis: a single centre 10-year experience* by Krasulová E, *et al* in Multiple Sclerosis (2010)[107] with permission from SAGE Publications. (B) *Autologous haematopoietic stem cell transplantation with an intermediate intensity conditioning regimen in multiple sclerosis: the Italian multi-centre experience* by Mancardi GL, *et al* in Multiple Sclerosis (2012)[108] with permission from SAGE Publications. (C) *Autologous hematopoietic stem cell transplantation with reduced-intensity conditioning in multiple sclerosis* by Shevchenko JL, *et al* in Experimental Hematology (2012)[122] with permission from Elsevier. (D) *Autologous haematopoietic stem cell transplantation for aggressive multiple sclerosis: the Swedish experience* by Burman J, *et al* in Journal of Neurology, Neurosurgery, and Psychiatry (2014)[I] with permission from BMJ Publishing Group.

Figure 4.2
Reprinted from *Long-term results of stem cell transplantation for MS: A single-center experience* by Fassas, A, *et al* in Neurology (2011)[119] with permission from Wolters Kluwer Health.

Figure 4.3
Reprinted from *Autologous haematopoietic stem cell transplantation for aggressive multiple sclerosis: the Swedish experience* by Burman J, *et al* in Journal of Neurology, Neurosurgery, and Psychiatry (2014)[I] with permission from BMJ Publishing Group.

Figure 4.4
Reprinted from *Thymic output generates a new and diverse TCR repertoire after autologous stem cell transplantation in multiple sclerosis patients* by Muraro PA, *et al* in Journal of Experimental medicine (2005)[136] with permission from Rockefeller University Press.

Figure 4.5
Reprinted from *T cell repertoire following autologous stem cell transplantation for multiple sclerosis* by Muraro PA, in Journal of Clinical Investigation (2014)[137] with permission from CCC republication.

Bibliography

1. Ahlgren C, Oden A and Lycke J. High nationwide prevalence of multiple sclerosis in Sweden. *Multiple Sclerosis*. 2011; 17: 901-8.
2. Compston A and Coles A. Multiple sclerosis. *The Lancet*. 2002; 359: 1221-31.
3. Brønnum-Hansen H, Koch-Henriksen N and Stenager E. Trends in survival and cause of death in Danish patients with multiple sclerosis.pdf. *Brain*. 2004; 127: 844-50.
4. Daumer M, Griffith LM, Meister W, Nash RA and Wolinsky JS. Survival, and time to an advanced disease state or progression, of untreated patients with moderately severe multiple sclerosis in a multicenter observational database: relevance for design of a clinical trial for high dose immunosuppressive therapy with autologous hematopoietic stem cell transplantation. *Multiple Sclerosis*. 2006; 12: 174-9.
5. Hader W. Disability and survival of multiple sclerosis in Saskatoon, Saskatchewan. *Can J Neurol Sci*. 2010; 37: 28-35.
6. Skoog B, Runmarker B, Winblad S, Ekholm S and Andersen O. A representative cohort of patients with non-progressive multiple sclerosis at the age of normal life expectancy. *Brain*. 2012; 135: 900-11.
7. Lublin F and Reingold S. Defining the clinical course of multiple sclerosis: results of an international survey. *Neurology*. 1996; 46: 907-11.
8. Filippi M, Rossi P, Campi A, Colombo B, Pereira C and Comi G. Serial contrast-enhanced MR in patients with multiple sclerosis and varying levels of disability. *American Journal of Neuroradiology*. 1997; 18: 1549-56.
9. Koziol J, Wagner S and Adams H. Assessing information in T2-weighted MRI scans from secondary progressive MS patients. *Neurology*. 1998; 51: 228-33.
10. Confavreux C, Vukusic S and Adeleine P. Early clinical predictors and progression of irreversible disability in multiple sclerosis: an amnesic process. *Brain*. 2003; 126: 770-82.
11. inflammation. *Encyclopædia Britannica Online*. 2014.
12. autoimmunity. *Encyclopædia Britannica Online*. 2014.

