

Rituximab treatment of MS: a single centre retrospective observational study

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Master Thesis in Medicine

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List of abbreviation

AE	Adverse Event
APC	Antigen Presenting Cells
BBB	Blood Brain Barrier
CDP	Confirmed Disease Progression
CIS	Clinically Isolated Syndrome
CNS	Central Nervous System
CSF	Cerebro Spinal Fluid
DIS	Dissemination in Space
DIT	Dissemination in Time
DMT	Disease Modifying Therapies
EAE	Experimental Autoimmune Encephalomyelitis
EDSS	Expanded Disability Status Score
FASS	Pharmaceutic Specialties – Corporate Drug Industry Service in Sweden
FDA	Food and Drug Administration (USA)
JCV	John Cunningham Virus (Polyomavirus)
MRI	Magnet Resonance Imaging
MS	Multiple Sclerosis
MSSS	Multiple Sclerosis Severity Scale
NAB	Neutralizing Antibodies
NMO	Neuromyelitis Optica
PML	Progressive multifocal leukoencephalopathy
PMS	Progressive Multiple Sclerosis
PPMS	Primary Progressive Multiple Sclerosis
RRMS	Relapsing Remitting Multiple Sclerosis
RTX	Rituximab
SPMS	Secondary Progressive Multiple Sclerosis

Abstract

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Rituximab treatment of MS: a single centre retrospective observational study

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Background

Monoclonal antibodies targeting B-cells has become one of the most promising options in the treatment of autoimmune diseases. In multiple sclerosis (MS), rituximab (RTX) has been evaluated in two phase II studies. While RTX showed beneficial effects in relapsing-remitting (RR) MS, the study of primary progressive (PP) MS was negative. Yet, a subgroup analysis revealed lower rate of progression in younger patients with ≥ 1 contrast enhancing lesion on MRI (Magnetic Resonance Imaging). RTX is not approved for MS, but the off-label use has over recent years increased in Sweden and comprises 19,2% (May 2015) of all MS treatments.

Objectives

To evaluate the reason for use, efficacy, safety and tolerability of RTX treatment in MS.

Method

In this retrospective study we searched the Swedish MS register (SMSreg) and treatment registers for MS patients treated with RTX between 2008 to 2014. We

identified 105 patients; 41 RRMS, 41 secondary progressive (SP) MS and 23 PPMS. Data were extracted from the SMSreg and medical chart review. RTX was initiated with 2 infusions of 1000 mg, 2 weeks apart, and then as single infusions at 6 months intervals.

Results

Reasons for switching to RTX varied from one to several combined causes. In RRMS patients (n=41) the reason was treatment failure on other disease modifying therapies (DMTs, 56%), JC virus antibody (JCV+) in natalizumab treated patients (34%), adverse events (AE) from previous DMT (Disease Modifying Treatment)(20%), and neutralizing antibodies (NAB) against interferon beta or natalizumab (7%). The corresponding reasons for RTX in the PMS group (n=64, both SPMS and PPMS) was disease activity and progression (90%), JCV+ (12%), AE (6%) and NAB (3%). Comparing the number of patients having “relapses the last two years” and “relapses after RTX start” shows a reduction in both the RRMS patients (from 36.6% to 9.8%) and PMS patients (from 21.8% to 7.8%).

RTX median treatment time was 13 (3-74) months. During RTX treatment of RRMS and PMS the median expanded disability status scale (EDSS) increased (+0.5, range 0-8.0 and +0.5, range 1.5-8.5, respectively), median multiple sclerosis severity scale (MSSS) decreased (-0.66, range 0.86-9.94 and -0.19, range 1.43-9.95, respectively). However the MSSS-score did not show statistical significance, $p=0.077$. Infusion related reactions was 49.5% at first infusion, 13.3% at 2nd infusion, and 9.5% at 3rd RTX infusion. No severe AE was recorded.

16 patients stopped RTX treatment; 6 due to AE, 4 due to treatment failure and 6 of unknown reasons.

Conclusions

RTX was used as treatment primarily as a last line of treatment after having disease activity in previous treatments. RTX was well tolerated with no severe AE. RTX had no obvious effect on EDSS progression but decreased relapse rate and MSSS which could indicate a beneficial effect in both RRMS and PMS.

Key words: *rituximab, Multiple Sclerosis, off-label*

Introduction

According to the Swedish MS Association there are approximately 17000 people with Multiple Sclerosis (MS) in Sweden today. [1] Sweden has one of the highest prevalence and incidence in the world and MS is the main non-traumatic cause of neurological disability among young adults. [2]

There is no curative treatment for MS today, but there are several disease modifying therapies (DMT) available, all of which primarily target the immune process either unspecific or associated to T-cell function (e.g. dimeylfumarate, teriflunomide, fingolimod, natalizumab, interferon-beta and glatirameracetate).[3, 4] These therapies can only in part affect the disease in RRMS, and has not showed any significant effect in the progressive stage of MS. [5-7]

The role of the B-cell in the disease process of MS has been explored the last decade and is now believed to play a key role in MS, as the B-cell takes part in multiple stages in the inflammatory activation, through antibody production, T-cell activation and the release of cytokines.

