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Pharmacoepidemiological Studies of Rituximab and Other Recent Therapies in Multiple Sclerosis

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Stockholm 2022

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Published by Karolinska Institutet.

Printed by Universitetsservice US-AB Tryckeriet, 2022

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ISBN 978-91-8016-795-6

Pharmacoepidemiological Studies of Rituximab and Other Recent Therapies in Multiple Sclerosis

THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

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The thesis will be defended in public at CMM Lecture Hall, L8:00, Visionsgatan 18, Solna, 2022-11-03 14:00

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Abstract

Multiple Sclerosis (MS) is a chronic autoimmune disorder of the central nervous system, characterized by the accumulation of demyelinating inflammatory lesions. Milder cases exist, but most patients eventually develop severe neurological symptoms and disability. However, this dire prognosis is likely to change with the increasing availability of modern, highly effective disease-modifying therapies (DMTs).

The rapid development of new therapies for MS has resulted in a quickly changing treatment landscape. Recently, the use of off-label rituximab has become the most used DMT for MS in Sweden. In addition to the purely pharmacological therapies, autologous haematopoietic stem cell transplantation (AHSCT) has emerged as an option for patients with severe disease.

The aim of this PhD project was to investigate risk-benefit aspects of DMTs for MS with a special focus on rituximab.

Study I was a cohort study comparing the effectiveness of rituximab and fingolimod in patients switching from natalizumab due to JC-virus positivity. In 256 patients, fewer relapses and contrast-enhancing lesions were observed in the rituximab group, within the first 1.5 years of the therapy switch. The rituximab group also reported fewer adverse events and better drug survival, compared with fingolimod. Rituximab appears to be the superior choice in this patient population and likely for highly active MS in general.

Study II was a validation of the Swedish MS Register, the main data source for MS research in Sweden. Among 3012 patients, data on treatment and Expanded Disability

Status Scale (EDSS) was reasonably complete. Observations of Magnetic Resonance Imaging (MRI) were often missing or incomplete, but could partly be recovered from the medical records. Missingness varied by MS clinic and treatment. The proportion of missing was higher for the older injectable therapies, compared with newer DMTs, which has implications for comparative studies.

Study III was a cohort study assessing cancer risk in patients treated with rituximab, natalizumab, or fingolimod. Among 7477 therapy starts, we identified 78 cancers. After controlling for possible confounding, we found an increased risk of cancer for the fingolimod group, compared with both the rituximab group and the general population. It was not possible to assess if any specific type of cancer was driving this difference. No increased risk was found for rituximab or natalizumab, compared with the general population. These findings were in line with previous studies of rituximab in rheumatoid arthritis, but interestingly with a lower risk of breast cancer on rituximab than in the trial programme for ocrelizumab.

Study IV was a cohort study assessing safety outcomes for patients treated with the induction therapies AHSCT or alemtuzumab. Among 271 patients, one death occurred in the AHSCT group and four deaths occurred in the alemtuzumab group. The mortality rate for AHSCT was lower in our study than in most previous reports. AHSCT was associated with an increased risk of infection that diminished a few months after treatment. The incidence of thyroid disease with alemtuzumab was higher in our study than previously reported in clinical trials, but in line with other observational data. The rate of starting a new therapy was lower with both AHSCT and alemtuzumab, compared with conventional MS therapies.

Study V was a cohort study assessing cost-effectiveness for patients treated with rituximab, natalizumab, fingolimod, or dimethyl fumarate. Among 5924 therapy starts, rituximab was superior to the other therapies, resulting in both lower overall healthcare costs and fewer relapses. Mean cost savings per patient over five years were €34 000–€66 000 and mean number of prevented relapses 0.12–0.21, which translates to total cost savings of €307 000 000 and 1063 prevented relapses, over five years. The cost savings and lower number of relapses with rituximab were possible without any increase in other

individual healthcare costs, such as prescription drugs, specialized outpatient visits, or inpatient care.

In summary, the data in the Swedish MS Register was of generally good quality, especially for data relating to therapy use, making the register a good resource for pharmacoepidemiologic studies. We found that AHSCT is an effective and safe induction-type treatment for MS, with treatment-related mortality similar to other DMTs. However, there was an increased risk of infection around the time of treatment and a more long-lasting risk of autoimmune thyroid disease. We found rituximab to be a cost-effective, safe, and well tolerated treatment for MS, resulting in fewer clinical relapses, fewer contrast-enhancing lesions on MRI, better drug survival, and no increased risk of cancer. Rituximab should be considered as an alternative first-line therapy for most MS patients with active, non-progressive disease. AHSCT should be considered for younger patients without severe disabilities, with clinical worsening and inflammatory signs not adequately controlled by regular therapies. Rituximab also appears to be the most attractive treatment option for MS patients in low-resource settings, where MS-approved alternatives are might be unavailable due to their high costs.

Popular Science Summary

Multiple Sclerosis (MS) is a lifelong, autoimmune disease affecting the brain and spinal cord. In most cases, persons affected by MS experience repeated inflammatory activity that causes neurological damage and can result in a wide range of possible symptoms. Some people with MS have milder disease, but many develop severe disability as the disease progresses. However, this bleak outlook is likely to change as there have been great advancements in the development of new treatments.

The antibody therapy rituximab has quickly become the most commonly used treatment for MS in Sweden, even though it is not approved specifically for MS. Another therapeutic intervention that is not formally approved for MS is Autologous Haematopoietic Stem Cell Transplantation (AHSCT), which is mainly used for patients with severe disease. Therapies approved for MS include the oral medications fingolimod and dimethyl fumarate (pills), and the antibody therapies natalizumab and alemtuzumab (intravenous infusion).

The main objective of this PhD project and its five studies has been to investigate risk-benefit aspects of treatments for MS, with a special focus on rituximab.

Study I compared the effectiveness of rituximab and fingolimod in MS patients switching from natalizumab because of the risk for severe treatment-related side effects. We found that patients receiving rituximab had less disease activity, fewer negative side effects of treatment, and remained longer on the same treatment, compared with fingolimod.

