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Treatment with the monoclonal antibody rituximab in Multiple Sclerosis

-a study based on an academic clinical trial

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*“No matter where you’re longing,
there is a limit to how far you’re able to bend,
without the option of an end”*

Slowly Summer Sighed, Voices of Eden, Andreas Mattson, Fläskkvartetten

*To Anders Gard and Lars Johan Liedholm for guiding me into the fields of
Neurology*

To my mentors who tempted me to discover science

To Anna, Jacques and André for being alongside on the journey

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Abstract

Background: Multiple sclerosis (MS) is a chronic, inflammatory disease, affecting the central nervous system. A growing number of disease modifying treatment alternatives entails a need for an individualised risk-benefit-convenience analysis in the counselling of patients and methods to monitor the treatment effect, including markers for subclinical inflammation. Today, MRI and the biomarker neurofilament light chain (NFL) in cerebrospinal fluid (CSF-NFL) are commonly used. The development of new techniques for analysing NFL in very low concentrations in serum or plasma provides a promising opportunity for a less invasive method. Rituximab is a chimeric monoclonal antibody with B-cell depleting properties vastly used in rheumatological disease and certain haematological malignancies. Phase II studies have shown a beneficial effect on inflammation also in MS, the detailed mechanisms of action yet to be explained.

Aims: The aims of this thesis were to evaluate rituximab as a treatment alternative in relapsing remitting MS (RRMS) by describing the clinical effect and patient related outcome measures after a switch of therapy from first-line injectables to rituximab and to explore possible immunological mechanisms of B cell depletion as well as to evaluate the use of neurofilament in plasma (p-NFL) as an end-point in a clinical trial setting.

Methods: The thesis is based on the open-label phase II multicentre clinical trial Switch-To-RituXimab in MS (STRIX-MS; EudraCT 2010-023021-38), in which 75 patients completed a therapy switch from first-line injectables to rituximab, and, to some part, the extended follow-up study, STRIX-MS extension (EudraCT 2013-002378-26). The disease modifying effect was evaluated by regular clinical evaluations, MRI and analyses of CSF-NFL. The clinical outcome was evaluated by the EDSS and SDMT scales. The questionnaires MSIS-29, FSMC and TSQM were used for the evaluation of patient related outcome measures. Immunological mechanisms of the B cell depletion were explored by the analysis of a broad panel of cyto- and chemokines in CSF by an electrochemiluminiscens method before and after therapy switch, and in comparison to healthy controls. The concentration of p-NFL was measured by an in-house NF-light assay on the Simoa platform with a Homebrew kit and explored for the use as a clinical trial end-point.

Results: During the follow-up, signs of inflammatory activity decreased. Both the mean number of Gd enhancing lesions (0.03 vs 0.36, $p=0.029$) and the number of new or enlarged T2 lesions were reduced (0.01 vs 0.28, $p=0.01$). The mean concentration of CSF-NFL was reduced during the first year (491 vs 387, $p=0.01$). The corresponding reduction in plasma did not reach the level of statistical

significance. The rating of overall treatment satisfaction improved significantly (6.3 vs 4.8, scale range 1-7, $p < 0.001$). In the explorative immunological study, the immunological profile was altered after therapy switch with the most prominent reduction observed in the concentrations of IP-10 and IL-12/23p40.

Conclusions: The results indicate a disease modifying effect of rituximab in line with other studies and provide support for a superior treatment satisfaction with rituximab as compared with injectable therapies. However, the lack of control group hampers the possibility to draw definite conclusions on the therapy effect. The immunological effects of B cell depletion need to be further explored.

Original papers

Paper I. de Flon P, Gunnarsson M, Laurell K, Söderström L, Birgander R, Lindqvist T, et al. Reduced inflammation in relapsing-remitting multiple sclerosis after therapy switch to rituximab. *Neurology*. 2016;87(2):141-7.

Paper II. de Flon P, Laurell K, Söderström L, Gunnarsson M, Svenningsson A. Improved treatment satisfaction after switching therapy to rituximab in relapsing-remitting MS. *Mult Scler*. 2017;23(9):1249-57.

Paper III. de Flon P, Söderström L, Laurell K, et al. Immunological profile in cerebrospinal fluid of patients with multiple sclerosis after treatment switch to rituximab and compared with healthy controls. *PLoS One* 2018;13:e0192516.

Paper IV. de Flon P, Laurell K, Sundström P, Blennow K, Söderström L, Zetterberg H, Gunnarsson M, Svenningsson A. Comparison of plasma and CSF Neurofilament light as outcome in a multiple sclerosis trial (Prepared manuscript)

Reprints were made with permission from the publishers. The manuscript for paper IV has been approved for publication in this thesis by all authors.

Abbreviations

AE	Adverse event
APC	Antigen presenting cell
ARR	Annualized relapse rate
BCDT	B cell depleting therapy
CIS	Clinically isolated syndrome
CNS	Central nervous system
CSF	Cerebrospinal fluid
CSF-NFL	Neurofilament light chain in cerebrospinal fluid
DMT	Disease modifying therapy
EDSS	Expanded Disability Status Scale
FSMC	Fatigue Scale for Motor and Cognitive Functions
GA	Glatirameracetate
Gd	Gadolinium
HC	Healthy control
IFN-beta	Interferon beta
IQR	Interquartile range
IV	Intravenous
kDa	kilo Dalton
LLoQ	Lowest level of quantification
MAGNIM	“MAGNetic Resonance Imaging in Multiple Sclerosis”, an independent European network of academic experts in the field

MRI	Magnetic resonance imaging
MS	Multiple sclerosis
MSIS-29	Multiple Sclerosis Impact Scale
NFL	Neurofilament light chain
p-NFL	Neurofilament light chain in plasma
PROM	Patient related outcome measure
RCT	Randomised controlled trial
RIS	Radiologically isolated syndrome
RRMS	Relapsing-remitting multiple sclerosis
SDMT	Symbol Digit Modalities Test
s-NFL	Neurofilament light chain in serum
STRIX-MS	The “Switch-To-RItuXimab-in-MS” trial
TSQM	Treatment Satisfaction Questionnaire for Medicine

Enkel sammanfattning på svenska

Vid multipel skleros (MS) angriper kroppens eget immunförsvar vävnad i hjärna och ryggmärg, dvs det centrala nervsystemet (CNS). Angreppet orsakar härdar av inflammation som kan upptäckas vid magnetkameraundersökning (MR) och, beroende på lokalisering, också upplevas som episoder av funktionspåverkan, benämnda skov. Sjukdomen medför därtill en successivt tilltagande nervcellsundergång som efter en längre tids sjukdom kan ge upphov till en tilltagande funktionsnedsättning, en progressiv sjukdomsbild. Hos ca 15% startar sjukdomen med en progressiv bild utan föregående skov. Sjukdomen debuterar vanligen vid 20-40 års ålder och i Sverige finns idag ca 18 000 personer med diagnosen MS (prevalens 200/100 000 invånare).

Sedan drygt tjugo år tillbaka är det möjligt att påverka sjukdomsförloppet med "bromsmediciner", läkemedel som minskar de inflammatoriska angreppen och därmed också risken för långsiktig funktionsnedsättning. De tidiga preparaten, som numera används i begränsad omfattning i Sverige, ges som injektioner i underhud eller muskel en eller flera gånger per vecka. Det har, ffa under de sista tio åren, tillkommit ett betydande antal nya preparat med olika administrationsätt, såväl tabletter som injektioner och dropp. De nya läkemedlen har olika profiler avseende effekt, risker och biverkningar liksom olika rutiner för uppföljande säkerhetskontroller.

Rituximab är en substans (i Sverige med läkemedelsnamnet Mabthera[®]) som sedan länge använts för behandling av bland annat reumatoid artrit (RA) men som också har visat en inflammationsdämpande effekt vid MS. Rituximab verkar genom att minska antalet av en viss typ av vita blodkroppar, B-lymfocyter, men de exakta mekanismerna som bidrar till effekten vid MS är inte klarlagda.

Denna avhandling utgår från en läkemedelsstudie av rituximab, STRIX-MS, och dess förlängningsstudie STRIX-MS extension. Studierna är genomförda i samarbete mellan MS-mottagningarna i Umeå, Örebro och Östersund. Studien är helt finansierad av deltagande landsting och regioner. I STRIX-MS studien har 75 patienter med känd skovvis förlöpande MS och pågående injektionsbehandling fått byta behandling till rituximab efter en tre månader lång observationsfas. Rituximab gavs som intravenös infusion vid två tillfällen med två veckors mellanrum. Uppföljningen pågick därefter i två år, följt av ytterligare tre år i förlängningsstudien. Under studietiden har uppföljningen inkluderat kliniska kontroller, magnetkameraundersökningar (MR) och prover tagna i blod och cerebrospinalvätska (folkligt benämnd "ryggmärgsvätska"). De kliniska kontrollerna har innefattat bedömning av funktionspåverkan, eventuella biverkningar samt skattningsskalor avseende upplevelse av sjukdom och nöjdhet

med behandling. Magnetkameraundersökningarna har kartlagt graden av inflammatorisk aktivitet i CNS. En markör för nervcellskada, neurofilament, har analyserats i cerebrospinalvätska och blod. Analysen av cerebrospinalvätskan har dessutom innefattat ett antal immunologiskt aktiva substanser.

Avhandlingen omfattar fyra delarbeten och sammantaget har behandlingseffekt, upplevelse av behandling och immunologiska effekter av behandlingen belysts. I avhandlingens sista arbete har möjligheten att ersätta analys av neurofilament i ryggmärgsvätska med motsvarande analys i blod utforskats.