In line with these theories the chimeric monoclonal antibody, rituximab (RTX) has been tested and examined as a possible treatment against MS. RTX was originally manufactured for diseases with a B-cell-associated pathogenicity, such as Hodgkins

Lymphoma and Rheumatoid Arthritis (RA). RTX targets hocytes expressing the CD20-antigen, which is expressed on B-cells and induces cell death in the entire germline, with exception for the plasma cells. [8-10]

In multiple sclerosis (MS) RTX has been evaluated in two phase II studies.

In these studies a beneficial effect was shown in relapsing-remitting (RR) MS, but showed no positive effect in primary progressive (PP) MS. However, a subgroup analysis discovered lower rate of progression in patients of younger age, ≤ 51 years and with ≥ 1 contrast enhancing lesion on MRI.[7]

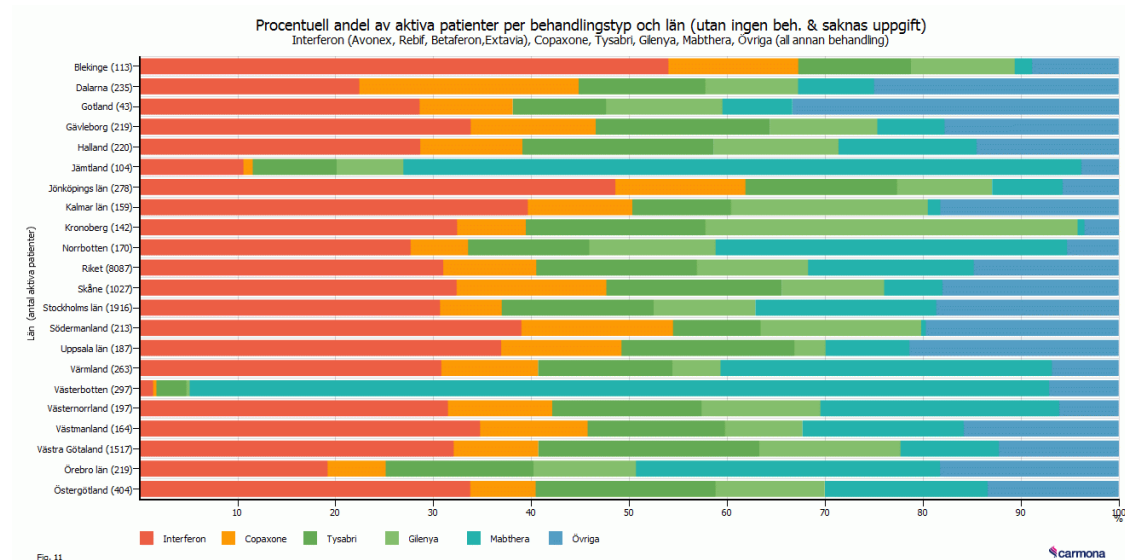


Figure 1. Treatment dispersion across Sweden in percentages and county. Rituximab = Mabthera – here represented in turquoise. [11]

There is an extensive use of RTX, off label, against MS in all regions of Sweden and comprises a total of 19.2% of all MS treatments in the country. In Gothenburg RTX has been used since 2008 with a steady increase. [1]

Why remains to be explained as well as to evaluate outcomes for these patients.

In order to correctly examine and evaluate important aspects of patients being treated with RTX we performed a retrospective study of all patients diagnosed with RRMS, PPMS and SPMS treated with Mabthera (Rituximab) between 2008 and early 2015 in the city of Gothenburg, Sweden.

Epidemiology/Aetiology

Alike the other Scandinavian countries, Sweden has one of the highest MS prevalence in the world, with a prevalence of 189/100000 and with an estimated incidence of circa 10/100000. (Ahlgren et al.) Today there are approximately 17500 registered patient with MS in Sweden. In MS there is a 2.35 ratio between women and men with a more then double risk for women compared to men. The risk ratio is less obvious in PPMS. [2] Disease onset is most common around the age of 20-40.

There is a correlation between latitude and risk of developing MS, in other words the risk of MS increases the farther from the equator one lives. [12] There are also theories correlating the lack of sun exposure and therefore lower levels of vitamin-D with MS, which in turn can be associated with geographical distribution. [13]

Being a smoker or living with a smoker seems to have a worsening impact on risk and prognosis of MS. [14-17]

It is believed that being infected by EBV at a young age increases the risk of developing MS. [18] Some studies even show that elevations in antiEBV-antibody titers occur prior to the MS-debut and that this is a sign of early MS. [19]

The theory is strengthened by the fact that people who migrate from high to low risk regions during childhood have a decreased risk profile and vice versa. [20] [21]

Clinical Course of MS

The clinical onset of multiple sclerosis is in 85% of cases preceded by a clinically isolated syndrome (CIS), an acute or sub-acute neurological disturbance due to white-matter lesions. [22] If in addition CIS is accompanied by abnormalities in unaffected white matter the risk of a second attack is high. With a secondary relapse the McDonald-criteria (see Diagnostic Criteria) for the diagnosis MS are met.[23, 24]