Rituximab appeared to be the better treatment choice for this group of patients and likely for patients with highly active MS in general.

Study II was a validation of the main data source for MS research in Sweden: the Swedish MS Register. We found that data in the MS register overall had good quality, especially for data directly relating to MS therapy use. However, some other types of data were missing to a larger extent, but could partly be recovered from medical records.

Study III investigated the risk of cancer for MS patients treated with rituximab, natalizumab, or fingolimod. We found an increased risk of cancer for the fingolimod group, compared with both the rituximab group and a group with neither MS nor MS treatment. No increased risk was found for rituximab or natalizumab, compared with the non-MS group.

Study IV investigated the safety of treatment with AHSCT or alemtuzumab in MS patients. We found fewer deaths with AHSCT than in most previous studies. However, there was an increased risk of infection close after treatment start. Disease of the thyroid gland was more common with alemtuzumab, but the risk was also increased for patients with AHSCT. Many patients in both groups remained without a need of further MS therapies, indicating long-lasting effects on disease activity.

Study V assessed the cost-effectiveness of the therapies rituximab, natalizumab, fingolimod, and dimethyl fumarate. We found that rituximab was more cost-effective than the other therapies, with both lower costs and lower risk of disease activity. If everyone in the study had received rituximab instead of MS-approved therapies, €307 000 000 would have been saved and 1063 relapses prevented, over five years. The cost savings and decreased disease activity with rituximab were possible without any increase in other individual healthcare costs.

In summary, we found that AHSCT is an effective and safe treatment for MS, but with a temporarily increased risk of infection around treatment start and an increased risk of autoimmune thyroid disease. We found rituximab to be a cost-effective, safe, and well tolerated treatment for MS, resulting in less disease activity, lower risk of having to switch therapy, and no increased risk of cancer. Rituximab should be considered as an alternative for most MS patients with active disease, while AHSCT should be considered for younger patients with aggressive, active MS, where the disease cannot be controlled adequately with regular therapies.

Populärvetenskaplig Sammanfattning

Multipel Skleros (MS) är en livslång, autoimmun sjukdom som påverkar hjärnan och ryggmärgen. I de flesta fall drabbas personer med MS av återkommande inflammatorisk aktivitet som orsakar neurologisk skada och kan resultera i en rad olika typer av symtom. Vissa personer med MS har en lindrigare sjukdom, men många utvecklar svåra funktionsnedsättningar allt eftersom sjukdomen fortskrider. Prognosen för MS håller dock sannolikt på att förändras, i och med de stora framsteg som har gjorts i utvecklingen av nya behandlingar.

Antikroppsläkemedlet rituximab har snabbt blivit den mest använda behandlingen för MS i Sverige, trots att den inte är godkänd specifikt för MS. En annan behandling som inte är formellt godkänd för MS är autolog hematopoietisk stamcellstransplantation (AHSCT), som framför allt används till patienter med allvarlig sjukdom. Behandlingar som är godkända för MS inkluderar de orala läkemedlen fingolimod och dimetylfumarat (tabletter), samt antikroppsläkemedlen natalizumab och alemtuzumab (intravenös infusion).

Det huvudsakliga syftet med detta doktorandprojekt och dess fem studier har varit att undersöka risk-nyttoaspekter av behandlingar för MS, med ett särskilt fokus på rituximab.

Studie I jämförde effektiviteten av rituximab och fingolimod hos MS-patienter som bytt från natalizumab på grund av risken för allvarliga behandlingsrelaterade biverkningar.

Vi fann att patienter som fick rituximab hade mindre sjukdomsaktivitet, färre negativa biverkningar av behandlingen och stannade längre på samma behandling, jämfört med fingolimod. Rituximab verkade vara det bättre valet av behandling för denna grupp patienter och sannolikt för patienter med högaktiv MS i allmänhet.

Studie II var en validering av den huvudsakliga datakällan för MS-forskning i Sverige: Svenska MS-registret. Vi fann att uppgifterna i MS-registret överlag var av god kvalitet, särskilt för data som var direkt relaterade till användningen av MS-behandling. Vissa andra typer av uppgifter saknades dock i högre grad, men kunde delvis återhämtas från journaler.

Studie III undersökte risken för cancer hos MS-patienter som behandlats med rituximab, natalizumab eller fingolimod. Vi fann en ökad risk för cancer i fingolimod-gruppen, jämfört med både rituximab-gruppen och med en grupp utan varken MS eller MS-behandling. Ingen ökad risk hittades för rituximab eller natalizumab, jämfört med gruppen utan MS.

Studie IV undersökte säkerheten av behandling med AHSCT eller alemtuzumab hos MS-patienter. Vi hittade färre dödsfall med AHSCT än vad man har gjort i de flesta tidigare studier. När AHSCT-behandling påbörjades fanns det dock en ökad risk för infektion strax efter behandlingsstart. Sjukdomar i sköldkörteln var vanligare med alemtuzumab, men risken var även ökad för patienter med AHSCT. Många patienter i båda grupperna förblev utan ett behov av ytterligare MS-behandlingar, tydandes på långverkande effekter på sjukdomsaktivitet.

Studie V utvärderade kostnadseffektiviteten för behandlingarna rituximab, natalizumab, fingolimod och dimetylfumarat. Vi fann att rituximab var mer kostnadseffektivt än de övriga behandlingarna, med både lägre kostnader och lägre risk för sjukdomsaktivitet. Om alla i studien hade fått rituximab istället för MS-godkända läkemedel, så skulle €307 000 000 ha sparats och 1063 skov undvikits, under fem års tid. Kostnadsbesparingarna och den minskade sjukdomsaktiviteten med rituximab var möjliga utan någon ökning av andra enskilda sjukvårdskostnader.