13. Witebsky E, Rose NR, Terplan K, Paine JR and Egan RW. Chronic thyroiditis and autoimmunization. *Journal of the American Medical Association*. 1957; 164: 1439-47.
14. Rose NR and Bona C. Defining criteria for autoimmune diseases (Witebsky's postulates revisited). *Immunology today*. 1993; 14: 426-30.
15. Matzinger P. The danger model: a renewed sense of self. *Science*. 2002; 296: 301-5.
16. Matzinger P and Kamala T. Tissue-based class control: the other side of tolerance. *Nat Rev Immunol*. 2011; 11: 221-30.
17. Uhthoff W. Untersuchungen über die bei der multiplen Herdsklerose vorkommenden Augenstörungen. *Archiv für Psychiatrie und Nervenkrankheiten*. 1890; 21: 55-116; 303-410.
18. Guthrie T. Visual and motor changes in patients with multiple sclerosis; a result of induced changes in environmental temperature. *Arch Neurol Psychiatry*. 1951; 65: 437-51.
19. Filippi M, Rovaris M, Capra R, et al. A multi-centre longitudinal study comparing the sensitivity of monthly MRI after standard and triple dose gadolinium-DTPA for monitoring disease activity in multiple sclerosis. Implications for phase II clinical trials. *Brain*. 1998; 121: 2011-20.
20. Vellinga MM, Oude Engberink RD, Seewann A, et al. Pluriformity of inflammation in multiple sclerosis shown by ultra-small iron oxide particle enhancement. *Brain*. 2008; 131: 800-7.
21. Tourdias T, Roggerone S, Filippi M, et al. Assessment of disease activity in multiple sclerosis phenotypes with combined gadolinium- and superparamagnetic iron oxide-enhanced MR imaging. *Radiology*. 2012; 264: 225-33.
22. Lamers KJ, de Reus HP and Jongen PJ. Myelin basic protein in CSF as indicator of disease activity in multiple sclerosis. *Multiple Sclerosis*. 1998; 4: 124-6.
23. Schumacker GA, Beebe G, Kibler RF, et al. Problems of Experimental Trials of Therapy in Multiple Sclerosis: Report by the Panel on the Evaluation of Experimental Trials of Therapy in Multiple Sclerosis. *Annals of the New York Academy of Sciences*. 1965; 122: 552-68.
24. Polman CH, Reingold SC, Banwell B, et al. Diagnostic criteria for multiple sclerosis: 2010 revisions to the McDonald criteria. *Annals of Neurology*. 2011; 69: 292-302.
25. Burt RK, Cohen BA, Russell E, et al. Hematopoietic stem cell transplantation for progressive multiple sclerosis: failure of a total body irradiation-based conditioning regimen to prevent disease progression in patients with high disability scores. *Blood*. 2003; 102: 2373-8.

26. Martinelli Boneschi F, Vacchi L, Rovaris M, Capra R and Comi G. Mitoxantrone for multiple sclerosis. *The Cochrane database of systematic reviews*. 2013; 5: CD002127.
27. De Stefano N, Matthews P, Fu L, et al. Axonal damage correlates with disability in patients with relapsing-remitting multiple sclerosis. Results of a longitudinal magnetic resonance spectroscopy study. *Brain*. 1998; 121: 1469-77.
28. Norgren N, Sundström P, Svenningsson A, Rosengren L, Stigbrand T and Gunnarsson M. Neurofilament and glial fibrillary acidic protein in multiple sclerosis. *Neurology*. 2004; 63: 1586-90.
29. Prineas J. The neuropathology of multiple sclerosis. In: Koetsier J, (ed.). *Handbook of clinical neurology*. Amsterdam: Elsevier, 1985, p. 213–57.
30. International Multiple Sclerosis Genetics Consortium, Beecham AH, Patsopoulos NA, et al. Analysis of immune-related loci identifies 48 new susceptibility variants for multiple sclerosis. *Nature genetics*. 2013; 45: 1353-60.
31. International Multiple Sclerosis Genetics Consortium, Hafler DA, Compston A, et al. Risk alleles for multiple sclerosis identified by a genomewide study. *N Engl J Med*. 2007; 357: 851-62.
32. Hansen T, Skytthe A, Stenager E, Petersen HC, Bronnum-Hansen H and Kyvik KO. Concordance for multiple sclerosis in Danish twins: an update of a nationwide study. *Mult Scler*. 2005; 11: 504-10.
33. Westerlind H, Ramanujam R, Uvehag D, et al. Modest familial risks for multiple sclerosis: a registry-based study of the population of Sweden. *Brain*. 2014; 137: 770-8.
34. Ascherio A. Environmental factors in multiple sclerosis. *Expert review of neurotherapeutics*. 2013; 13: 3-9.
35. Pette M, Fujita K, Wilkinson D, et al. Myelin autoreactivity in multiple sclerosis: recognition of myelin basic protein in the context of HLA-DR2 products by T lymphocytes of multiple-sclerosis patients and healthy donors. *Proc Natl Acad Sci U S A*. 1990; 87: 7968-72.
36. Sospedra M and Martin R. Immunology of multiple sclerosis. *Annual Review of Immunology*. 2005; 23: 683-747.
37. Smith K, McDonald I, Miller D and Lassmann H. The pathophysiology of multiple sclerosis. In: Compston A, Confavreux C, Lassmann H, et al., (eds.). *McAlpine's multiple sclerosis*. 4th ed. London: Churchill Livingstone, 2006.
38. Pryce G, O'Neill JK, Croxford JL, et al. Autoimmune tolerance eliminates relapses but fails to halt progression in a model of multiple sclerosis. *Journal of Neuroimmunology*. 2005; 165: 41-52.
39. Lindner M, Fokuhl J, Linsmeier F, Trebst C and Stangel M. Chronic toxic demyelination in the central nervous system leads to axonal damage despite remyelination. *Neuroscience Letters*. 2009; 453: 120-5.