Onset of disease may be abrupt or insidious. The severity of onset symptoms varies within a wide range, from trivial to severe. [25] The most common disease course is relapsing-remitting MS (RRMS), and is represented in 85% of patients in the initial stage of disease. An average of 65% of patients with RRMS enter the progressive phase of MS (SPMS). This usually occurs within 10-15 years (in an untreated population). For around 10-15% of patients the course is progressive from start, then called primary progressive (PPMS). This progressive course of disease is characterized by less evident inflammation and a more gradual worsening of symptoms without obvious fluctuations or discernible relapses. [23, 26, 27]

Initial symptoms of MS may have mono- or multifocal basis and symptoms are dependent on location of the lesions in the central nervous system (CNS). Even though location of the lesions are to some extent random they more frequently involve certain areas including the optic nerve (optic neuritis, 25%), the spinal cord (40%) or the brain stem (25%). Other early symptoms are weakness, sensory disturbance such as diminished tactility, diplopia, gait instability, and ataxia. [25, 26]

In RRMS there is possibility of a complete or partial recovery from symptoms is possible. Relapses usually last weeks to months. With time the symptoms persist and accumulate. Typical symptoms of progressive MS (PMS) consist of over months to years development of spastic para- or tetraparesis, cerebellar ataxia, spastic hemiparesis. Disability also manifests through symptoms such as bladder dysfunction, fatigue, heat sensitivity, Lhermitte's symptom (electric sensory sensation down the spine evoked by neck flexion), hemifacial weakness or pain, vertigo, tonic spasms and other paroxysmal symptoms, add to the complex disease progress.

Approximately 50% of untreated patients will eventually become dependent of a wheel chair 30 years after diagnosis. [28, 29]

Cognitive and neurophysiological deficits (e.g. memory loss, attention disorders, slowed problem solving), accompany the disease especially in advanced cases. [30, 31] Depression is expressed by around 60% of patients and therefore the suicide risk is 7.5-fold more common. [25]

Patients diagnosed with MS are usually expected to have a reduction in life expectancy of up to 5-10 years, with an additional risk of committing suicide as mentioned. Death is attributable to MS in 2/3 of cases, where the patients susceptibility to infections is increased.[32-34]

Diagnostic Criteria in MS

As MS has a wide spectrum of phenotypes and clinical appearances diagnosis is not always easy to establish. A fast and precise diagnosis is essential in an era with a plethora of DMT's preventing neurological disability.

The core criterion in diagnosing MS has long been to establish evidence of dissemination in time (DIT) and in space (DIS) between, at least, two white matter inflammations in the CNS, that have objective clinical evidence. [35]

The McDonald criteria help establish ground rules on how these diagnostic parameters should be accounted for.

The principal goal is to simplify the MS diagnostic criteria without compromising in sensitivity and specificity, and so since its acknowledgement the McDonald criteria has been modified and revised twice. [35-37] These improvements have resulted in the possibility of earlier diagnosis and therefore also earlier interventions and/or treatments. [38, 39]

In struggle to deliver a fast diagnosis it is vital to consider and reject alternative diagnosis, as e.g. neuromyelitis optica (NMO). These diseases often resemble MS but treatments generally have no or less effect.

In the McDonald criteria latest revision PPMS criteria is now included and requires one year of disease progression and two of the following three findings: positive brain MRI; positive spinal cord MRI; or positive CSF. [35]

It is not evident to see the transition between RRMS and SPMS since relapses and exacerbations can leave remaining disabilities and also superimpose. However the SPMS diagnosis is coupled with unremitting worsening for at least six months.

Measurements of disease activity and progression

Disease activity is best measured in clinical manifestations and everyday functionality. In addition, a prognostic score is available to show what is to expect in terms of worsening and amelioration.

Expanded Disability Status Scale, EDSS

Ever since J.F.Kurtzke introduced the Expanded Disability Status Scale (EDSS) in 1983 it's been the golden standard when it comes to rating disease disability in clinical routine.

The EDSS evaluates seven functional systems (FS) and ambulatory abilities, through a standardized set of neurological tests. The scale ranges from 0 to 10.

The FS consider:

- Pyramidal motor functions
- Cerebellar function
- Brainstem
- Sensory abilities
- Bowel and bladder
- Visual
- Cerebral or mental
- Motor function

Zero constitutes a normal neurological examination. A score of 1.0 to 3.5 represents people with some FS symptoms but with complete ambulatory capabilities. 4.0 until 6.5 include people with ambulatory restrictions. A score more than 6.5 will mainly

incorporate patients in need of assistance or even wheelchair. Score 10 means death caused by MS.[40, 41]

Multiple Sclerosis Severity Scale, MSSS

In comparison to EDSS the MSSS aims towards predicting disability progression in MS. The EDSS development over the course of MS in 9892 patients from eleven countries helped establish a general disease progression rate, plotted into a standardized matrix. By inserting the EDSS and the duration of disease in each axis of the matrix one can find its MSSS-score, 0.01 to 9.99.

Though the MSSS is based on a large population some question if it is applicable on today's population influenced by modern therapies.