I resultaten har vi, utifrån utfallet av magnetkameraundersökningar och uppmätta nivåer av neurofilament, kunnat visa en minskad inflammatorisk aktivitet efter behandlingsbytet (delarbete 1). De resultat som avspeglar patienternas upplevelse av behandlingen visar en ökad nöjdhet efter genomfört behandlingsbyte (delarbete 2). Två immunologiskt aktiva substanser av möjlig betydelse för verkningsmekanismen för rituximab har identifierats (delarbete 3). Slutligen beskrivs i det fjärde arbetet att de förutsättningar som finns för att ersätta nuvarande analys av neurofilament i cerebrospinalvätska med ett blodprov behöver kompletteras med en fördjupad kunskap om analysen inför användning i såväl kliniska studier som i klinisk vardag.

Introduction / Background

Multiple Sclerosis

Multiple sclerosis (MS) is a chronic inflammatory disease affecting the central nervous system (CNS) causing a broad spectrum of neurologic deficits(1). The prevalence differs markedly between different areas of the world(2). In Sweden, the prevalence is estimated to approximately 200/100 000, with twice as high numbers for women as for men and a reported increase over time(3, 4). The incidence is age dependent with a peak around thirty years of age(5). MS does not only represent a challenge for the afflicted but also for the health care system and the society due to substantial costs(6, 7).

The cause of MS is unknown but various genetic and environmental risk factors have been identified(8). The mechanisms by which possible genetic or environmental factors interact in the development of MS are still investigated intensely(9).

The diagnosis of MS is based on criteria demonstrating a dissemination of disease related lesions to various locations in the CNS that has occurred at different time points (dissemination in space and time), with no other plausible explanation. The diagnosis can be made solely on clinical grounds but according to the latest versions of the continuously up-dated diagnostic criteria, magnetic resonance imaging (MRI) and cerebrospinal fluid (CSF) can support and, in some circumstances, replace the clinical criteria in order to obtain an earlier diagnosis(10, 11).

The clinical course is variable and it has been an important task to define the terms used for the description of the clinical evolution over time. The core description of the clinical phenotypes as either relapsing-remitting (RRMS) or progressive (PMS) was emphasised in the latest revision of the definitions(12). It was clarified that the phenotype is dynamic and that the classification for a specific patient may change over time, e.g. RRMS may develop into SPMS(12). Some new terms have been added as complements. The term clinically isolated syndrome (CIS) is describing an isolated clinical syndrome compatible with MS and the term radiologically isolated syndrome (RIS) specifies the incidental MRI finding suggestive of inflammatory demyelination despite the absence of clinical symptoms. For the majority of the patients, the natural history of the disease displays an initially relapsing-remitting course with subsequent transition to a progressive clinical course(1). For the remaining minority, the course is progressive from the clinical start of the disease.

Pathogenesis

The pathologic hallmark of MS, focal areas of inflammatory demyelination, called plaques, was described already in the late 19th century(13, 14) The features of the plaques have since then been further described and categorised in different ways in order to better understand the underlying process of the tissue damage(15, 16). An extensive use of animal models has made a significant contribution to the insights in potential immunological mechanisms but have also contributed to an opinion of MS being an exclusively T cell mediated disease(17). This concept was strongly challenged when the B cell depleting therapy (BCDT) rituximab proved to be highly efficacious in a clinical trial in MS(18).

MS is mainly recognised as a demyelinating disease but the pathology within the plaques also includes axonal injury(19). Axonal damage is identified as the major determinant of irreversible neurological deficit. The processes leading to axonal injury are not fully understood but in principle two different main mechanisms are outlined(20). In the early phase of the disease the inflammatory attack is the main cause followed by a chronic degeneration independent of inflammation in the later stages. The great individual variation in time to develop a moderate disability level, corresponding to discrete inflammatory attacks, is followed by a more uniform progressive course from moderate to severe disability, corresponding to chronic degeneration(21). An important observation is the presence of persistent axonal damage in the absence of clinical manifestations already early in the disease (20) emphasizing the need for surrogate markers in both therapeutic trials and the follow-up in clinical routine.

The tissue damage in MS is a result of a complex interaction between cells of the immune system and CNS cells, eg glial cells and neurons(9). Briefly, a well-established stepwise outline of the pathogenic process involves an increased migration of autoreactive lymphocytes across the blood-brain barrier, followed by a local activation of autoreactive T cells that cause focal sites of inflammation. This process may be amplified by recruitment and activation of microglia. During the course of the disease, B lymphoid follicles tend to accumulate in the meninges promoting a compartmentalised humoral immune response that may be a leading cause of the progressive course of the disease(1, 22). A growing knowledge of new T cell subsets(23), the contribution of glial cells to the process(9), new insights in the pathology of cortical demyelination and meningeal pathology (24-26), the complexity of cytokine networks(27, 28) and last but not least, new insights in the role of B cells, have recently added further to the complexity.

Cytokines and chemokines

Cytokines are low-molecular weight proteins (8-30 kDa), acting as regulators of the immune response. They have been of interest as a possible pathway to a better

understanding of the mechanisms in the pathogenesis of MS, and also to identify possible treatment strategies, since more than thirty years(29). The complexity in analysing and interpreting their overlapping actions and their property to elicit different response within different target cells is recognised for equally long time. Another group of small (8-14 kDa) proteins called chemokines, characterised by attracting leukocytes, was more recently added to the picture(28).

The development of new, highly sensitive, methods permitting analyses of multiple samples in very small volumes of body fluid have provided more easily available tools for research on cyto- and chemokines, including their profiles in various settings(30). Efforts have been made to characterise the immunological profiles in relation to different subtypes of MS(31) and in response to treatments, including rituximab(32, 33). However, difficulties in comparing and interpreting the results have also been acknowledged(34).

B cells

Lymphocytes from the B cell lineage are classically considered as antibody-secreting cells. Cells with immunoglobulin-producing capacity first appear as plasmablasts that become long-lived plasma cells if they find a niche providing mediators essential for their survival. Plasma cells are the source of circulating IgG(35). The possible contribution of B cells to the pathogenesis of MS has been recognised since long through the persistent occurrence of intrathecal synthesis of immunoglobulins forming oligoclonal bands (OCB) detected in the CSF immune electrophoresis(36). The detection of intrathecal immunopathy is well described as an important part of the early MS diagnostic work-up(37). After the discovery of the marked treatment effect of B cell depleting therapy(18), leaving the intrathecal levels of OCB unchanged(38), the role of the B cells as mainly antibody producing cells has been re-assessed. It is now evident that the contribution of the B cells is complex and involves the characteristics of antigen presenting cells (APC) as well as the production of both pro- and anti-inflammatory cytokines(39). Interestingly, several therapeutic agents, apart from the B cell depleting therapies, seem to influence the function of the B cells(39, 40).

Disease modifying treatment

During the last two decades, there has been an extensive evolution of immunomodulating therapies with an increasing efficacy in reducing the inflammatory activity in MS. The development justifies the designation “a new era” in MS care(41, 42). The number of disease modifying therapies (DMT) reaching the market have escalated in the last years, see Fig 1. The short-term efficacy and benefit from DMTs is well known from the pivotal trials. The evaluation of the long-term effect, especially a possible delay of the transition into

a secondary progressive course, has been more complicated to prove. Despite the methodological problems, there is an increasing support for the beneficial therapeutic effect also on the long-term prognosis(43, 44).

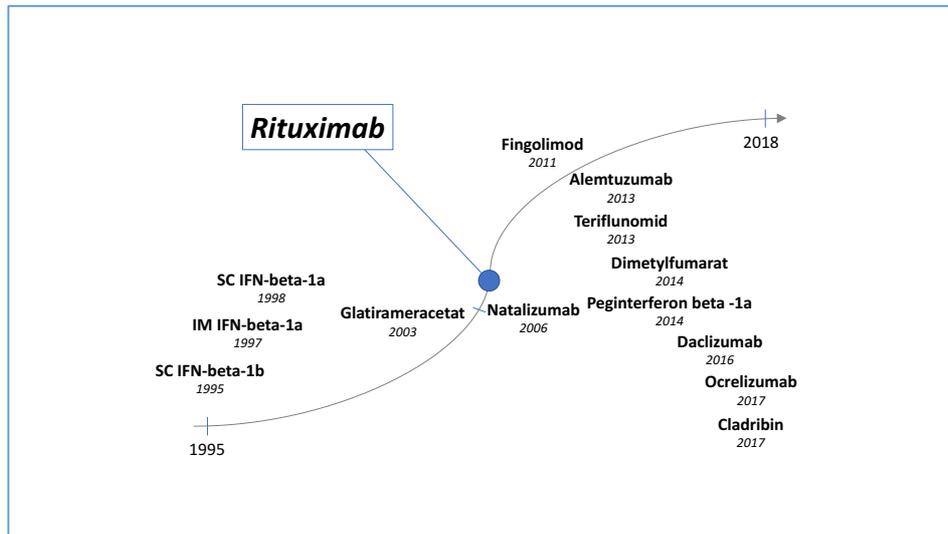


Figure 1. Schematic time line over the introduction of new disease modifying therapies for RRMS. The therapies are named by their active substances and the the numbers in italics below indicate the year of approval by regulatory authorities. Note that no application for approval of rituximab has been filed by the marketing company at the time of publication of this thesis.

SC IFN-beta= Interferon beta for subcutaneous administration, IM IFN-beta= Interferon beta for intramuscular administration, Peginterferon beta= Pegylated interferon beta.