Sammanfattningsvis så fann vi att AHSCT är en effektiv och säker behandling för MS, men med en tillfälligt ökad risk för infektioner runt behandlingsstart och en ökad risk för autoimmun sköldkörtelsjukdom. Vi fann att rituximab är en kostnadseffektiv, säker och väl tolererad behandling för MS, som resulterar i mindre sjukdomsaktivitet, mindre risk att behöva byta behandling och ingen ökad risk för cancer. Rituximab bör övervägas som ett alternativ för de flesta MS-patienter med aktiv sjukdom, medans AHSCT bör övervägas för yngre patienter med aggressiv, aktiv MS, där sjukdomen inte kan kontrolleras adekvat med vanliga behandlingar.

Περίληψη Εκλαϊκευμένης Επιστήμης

Η σκλήρυνση κατά πλάκας (ΣΚΠ) είναι μια δια βίου, αυτοάνοση ασθένεια που επηρεάζει τον εγκέφαλο και το νωτιαίο μυελό. Στις περισσότερες περιπτώσεις, τα άτομα που πάσχουν από ΣΚΠ εμφανίζουν επαναλαμβανόμενη φλεγμονώδη δραστηριότητα που προκαλεί νευρολογική βλάβη και μπορεί να οδηγήσει σε ένα ευρύ φάσμα πιθανών συμπτωμάτων. Μερικοί άνθρωποι με ΣΚΠ έχουν ηπιότερη νόσο, αλλά πολλοί αναπτύσσουν σοβαρή αναπηρία καθώς η νόσος εξελίσσεται. Ωστόσο, αυτή η θλιβερή προοπτική είναι πιθανό να αλλάξει καθώς έχουν σημειωθεί μεγάλες εξελίξεις στην ανάπτυξη νέων θεραπειών.

Η θεραπεία με αντισώματα rituximab έχει γίνει γρήγορα η πιο συχνά χρησιμοποιούμενη θεραπεία για τη ΣΚΠ στη Σουηδία, παρόλο που δεν είναι εγκεκριμένη ειδικά για τη ΣΚΠ. Μια άλλη θεραπευτική παρέμβαση που δεν είναι επίσημα εγκεκριμένη για τη ΣΚΠ είναι η Αυτόλογη Μεταμόσχευση Αιμοποιητικών Βλαστοκυττάρων (AHSCT), η οποία χρησιμοποιείται κυρίως σε ασθενείς με σοβαρή νόσο. Οι θεραπείες που έχουν εγκριθεί για τη ΣΚΠ περιλαμβάνουν τα από του στόματος φάρμακα fingolimod και dimethyl fumarate (χάπια) και τις θεραπείες με αντισώματα natalizumab και alemtuzumab (ενδοφλέβια έγχυση).

Ο στόχος αυτού του διδακτορικού έργου και των πέντε μελετών του ήταν η διερεύνηση πτυχών κινδύνου-οφέλους των θεραπειών για τη ΣΚΠ, με ιδιαίτερη έμφαση στο rituximab.

Η **Μελέτη I** συνέκρινε την αποτελεσματικότητα του rituximab και fingolimod σε ασθενείς με ΣΚΠ που άλλαξαν από το natalizumab λόγω του κινδύνου για σοβαρές παρενέργειες που σχετίζονται με τη θεραπεία. Βρήκαμε ότι οι ασθενείς που έλαβαν rituximab είχαν λιγότερη δραστηριότητα της νόσου, λιγότερες αρνητικές παρενέργειες της θεραπείας και παρέμειναν περισσότερο καιρό στην ίδια θεραπεία, σε σύγκριση με το fingolimod. Το rituximab φάνηκε να είναι η καλύτερη θεραπευτική επιλογή για αυτήν την ομάδα ασθενών και πιθανότατα για ασθενείς με πολύ ενεργή ΣΚΠ γενικά.

Η **Μελέτη II** ήταν μια επικύρωση της κύριας πηγής δεδομένων για την έρευνα της ΣΚΠ στη Σουηδία: του Σουηδικού Μητρώου ΣΚΠ. Διαπιστώσαμε ότι τα δεδομένα στο μητρώο ΣΚΠ είχαν συνολικά καλή ποιότητα, ειδικά για δεδομένα που σχετίζονται άμεσα με τη χρήση θεραπείας για τη ΣΚΠ. Ωστόσο, κάποια άλλα είδη δεδομένων έλειπαν σε μεγαλύτερο βαθμό, αλλά μπορούσαν εν μέρει να ανακτηθούν από τα ιατρικά αρχεία.

Η **Μελέτη III** διερεύνησε τον κίνδυνο καρκίνου για ασθενείς με ΣΚΠ που έλαβαν θεραπεία με rituximab, natalizumab ή fingolimod. Διαπιστώσαμε αυξημένο κίνδυνο καρκίνου για την fingolimod ομάδα, σε σύγκριση τόσο με την rituximab ομάδα όσο και με μια ομάδα που ούτε είχε ΣΚΠ ούτε λάμβανε θεραπεία για ΣΚΠ. Δεν βρέθηκε αυξημένος κίνδυνος για το rituximab ή το natalizumab, σε σύγκριση με την ομάδα χωρίς ΣΚΠ.

Η **Μελέτη IV** διερεύνησε την ασφάλεια της θεραπείας με AHSCT ή alemtuzumab σε ασθενείς με ΣΚΠ. Βρήκαμε λιγότερους θανάτους με AHSCT σε σχέση με τις περισσότερες προηγούμενες μελέτες. Ωστόσο, υπήρχε αυξημένος κίνδυνος μόλυνσης μετά την έναρξη της θεραπείας. Η νόσος του θυρεοειδούς αδένα ήταν πιο συχνή με το alemtuzumab, αλλά ο κίνδυνος ήταν επίσης αυξημένος για τους ασθενείς με AHSCT. Πολλοί ασθενείς και στις δύο ομάδες παρέμειναν χωρίς την ανάγκη περαιτέρω θεραπειών για ΣΚΠ, γεγονός που υποδεικνύει μακροχρόνιες επιπτώσεις στη δραστηριότητα της νόσου.