Magnetic Resonance Imaging - MRI

During the last decade, the radiologic magnetic resonance imaging (MRI) has become an important tool in measuring therapeutic efficacy, disease activity and progression of MS.

By “weighting” MRI-images, adjusting properties, it is possible to acquire distinctive information when it comes to pathological severity, presence of blood brain barrier damage, BBB-damage, or to visualizing disease expansion.

MRI also has predictive capabilities in that the number of lesions found at disease onset may predict the disability development. [42]

Clinical evaluation together with MRI, make up the diagnostic foundation for MS and have made other paraclinical tools such as biochemical biomarkers and neurophysiological examinations less significant. [36, 43-45] The latest revision of

the MRI criteria (MAGNIMS) allows MS diagnosis after one clinical episode in addition to signs of activity on one MRI (with Gadolinium enhancing lesions).[46] However MRI cannot be used as an evaluation tool of the pathophysiological developments nor is it optimal for evaluating the PMS progression as changes in brain tissue is not as evident in this form of MS.

Genes

The familial recurrence rate in MS is around 20%, but the heredity is complex. More than 50 risk genes have been identified. The genotype called “HLA class II genotype DRB1*15:03”, carried by 28-33% of northern Caucasian MS patients versus 9-15% of healthy controls has the strongest association of all genes.[47] Risk reducing genes have also been found. Most genes are located in or in close proximity of the immune-regulating genes. Most genes have association with other autoimmune diseases. [48, 49]

B-cell

Over the last couple of years several studies have targeted the B-cell to be a key component in MS. [50] The humoral immune system and the B-cell involvement has been known for decades but has been regarded as less important for the process . However, recently this matter has been subject for further investigation, as the T-cell branch of the immune system no longer is believed to be sufficient for full expression of MS. [8, 51]

The B-cell may play a key role in MS, here follows some evidence of such a postulations: Histopathological studies that have shown presence of b-cell, b-cell derived plasma cells and antibodies in active and chronic plaques in patients

diagnosed with MS.[52] The intrathecal IgG synthesis is consistent with B-cell expansion.[53] The presence of markers of B-cell activity, such as (nonpathogenic myelin basic protein), B-cell chemokines (CXCL 13) and antibodies.[54] The discovery of the B-cells fundamental role in the induction of the animal equivalent to MS, experimental autoimmune encephalomyelitis (EAE). [55]

As implied B-cells have not been the main target of elimination in therapeutic treatments, “until now”. But established treatments have unintentionally displayed anti B-cell action through several coherent mechanisms, e.g. (a) interferon-beta inhibits antibody secretion through anti-CD40/80 effect.[56-58] (b) Glatiramer acetate is known to inhibit antigen-presenting cells (APC), including B-cells. [59] (c) Antiproliferative medications such as Mitoxantrone affects the total number of B-cells and their cytokine production. [60] (d) Through the mechanism of altering the APCs capabilities Corticosteroids affect the immunoglobulin synthesis. [61, 62]And finally (e) Nataluzimab alters the expression of very late antigen 4(VLA-4), which is expressed on and responsible for the B-cells transmigration brain endothelial sites.[63]

As mentioned earlier the EBV may be implicated in triggering the autoimmunity in MS, furthermore the EBV mainly hibernates in B-lymphocytes.

The role(s) played by B-cells, plasma cells, and antibodies in CNS inflammatory demyelinating diseases are likely to be multifactorial and complex, involving distinct and perhaps opposing roles for B cells versus antibody. [3]

Rituximab

The Food and Drug Administration (FDA, USA) in 1997 first approved RTX for use against B-cell Lymphomas and later treatment resistant Rheumatoid Arthritis.

However RTX has been used for several years as an off-label treatment against other autoantibody-mediated diseases.[64]

RTX is a genetically engineered chimeric IgG monoclonal antibody. It targets B-lymphocytes in all development stages, with exception for the plasma cell, through the trans-membrane antigen CD20. The Fab domain of the monoclonal antibody binds the CD20 antigene of the B-cell. The antibody then induces cytotoxicity through apoptosis or complement-dependent cytotoxicity, resulting in lysis. [8, 9, 65]

The depletion of the B-lymphocytes will affect development of new antibody producing cells, all B-cell mediated, antigene presentation, activation and propagation of T-cells, macrophages and cytokine networks. [4]

In the phase II trial OLYMPUS, Hawker and co-workers investigated RTX in treatment of MS. They discovered no significant effect in their primary end point, “time to confirmed disease progression” (CDP), but patients had an overall diminished lesion progression in all subjects. Further and foremost new evidence for the usage towards a subgroup of patients, patients aged ≤ 51 years old was found, were subjects in the subgroup had an increased time to CDP and a reduction in gadolinium-enhanced lesions.

Questions have been raised against the effectiveness of RTX since the B-cell activation and IgG-synthesis is intrathecal, but its been shown that RTX penetrates the CNS, through an intact blood brain barrier (BBB). [66]

The usage of RTX has a positive risk profile since the drug has long documentation of AE's on treatment against RA. [67] Several studies mostly show mild to moderate AE's. [7] [68] The most common AE's documented are itching of skin and throat, flushing, chills, fever and/or diarrhea.[68, 69]

Contraindications for treatment with RTX is know immune-deficiency syndrome due to immune-suppressive treatment, on going pregnancy and breast-feeding.