40. Serafini B, Rosicarelli B, Magliozzi R, Stigliano E and Alois iF. Detection of ectopic B-cell follicles with germinal centers in the meninges of patients with secondary progressive multiple sclerosis. *Brain Pathology*. 2004; 14: 164-74.
41. Karni A, Abraham M, Monsonego A, et al. Innate immunity in multiple sclerosis: myeloid dendritic cells in secondary progressive multiple sclerosis are activated and drive a proinflammatory immune response. *J Immunol*. 2006; 177: 4196-202.
42. Anderson AC, Anderson DE, Bregoli L, et al. Promotion of tissue inflammation by the immune receptor Tim-3 expressed on innate immune cells. *Science*. 2007; 318: 1141-3.
43. Tedeholm H, Lycke J, Skoog B, et al. Time to secondary progression in patients with multiple sclerosis who were treated with first generation immunomodulating drugs. *Mult Scler*. 2013; 19: 765-74.
44. Kabat E, Freedman D, Murray J and Knaub v. A study of the crystalline albumin, gamma globulin and total protein in the cerebrospinal fluid of 100 cases of multiple sclerosis and in other diseases. *Am J Med Sci*. 1950; 219: 55-64.
45. Walsh M and Tourtellotte W. Temporal invariance and clonal uniformity of brain and cerebrospinal IgG, IgA, and IgM in multiple sclerosis. *J Exp Med*. 1986; 163: 41-53.
46. Link H and Tibbling G. Principles of albumin and IgG analyses in neurological disorders. III. Evaluation of IgG synthesis within the central nervous system in multiple sclerosis. *Scandinavian journal of clinical and laboratory investigation*. 1977; 37: 397-401.
47. Trotter J and Rust R. *Human cerebrospinal fluid immunology*. Amsterdam: Martinus Nyhoff, 1989, p.179-226.
48. Bourahoui A, De Seze J, Guttierez R, et al. CSF isoelectrofocusing in a large cohort of MS and other neurological diseases. *European journal of neurology : the official journal of the European Federation of Neurological Societies*. 2004; 11: 525-9.
49. Lily O, Palace J and Vincent A. Serum autoantibodies to cell surface determinants in multiple sclerosis: a flow cytometric study. *Brain*. 2004; 127: 269-79.
50. Mosmann TR, Cherwinski H, Bond MW, Giedlin MA and Coffman RL. Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. *J Immunol*. 1986; 136: 2348-57.
51. Segal BM. Th17 cells in autoimmune demyelinating disease. *Seminars in Immunopathology*. 2010; 32: 71-7.
52. Barr TA, Shen P, Brown S, et al. B cell depletion therapy ameliorates autoimmune disease through ablation of IL-6-producing B cells. *Journal of Experimental Medicine*. 2012; 209: 1001-10.
53. Hartung H and Kieseier B. Atacicept: targeting B cells in multiple sclerosis. *Ther Adv Neurol Disord*. 2012; 3: 205-16.