Η **Μελέτη V** αξιολόγησε τη σχέση κόστους-αποτελεσματικότητας των θεραπειών rituximab, natalizumab, fingolimod και dimethyl fumarate. Βρήκαμε ότι το rituximab ήταν πιο οικονομικό από τις άλλες θεραπείες, με χαμηλότερο κόστος και χαμηλότερο κίνδυνο δραστηριότητας της νόσου. Εάν όλοι στη μελέτη είχαν λάβει rituximab αντί για τις εγκεκριμένες θεραπείες για την ΣΚΠ, θα είχαν εξοικονομηθεί €307 000 000 και θα είχαν προληφθεί 1063 υποτροπές, σε διάστημα πέντε ετών. Η εξοικονόμηση κόστους και η μειωμένη δραστηριότητα της νόσου με το rituximab ήταν δυνατή χωρίς καμία αύξηση σε άλλα ατομικά έξοδα υγειονομικής περίθαλψης.

Συνοπτικά, βρήκαμε ότι το AHSCT είναι μια αποτελεσματική και ασφαλής θεραπεία για τη ΣΚΠ, αλλά με προσωρινά αυξημένο κίνδυνο μόλυνσης κατά την έναρξη της θεραπείας και αυξημένο κίνδυνο αυτοάνοσης νόσου του θυρεοειδούς. Βρήκαμε ότι το rituximab είναι μια οικονομικά αποδοτική, ασφαλής και καλά ανεκτή θεραπεία για τη ΣΚΠ, με αποτέλεσμα λιγότερη δραστηριότητα της νόσου, μικρότερο κίνδυνο αλλαγής θεραπείας και μη αυξημένο κίνδυνο καρκίνου. Το rituximab θα πρέπει να θεωρείται ως εναλλακτική λύση για τους περισσότερους ασθενείς με ΣΚΠ με ενεργό νόσο, ενώ το AHSCT θα πρέπει να λαμβάνεται υπόψη για νεότερους ασθενείς με επιθετική, ενεργή νόσο, όπου η νόσος δεν μπορεί να ελεγχθεί επαρκώς με τακτικές θεραπείες.

— Translated by Marina Dehara

Acknowledgements

There are so many people that have supported me during my PhD and in my life in general. I want to thank each and every one of you for making this a more exciting and rewarding experience.

Fredrik Piehl and **Thomas Frisell**, my fantastic supervisors. Without you, none of this would have been possible. Your support, encouragement, and expertise has been invaluable. You have let me grow and experiment, while also making sure that I did not stray too far from the path. I am truly grateful and I look forward to future collaborations.

Marina Dehara, for all your support, love, and patience. You make me a better and happier person. I cannot imagine how I would have managed the sprint of writing this thesis without you.

Renata Zelic, for looking out for me (and my office plant), providing good hiking company, and tolerating me constantly asking to have a fika.

Andrei Barbulescu, for always being supportive, sharing your excitement, and never missing an opportunity to discuss interesting statistical conundrums.

Kelsi Smith, for being a great desk buddy, always helping out if needed, and making the long days at work more fun.

Carl-Martin Pethrus and **John Svensson**, my unofficial mentors, for helping me grow outside KI and see that there is more than one path forward.

My friends, colleagues, and family that took the time to provide feedback on this thesis, when I needed it the most: **Marina Dehara**, **Renata Zelic**, **Andrei Barbulescu**, **Kelsi Smith**, **Suvi Virtanen**, **Matilda Morin**, **Anton Öberg Sysojev**, **Felix Wittström**, **Malik Cakir**, **Simon Steiger**, **Emilia Schwertner**, **Markus Janasch**, **Arne Alping**, and **Annika Lundin**.

My friends from work that have made research more fun: **Arda Yilal**, **Joshua Entrop**, **Simon Englund**, **Eleni Tsamantioti**, **Viktor Molander**, **Viktor Wintzell**, **Huiling Xu**, and **Maxime Cuijpers**.

My friends from medical school, for not allowing me to forget the clinic, helping me navigate AT, and just being awesome people: **Sebastian Samuelsson**, **Marius Matusevicius**, **Martin Östholm**, and **Emil Sternegård**.

My childhood friends, for cheering me on and bringing me happiness, for the better part of my life: **Rickard Haeggman**, **Wictor Enquist**, **Björn Svensson**, **Tobias Palmqvist**, and **Malin Enberg**.

My brother **Mikael Alping**, for being my best friend, always standing by my side, and never hesitating to lend an ear or a hand when needed. My parents **Arne Alping** and **Annika Lundin**, for making me the person I am today, supporting me in all my endeavours, and giving me so much. My family **Marianne Lundin**, **Tove Lundin**, **David Lundin**, **Britten Årsjö**, **Sören Årsjö**, **Mikael Årsjö**, **Tage Alping**, **Ann Lindsgård**, and **David Alping**.

Finally, thank you to all the people at KEP, for your support, feedback, and company: **Johan Askling**, **Martin Neovius**, **Elisa Longinetti**, **Bénédicte Delcoigne**, **Elizabeth Arkema**, **Hannah Bower**, **Björn Pasternak**, **Gustaf Bruze**, **Helga Westerlind**, **Daniela Di Giuseppe**, and many more.

I would also like to express my gratitude to the **Department of Clinical Neuroscience** for providing me with administrative support, and the **Division of Clinical Epidemiology** for adopting me into their research environment.

Thank you — Peter

Funding

This PhD project was partly funded through external sources. The funding sources had no role in the study designs; in the collection, analysis, or interpretation of data; in the writing of the reports; or in the decision to submit any of the papers for publication.

Clinical Scientist Training Programme (CSTP) Was a programme by Karolinska Institutet with the aim to stimulate research interest among the university's students. It directly sponsored half of this PhD project.

Patient-Centered Outcomes Research Institute (PCORI) A US non-profit institute that indirectly sponsored this PhD project through the Award (MS-1511-33196) for the COMparison Between All immunoTherapies for Multiple Sclerosis (COMBAT-MS) project. "The statements presented in this publication are solely the responsibility of the authors and do not necessarily represent the views of the Patient-Centered Outcomes Research Institute, its Board of Governors or Methodology Committee".