Antibody production against RTX is documented and quite common, in some cases with frequencies around 30-40%. However these antibodies are usually none-neutralizing and do not affect the intended treatment.

No significance was been shown in studies when testing the increased risk of common infections whilst being under anti-CD20 antibody treatments. Progressive Multifocal Leukoencephalopathy (PML) has previously been mentioned in correlation with Mabthera (Rituximab) treatments but none in the field of neurology under indications of MS.[70]

In using RTX it is expected that B-cells play an important role in the pathogenesis of multiple sclerosis and links having an abnormal B-cell activation to T-cell mediated immunopathology. In fact increasing evidence show that antibodies may play a role in the initiation of plaques and the demyelination process in MS. [64]

Indications

The usage of RTX is still quite arbitrary in the neurological field and in MS, but there does exist local indications. In RRMS, RTX can be introduced when there is evidence of either clinical or radiological activity whilst being under treatment of a more established immunomodulating treatment (beta-interferon, glatirameracetat, fingolimod, natalizumab, mitoxantrone). [71]

In PPMS and SPMS, indications of inflammatory activity either noted through clinical relapses or by new MRI lesions. [72]

Aims

The purpose of this study is to evaluate reasons for using of RTX, its efficacy, safety and tolerability among patients with MS.

Method/Materials

Demographics

All patients had been diagnosed according to the McDonald's Criteria [1] with subgroups RRMS, PPMS or SPMS. [73] All individuals started treatment between 2008 and early 2015.

All patients treated with RTX, on the indication MS, at the MS-Center, Sahlgrenska University Hospital were included. 105 patients were selected after discarding patients with NMO, patients with inconclusive diagnosis and patients lacking essential data.

Demographic data shown in *Table 2*. RRMS 41 patients (39%), SPMS also 41 patients (39%) and PPMS 23 patients (21.9%), *figure 3*.

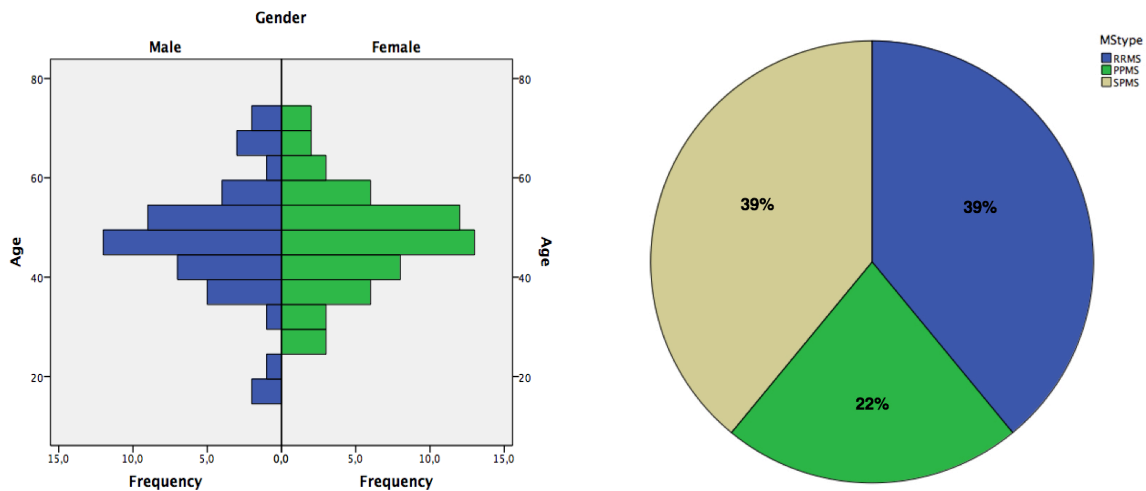


Figure 2-3. To the left - demographics gender distribution in the group and age. To the right - Disease form representation, shown in percentage. RRMS, SPMS and PPMS.

Clinical Assessments

All patients were treated with RTX in accordance with guidelines established at the MS-Center (The Department of Clinical Neuroscience and Rehabilitation) Sahlgrenska University Hospital, Gothenburg – Mabthera (Rituximab) – treatment of Multiple Sclerosis.[71] These guidelines are mainly based on the OLYMPUS phase II study by Hawker et al. [7]

1. RRMS-patients – Initial treatment dose consists of 2 infusions a 1000mg x2 with 14 days apart. Thereafter infusions every 6 month a 1000mg during a period of 3 years. After which an annual infusion is made if no signs of disease progression, usually evaluated through MRI.
2. PPMS and SPMS-patients - Same dosage as for RRMS-patients but with evaluations after 3 years. After 3 years patients will only be subject to

infusions if new progression is shown. MRI-evaluations after 3-6 months and then each 12 months.

Patients who underwent the Mabthera (Rituximab) treatment were also subjects to testing prior and after treatment. See *Table 1*.

Table 1. An extract from the Mabthera treatment protocol showing tests taking prior and during Rituximab treatment.