54. Hauser S, Waubant E, Arnold D, et al. B-cell depletion with rituximab in relapsing-remitting multiple sclerosis. *N Engl J Med*. 2008; 358: 676-88.
55. Hawker K, O'Connor P, Freedman MS, et al. Rituximab in patients with primary progressive multiple sclerosis: Results of a randomized double-blind placebo-controlled multicenter trial. *Annals of Neurology*. 2009; 66: 460-71.
56. Yuseff MI, Pierobon P, Reversat A and Lennon-Dumenil AM. How B cells capture, process and present antigens: a crucial role for cell polarity. *Nat Rev Immunol*. 2013; 13: 475-86.
57. Magliozzi R, Howell O, Vora A, et al. Meningeal B-cell follicles in secondary progressive multiple sclerosis associate with early onset of disease and severe cortical pathology. *Brain*. 2007; 130: 1089-104.
58. Pettinelli C and McFarlin D. Adoptive transfer of experimental allergic encephalomyelitis in SJL/J mice after in vitro activation of lymph node cells by myelin basic protein: requirement for Lyt 1+ 2- T lymphocytes. *J Immunol*. 1981; 127: 1420-3.
59. Lassmann H and Wekerle H. The pathology of multiple sclerosis. *McAlpine's multiple sclerosis*. 4th ed. London: Churchill Livingstone, 2005, p. 557-59.
60. Madsen LS, Andersson EC, Jansson L, et al. A humanized model for multiple sclerosis using HLA-DR2 and a human T-cell receptor. *Nature genetics*. 1999; 23: 343-7.
61. Quandt JA, Baig M, Yao K, et al. Unique clinical and pathological features in HLA-DRB1*0401-restricted MBP 111-129-specific humanized TCR transgenic mice. *J Exp Med*. 2004; 200: 223-34.
62. Bielekova B, Goodwin B, Richert N, et al. Encephalitogenic potential of the myelin basic protein peptide (amino acids 83-99) in multiple sclerosis: results of a phase II clinical trial with an altered peptide ligand. *Nat Med*. 2000; 6: 1167-75.
63. van Oosten B, Lai M, Hodgkinson S, et al. Treatment of multiple sclerosis with the monoclonal anti-CD4 antibody cM-T412 - results of a randomized, double-blind, placebo-controlled, MR-monitored phase II trial. *Neurology*. 1997; 49: 351-57.
64. Crome SQ, Wang AY and Levings MK. Translational mini-review series on Th17 cells: function and regulation of human T helper 17 cells in health and disease. *Clin Exp Immunol*. 2010; 159: 109-19.
65. Havrdová E, Belova A, Goloborodko A, et al. Positive proof of concept of AIN457, an antibody against interleukin-17A, in relapsing-remitting multiple sclerosis. *ECTRIMS*. Lyons, 2012.
66. International Multiple Sclerosis Genetics Consortium, Wellcome Trust Case Control Consortium, Sawcer S, et al. Genetic risk and a primary role for cell-mediated immune mechanisms in multiple sclerosis. *Nature*. 2011; 476: 214-9.

67. Hoftberger R, Aboul-Enein F, Brueck W, et al. Expression of major histocompatibility complex class I molecules on the different cell types in multiple sclerosis lesions. *Brain Pathol*. 2004; 14: 43-50.
68. Neumann H, Medana IM, Bauer J and Lassmann H. Cytotoxic T lymphocytes in autoimmune and degenerative CNS diseases. *Trends in neurosciences*. 2002; 25: 313-9.
69. Junker A, Ivanidze J, Malotka J, et al. Multiple sclerosis: T-cell receptor expression in distinct brain regions. *Brain*. 2007; 130: 2789-99.
70. Annibali V, Ristori G, Angelini DF, et al. CD161(high)CD8+T cells bear pathogenetic potential in multiple sclerosis. *Brain*. 2011; 134: 542-54.
71. Abrahamsson SV, Angelini DF, Dubinsky AN, et al. Non-myeloablative autologous haematopoietic stem cell transplantation expands regulatory cells and depletes IL-17 producing mucosal-associated invariant T cells in multiple sclerosis. *Brain*. 2013.
72. Taylor AL and Llewelyn MJ. Superantigen-Induced Proliferation of Human CD4+CD25- T Cells Is Followed by a Switch to a Functional Regulatory Phenotype. *The Journal of Immunology*. 2010; 185: 6591-8.
73. Thornton AM, Korty PE, Tran DQ, et al. Expression of Helios, an Ikaros Transcription Factor Family Member, Differentiates Thymic-Derived from Peripherally Induced Foxp3+ T Regulatory Cells. *The Journal of Immunology*. 2010; 184: 3433-41.
74. Wang J, Ioan-Facsinay A, van der Voort E, Huizinga T and Toes R. Transient expression of FOXP3 in human activated nonregulatory CD4+ T cells. *Eur J Immunol*. 2007; 37: 129-38.
75. Allan SE, Crome SQ, Crellin NK, et al. Activation-induced FOXP3 in human T effector cells does not suppress proliferation or cytokine production. *International Immunology*. 2007; 19: 345-54.
76. Viglietta V, Baecher-Allan C, Weiner HL and Hafler DA. Loss of functional suppression by CD4+CD25+ regulatory T cells in patients with multiple sclerosis. *J Exp Med*. 2004; 199: 971-9.
77. Lowther D and Hafler D. Regulatory T cells in the central nervous system. *Immunol Rev*. 2012; 248: 156-69.
78. Fransson M, Burman J, Lindqvist C, Atterby C, Fagius J and Loskog A. T regulatory cells lacking CD25 are increased in MS during relapse. *Autoimmunity*. 2010; 43: 590-7.
79. Dalla Libera D, Di Mitri D, Bergami A, et al. T regulatory cells are markers of disease activity in multiple sclerosis patients. *PLoS One*. 2011; 6: e21386.
80. Getnet D, Grosso JF, Goldberg MV, et al. A role for the transcription factor Helios in human CD4+CD25+ regulatory T cells. *Molecular Immunology*. 2010; 47: 1595-600.