NEURO Sweden (Neuroförbundet) A Swedish non-governmental organization that directly sponsored this PhD project through two grants from their research fund (2017 and 2018).

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II Peter Alping et al. 'Validation of the Swedish Multiple Sclerosis Register: Further Improving a Resource for Pharmacoepidemiologic Evaluations'. In: *Epidemiology* 30.2 (Mar. 2019), pp. 230–233. DOI: 10/gfvpd7.

III Peter Alping et al. 'Cancer Risk for Fingolimod, Natalizumab, and Rituximab in Multiple Sclerosis Patients'. In: *Annals of Neurology* (13th Feb. 2020). DOI: 10/ggk2dq.

IV Peter Alping et al. 'Safety of Alemtuzumab and Autologous Hematopoietic Stem Cell Transplantation Compared to Non-induction Therapies for Multiple Sclerosis'. In: *Neurology* (29th Jan. 2021). DOI: 10.1212/WNL.0000000000011545.

V Peter Alping et al. 'Effectiveness and Costs with Rituximab versus Approved Disease-Modifying Therapies in Multiple Sclerosis: A Nationwide Cost-Effectiveness Study Using Real-World Data'. Manuscript. 2022.

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VI Jonatan Salzer et al. 'Rituximab in Multiple Sclerosis: A Retrospective Observational Study on Safety and Efficacy'. In: *Neurology* 87.20 (15th Nov. 2016), pp. 2074–2081. DOI: 10.1212/WNL.0000000000003331.

VII Gustavo Luna et al. 'Infection Risks Among Patients With Multiple Sclerosis Treated With Fingolimod, Natalizumab, Rituximab, and Injectable Therapies'. In: *JAMA Neurology* (7th Oct. 2019). DOI: 10/ggk2dr.

Abbreviations

AHSCT Autologous Hematopoietic Stem Cell Transplantation.

ATC Anatomical Therapeutic Chemical Classification System.

CI Confidence Interval.

COMBAT-MS COMparison Between All immunoTherapies for Multiple Sclerosis.

COVID-19 Coronavirus Disease 2019.

DAG Directed Acyclic Graph.

DMT Disease-Modifying Therapy.

EBV Epstein-Barr Virus.

EDSS Expanded Disability Status Scale.

EMA European Medicines Agency.

FDA U.S. Food and Drug Administration.

GDPR General Data Protection Regulation.

HR Hazard Ratio.

HSC Haematopoietic Stem Cell.

ICD International Classification of Diseases.

IR Incidence Rate.

IRR Incidence Rate Ratio.

JCV John Cunningham Virus.

LIF Läkemedelsindustriföreningen.

LISA Longitudinal Integrated Database for Health Insurance and Labour Market Studies.

MAR Missing at Random.

MCAR Missing Completely at Random.

MICE Multiple Imputation by Chained Equations.

MNAR Missing Not at Random.

MRI Magnetic Resonance Imaging.

MS Multiple Sclerosis.

MSFC Multiple Sclerosis Functional Composite.

MSIS-29 Multiple Sclerosis Impact Scale.

OR Odds Ratio.

PCORI Patient-Centered Outcomes Research Institute.

PML Progressive Multifocal Leukoencephalopathy.

PPMS Primary-Progressive Multiple Sclerosis.

QALY Quality-Adjusted Life-Year.

RRMS Relapsing-Remitting Multiple Sclerosis.

SARS-CoV-2 Severe Acute Respiratory Syndrome Coronavirus 2.

SDMT Symbol Digit Modalities Test.

SPMS Secondary-Progressive Multiple Sclerosis.

Chapter 1

Introduction

Descriptions of Multiple Sclerosis (MS) date back to the 14th century, but it would take until 1868 before it was recognized as a distinct disease, by neurologist Jean-Martin Charcot. Since its recognition, a multitude of different treatments have been tried without much success, until 1951, when cortisone was found to reduce the severity of MS inflammation. Still today, cortisone is the main treatment for acute inflammation in MS. However, it was only in 1993 that the first therapy was introduced that could affect the long-term course of the disease, interferon-beta-1b. Since then, several other, disease-modifying therapies have been developed, steadily improving the lives of those living with MS.

The rapid development of new therapies for MS has resulted in quickly changing guidelines and treatment patterns in Sweden and around the world. With increasingly potent therapies available, the prognosis of MS has improved, and more patients start these highly effective therapies earlier in their disease course. It is likely that what we have known about the progression and long-term outcomes of MS will change drastically for the better, to much rejoice of patients and physicians alike.

1.1 Multiple Sclerosis

MS is a chronic autoimmune disorder of the central nervous system (brain and spinal cord), characterized by the accumulation of demyelinating inflammatory lesions.[1,2] It is more than twice as common in females as in males, with disease onset usually between 20 and 40 years of age, although 10% of patients experience their first demyelinating event before the age of 18.[2,3] MS affects over two million people worldwide and the incidence has been increasing since the 1950s, especially in females.[4–6] In Sweden, the prevalence of MS is around 190/100 000, with an incidence rate of 10/100 000 person-years.[7–9] This is much higher than the estimated world average prevalence of 36/100 000 and incidence rate of 2/100 000 persons-years, but there is great variation between countries.[10] Although the clinical spectrum includes milder cases, a large proportion of patients with MS will eventually develop severe neurological symptoms. The life expectancy in the MS population is estimated to be reduced by seven to 14 years, compared with the general population, and more than 50% of patients die from directly MS-related causes.[11] However, this dire prognosis is likely to change with the increasing availability of highly effective disease-modifying therapies.