Tests/Control	Prior to treatment	Each 6 months	Annually
Blood/Differential Count and IgG	YES	YES	
Immunophenotyping and B-lymphocyte count (%)	YES		YES
Pregnancy testing	Before each infusion		

Collected Data

The patient data and information was collected through the VGR (Västra Götalands Regions) administrative system – *Melior* and through the “*Swedish National Multiple Sclerosis Register*”. Two separate data extraction dates were put in place, 01/08/14 and 01/02/15.

The following parameters were recorded for each individual:

- Age
- MS-type
- Reasons for treatment change
- EDSS-scores one year before RTX at start and at data extraction date
- Sex
- Start & stop dates of treatment
- Number of doses received
- MSSS-scores at start and at data extraction date

- New MRI-lesions
- Reoccurrence of B-cells
- Relapse rate the last two years and after RTX start
- Adverse events

The MS-debut and diagnose dates for each patient was collected as well as dates for the transition to SPMS for concerned parties. Emphasis was put on the MS-debut, which was based on first symptom presentation.

The reasons for change to Rituximab from other treatment were categorized in following groups:

- Treatment refractivity of other treatments
- Neutralizing antibodies (NAB) against the previously given medication
- Adverse effects against previously given medication
- Disease progression in general.
- JCV-positive or PML patients
- No other treatment is available – all other possibilities had been considered
- Other – patients whom for special reasons changed to Rituximab.

RRMS patient- and PMS patient (SPMS and PPMS together) results were kept apart to abridge evaluation.

Within these topics sub-groups were made to evaluate common factors for change, whether it was a drug or an adverse effect, against one or several of these drugs, that had caused the clinician and patient to choose rituximab. More over the mere occurrence of adverse effects was registered from first administration and until the 7th injection. The attributes of the adverse effects were registered separately.

Reoccurrence of B-cells was tested before and after every treatment.

Calculation and Analyses

All numeric data was plotted into SPSS Statistics version 22.0. Median and endpoints were calculated for the distribution of age, sex, disease duration, disease progression and severity. For SPMS, years in progress were also calculated.

Ethics

The patients when being registered to the Swedish MS-register gave consent to the use and analysis of their journals and data.

Results

The median *Disease Duration* was overall longer in patients diagnosed with SPMS, a median of 19 years compared with compared to 12 and 11 years in median for RRMS and PPMS, respectively. Years in progression were for SPMS, a median of 6 years.

The Number of Treatments was 3 (median) per patient, ranging from 1-10.

Treatment Length varied from 3-74 months, with a median of 13 months. The PPMS-group had a median time of 15 months, RRMS – 9months and SPMS – 13months.

MS-type	Number of patients	Gender (f:m)	Age, yr* Median	Duration, yr Median	Years in progress (median)
All MS	105	58:47	48 (17-74)	14 (1-44)	NA**
RRMS	41 (39%)	28:13	45 (17-72)	12 (1-32)	NA
SPMS	41(39%)	21:20	50 (24-71)	19 (6-44)	6 (1-24)
PPMS	23(21.9%)	9:14	48 (26-74)	11 (2-21)	NA

Table 2. Patients Demographics and disease duration.

*Yr – years. **NA – not applicable. Within parenthesis – range and percentage.

Change of treatment

The heterogeneous patient group all had different *reasons for change of treatment*, some with several combined reasons. The largest portion;

- Out of 64 PMS-patients (SPMS and PPMS) 58 (90%) were offered Rituximab due to *detectable disease progression* under previous DMT. In the RRMS-group 22 patients (56%) had relapses under previous DMT. Previous treatments were *nataluzimab, beta-interferon, fingolimod, novantrone, mitoxantrone*.
- 14 patients (34%) having RRMS had shown JC virus antibodies (JCV) under nataluzimab treatment and therefore also risk of PML. In PMS, 8 patients (12%) were at risk.
- 8 subjects (20%) with RRMS and 4 patients (6%) with PMS had shown AE's against one or several previous DMT's.
- 3 patients (7%) showed productions of NAB against either *Beta-interferon* or *nataluzimab*. In PMS the number was 2 patients (3%).
- 13 patients (12%) had cardiovascular or compliance problems, and therefore Rituximab was more suitable.

Efficacy

Relapses the last two years – Amongst the RRMS patients 15 (36.6%) underwent episodes of relapses, 25 were in steady state and 3 had missing data.

In the PMS-group, 14 patients (21.8%) had episodes of relapses, 44 in steady state (68.7%) and 6 with missing data.

Out of the 41 patients having RRMS 4 (9.8%) had had episodes of neurological disturbances lasting for at least 24h, classified as *relapses after RTX start*. 3 patients had no record on this matter.

In the equivalent PMS-group, 5 (7.8%) had relapses after start and a total of 9 had missing data.

Table 3. Collected Data – including: number of treatments, treatment length, EDSS-values, MSSS-values. In parenthesis are range and number of patients included.