81. Kaur G, Trowsdale J and Fugger L. Natural killer cells and their receptors in multiple sclerosis. *Brain*. 2012.
82. Bielekova B, Catalfamo M, Reichert-Scrivner S, et al. Regulatory CD56(bright) natural killer cells mediate immunomodulatory effects of IL-2Ralpha-targeted therapy (daclizumab) in multiple sclerosis. *Proc Natl Acad Sci U S A*. 2006; 103: 5941-6.
83. Huang D, Shi FD, Jung S, et al. The neuronal chemokine CX3CL1/fractalkine selectively recruits NK cells that modify experimental autoimmune encephalomyelitis within the central nervous system. *Faseb J*. 2006; 20: 896-905.
84. Eriksson M, Andersen O and Runmarker B. Long-term follow up of patients with clinically isolated syndromes, relapsing-remitting and secondary progressive multiple sclerosis. *Multiple Sclerosis*. 2003; 9: 260-74.
85. Lukas C, Minneboo A, de Groot V, et al. Early central atrophy rate predicts 5 year clinical outcome in multiple sclerosis. *J Neurol Neurosurg Psychiatry*. 2010; 81: 1351-6.
86. Rovaris M, Rocca MA, Barkhof F, et al. Relationship between brain MRI lesion load and short-term disease evolution in non-disabling MS: a large-scale, multicentre study. *Multiple Sclerosis*. 2011; 17: 319-26.
87. Popescu V, Agosta F, Hulst HE, et al. Brain atrophy and lesion load predict long term disability in multiple sclerosis. *J Neurol Neurosurg Psychiatry*. 2013; 84: 1082-91.
88. Salzer J, Svenningsson A and Sundstrom P. Neurofilament light as a prognostic marker in multiple sclerosis. *Multiple Sclerosis*. 2010; 16: 287-92.
89. The IFNB Multiple Sclerosis Study Group and The University of British Columbia MS/MRI Analysis Group. Interferon beta-1b in the treatment of multiple sclerosis: final outcome of the randomized controlled trial. *Neurology*. 1995; 45: 1277-85.
90. Sydenham T. Observationes medicae, 1676. In: Caplan AL, Engelhardt T and McCartney J, (eds.). *Concepts of Health and Disease: Interdisciplinary Perspectives*. Reading, Massachusetts, 1981.
91. McWhinney IR. Are we on the brink of a major transformation of clinical method? *CMAJ : Canadian Medical Association journal = journal de l'Association medicale canadienne*. 1986; 135: 873-8.
92. Foucault M. *The Birth of the Clinic. An Archaeology of Medical Perception*. New York: Vantage, 1975.
93. Barondess JA. Disease and illness--a crucial distinction. *The American journal of medicine*. 1979; 66: 375-6.
94. Szasz T. *The Second Sin*. New York: Doubleday, 1973.
95. WHO. Preamble to the Constitution of the World Health Organization. *International Health Conference*. New york, 1946.