1.1.1 Aetiology and Pathophysiology

The aetiology of MS is not fully understood, but it is clear that both genetic and environmental factors have important roles.[12] Predisposition for MS is highly polygenic and the currently identified risk alleles carry only a slight increase in risk individually. The highest risk is conveyed by polymorphisms in the genes for human leukocyte antigen class I and II.[13] Infection with Epstein-Barr Virus (EBV) has recently been identified as the leading environmental cause of MS, increasing the risk of MS 32-fold after infection.[14] This is not seen after infection with other viruses, including the similar cytomegalovirus. Other environmental risk factors include living at high latitude, smoking, low levels of vitamin D, and obesity during adolescence.[12]

The pathophysiology of MS is also incompletely understood, but it is clear that the inflammatory lesions in the central nervous system have a central role and the root cause is thought to be an autoimmune process.[15] Traditionally, dysfunctional CD4+ and

CD8+ T cells with specificity for the myelin protein were suspected to be the main culprits in the central nervous system inflammation. These T cells are thought to be primed in the periphery, possibly through cross-recognition between EBV epitopes and neuroglial antigens (molecular mimicry), cross the blood-brain barrier, and contribute to the development of new inflammatory lesions.[15,16] However, new research and the success of several anti-CD20 therapies have made it clear that B cells also have an important role. Possible mechanisms for B cell involvement include the presentation of antigens to T cells and high-capacity secretion of cytokines, such as interleukin-6.[15,17,18] Additionally, after primary infection with EBV, the virus establishes itself as a chronic latent infection in the memory B cell pool. The innate immune system is also thought to be involved in the disease, with both protective and damaging effects, but the functional relevance remains to be established.[15]

1.1.2 Classification

MS is divided into three groups based on the characteristics of the disease course: Relapsing-Remitting Multiple Sclerosis (RRMS), Secondary-Progressive Multiple Sclerosis (SPMS), and Primary-Progressive Multiple Sclerosis (PPMS).[19,20] RRMS is the most common type of MS in newly diagnosed patients and is characterized by bouts of acute focal inflammatory activity, demyelination, and axonal damage, lasting days to weeks, called a relapse, followed by periods of recovery and reversal of symptoms. A substantial proportion of patients eventually develop severe neurological symptoms and after 10 to 20 years many patients proceed to a progressive disease course, SPMS.[21] Approximately 10%–15% of patients are diagnosed with a progressive disease course from the start, PPMS, often with a later onset than RRMS.

1.1.3 Diagnosis and Follow-Up

The diagnosis of MS is based on the revised McDonald criteria, including patient history, clinical findings, Magnetic Resonance Imaging (MRI), and/or testing of cerebrospinal fluid, including measuring levels of neurofilaments.[19] The diagnosis is based on finding evidence of at least two periods of disease activity, separated in both time and space, i.e.

different affected regions of the central nervous system at different time points. However, as it is becoming easier to find signs of MS earlier in the disease course, primarily due to better diagnostic tools such as MRI and higher awareness of the disease, it is increasingly common that patients only fulfil parts of the diagnostic criteria, although the suspicion of MS remains. If a patient presents with an acute or subacute episode of neurological symptoms due to a single white-matter lesion, but does not fulfil the diagnostic criteria of separation in time and space, this is called a clinically isolated syndrome. Similarly, a radiologically isolated syndrome is when the first sign of disease is found incidentally through imaging of an asymptomatic patient whom does not fulfil the diagnostic criteria for MS. Follow-up of diagnosed patients include regular visits to a neurologist, MRI scans, and measuring of different clinical scales. The main goals of follow-up are to monitor disease activity, identify and treat any emerging relapses, continually evaluate treatment effectiveness and safety, alleviate any MS-related symptoms, and ensure a high quality of life for the patient.

Magnetic Resonance Imaging

MRI is a radiological imaging technique that uses strong magnetic fields to generate a detailed three-dimensional representation of the human body. MRI is used in the diagnosis of MS, as well as to follow disease progression and response to therapy.[22] Different MRI protocols can be used to visualize specific aspects of the investigated tissues and a contrast agent can be administered to further enhance the imaging. In MS, the T2 protocol is often used to assess the total number of lesions in the brain and spinal cord. The T1 protocol, together with gadolinium contrast, is used to better visualize active lesions with blood-brain barrier leakage, as the contrast leaks from the blood vessels to the surrounding brain or spinal cord tissue. In Sweden, guidelines state that patients with active MS should have at least one MRI scan per year.

Relapses

A relapse is defined as any episode of new neurological signs or symptoms, lasting for more than 24 hours and occurring in the absence of fever, infection, or encephalopathy.[2] The onset is acute or sub-acute, with peak dysfunction at two to three weeks, followed by weeks or months of full or partial recovery. The signs and symptoms presented during an inflammatory episode depend on what part of the central nervous system is affected. The typical onset of MS is characterized by an initial acute episode of neurological dysfunction due to lesions of the optic nerve (optical neuritis), spinal cord (myelitis), cerebral hemispheres, brainstem, or cerebellum.

Clinical Scales

Several clinical scales have been developed in an effort to quantify different aspects of the MS disease. These scales are valuable both for following MS patients in the clinic and in research, as baseline characteristics and outcome measures.

Expanded Disability Status Scale (EDSS)[23–26] is one of the oldest clinical scales for assessing disease activity in persons with MS and is still frequently used in both the clinic and research. EDSS evaluates eight different functional systems of the patient, with a final score that ranges from 0 (no signs or symptoms) to 10 (death due to MS). Focuses heavily on motor symptoms and mobility.

Multiple Sclerosis Impact Scale (MSIS-29)[27] is a newer scale that combines patient perspectives with psychometric methods, and measures both the physical and psychological impacts of MS. It has been reported to be more responsive to change than the EDSS.

Multiple Sclerosis Functional Composite (MSFC)[28,29] is a composite measure of arm, leg, and cognitive function, developed as improvement over EDSS for use in clinical trials. It has been reported to be more responsive to change than the EDSS.

Symbol Digit Modalities Test (SDMT)[30] is a test to screen for impairment in cognitive processing speed, a cerebral function that is often affected in persons with MS. In this test, the patient substitutes geometric shapes with numbers, with use of a reference key.