The follow-up of MS in clinical practice

The increasing number of treatments brings new challenges in the therapeutic decision-making process and the clinical follow-up. The differences in efficacy and safety profile, adverse effects, implications on fertility, practical aspects regarding administration and safety monitoring have to be taken into account in the counselling of patients. Definition of treatment goals and evaluation of treatment effects need to be addressed. The follow-up should cover all aspects of the disease; inflammatory activity (manifested as relapses and/or MRI lesions), progress of disability (a manifestation of axonal loss) and the overall functionality of the patients (a combination compensatory mechanisms and symptom control)(45). The patient's experience of the treatment is crucial for compliance and long-term adherence to therapy(46-48). And since adherence is an obvious prerequisite for treatment effect, regular evaluation of these aspects is of great importance.

Magnetic Resonance Imaging

The use of MRI in MS, introduced in the 1980`s(49), has evolved to a leading role in the diagnostic work-up and the assessment of therapy effect. The technique has become increasingly available and the investigation is now relatively convenient albeit time consuming to use. The association between pathology and MRI findings is well described(50). Inflammatory plaques are seen in the white matter as areas of increased signal intensity on T2 weighted images. The lesions are per se non-specific but harbour more characteristic features if evaluated in relation to their appearance, location and signal behaviour. In the acute stage of a lesion, active inflammation can disrupt the blood-brain barrier and appear as gadolinium (Gd) enhancement on MRI during 2-6 weeks. Grey matter demyelination is typically not seen on conventional MRI.

MRI serves multiple purposes in the follow-up of MS. Firstly, the baseline MRI provides prognostic value. Secondly, repeated scans after initiation of therapy monitor treatment effect and, lastly, MRI is an important tool for detection of treatment related adverse events(51). It is demonstrated that the effect of treatment on relapses can be accurately predicted by the treatment effect on MRI lesions(52). By identifying not only relapses but also clinically silent lesions, i.e. new or enlarged T2 lesions on MRI, patients with insufficient treatment response can be identified and evaluated for therapy switch to a more efficient treatment strategy. Therefore, regular monitoring with MRI is now recommended in the MAGNIMS consensus guidelines and is routine practice in the follow-up of MS patients in Sweden(51).

Biomarker for axonal damage

The search for a biomarker to monitor the axonal damage not detectable by MRI or clinical evaluation has been extensive. The most widely used marker today is neurofilament light chain (NFL), a structural component of the axonal cytoskeleton. Neurofilament is composed of three chains of different molecular weight, light (NFL, 68 kDa), medium (NFM, 150 kDa) and heavy (NFH, 190-210 kDa)(53, 54). NFL is released into the extracellular space upon acute axonal damage, described for several conditions affecting the CNS(55-63) and NFL is thus a marker for ongoing axonal pathology regardless of the cause. It has been investigated as a potential biomarker for inflammatory activity in MS since the 1990`s(64). It is now well described how NFL in cerebrospinal fluid (CSF-NFL) correlates with clinical and radiological manifestations of disease activity in MS(65-67). The usefulness of CSF-NFL as a marker for treatment response is also recognised(67, 68). Unfortunately, the use of CSF-NFL is limited by the inconvenience of the lumbar puncture (LP) needed to obtain samples. Therefore, the finding of NFL also being measurable in peripheral blood has attracted much attention. The development of highly sensitive analytical techniques(69) have

been followed by a growing body of information on NFL analysed in either serum or plasma in MS. NFL in peripheral blood correlates well with NFL in CSF(70-72), but also with the inflammatory activity in RRMS (71-73) and the response to disease modifying drugs (74). Based on the rapid development on the field, the possibility of replacing CSF-NFL with a blood sample is probably to be expected within the near future.

Clinical and patient related outcome measures

While MS still is a chronic, non-curable, disease, MS treatment and care need to include, and in the early stages of the disease focus on, prevention and delay of disability(45). The clinical manifestations of disease activity need to be monitored regularly regarding relapses and progression by a careful medical history and neurologic examination. The most widely used instrument to evaluate progression is the Expanded Disability Status Scale (EDSS), introduced in the 1980's and included as a primary end-point in clinical trials since 1990's(75, 76).

A treatment regimen can, from a patient perspective, be a failure if it produces adverse effects that impairs quality of everyday life to a greater extent than the disease itself. In this perspective, the incorporation of patient related outcome measures (PROM) in the follow-up and evaluation of treatment is necessary. The flora of scales measuring different aspects of patient related parameters have increased during the last decade(77). The characteristics needed in the clinical practice compared to the need in the research setting differ. The requirement of clinically relevant instruments, easy to use, needs to be balanced against the researchers' need of sensitivity and ascertained validity.

Rituximab

Rituximab is a mouse-human chimeric monoclonal IgG1 antibody directed against the surface antigen CD20. When attached to the CD20 molecule, rituximab induces lysis of the CD20-expressing cell by activating a combination of cell-mediated and antibody dependent cytotoxic effects(78). CD20 is expressed on B lymphocytes, with the exception of the earliest and latest stages of the life-cycle (pro-B cells and matured plasma cells) and to a small extent on certain T lymphocytes.

Rituximab was synthesised in the 1980's, first approved by the FDA for treatment of B cell lymphoma in the late 1990's and later for rheumatoid arthritis(79). The first papers on a beneficial effect in MS were published more than a decade ago(38, 80, 81) and the only randomised controlled trial (RCT) in RRMS was published in 2008(18). Taken all together, the results clearly showed that B cell depletion had a pronounced effect on MS inflammation. Further studies on rituximab by the marketing holder were halted in favour of the

development of a fully humanised anti-CD20 monoclonal antibody, ocrelizumab(79). The concept of anti-CD20 therapy in MS has now been further established by the results from the pivotal studies on ocrelizumab(82, 83) and the trials on new compounds such as ofatumumab(84).

The experience of rituximab treatment, especially from the treatment of rheumatoid arthritis, has provided long-term experience of safety(85, 86). This, in combination with the favourable therapeutic effect and relatively convenient administration routine has formed the basis for an extensive use of rituximab as off-label treatment in Sweden. One of the first steps towards this development was our own phase II study, Switch-To-RITuXimab in MS (the STRIX-MS trial), presented in detail below and in Paper I. Several studies have followed, confirming the observations on efficacy and safety(87-89).

The STRIX-MS trial

As illustrated in Fig 1, the first results on rituximab treatment in MS were published at a time when neurologists treating MS were on the doorstep to a new era with new compounds about to enter the market, including the first oral formulations. With the knowledge of the safety profile of rituximab from the use in RA(90) and the promising results on efficacy in MS(18), it was highly motivated to pursue further studies, despite the reluctance of the pharmaceutical company to investigate the compound further for MS. This was the background for the planning and implementation of the STRIX-MS trial and the extended follow-up in the STRIX-MS extension trial. The trials were fully funded by the participating counties. Details on the trials are outlined below in the Method section.

Aims

The aims of this thesis were:

-to describe the effect on inflammatory activity in RRMS of a therapy switch from first-line injectables to rituximab measured by clinical evaluation, MRI and the concentration of CSF-NFL.

-to describe the changes in patient related outcome measures and disability scores after a therapy switch to rituximab in comparison to a run in-period before the change of treatment.

-to explore and describe changes in the immunological profile of patients with RRMS after therapy switch to rituximab and in relation to healthy controls.

-to describe the correlation of NFL in plasma and CSF and to evaluate the use of plasma NFL as an end point in a clinical trial.

Materials and Methods

This thesis is based on the clinical trial STRIX-MS (EudraCT 2010-023021-38) and partly on the succeeding extension trial STRIX-MS extension (EudraCT 2013-002378-26). In summary, the STRIX-MS trial was an open-label, multicentre phase II trial where patients with clinically stable RRMS while on treatment with first-line injectables were switched to rituximab and then followed for two years. The STRIX-MS trial was followed by another three-year follow-up in the extension study. Inclusion was begun in November 2011 and the last patient completed the STRIX-MS trial by March 2015. The extension trial is expected to complete the follow-up during the second quarter of 2018. The trials are conducted in collaboration between the Neurological Departments in Umeå, Östersund and Örebro. Both studies are funded by the councils in Västerbotten, Jämtland and Örebro with no involvement from pharmaceutical companies.

The STRIX-MS and the STRIX-MS extension trials

Study design of the STRIX-MS and the STRIX-MS extension trials

The outlines of the study design of the STRIX-MS and STRIX-MS extension trials are presented in Fig 2. At the inclusion in the STRIX-MS extension trial, lumbar punctures were optional.

Study population in the STRIX-MS trial

The inclusion and exclusion criteria of the STRIX-MS trial are outlined in Table 1. In the inclusion process, all patients registered in the Swedish MS registry, at the three participating centres, with the diagnosis of RRMS and ongoing therapy with first-line injectables, were identified. A structured sampling strategy was used to select patients for screening in order to avoid selection bias. The inclusion was terminated when the predefined number of participants, according to the power analysis, was reached, see section on statistical methodology below. The outcome of the screening process is presented in Table 2.

Of the 77 patients included, two patients withdrew consent before therapy switch and thus 75 patients received the initial treatment with rituximab. Thirty patients with an initial acceptance to perform LP had completed the extension trial at the centers of Umeå and Östersund by the end of September 2017. These patients were included in paper IV. For demographics, see Table 3. Some special circumstances were noted among the study participants; one patient had a ventriculo-peritoneal shunt and one patient was re-diagnosed as CADASIL during the follow-up in the extension trial. Details regarding drop outs and the

use of data from these patients are outlined for each paper, respectively, below and summarised in Fig 3.