96. Boyd W. *Textbook of Pathology*. 7th ed. Philadelphia: Lea & Febiger, 1965.
97. Huttner HB, Bergmann O, Salehpour M, et al. The age and genomic integrity of neurons after cortical stroke in humans. *Nature neuroscience*. 2014.
98. W. MF, Granville NB and Dameshek W. Autologous bone marrow infusion as an adjunct in therapy of malignant disease. *Blood*. 1959; 14: 503-21.
99. Thomas ED, Lochte HL, Jr., Cannon JH, Sahler OD and Ferrebee JW. Supralethal whole body irradiation and isologous marrow transplantation in man. *The Journal of clinical investigation*. 1959; 38: 1709-16.
100. Gratwohl A, Baldomero H and Passweg J. Hematopoietic stem cell transplantation activity in Europe. *Current opinion in hematology*. 2013; 20: 485-93.
101. Burt RK, Burns W, Ruvolo P, et al. Syngeneic bone marrow transplantation eliminates V beta 8.2 T lymphocytes from the spinal cord of Lewis rats with experimental allergic encephalomyelitis. *Journal of neuroscience research*. 1995; 41: 526-31.
102. Burt RK, Burns W and Hess A. Bone marrow transplantation for multiple sclerosis. *Bone Marrow Transplant*. 1995; 16: 1-6.
103. Fassas A, Anagnostopoulos A, Kazis A, et al. Peripheral blood stem cell transplantation in the treatment of progressive multiple sclerosis: first results of a pilot study. *Bone Marrow Transplant*. 1997; 20: 631-8.
104. Fagius J, Lundgren J and Oberg G. Early highly aggressive MS successfully treated by hematopoietic stem cell transplantation. *Mult Scler*. 2009; 15: 229-37.
105. Burt R, Loh Y, Cohen B, et al. Autologous non-myeloablative haemopoietic stem cell transplantation in relapsing-remitting multiple sclerosis: a phase I/II study. *Lancet Neurol*. 2009; 8: 244-53.
106. Hintzen RQ. Stem cell transplantation in multiple sclerosis: multiple choices and multiple challenges. *Multiple Sclerosis*. 2002; 8: 155-60.
107. Krasulova E, Trneny M, Kozak T, et al. High-dose immunoablation with autologous haematopoietic stem cell transplantation in aggressive multiple sclerosis: a single centre 10-year experience. *Multiple Sclerosis*. 2010; 16: 685-93.
108. Mancardi G, Sormani M, Di Gioia M, et al. Autologous haematopoietic stem cell transplantation with an intermediate intensity conditioning regimen in multiple sclerosis: the Italian multi-centre experience. *Multiple Sclerosis Journal*. 2012; 18: 835-42.
109. Atkins HL and Freedman MS. Hematopoietic stem cell therapy for multiple sclerosis: top 10 lessons learned. *Neurotherapeutics*. 2013; 10: 68-76.

110. Burt RK, Balabanov R, Voltarelli J, Barreira A and Burman J. Autologous hematopoietic stem cell transplantation for multiple sclerosis - if confused or hesitant, remember: 'Treat with standard immune suppressive drugs and if no inflammation, no response'. *Multiple Sclerosis Journal*. 2012; 18: 772-5.
111. Meng L, Ouyang J, Zhang H, Wen Y, Chen J and Zhou J. Treatment of an autoimmune encephalomyelitis mouse model with nonmyeloablative conditioning and syngeneic bone marrow transplantation. *Restor Neurol Neurosci*. 2011; 29: 177-85.
112. McAllister LD, Beatty PG and Rose J. Allogeneic bone marrow transplant for chronic myelogenous leukemia in a patient with multiple sclerosis. *Bone Marrow Transplant*. 1997; 19: 395-7.
113. Mandalfino P, Rice G, Smith A, Klein JL, Rystedt L and Ebers GC. Bone marrow transplantation in multiple sclerosis. *J Neurol*. 2000; 247: 691-5.
114. La Nasa G, Littera R, Cocco E, Battistini L, Marrosu MG and Contu L. Allogeneic hematopoietic stem cell transplantation in a patient affected by large granular lymphocyte leukemia and multiple sclerosis. *Annals of hematology*. 2004; 83: 403-5.
115. Openshaw H, Stuve O, Antel JP, et al. Multiple sclerosis flares associated with recombinant granulocyte colony-stimulating factor. *Neurology*. 2000; 54: 2147-50.
116. Mancardi G and Saccardi R. Autologous haematopoietic stem-cell transplantation in multiple sclerosis. *The Lancet Neurology*. 2008; 7: 626-36.
117. Farge D, Labopin M, Tyndall A, et al. Autologous hematopoietic stem cell transplantation for autoimmune diseases: an observational study on 12 years' experience from the European Group for Blood and Marrow Transplantation Working Party on Autoimmune Diseases. *Haematologica*. 2010; 95: 284-92.
118. Samijn JP, te Boekhorst PA, Mondria T, et al. Intense T cell depletion followed by autologous bone marrow transplantation for severe multiple sclerosis. *J Neurol Neurosurg Psychiatry*. 2006; 77: 46-50.
119. Fassas A, Kimiskidis VK, Sakellari I, et al. Long-term results of stem cell transplantation for MS: A single-center experience. *Neurology*. 2011; 76: 1066-70.
120. Saiz A, Blanco Y, Carreras E, et al. Clinical and MRI outcome after autologous hematopoietic stem cell transplantation in MS. *Neurology*. 2004; 62: 282-4.
121. Saccardi R, Kozak T, Bocelli-Tyndall C, et al. Autologous stem cell transplantation for progressive multiple sclerosis: Update of the European Group for Blood and Marrow Transplantation autoimmune diseases working party database. *Multiple Sclerosis*. 2006; 12: 814-23.