1.1.4 Treatment

Treatment for MS can be divided into three distinct types: therapies limiting the acute inflammation during a relapse, symptomatic treatment, and therapies modifying the disease course. In the event of a relapse, it is important to quickly control the inflammation to limit possible neurological damage. This is mainly done by administering oral or intravenous corticosteroids, e.g. intravenous methylprednisolone 1000 mg for three days. For severe relapses plasmapheresis can be considered. Lasting disability after a relapse can take many forms and is managed with symptomatic treatment. Common symptoms include spasticity, pain, fatigue, ataxia, tremor, neuropsychiatric symptoms, cognitive symptoms, bladder disorders, sexual dysfunction, and gastrointestinal symptoms. Finally, the disease-modifying therapies, which are the focus of this PhD project, alter the patient's immune system to reduce the risk of future relapses and disease progression.

1.2 Disease-Modifying Therapies

The field of MS treatment has seen drastic developments in the last three decades; from no effective long-term therapy options to the array of different Disease-Modifying Therapies (DMTs) available today. The first DMTs to be approved for MS were recombinant interferon-beta (1993) and glatiramer acetate (1997), both still commonly used, followed by several second-generation oral and parenteral therapies. Most treatments are only approved for use in RRMS, as few therapies have any proven effect for progressive MS. In recent years, use of off-label rituximab has seen increasing interest, and over the last decade it has become the most used MS DMT in Sweden (Figure 1.1).[9] In addition to the purely pharmacological therapies, Autologous Hematopoietic Stem Cell Transplantation (AHSCT) has emerged as a treatment option for patients with severe disease. Table 1.1 lists the currently available DMTs for MS.

1.2.1 Treatment Strategy

Treatment for MS generally follows an escalation strategy and patients with newly diagnosed RRMS are frequently started on less effective, but safer, first-line therapies These

Table 1.1: Currently available disease-modifying therapies for multiple sclerosis

				First Approved		Studies	
ATC	Generic Name	Brand Name/s	MAH/s	EMA	FDA	Trials	PhD
L03AB08	Interferon-beta-1b	Betaferon/Extavia	Bayer/Novartis	1995	1993	[31–38]	
L03AB07	Interferon-beta-1a	Avonex/Rebif	Biogen/Merck	1997	1996	[39–43]	
L03AX13	Glatiramer acetate	Copaxone	Teva	—[a]	1997	[44]	
L04AA23	Natalizumab	Tysabri	Biogen	2006	2004	[45–48]	I/III/V
L04AA27	Fingolimod	Gilenya	Novartis	2011	2010	[49–54]	I/III/V
L04AA31	Teriflunomide	Aubagio	Sanofi	2013	2012	[55–58]	
L04AA34	Alemtuzumab	Lemtrada	Sanofi	2013	2014	[59–61]	IV
L04AX07	Dimethyl fumarate	Tecfidera	Biogen	2014	2013	[62–64]	V
L03AB13	Peginterferon-beta-1a	Plegidry	Biogen	2014	2014	[65, 66]	
L01DB07	Mitoxantrone	Novantrone	Meda	2016	2000	[67–70]	
L04AC01	Daclizumab	Zinbryta	Biogen	~~2016~~[w]	~~2016~~[w]	[71–73]	
L04AA40	Cladribine	Mavenclad	Merck	2017	2019	[74–77]	
L04AA36	Ocrelizumab	Ocrevus	Roche	2018	2017	[78–80]	
L04AA42	Siponimod	Mayzent	Novartis	2020	2019	[81–84]	
L04AA38	Ozanimod	Zeposia	Celgene	2020	2020	[85, 86]	
L04AX09	Diroximel fumarate	Vumerity	Biogen	2021	2019	[87]	
L04AA52	Ofatumumab	Kesimpta	Novartis	2021	2020	[80, 88–90]	
L04AA50	Ponesimod	Ponvory	Janssen	2021	2021	[91, 92]	
L01XC02	Rituximab	MabThera/Rituxan	Roche	—[o]	—[o]	[93–97]	I/III/V
—	AHSCT	—	—	—[o]	—[o]	[98–103]	IV

[a] Glatiramer acetate is nationally authorized in Sweden since 2001
[w] Daclizumab was voluntarily withdrawn in 2018 by its MAH
[o] Rituximab and AHSCT are used off-label for MS in some countries
ATC=Anatomical Therapeutic Chemical Classification System
MAH=Marketing Authorisation Holder
EMA=European Medicines Agency
FDA=U.S. Food and Drug Administration
AHSCT=Autologous Hematopoietic Stem Cell Transplantation

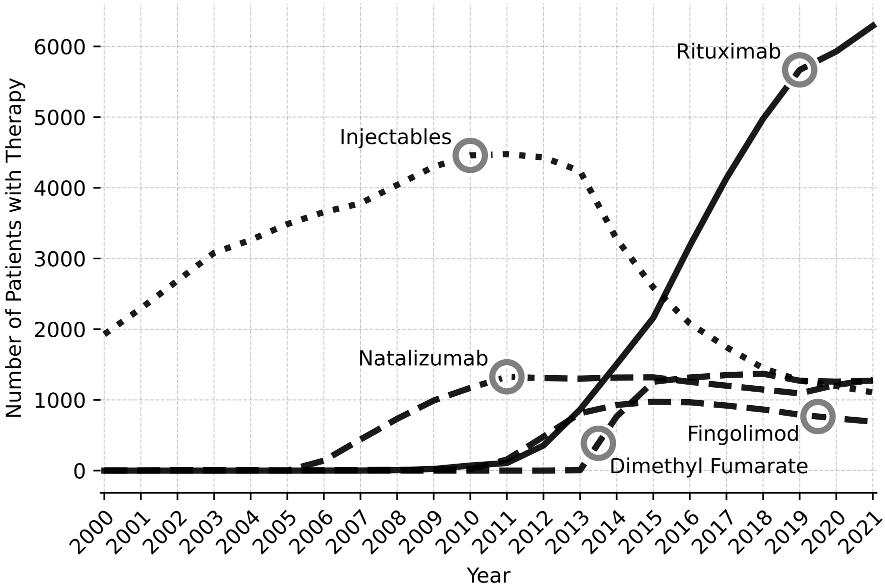


Figure 1.1: Disease-modifying therapies for multiple sclerosis in Sweden over time. Number of patients with ongoing therapy (vertical axis) by the end of the year (horizontal axis), for the four most common therapies in Sweden and injectables as a group consisting of interferon-beta, glatiramer acetate, and peginterferon-beta

generally include the older injectable therapies interferon-beta and glatiramer acetate, and more recently developed oral therapies, such as teriflunomide and dimethyl fumarate. If this initial treatment is not sufficiently effective or if the patient presents with aggressive disease from diagnosis, second- or even third-line therapies are considered. Preference of second-line therapy differs substantially between countries and even individual hospitals, but in Sweden, fingolimod, dimethyl fumarate, natalizumab, and (off-label) rituximab are often used (Figure 1.1).[9] Third-line options include the induction therapies, alemtuzumab and AHSCT, which provide long-term effects without the need for continuous administration.