Study design of the trials STRIX-MS and STRIX-MS extension

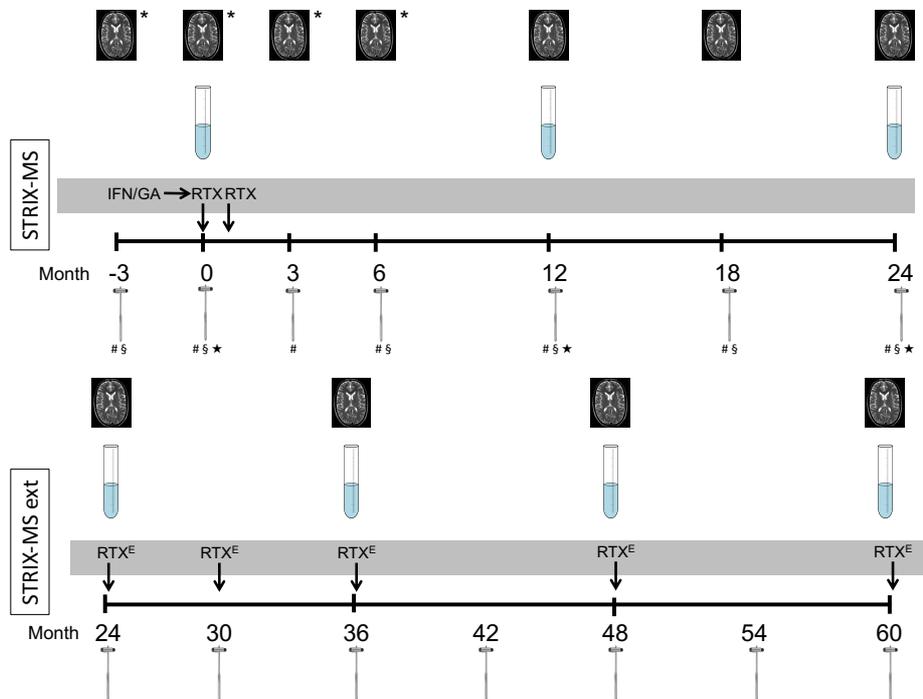


Figure 2. Overview of the study design of the STRIX-MS and the STRIX-MS extension trials. Treatment within the studies are marked by (RTX/RTX^E), where RTX^E indicates dosing according to the protocol of the extension study. Note that month 24 was the last visit in the STRIX-trial and at the same time the first visit in the extension trial with treatment according to the protocols of the extension trial.

MRI pictures indicate the timing of radiological evaluation, the asterisks marking investigations performed with double-dose contrast.

The sample tubes indicate the timing of samples of CSF and blood.

The reflex hammers indicate the timing of clinical evaluation (#= EDSS; §= MSIS-29, FSMC and SDMT; ★= TSQM)

STRIX-MS	
Inclusion criteria	Exclusion criteria
Between the age of 18 and 55	Diagnosis of Secondary Progressive MS
Diagnosis of RRMS according to revised McDonald criteria 2010 or one demyelinating episode in conjunction with at least two high intensity T2 lesions with size and location compatible with MS.	Pregnant or lactating women
Treatment with any of the first line injectable DMT:s, ie Avonex [®] , Betaferon [®] , Rebif [®] or Copaxone [®] for at least 6 months and during this time period being clinically stable without signs of relapses or clinical worsening	Patients not willing or able, from contraindications or other reasons, to comply with the protocol specified for this study
In fertile females, willingness to comply with effective contraceptive methods. These include birth control pills, surgical sterilization of patient or partner or consistent use of condom by partner. Non-fertile women are defined as more than 5 years since menopause or, in case of ambiguities, an FSH level above 30 IU/L	Documented vulnerability to infections
	Simultaneous treatment with other immunosuppressive drugs
	Received Mabthera [®] , MabCampath [®] , Novantrone [®] at any time
	Documented allergy or intolerance to Rituximab
	Severe psychiatric condition

Table 1. Overview of the criteria for inclusion and exclusion in the STRIX-MS trial.
DMT= disease modifying therapy.

Screening data for the STRIX-MS trial					
Centre	Number of patients screened	Number of patients included	Reasons for non-inclusion		
			Pregnancy	Unwilling to comply	Not fulfilling inclusion criteria
Umeå	64	37	4	12	11
Östersund	31	20	3	7	1
Örebro	80	20	0	9	51
Total	175	77	7	28	63

Table 2. Overview of the outcome of the screening for inclusion to the STRIX-MS trial.

	STRIX-MS (n=75)	Participants from STRIX-MS extension (n=30)*	Healthy controls (n=55)
Gender Female, n (%)	52 (69)	19 (63)	28 (51)
Age at inclusion, mean (SD)	41 (8.1)	39 (6.9)	37.6 (13.0)
EDSS, median (range)	1.5 (0-5)	1.75 (0-3.5)	
MRI activity during run-in, n (%)	17 (23)	7 (23)	

Table 3. Overview of the demographics of the STRIX-MS trial and HC. The middle column presents demographics for the 30 patients who continued to participate in the extension study, and contributed with data to Paper IV. Note that these patients are also included in the STRIX-MS study population.

*=one patient treated according to the low-dose protocol.
SD= standard deviation

Treatment within the studies

The treatment with first-line injectables (IFN-beta or GA) was ongoing since at least 6 months at the time of inclusion in the trial. This treatment continued during the three-month run-in period and was terminated at the time of the switch to rituximab (Mabthera[®], Roche), 1000 mg intravenously (IV) per dose, with two doses given two weeks apart. Further doses of rituximab, or other possible rescue therapies, were given according to predetermined criteria for insufficient treatment effect. Insufficient treatment effect was defined as either a clinical relapse or the occurrence of one Gd-enhancing lesion or more than one new T2 lesion on MRI. Fulfilment of these criteria during the first year after the therapy switch was classified as therapy failure and the patient was offered return to earlier injection therapy or an alternative treatment regimen according to clinical routine. If the criteria for insufficient treatment effect were fulfilled during the second year after therapy switch, it was defined as insufficient duration of therapy and the patient was treated with a repeated dose of rituximab 1000 mg IV.

The extension study included two different protocols for treatment. A low-dose protocol (rituximab 500 mg IV every six months for one year) was used for patients with age > 50 years, no Gd-enhancing lesions and no new T2 lesions during the run-in period of the STRIX trial, no relapses and no Gd-enhancing or new T2 lesions during the two-year follow-up. A high-dose protocol (rituximab 1000 mg IV every six months for one year and thereafter every 12 months) was applied for the remaining patients included in the extension trial.

Clinical assessment and patient related outcome measures

The repeated clinical assessments during the studies included evaluation of relapses and monitoring of adverse events. A battery of clinical and patient related outcome measures, summarised in Table 4, were used at time points illustrated in Fig 2. The questionnaire used for evaluation of treatment satisfaction was the same as applied in the Swedish MS Registry. It consists of a modified version of the Treatment Satisfaction Questionnaire for Medicine, version TSQM-9(91), supplemented with one question regarding side-effects. It is originally written in Swedish but was for the purpose of the publication of the study in Paper II translated by a professional translation service into English. The questionnaire is published in detail in paper II. The other evaluation tools were applied as described in the original references(75, 76, 92-94).

Clinical and patient reported outcome measures		
Parameter	Evaluation form	Reference
Treatment satisfaction	TSQM-10 (Treatment satisfaction questionnaire)	Atkinson et al(95) Bharmal et al(91)
Perceived impact of disease on daily life	MSIS-29 (Multiple Sclerosis Impact Scale)	Hobart et al(92)
Fatigue	FSMC (Fatigue Scale for Motor and Cognitive functions)	Penner et al(93)
Cognitive performance	SDMT (Symbol Digits Modalities Test)	Van Shependom et al(94)
Neurologic impairment	EDSS (Expanded Disability Status Scale)	Kurtzke(75) Meyer-Mooock et al(76)

Table 4. Overview of the clinical and patient reported outcome measures in the STRIX-MS trial and the evaluations forms used for assessment.

CSF and blood sampling

Samples of CSF and blood were obtained at time points indicated in Fig 2. Lumbar punctures were performed according to clinical routine. The CSF was centrifuged and the supernatant dispensed in aliquots of 1 mL. Blood samples were continuously analysed for safety monitoring and plasma was stored for later analyses of NFL. All stored samples were frozen to -80°C within one hour after the sampling.

Magnetic Resonance Imaging

All patients at the centre of Östersund performed MRI in Umeå for technical reasons. The Östersund and Umeå populations were examined with an 8-channel head coil in a 3T Achieva system (Philips Healthcare, Best, The Netherlands). The Örebro population was examined with an 8-channel head coil in a 1.5 T Achieva system (Philips Healthcare). In all patients, sequences were obtained after IV contrast administration (Magnevist; Bayer, Leverkusen, Germany). In the first four investigations (month -3 to month 6) a double dose of contrast was used to enhance the sensitivity for detection of inflammatory activity(96). During the trial, all MRI data were assessed unblinded for monitoring of safety and therapeutic effect. After termination of the trial all examinations were re-assessed, for the purpose of the study in Paper I, by two experienced neuro-radiologists blinded for clinical information.

Healthy Controls

Cerebrospinal fluid from 55 healthy controls (HC) were used for comparison in the study described in Paper III. The cohort of HC, without diagnosis of neurological disease and without first-degree relatives with such disease, were initially recruited for the purpose of an earlier study(97). The screening for inclusion and exclusion criteria were performed by an experienced research nurse. For demographics, see Table 3.