122. Shevchenko JL, Kuznetsov AN, Ionova TI, et al. Autologous hematopoietic stem cell transplantation with reduced-intensity conditioning in multiple sclerosis. *Exp Hematol*. 2012; 40: 892-8.
123. Sheremata W, Vollmer T, Stone L, Willmer-Hulme A and Koller M. A safety and pharmacokinetic study of intravenous natalizumab in patients with MS. *Neurology*. 1999; 52: 1072-4.
124. Coles AJ. Alemtuzumab therapy for multiple sclerosis. *Neurotherapeutics*. 2013; 10: 29-33.
125. Rudick R, Stuart W, Calabresi P, et al. Natalizumab plus interferon beta-1a for relapsing multiple sclerosis. *N Engl J Med*. 2006; 354: 911-23.
126. Coles AJ, Twyman CL, Arnold DL, et al. Alemtuzumab for patients with relapsing multiple sclerosis after disease-modifying therapy: a randomised controlled phase 3 trial. *Lancet*. 2012; 380: 1829-39.
127. Bloomgren G, Richman S, Hotermans C, et al. Risk of natalizumab-associated progressive multifocal leukoencephalopathy. *N Engl J Med*. 2012; 366: 1870-80.
128. CAMMS223 Trial Investigators, Coles AJ, Compston DA, et al. Alemtuzumab vs. interferon beta-1a in early multiple sclerosis. *N Engl J Med*. 2008; 359: 1786-801.
129. Cohen JA, Coles AJ, Arnold DL, et al. Alemtuzumab versus interferon beta 1a as first-line treatment for patients with relapsing-remitting multiple sclerosis: a randomised controlled phase 3 trial. *Lancet*. 2012; 380: 1819-28.
130. Snowden JA, Saccardi R, Allez M, et al. Haematopoietic SCT in severe autoimmune diseases: updated guidelines of the European Group for Blood and Marrow Transplantation. *Bone Marrow Transplant*. 2012; 47: 770-90.
131. Hamerschlak N, Rodrigues M, Moraes DA, et al. Brazilian experience with two conditioning regimens in patients with multiple sclerosis: BEAM/horse ATG and CY/rabbit ATG. *Bone Marrow Transplant*. 2010; 45: 239-48.
132. Popper K. *Conjectures and refutations : the growth of scientific knowledge*. London: Routledge, 1963.
133. Bonzano L, Roccatagliata L, Mancardi GL and Sormani MP. Gadolinium-enhancing or active T2 magnetic resonance imaging lesions in multiple sclerosis clinical trials? *Mult Scler*. 2009; 15: 1043-7.
134. Sicotte NL, Voskuhl RR, Bouvier S, Klutch R, Cohen MS and Mazziotta JC. Comparison of multiple sclerosis lesions at 1.5 and 3.0 Tesla. *Investigative radiology*. 2003; 38: 423-7.
135. Leppert IR, Narayanan S, Araujo D, et al. Interpreting therapeutic effect in multiple sclerosis via MRI contrast enhancing lesions: now you see them, now you don't. *J Neurol*. 2014.