	Number of treatments Median	Treatment Length Median (months)	EDSS One year prior Median	EDSS Start Median	EDSS *Date of data extraction Median	MSSS Start Median	MSSS *Date of data extraction Median
All MS	3 (median) 382 (total) (1-10)	13 (3-74)	4,5 (0-8) (n=84)	5 (0-8.5) (n=98)	6 (0-8.5) (n=87)	6.39 (0.86-9.83) (n=98)	6.33 (0.45-9.95) (n=87)
RRMS	3 (median) 141 (total)	9 (3-74)	2,5 (0-8) (n=33)	3 (0-8) (n=39)	3.5 (0-8) (n=35)	4.79 (0.86-9.74) (n=39)	4.13 (0.45-9.94) (n=35)
SPMS	3 (median) 138 (total)	13 (5-41)	6,0 (2.5-8) (n=35)	6.5 (2.5-8.5) (n=39)	6.25 (2.5-8.5) (n=34)	7.14 (1.43-9.57) (n=39)	6.92 (1.43-9.81) (n=34)
PPMS	4 (median) 89 (total)	15 (3-39)	6.0 (2-8) (n=16)	5.5 (1.5-8.5) (n=20)	6 (0-8.5) (n=18)	7.2 (3.65-9.83) (n=20)	7.19 (3.94-9.95) (n=18)

*Date of extraction – on the 010814 and 010215. Range showing within parenthesis as well as

There was an increase in EDSS when comparing parameters; *EDSS at start* and *EDSS at extraction date (010814 and 010215)*. In RRMS patients went from a median of EDSS-score 3 to 3.5, with a range of 0-8.0. In PMS the median EDSS going into the study was 6 and at extraction date 6.5.

The prognostic MSSS-score was instead decreased, from *MSSS at start* to *MSSS at extraction date* in both RRMS, -0.66 ranging 0.86-9.94 and in the PMS-group, -0.19

ranging 1.43-9.95. However it did not show statistical significance, in the entire group $p=0.077$ and in the RRMS group $p=0.052$.

New MRI lesions could be detected in 10 patients (24.4%) having RRMS and in 10 patients (15.6%) having PPMS. A total of 31 patients had no record of MRI after start.

Adverse Events

Adverse effects (AE) in correlation to administration of Rituximab. 52 patients (49.5%) felt AE's in relation to the first injection.

At the second injection the amount of patients feeling AE's had dropped to 14 (13.3%). Third injection, 10 patients were affected (9.6%).

After third injections there were few reported AE's.

Common AE's during injections included; Sensation of pressure over the chest, sensation of thickening in the gorge and tongue, with sometimes added itchiness.

Flush in face and ears, headache, nausea and general fatigue was also common. Rare symptoms with fever and shivering also appeared.

6 patients stopped the rituximab-treatment due to either subjective or objective AE's.

A total of 16 patients did *stop* their treatment. 6 Patients stopped due to adverse effects, 4 due to disease progression and 6 for unknown reasons.

Reoccurrence of B-cell was noticeable in 5 cases (12.2%) in RRMS and 5 cases (7.8%) in PMS. 3 RRMS and 4 PMS patients had not been examined, most of which had either stopped their treatment all together before testing or due to relocation.

Discussion

Indication and Efficacy

Our retrospective study shows that a majority of this highly selected group started treatment on indication disease progression under previous treatment or for the RRMS-group, relapses under previous treatment. A great number of the subjects had undergone several treatments before choosing RTX as a last line of treatment.

There is a median disability increase, shown through EDSS, in both RRMS and PMS whilst being under treatment of RTX. It is though difficult to estimate if RTX has given any effect on the disease activity. With no control group the efficacy could not be evaluated.

However at baseline 36% of RRMS and 22% of PMS had relapses the last two years when compared with relapses after RTX start showed a decrease, where only 9.8% of RRMS and 7.8% of PMS reported relapses.

It is also shown that the overall MSSS-score decreases, in both RRMS and under RTX treatment and that this may be an additional sign of treatment effect. However the score did not show statistical significance and since the MSSS score is based on a population with a majority not treated with immune-modulatory treatment it is debated if MSSS-score is representative for therapeutical effect, but since MSSS is predictive it may have importance.[74]

Twenty patients (19%) had developed new MRI-lesions since beginning treatment with RTX. This illustrates that a minority of the patients had disease activity during the treatment period. The MRI data will be further analysed according to time from treatment start activity was seen. A treatment delay was observed in the OLYMPUS study, from treatment start to effect and it is possible that this could also be the case in this study and could partly explain the EDSS increase and MRI-lesions. [7]

Is rituximab safe?

Despite the fact that 49.5% of the study group experienced adverse events (AE) upon first injection none of these AE's were life threatening. In addition, upon the second injection only 13.3% had AE's and at the subsequent infusions even less, 9.6%. Most AE were infusion related.

As has been concluded in other studies treatment with RTX is correlated with an increased risk of adverse effects but not serious adverse events.[75] It has also previously been shown that AE's lessen with subsequent infusions, which also was the case in this study, with AE being uncommon after third dose.