Analyses of cytokines in CSF

The CSF was analysed for a set of selected immunoactive components by the MesoScale Discovery V-PLEX® multiplex electrochemiluminiscens assay platform (MSD; MesoScale Discovery, USA) at the Umeå Centre of Molecular Medicine, Umeå, Sweden, according to the instructions from the manufacturer. All analytes were analysed as duplicates. The process was divided into two batches. For details regarding the batches, the selection process of immunoactive substances and methodology, please see the original article (Paper III). A quality control assessment procedure was applied including a quality control of the standard curve and the definition of the Lowest Level of Quantification (LLoQ), assessment of intra-assay accuracy for individual samples and an assessment of

the inter-assay accuracy. Analytes with >50% of the values below LLoQ were excluded from the final statistical analyses as were analytes with >25% of the values with a CV >25% in duplicates.

Neurofilament light in CSF

The concentration of NFL in CSF collected during the STRIX-MS trial was determined by a sandwich ELISA method (NF-light ELISA kit; UmanDiagnostics AB, Umeå, Sweden) at the Umeå Center of Molecular Medicine, Sweden, according to the instructions of the manufacturer. The concentrations of NFL in CSF from the extension trial was analysed by an in-house sandwich ELISA method, as previously described in detail(98), at the Clinical Neurochemistry Laboratory at the Sahlgrenska University Hospital, Sweden.

Neurofilament light in plasma

The concentration of NFL in plasma was determined by a NF-light assay adapted for the Simoa platform with a Homebrew kit (Quanterix Corp, Boston, MA, USA) at the Clinical Neurochemistry Laboratory at the Sahlgrenska University Hospital, Göteborg, Sweden, as previously described in detail(99).

Comments on details for each specific paper

Details on paper I

Data on the MRI were analysed regarding the number of patients displaying Gd-enhancing and/or new T2 lesions at each time-point. The sum of Gd-enhancing lesions at month -3 and month 0 (before treatment switch) was calculated per patient and the mean was compared with the corresponding value calculated on the MRI at month 3 and 6 (after treatment switch). The mean number of new or enlarged T2 lesions per patient were compared at month 0, 12 and 24. Details on drop-outs and missing values are displayed in Fig 3.

For the purpose of the CSF analyses in Paper I, one patient with a ventriculo-peritoneal shunt was excluded from all analyses. For details on the number of CSF samples available at each time point, see Fig 3. Note that the numbers differ slightly from the published paper. This is because, by mistake, all patients that performed LP were noted in the published version even though the results were not included in the final analyses. The reasons for not being included are described in Fig 3.

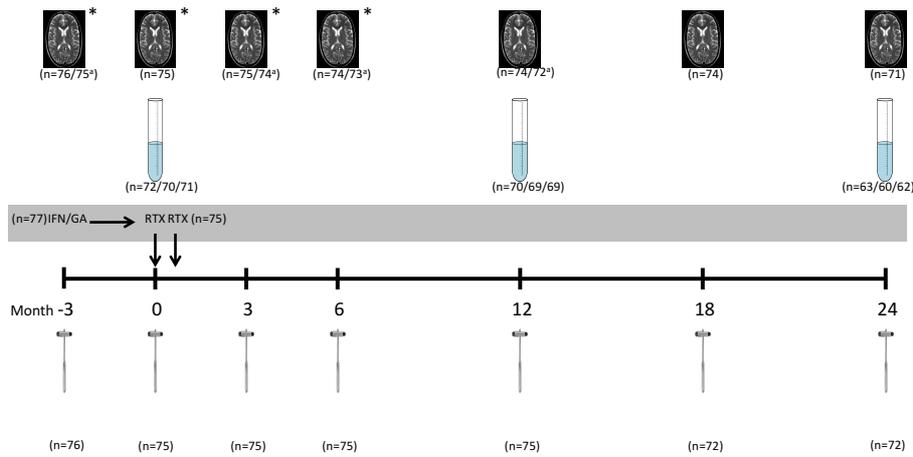


Figure 3. Overview of participants and available data.

Two patients withdraw consent for further participation before the treatment switch, one of them before the first clinical assessment. Further comments on missing data below, specified for each parameter and each study in the thesis.

Clinical assessment: The reflex hammers indicate clinical assessment. n = the number of patients being clinically assessed at each time-point. Three patients withdraw consent at month 18 (pregnancy $n=2$, reluctance to wait for re-treatment with rituximab $n=1$).

MRI data: The MRI picture indicate evaluation with MRI. n = the number of patients that performed MRI at each time-point. * represents the number investigations available for the blinded assessment in Paper I. One patient declined the investigations at month 6 and 18 and another at month 12. Four patients declined the investigation at month 24.

CSF data: The sample tubes indicate LP. n = the number of patients with CSF available for the analyses in Paper I/ III/ IV respectively. One patient with a VP-shunt was excluded from all three studies at all time points. Two patients (one re-diagnosed as CADASIL, one patient treated with Tysabri at month 8) were excluded from Paper III and IV at all time points. At month 0, the patient treated with Tysabri was included in Paper I. At month 0, no CSF was obtained from two patients (“dry-tap” or declined LP), at month 12, no CSF was obtained from three patients and at month 24, no CSF was obtained from ten patients. At month 24, two more patients were excluded from the analyses in Paper III due to re-treatment with rituximab because of inflammatory activity.

Details on paper II

All patients treated with rituximab were included for analyses in this paper regardless of any therapy changes, timing of visits or violation to the study protocol in an intention-to-treat strategy. At month 18, three patients withdrew consent to follow-up (pregnancy n=2, request for earlier retreatment with rituximab n=1). The number of patients with data available for evaluation of the clinical and patient related outcome measures at each time-point are shown in Fig 3.

Details on paper III

For the purpose of the study described in Paper III, three patients were excluded (reasons being re-diagnosed as CADASIL, VP-shunt and treatment with natalizumab as a rescue therapy during the first year of the STRIX-MS trial) from all of the analyses. Another two patients were excluded from the analyses at month 24 due to repeated infusion of rituximab after fulfilment of study criteria for therapy failure. These factors were considered confounding in relation to the aim of the study. Details on the number of patients available for analysis at each time point are shown in Fig 3. There was a difference regarding age and sex between the study population and the HC at a level of statistical significance but within a range that any crucial impact on the conclusions was assessed unlikely.

Details on paper IV

Data on concentrations of CSF-NFL at month 0, 12 and 24 from the study described in Paper I were also used in paper IV. CSF samples from month 36-60 (the extension trial) were analysed by an in-house sandwich ELISA method(98) at the Clinical Neurochemistry Laboratory at the Sahlgrenska University Hospital, Göteborg, Sweden. For details on the total number of patients available for analyses at each time point is shown in Fig 3.

Statistical methodology

The sample size for the STRIX-MS trial was calculated by the use of data from earlier published studies regarding comparable treatment effects on Gd-enhancing lesions(100) and levels of CSF-NFL(68).

Descriptive statistics was presented as mean, standard deviation(SD), standard error of the mean (SEM), median, range and interquartile range (IQR) as appropriate for the type of variables. The statistical significance for differences between results at different time-points was tested using paired t-test or Wilcoxon signed rank test as appropriate. Kruskal Wallis rank test was applied for testing the difference between MS patients at different time points and HC in Paper III. To test for differences in demographic data in Paper III, Pearson Chi-

square test was used for sex and Mann-Whitney for age. The level for statistical significance was set to $p < 0.05$ with adjustment according to Bonferroni and Holm-Bonferroni in Paper II and III respectively.

Statistical calculations were performed by the use of software of SPSS v23 and 24, Excel for Mac v15.33, SAS 9.4 (SAS Institute Inc, Cary, NC, USA) and MATLAB R2016 (MathWorks Inc, USA).

Ethical and regulatory statements

The STRIX-MS and the STRIX-MS extension trials were approved by the Regional Ethical Review Board in Umeå, Sweden (2010-315-31M and 2013-301-31). A supplementary application was made, and approved, for the purpose of including the comparison with healthy volunteers in paper III and the analyses of p-NFL in paper IV (2017-37-32M). The STRIX-MS and STRIX-MS extension trials are registered in the European Union (EU) Clinical Trials Register (EudraCT number 2010-023012-38 and 2013-002378-26). Written informed consent was obtained from each patient.

Results

General comments on the STRIX-MS trial

During the two-year follow-up in the STRIX trial, the measure of neurologic impairment, EDSS, was stable. The discrete improvement observed at year one did not reach the level of statistical significance. One patient experienced a clinical relapse (isolated optic neuritis) during the first year and the treatment was changed to natalizumab with no further signs of inflammatory activity during the rest of the study period. No patients fulfilled the MRI criteria for treatment failure during the first year. During the second year, one patient experienced a clinical relapse (myelitis) and the same patient, together with three others, fulfilled the MRI criteria for insufficient therapy duration. These four patients were re-treated with rituximab according to the study protocol. One of the re-treated patients was later re-diagnosed as CADASIL during the extension trial.

During the second year of the study period, two patients became pregnant and one patient withdraw consent due to reluctance to wait until the end of the trial to reinstitute treatment.

The treatment was generally well-tolerated. The most common side-effect was light to moderate infusion reactions as expected. Six serious adverse events (AE) were documented, three of which were assessed as possibly related to rituximab (pyelonephritis, n=2, influenza, n=1) and three assessed as non-related (stroke, cholangitis, and suicidal attempt by intoxication). The three possibly related AE's all required hospitalisation but were followed by full recovery. There were in total 17 non-serious AEs assessed as related or possibly related to rituximab comprising either infections or infusion related events.