136. Muraro P, Douek D, Packer A, et al. Thymic output generates a new and diverse TCR repertoire after autologous stem cell transplantation in multiple sclerosis patients. *Journal of Experimental Medicine*. 2005; 201: 805-16.
137. Muraro PA, Robins H, Malhotra S, et al. T cell repertoire following autologous stem cell transplantation for multiple sclerosis. *The Journal of clinical investigation*. 2014; 124: 1168-72.
138. Krumbholz M, Derfuss T, Hohlfeld R and Meinl E. B cells and antibodies in multiple sclerosis pathogenesis and therapy. *Nat Rev Neurol*. 2012; 8: 613-23.
139. Darlington PJ, Touil T, Doucet JS, et al. Diminished Th17 (not Th1) responses underlie multiple sclerosis disease abrogation after hematopoietic stem cell transplantation. *Annals of Neurology*. 2013; 73: 341-54.
140. Sun W, Popat U, Hutton G, et al. Characteristics of T-cell receptor repertoire and myelin-reactive T cells reconstituted from autologous haematopoietic stem-cell grafts in multiple sclerosis. *Brain*. 2004; 127: 996-1008.
141. Iliff JJ, Lee H, Yu M, et al. Brain-wide pathway for waste clearance captured by contrast-enhanced MRI. *The Journal of clinical investigation*. 2013; 123: 1299-309.
142. Saiz A, Carreras E, Berenguer J, et al. MRI and CSF oligoclonal bands after autologous hematopoietic stem cell transplantation in MS. *Neurology*. 2001; 56: 1084-9.
143. Nash RA, Bowen JD, McSweeney PA, et al. High-dose immunosuppressive therapy and autologous peripheral blood stem cell transplantation for severe multiple sclerosis. *Blood*. 2003; 102: 2364-72.
144. Henriksson A, Link H, Cruz M and Stiernstedt G. Immunoglobulin abnormalities in cerebrospinal fluid and blood over the course of lymphocytic meningoradiculitis (Bannwarth's syndrome). *Ann Neurol*. 1986; 20: 337-45.
145. Martin R, Martens U, Sticht-Groh V, Dorries R and Kruger H. Persistent intrathecal secretion of oligoclonal, Borrelia burgdorferi-specific IgG in chronic meningoradiculomyelitis. *J Neurol*. 1988; 235: 229-33.
146. Hansen K, Cruz M and Link H. Oligoclonal Borrelia burgdorferi-specific IgG antibodies in cerebrospinal fluid in Lyme neuroborreliosis. *The Journal of infectious diseases*. 1990; 161: 1194-202.
147. Matsushita T, Tateishi T, Isobe N, et al. Characteristic cerebrospinal fluid cytokine/chemokine profiles in neuromyelitis optica, relapsing remitting or primary progressive multiple sclerosis. *PLoS One*. 2013; 8: e61835.

148. Bielekova B, Komori M, Xu Q, Reich DS and Wu T. Cerebrospinal fluid IL-12p40, CXCL13 and IL-8 as a combinatorial biomarker of active intrathecal inflammation. *PLoS One*. 2012; 7: e48370.
149. Khademi M, Kockum I, Andersson ML, et al. Cerebrospinal fluid CXCL13 in multiple sclerosis: a suggestive prognostic marker for the disease course. *Mult Scler*. 2011; 17: 335-43.
150. Sellebjerg F, Bornsen L, Khademi M, et al. Increased cerebrospinal fluid concentrations of the chemokine CXCL13 in active MS. *Neurology*. 2009; 73: 2003-10.
151. Mellergard J, Edstrom M, Vrethem M, Ernerudh J and Dahle C. Natalizumab treatment in multiple sclerosis: marked decline of chemokines and cytokines in cerebrospinal fluid. *Mult Scler*. 2010; 16: 208-17.
152. Petzold A, Michel P, Stock M and Schluep M. Glial and axonal body fluid biomarkers are related to infarct volume, severity, and outcome. *J Stroke Cerebrovasc Dis*. 2008; 17: 196-203.
153. Lee M and Cleveland D. Neuronal intermediate filaments. *Annu Rev Neurosci*. 1996; 19: 187-217.
154. Eng L, Ghirnikar R and Lee Y. Glial fibrillary acidic protein: GFAP-thirty-one years (1969-2000). *Neurochem Res*. 2000; 25: 1439-51.
155. Kurtzke JF. Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). *Neurology*. 1983; 33: 1444-52.

Acta Universitatis Upsaliensis

Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine 1005

Editor: The Dean of the Faculty of Medicine

A doctoral dissertation from the Faculty of Medicine, Uppsala University, is usually a summary of a number of papers. A few copies of the complete dissertation are kept at major Swedish research libraries, while the summary alone is distributed internationally through the series Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine. (Prior to January, 2005, the series was published under the title "Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine".)

Distribution: publications.uu.se
urn:nbn:se:uu:diva-221888

ACTA
UNIVERSITATIS
UPSALIENSIS
UPPSALA
2014