It is natural to assume that the cytokine-release upon lysis of B-cells, which is the greatest upon first infusions, decrease in line with lesser amount of circulating B-cells. [76]

RTX should be considered safe when used properly, never the less we can only account for a median treatment time of 13 months. Long-term effects have not yet been evaluated. We do however have a range of 3-74 months in treatment length, which then includes 21 patients (20%) having been under treatment for more than

two years without unpredicted AE. More studies evaluating AE's on larger populations and during longer periods of time is still needed.

The risk of JCV and PML in MS-patients treated with RTX has not yet been tested, but the incidence in Rheumatoid Arthritis patients treated with RTX is 1/25000. [77] Comparing this with the incidence of PML when being under (monoclonal antibody) natalizumab treatment, 11/1000, shows a much lower risk. (Biogen – tysabri fact sheet). In studies of natalizumab it has been observed that some adverse events such as PML appear more frequently after two full years of treatment. [75] So far no indications are seen that this problem is the same for RTX.

A total of 16 patients stopped the treatment, 6 due to AE, 4 due to treatment failure shown on MRI and/or clinically. 6 stopped due to relocations or unknown reasons. A dropout rate of 15% should be considered low and is in line with other treatment on label for MS, especially when taking into account the different reasons for stopping treatment. [78, 79]

Five RRMS and five PMS patients had reoccurrence of B-cells whilst being under RTX treatment. It would be valued if reoccurrence would mirror a more aggressive disease or lack of treatment response. We could not find any correlation between this phenomenon and disease activity. The role of this population of B-cells in the disease process is obscure and needs to be investigated further.

Strengths and weaknesses

The group itself is challenging to analyze since they've shown to be particularly difficult to treat, hence being patients trying a second or last line of treatment available. Therefore the study results of progression rate, treatment effect, adverse effect can and will only be applicable to a small group of people. A sufficiently qualified control group would have been difficult to find, none the less as is said this is a group that needs more attention and is in need of sustainable treatment.

Missing data for several patients throughout the study.

Due to the character of the retrospective study, it was preferable to extract the patient data so that as many as possible would be included. In doing so some patients had not yet acquired certain information, e.g. MRI-scans or B-cell reoccurrence test results.

This can only explain in part missing data.

When retrieving information from medical charts and registers there can be bias in the chart itself, when transferring data and handling data.

With an average of one year of treatment, outcomes and AE could properly be addressed. By using both journals and the MS-reg we were able to, to some extent, substantiate information.

Use and Future

To our knowledge a retrospective study of the outcome for patients, in Gothenburg, Sweden, treated for MS with RTX has never before been done.

In order to have a better perspective of the prescription of Rituximab in Multiple Sclerosis, this study is a small step in obtaining increased knowledge.

Adding knowledge to a treatment form where not even the pathophysiology is clearly understood can help in making this treatment safer and better.

Finding patterns, standardizing and creating criteria on which physicians can seek guidance can be of help.

There are ongoing studies towards usage of Rituximab as a generic, called Ocrelizumab, and therefore it is acknowledged that anti-CD20 antibodies has a place and a purpose in the treatment of MS. However with Ocrelizumab on the horizon, questions arise concerning the future off-label use of Rituximab.

Conclusion

RTX was used as treatment primarily as a last line of treatment after having disease activity in previous treatments. RTX was well tolerated with no severe AE. RTX had no obvious effect on EDSS progression but decreased relapse rate and MSSS, though not statistically significant ($p=0,077$), could indicate a beneficial effect in both RRMS and PMS.

Polulärvetenskaplig sammanfattig (Swedish)

I denna studie tittade vi på multipel skleros (MS) -patienter som står under behandling av det oregistrerade läkemedlet rituximab. Det finns idag teorier om att andra delar av immunförsvaret är delaktiga i MS-sjukdomen än vad man tidigare trott. Rituximab är ett läkemedel som angriper en viss sorts celler, B-celler, som är delaktiga i immunförsvaret. Detta läkemedel har visat effekt speciellt på en subgrupp patienter med MS i två mindre förstudier.

Vi inhämtade data och information från MS-registret och patientjournaler. Patienterna som fick rituximab hade till största del haft sjukdomsaktivitet, exv anfall, i sin MS när det stått på annan behandling och därför valt att byta. Andra orsaker var relaterade till allergi och livshotande följd tillstånd.

Parametrar så som funktionsnedsättningsskalan, EDSS, visade en ökad invaliditet från start till i genomsnitt ett års behandling, men den prognostiska skalan MSSS visade förbättrad prognos. Vi såg även minskad sjukdoms-attacker jämfört med tidigare. Dock visar dessa resultat bara en del av bilden då vi inte har någon kontrollgrupp.

Vi såg även att läkemedlet var väl tolererat i patientgruppen. Patienterna fick biverkningar, men dessa var ej allvarliga och var uppkom främst vid första behandlingstillfället.

En uppföljande studie på patienter som behandlas med rituximab har inte tidigare gjorts och detta är ett litet steg i att få bättre insikt i biverkningar, anledningar till insättande av läkemedel och att se den effekt som behandlingen ger.

Med liknande preparat på väg ut på marknaden så är det än viktigare att hitta rätt indikationer och vad biverkningsprofilen är, för att rätt patienter ska få behandlingen.

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