The final evaluation of the data from the extension trial is not yet available as the study is still in progress at time for this thesis.

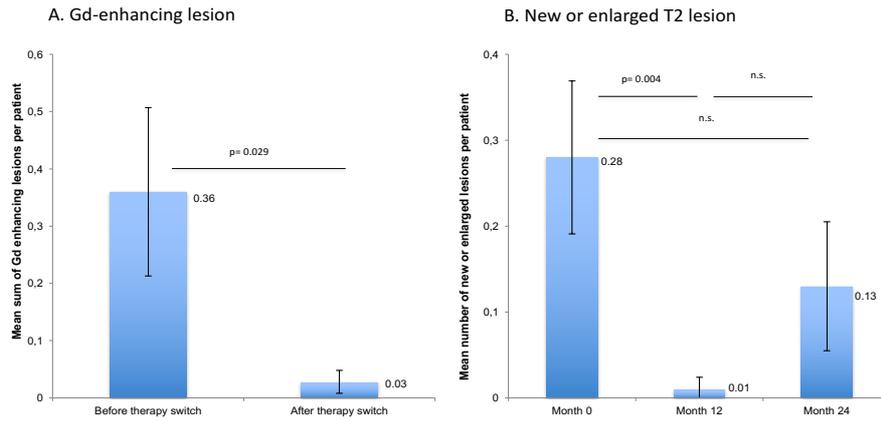


Figure 4. MRI lesions before and after therapy switch.
 Diagram A. Gd-enhancing lesions on MRI scan before therapy switch (month -3 and 0) and after therapy switch (month 3 and 6).
 Diagram B. New or enlarged T2 lesions, compared to the previous scan, at month 0, 12 and 24 respectively.
 The whiskers outline the SEM. The level for statistical significance is set to $p < 0.05$.

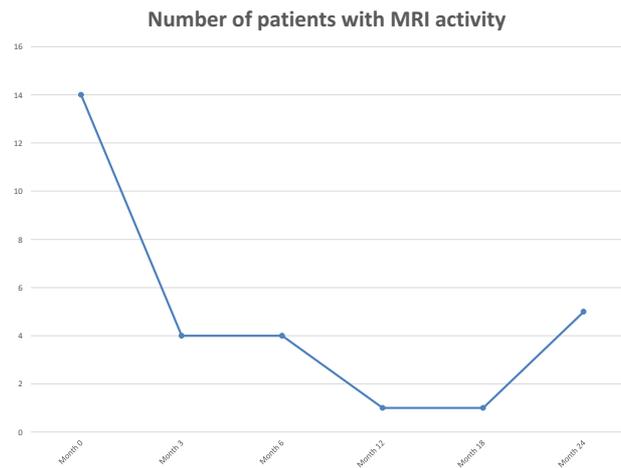


Figure 5. The number of patients displaying signs of inflammatory activity on MRI (Gd-enhancing lesions and/or new or enlarged T2 lesions) at each time point during the STRIX-MS trial.

Clinical and subclinical inflammation- Paper I

As commented above, two patients displayed clinical signs of inflammatory activity (relapses) during the STRIX-MS trial, one during the first and another during the second year of follow-up.

MRI parameters measuring subclinical inflammation were reduced at a level of statistical significance after the therapy switch, see Fig 4. Data for a paired comparison of the measure of Gd-enhancing lesions were available for seventy-two patients. The mean sum of Gd enhancing lesions per patient was reduced after therapy switch (0.36 vs 0.028; $p=0.029$). The mean number of new or enlarged T2 lesions at month 12 was reduced compared to month 0 (0.28 vs 0.01; $p=0.004$). An increase was noted at month 24 but without reaching the level of statistical significance.

The number of patients with MRI activity (new/enlarged T2 lesion and/or Gd enhancing lesion) at each time point are shown in Fig 5.

For the analyses of CSF-NFL, data was available for seventy patients for a paired comparison between month 0 and 12. The mean level of CSF-NFL was reduced from 491 ng/L (SEM 53.5) at baseline to 387 ng/L (SEM 39.4) at month 12 ($p=0.01$). There was an increase in the mean level of CSF-NFL at month 24 (418, SEM 43.6) albeit not statistically significant, Fig 6.

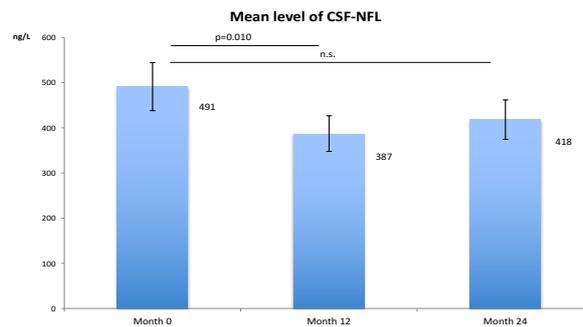


Figure 6. The mean level of CSF-NFL in the STRIX-MS trial. The whiskers outline the SEM. The level of statistical significance is set to $p<0.05$.

Clinical and patient reported outcome measures- Paper II

Regarding clinical outcome measures, the improvement in the Symbol Digit Modalities Test (SDMT) was statistically significant at month 12 and 24 ($p < 0.001$) but the changes were small in absolute values, median 53.5 points (IQR 14) at month 0 versus 57 points at month 12 and 24 (IQR 15 and 13 respectively), with 110 points being the highest possible score. Neurologic impairment assessed by the EDSS scale did not show any progression or improvement of statistical significance. The reduction at month 12 was within the range of variation in the method.

Among the patient reported outcome measures, only TSQM changed significantly after therapy switch. The median value for TSQM 1-9 (summarised score) improved from 40 points (IQR 12) at month 0, to 52 points (IQR 8) at month 12 ($p < 0.001$) and remained unchanged at month 24 (52; IQR 9.5). For TSQM question 10 (global satisfaction) the fraction of patients scoring 6 or 7 (“very” or “extremely” satisfied) increased from 24% at month 0 with injectable therapies to 87% and 85% at 12 and 24 months, respectively. For both scales, higher points indicate a better outcome, highest possible score being 59 points (summarised score 1-9) and 7 points (global score).

When tested separately, the ratings on all sub-questions included in TSQM improved significantly after therapy switch. The two questions with the most prominent changes during the first year were Question 4 (How easy or difficult is it to use the medication...), with mean (SD) 4.4 points (1.1) versus 6.5 points (0.8), $p < 0.001$, and Question 7 (How easy or difficult is it to live with the side effects...), with mean 4.0 points (1.4) versus 6.3 points (0.9), ($p < 0.001$).

The ratings of the Multiple Sclerosis Impact Scale (MSIS-29) and the Fatigue Scale for Motor and Cognitive Functions (FSMC) did not change at the level of statistical significance after treatment change.

Changes in immunological profile- Paper III

Fourteen cytokines/chemokines passed the quality assessment procedure and were accepted for the final statistical analyses. At the follow up one year after therapy switch the reduction in median level reached the level of statistical significance for IP-10, IL-12/23p40, IL-6, sVCAM-1, IL-15, sICAM-1 and IL-8 with the most pronounced relative difference for IP-10 and IL-12/23p40 (34% and 28%, respectively; $p < 0.001$). Before the therapy switch, the study population was found to have significantly higher levels of IP-10, IL-12/23p40, sVCAM-1, IL-8, MIP1 β , CRP, IL-15, sICAM-1 and SAA compared with HC. Also in this aspect, the difference was most prominent for IP-10 and IL12/23p40 ($p < 0.001$).

There was a statistically significant difference between the study population and the HC regarding age and gender (Table 3) but within a range making any major impact on the conclusions unlikely.

The use of NFL in plasma- Paper IV

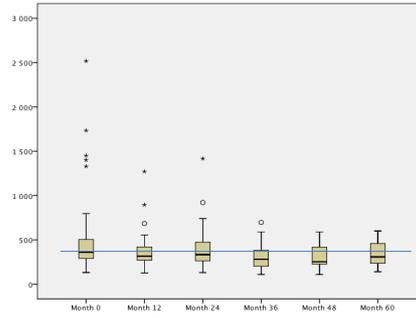
In addition to the data from the STRIX-MS trial, thirty patients contributed with data also from the extension trial in paper IV. Of these, one patient received an extra treatment with rituximab outside the protocol at month 42 as a precautionary measure due to MRI activity earlier in the STRIX-MS trial. One patient stopped treatment after month 36 due to side effects. Regarding inflammatory activity, one patient experienced a clinical relapse (isolated optic neuritis verified by an ophthalmologist) at month 54. Therapy was left unchanged at the request of the patient. One patient was evaluated with one new T2 lesion without Gd-enhancement or any clinical manifestations at month 36.

The overall correlation between NFL in plasma and CSF was moderate, $\rho=0.445$ ($p<0.01$).

As demonstrated in Paper I, there was a reduction of the mean level of CSF-NFL at month 12 ($p=0.006$). A corresponding reduction was detected in plasma and the relative difference at month 12 in CSF and plasma was 25 and 18% respectively. The reduction in plasma did not reach the level of statistical significance ($p=0.055$), for details see Fig 7.

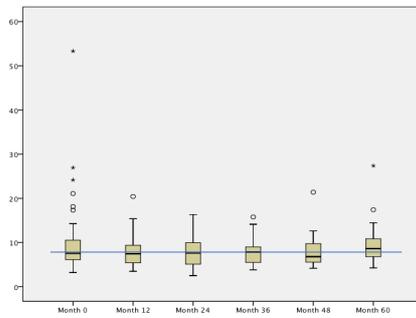
Correlation analyses, calculated individually for each of the patients followed for all the 60 months, did not provide any statistically significant results.

CSF-NFL



CSF	Month 0	Month 12	Month 24	Month 36	Month 48	Month 60
Available samples	71	69	62	29	26	23
Mean (SD)	471 (393)	354 (174)	382 (202)	303 (140)	310 (130)	346 (136)
Median (min-max)	367 (132-2518)	315 (127-1270)	334 (130-1417)	281 (109-697)	253 (109-590)	308 (140-600)
p-value versus month 0	-	p= 0.006	p= 0.083	p= 0.032	p= 0.129	p= 0.487

Plasma-NFL



Plasma	Month 0	Month 12	Month 24	Month 36	Month 48	Month 60
Available samples	71	68	62	29	26	23
Mean (SD)	9.73 (7.04)	7.94 (3.36)	7.99 (3.36)	8.04 (3.12)	7.87 (3.67)	9.69 (5.01)
Median (min-max)	7.60 (3.20-53.3)	7.44 (3.48-20.4)	7.62 (2.50-16.32)	7.85 (3.81-15.8)	6.80 (4.17-21.4)	8.62 (4.24-27.4)
p-value versus month 0	-	p= 0.055	p= 0.046	p= 0.088	p= 0.052	p= 0.296

Figure 7. Levels of NFL(pg/mL) in CSF (top) and plasma (bottom). The boxes represent the IQR with the line within the box marking the median and the whiskers marking the levels for upper and lower extreme. o= outliers, *=extreme outliers. The blue horizontal line represents the median at month 0 for visual clarity. SD=standard deviation.

Summary of the main results

In summary, this thesis provides results congruent with a reduced inflammatory activity, measured by MRI and CSF-NFL, as a result of therapy switch, from first-line injectables to rituximab, in patients with RRMS in a clinically stable phase at the time of inclusion. The results in Paper I are in line with earlier studies and constitute a prerequisite for the interpretation of the results in Paper II-IV.

The increase in TSQM noted at year one and two in the STRIX-MS trial indicates a substantial improvement in the experience of treatment satisfaction after therapy switch from injectable MS drugs to rituximab.

The cytokine profile in CSF was altered after therapy switch from injectable MS drugs to rituximab. The change in profile indicates a possible role for IP-10 and IL-12/23p40 in the immunopathogenesis of MS, affected by rituximab treatment.

The concentration of NFL in plasma correlated moderately with NFL in CSF. NFL in plasma appears to be less sensitive as end-point in an MS clinical trial setting.

Discussion

General reflections

In this thesis, several aspects of the use of rituximab in RRMS are described. The results indicate an improved treatment effect on the subclinical inflammatory activity, and an increased treatment satisfaction after the therapy switch from first-line injectables to rituximab. The demonstrated changes in the immunological profile can provide clues for the continuous search for understanding of the role of the B cells in the pathogenesis of MS.

The modern era of MS care is providing an increasing access to effective treatment options. In the clinical care of individuals affected by RRMS, the follow-up routine should not only be restricted to treatment efficacy. Information on treatment satisfaction, compliance and adherence ought to be included. These aspects are highly dependent on the perception of side effects, routines for treatment administration and safety monitoring. The STRIX-trial was designed in a time when a large number of new treatment options for RRMS were about to be launched to the market but still with a lack of first-line treatment devoid of significant side effects or safety issues. At that time, the effect of rituximab in RRMS was reported in a phase II trial(18), with a striking effect on MRI outcome measures. This, in combination with the experience and information on the safety profile derived from the treatment of RA, set high expectations among MS treating neurologists. But instead of initiating a pivotal trial with rituximab, the marketing pharmaceutical company decided to initiate a phase IIB trial on the humanised CD20-depleting monoclonal antibody ocrelizumab(79). Further studies on rituximab by the marketing holder was thus not to be expected. Initiation of a trial funded by the care giver organisations was an alternative to overcome this obstacle.

This thesis has provided possibilities to further explore different aspects of treatment with rituximab beyond those included as primary outcomes of the STRIX-MS trial. The ambition was to provide a broader perspective on rituximab treatment in RRMS in order to position the treatment among the contemporary MS medications with an approved formal indication and to gain a better understanding of the immunological mechanisms possibly involved in the therapeutic effect. During the time of the STRIX-MS trial, the development of techniques for analyses of p-NFL made it possible to design the study in paper IV in order to evaluate a more convenient method to obtain information on axonal injury.

Treatment efficacy and safety of rituximab

The main purpose of Paper I was to ascertain the disease modifying effect, a prerequisite for the interpretation of the following studies in the thesis. As a result of limited funding, a base-line-to-treatment design(101) was applied with a 3-month run-in period. MRI measures and CSF-NFL were used as parameters for subclinical disease activity and the results demonstrated a reduction of inflammatory activity in both parameters. The congruence between the results of NFL and MRI strengthens the interpretation that the inflammatory activity was reduced the first year after therapy switch to rituximab, indicative of a therapy effect.

The use of MRI as a surrogate marker for treatment effect is well established(52, 102) and the correlation of CSF-NFL to treatment effect has also been described(67, 68). The use of double dose Gd contrast in the first four MRI investigations increased the sensitivity for new Gd-enhancing lesions(96). The observational period for new or enlarged T2 lesions after the therapy switch in Paper I was longer (6 months) than the reference period before (3 months), with the potential bias in the comparison rather to underestimate the treatment effect. Due to the biologic dynamics in turn-over, CSF-NFL is a measure of the ongoing inflammation during a shorter time span (approximately 3 months)(70) and serves more like a marker for ongoing subclinical inflammation in the study population at time of sampling. These aspects were taken into account when evaluating the results on treatment effect.

Clinical manifestations of inflammatory activity and the occurrence of progressive neurologic impairment was evaluated regularly during the study by the occurrence of relapses and changes in EDSS, respectively, even though these parameters were not formal end-points of the STRIX-MS trial. In this thesis, the results of these clinical parameters of disease activity are presented in Paper I and II. The commonly used endpoint annualized relapse rate (ARR) before and after treatment shift was determined not to be possible to include in the design of the trial because of the necessity of a longer run-in period needed to enable a reliable comparison. However, only one patient fulfilled the predefined study criteria of treatment failure due to a clinical relapse (optic neuritis) during the first year and one during the second year (myelitis). In total, three patients (one patient later re-diagnosed as CADASIL not counted) fulfilled the criteria for insufficient treatment effect duration during the second year. These findings corresponded to only 5% of the study cohort experiencing any form of significant inflammatory activity during the two years after a single course of two doses of rituximab 1000 mg given two weeks apart.

There was no progressive worsening on the EDSS scale on a group level during the study period.

There were no unexpected adverse events during the study period. Infusion related AEs, usually mild to moderate, are well known(86). Data regarding the long-term follow-up in the extension trial are not yet available. Hence a complete analysis of the described risk for development of hypogammaglobulinaemia(103) or the more rare late onset neutropenia(104) cannot be evaluated within the frame of this thesis. Still, no cases were observed of either of these two severe side-effects during the available observation period.

Taken all together, the results on treatment effect are in agreement with earlier published data(18) and provide a base for the interpretation of the results of PROMs and immunologic profiling.

Treatment satisfaction and patient related outcome measures

Since MS is not a curable disease, management is focused on limiting disease progression, management of symptoms and optimizing quality of life(105). When evaluating modern MS treatment, it is of importance to recognise that treatment, from a patient perspective, may be considered as a failure if it implies adverse effects that interfere with quality of life(45). Changes in patient reported outcome measures are thus important to include as outcome measures in both clinical trials and as a part of the clinical follow-up routine. One of the first studies on the effects of MS on quality of life was published in 1992(106). In the following years, the assessment of MS mainly encompassed disability and functional impairment(77). During the epoch when treatment with injectables was the only option, a great focus was directed to adherence and factors affecting adherence to therapy(107) since an obvious basis for the treatment effect was that the patient actually was using the medication. Despite that treatment satisfaction was shown to be an independent predictor of adherence to treatment, studies on patients' perception of treatment satisfaction were scarce. At the time of publication of Paper II, no study exploring treatment satisfaction with rituximab in MS was published. Given the design of the STRIX-MS trial, with the patients constituting their own controls, TSQM was found to be a relevant parameter for this purpose. As one of the inclusion criteria was a clinically stable disease, the low measures on disability score (EDSS median 1.5), the lack of improvement in EDSS as well as in MSIS-29 was rather expected. It leaves the results of TSQM to be fairly unaffected by disease-related factors and therefore a valid measure of factors related to the treatment and its administration per se. The results are congruent with the abundant clinical observations made during the STRIX-trial with patients spontaneously reporting a feeling of relief when not having to continue with the self-administered injections. This is also supported by the most

prominent changes in TSQM being noted in the sub-questions regarding use and side effects of the current medication. A specific property of depleting and long lasting treatment regimes in MS is the possibility to provide an ongoing disease modifying effect without the patient having to be reminded about the disease by daily or weekly medication administrations. Of course, this necessitates a secured treatment effect, in order not to merely reflect the convenience of being relieved from continuous medication, and an easy-to-follow monitoring for late side effects.

Neurofilament light in plasma as a way to improve treatment evaluation

With the persistent neurological disability in MS being a result of axonal injury(1, 20), the efforts to find a biomarker assessing the axonal damage are logical. A method to obtain samples with less inconvenience for the patient than the LP would optimize the possibility to monitor axonal damage over time in both clinical trials and routine care.

In this thesis, the use of p-NFL was evaluated by comparison with CSF-NFL as an end-point in the STRIX-trial. The results of Paper IV showed a similar pattern of decrease in p-NFL as in CSF-NFL at month 12, but it did not reach the level of statistical significance. There might be several reasons for this. A plausible explanation is that the sensitivity is less when NFL is measured in plasma. A difference in the magnitude of the changes in CSF-NFL compared with NFL in peripheral blood has been described, e.g. a rise in CSF-NFL of 10 % was associated with a 5.9 % increase in NFL in serum(72). The age-related increase in NFL in CSF(97) seems to be “mirrored” in plasma(72) but this relationship has not been compared within the same study. During the STRIX-extension trial, one patient experienced an optic neuritis and CSF-NFL was measured in close relation to this relapse without increase (unpublished data). This observation raises the question whether lesion locations are of importance for the NFL levels in both CSF and plasma. The exact mechanisms involved in the passage of NFL to blood are unknown, but the permeability of the blood-brain barrier does not seem to be of primary importance(108).

Regarding evaluation of p-NFL on an individual level, a recent study(109) clarified the need of an increased knowledge of the magnitude of change in p-NFL being of relevance in the clinical evaluation as well as of the importance of the time frame between the inflammatory activity and the time for the sample. The design of the STRIX-trial did not provide conditions for further studies of these aspects.

Immunological profile

The complexity of the immunological network and the search for a conceptual framework clarifying the pathogenesis of MS has been a longstanding challenge(110, 111). Likewise, we still do not fully understand the immunological mechanisms of rituximab in autoimmune disorders. Using modern techniques for analysing broad panels of immuno-active substances provides great challenges in interpreting the results(34). The easily available kits for testing multiple analytes necessitates a broader immunological experience and competence for interpretation than usually is at hand in a clinical research setting. This provides a challenge in establishing cooperative efforts between clinical scientists and immunologists in order to draw the correct conclusions from the large datasets generated in this type of studies.

The strategy used for the study in Paper III, analysing a broad panel of analytes without a pre-specified hypothesis, can be regarded as potential “data-fishing”. But given the scarce knowledge on the immunological mechanisms for rituximab in RRMS and the opportunity provided by the regular CSF sampling in the STRIX-MS trial, an explorative study was considered justified. The intention was to obtain initial observations to be more rigorously tested in future studies. The finding of reduced levels of IP-10 has earlier been described after treatment with natalizumab(32). With the IP-10 receptor preferentially expressed on activated Th1 cells(112) the result implies a possible mechanism whereby B cells may, indirectly, affect T cell function.

Limitations

Apart from the discussions and comments made in each of the published papers, some further remarks are justified.

Firstly, the lack of control group entails several methodological challenges, i.e. a risk for the “regression to the mean”-phenomenon. Secondly, there could have been a risk of bias in the selection process and, thirdly, the evaluation of non-objective outcome measures involves a risk to capture placebo effects rather than real effects. These factors are further discussed below.

Regression to the mean

Regression to the mean is a phenomenon that inevitably occur every time the frequency of a specific event is studied and, simultaneously, the same event is used as an inclusion criterion for the study. A typical example is to look at relapse frequency while using the occurrence of a relapse as an inclusion criteria. In such a situation, the patients are included because of a high clinical disease activity and this will, by statistical law, be followed by a period of lower disease activity

unless the disease activity is not assumed to increase over time, which is rarely the case in MS. By doing *the opposite*, namely select patients that were in a *clinically stable* period and apply objective paraclinical outcome measures, this bias was avoided. This was possible to do since, at the time of the initiation of the STRIX-MS trial, disease activity demonstrated as clinical neurological symptoms, was still the major determinant for change of treatment. It was thus ethical to follow the patients by MRI during a run-in period without changing therapy despite the finding of inflammatory activity on MRI.

Furthermore, the use of MRI as an outcome measure in studies with a baseline-to-treatment design has been further studied since the initiation of the STRIX-trial(101). The referred study describes the effect of the regression to the mean phenomenon and provides support for the relevance of a baseline-to-treatment design even in a trial where regression to the mean can be assumed to affect the results. With this in mind, it is reasonable to draw the conclusion that the results presented in Paper I actually describe a real treatment effect. However, the fact that there was no control group in the trial still calls for a great deal of caution in interpreting the data.

Selection bias

The importance of the process by which the study population is selected is well known as is the impact of the selection on the interpretation and generalisability of the results.

The main inclusion criteria in the STRIX-MS trial was the diagnosis of RRMS in combination with a clinically stable course while on treatment with injectable DMT. The reasons for this conscious selection were several. Firstly, as mentioned above, it was a strategy to avoid the regression to the mean phenomenon. Secondly, at the time of initiation of the STRIX-MS trial, several new compounds, including preparations for oral use were about to be launched to the market. The general perception in the clinical practice was that there was a wish among a significant part of the patients to, if possible, change DMT to a preparation easier to use. Thus, it was of interest to explore rituximab, not only as a high efficient therapy to be used in case of therapy failure on injectables, but also as an alternative for patients searching for a more convenient therapeutic alternative. By selecting a study population with a clinically stable disease the latter could be achieved.

Another aspect on selection bias is the bias that occur every time a population to be included in a study is not chosen randomly or by some other objective principle. This risk was reduced by the use of systematic sampling. Each patient holds a unique ID number in the MS registry, which by itself has no identifying

property. Each centre had, at that time, an estimated coverage in the registry of over 90% of the target population. In the first step, all patients with the diagnosis RRMS were organised in order using these ID numbers. From this list, every fifth patient was asked for participation and, if willing to participate, screened for inclusion. The process was continued up to the point where the calculated total number of patients were included. This procedure was described in the study protocol before the selection of patients was started.

Outcome measures

The two primary outcome measures, the mean number of new or active MRI lesions and the mean level of CSF-NFL before and after treatment shift, used in Paper I, were evaluated blinded to clinical information and thus fairly objective measures. For the PROMs, several factors with a possible influence on the results are outlined in Paper II. The fact that the study population was aware of the termination of treatment with injections within a limited time could be added to that list. Repeated testing during the run-in period might have been, at least partly, a way to compensate for this.

In this context, the dosing regimen could also be discussed. The treatment with two doses of rituximab 1000 mg IV two weeks apart was adopted from the HERMES trial(18). It has since then been adjusted in clinical practice both regarding the dose and the dosing interval. An updated regimen was therefore applied in the STRIX-MS extension trial. Whether the initial regimen affected the outcome of the PROMs could be tested for by a later comparison with the corresponding data from the STRIX-MS extension trial.

In addition, the approach to explore areas of interest outside the primary aims of the STRIX-MS trial in Paper III and IV causes problems related to the lack of preformulated hypotheses. Thus, there was no pre-calculated power analysis and the sampling routine was not adjusted to a predefined question of interest. Despite these limitations, the use of the information available in the STRIX-trial was justified by the scarce, but well needed, knowledge on both the immunological profile in MS after CD20 depleting therapy as well as the effects of implementation of p-NFL in a trial setting, respectively.

Further research and final remarks

After the initiation of the STRIX-MS and the STRIX-MS extension trial, there has been an increased use of rituximab as an off-label treatment for MS, especially in Sweden(113).The initial reason for the interest in rituximab as a treatment of MS was, as already mentioned, the combination of a favourable therapeutic effect, a long-term experience of safety in other diseases and a treatment routine which entails a high level of convenience for the patient. During the course of the

STRIX-trial several studies have, by the use of structured clinical data from the Swedish MS registry, confirmed the initial observations(87-89). Together with the results on new B cell depleting compounds, i.e. the pivotal trials on ocrelizumab(82) and the results from studies on ofatumumab(84), this has provided a solid ground for the efficacy and safety of CD20 depleting therapies in MS.

Two important and urgent areas for further clarification are the long-term dosage with possible strategies for dose reduction or discontinuation and the structured monitoring for long-term side effects. The optimal dosing regimen for a longer time-span is not yet studied for any of the CD20 depleting therapies. With the unique experiences from rituximab treatment since almost 10 years now, Sweden appears to be an ideal place to perform these studies.

The lack of interest from pharmaceutical companies to pursue formal registration studies for rituximab necessitates additional knowledge to be reliant on purely academic trials. Given the well described efficacy and safety profile favouring rituximab treatment before some of the registered MS drugs, the use of rituximab now appears to be largely dependent on varying national regulatory aspects on treatment off-label. This perspective was clearly revealed by the approval of ocrelizumab, as recently debated(114-116). To overcome this obstacle two large trials are currently ongoing in Sweden, the RIFUND trial (Rituximab versus Fumarate in newly diagnosed MS; ClinicalTrials.gov Identifier: NCT02746744) and COMBAT-MS (COMparison Between All immunoTherpies for Multiple Sclerosis; ClinicalTrials.gov Identifier: NCT03193866). A further discussion on the regulatory, and health economic implications, is of great relevance but far beyond the purpose of this thesis.

Conclusions

In summary, the following conclusions can be made from the results presented in this thesis:

-the results are congruent with a reduced inflammatory activity, measured by MRI and CSF-NFL, after therapy switch from first-line injectables to rituximab.

-the findings reveal a substantial increase in treatment satisfaction measured by TSQM, one and two years after therapy switch to rituximab.

-there was a change in cytokine profile in the CSF after therapy switch to rituximab indicating a possible role for IP-10 and IL-12/23p40 in the immunopathogenesis of MS.

-there was a moderate correlation between NFL in plasma and CSF, and the results indicate that p-NFL is less sensitive than CSF-NFL if used as an end-point in clinical trials.

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