In recent years, the escalation approach to MS treatment has been questioned, as the neurological damage caused by relapses and subclinical inflammatory disease activity are, at least in part, irreversible. There is now enough clinical experience with the newer more effective therapies that these could be used safely early in the disease course. Particularly in Sweden, there is a trend of moving away from the old first-line therapies for newly diagnosed MS patients and instead start treatment with one of the newer, more potent alternatives.[9]

1.2.2 Evidence

Most of the data we have, especially on the newer MS therapies, come from randomized controlled trials. These are the gold standard for establishing the clinical efficacy and safety of a treatment, due to the ability to control variability in the study population, ensure high-quality data, and avoid confounding through randomization. However, these studies often have strict inclusion criteria, excluding the youngest and oldest age-groups, as well as people with multiple comorbidities. This make them less generalizable, as the studied populations will likely differ from those encountered in clinical practice. The high cost of clinical trials also imposes practical limits on their size and follow-up time, making it difficult to use them for studying rare or long-term outcomes. These drawbacks make register-based observational studies an important complement to the traditional randomized controlled trials, that allow for the study of rare, long-term outcomes in clinically relevant patient populations.

1.2.3 Interferon-Beta and Glatiramer Acetate

Interferon-beta, followed by glatiramer acetate, revolutionized the treatment of MS as the first DMTs made available in the 90s, and they are still frequently used today. Both are delivered by self-administered injections once a day to once a week.

Interferon-Beta

Interferon-beta is a protein with potent anti-inflammatory properties, secreted by cells, mainly fibroblasts, in response to infection.[104] Interferon-beta exists in two forms, interferon-beta-1a and interferon-beta-1b. The two forms have minor differences in their molecular structures, but similar biological effects. These effects are mediated by binding to cell surface receptors, inducing a cascade of signalling pathways that result in the production or secretion of a number of compounds with immunomodulatory, antiviral, and proliferative effects.[104] The mechanism of action for interferon-beta in MS is thought to include reduction in T cell activation, inhibition of the effects of interferon-gamma, induction of immune deviation, and inhibition of blood-brain barrier leakage.[104]

Glatiramer Acetate

Glatiramer acetate (also known as copolymer-I) is a synthetic mixture of random oligomers of the basic amino acids glutamine, lysine, alanine, and tyrosine.[104] These amino acids are highly represented in myelin basic protein, an antigen thought to be involved in the induction of autoimmunity in MS. The mechanism of action in MS is largely unknown, but it has been hypothesized that the oligomers induce immunological tolerization by binding to human leukocyte antigen class II molecules, inducing anergy in myelin-reactive T cells and activation of anti-inflammatory Th2 cells.[104] This leads to bystander suppression of inflammation in the central nervous system.

Safety and Efficacy

Interferon-beta has been shown to moderately reduce the annualized relapse rate in patients with RRMS,[31–43] with similar results for glatiramer acetate.[44,105,106] Both therapies have also been shown to reduce the risk of developing definite MS in patients with clinically isolated syndrome, although no effect has been demonstrated on the risk of permanent disability, risk of conversion to progressive MS, or the rate at which the disease advances in progressive MS. Long-term follow-up of the clinical trials, together with extensive clinical experience, indicate that both interferon-beta and glatiramer acetate have generally good long-term safety. However, therapy adherence is problematic, with more than 25% of patients discontinuing treatment within one to two years,[107] and two-thirds of patients starting interferon-beta or glatiramer acetate have an inadequate response within two years.[33,34,40,44]

1.2.4 Fingolimod (I, III, V)

Fingolimod was the first oral therapy to be approved for RRMS, as a second-line treatment, or for patients with highly active disease. It is taken once daily and targets the sphingosine-1-phosphate receptors expressed on a variety of cells, with the main mechanism of action in MS thought to be the entrapment of lymphocytes in lymph nodes.[108]

Clinical trials have shown good effect of fingolimod in patients with RRMS, lowering the annualized relapse rate compared with both placebo and interferon-beta.[50–52] It also reduced the risk of disability progression compared with placebo in one of the studies,[50] but not the other.[52] However, fingolimod does not appear to be an effective treatment for patients with PPMS.[53] In a paediatric population, fingolimod reduced the relapse rate and accumulation of lesions on MRI, compared with interferon-beta, but was also associated with a greater risk of serious adverse events.[54] Collectively in the trials, adverse events included bradycardia and atrioventricular block at treatment initiation, macular oedema, elevated liver-enzyme, hypertension, lymphopenia, and herpes virus infections. Serious adverse events included serious infections, basal-cell carcinoma, and malignancies. Two fatal infections occurred in the fingolimod group of one of the studies (disseminated primary varicella zoster and herpes simplex encephalitis).[51]

It is recommended that patients are monitored for cardiological adverse effects during the first administration of fingolimod and followed up with ophthalmological examination. Physicians need to consider carefully before prescribing fingolimod to patients with diabetes mellitus or heart conditions.

1.2.5 Dimethyl Fumarate (V)

Dimethyl fumarate (also known as BG-12) is a dimethyl ester of fumaric acid, an intermediate in the citric acid cycle, taken orally twice daily. The main mechanism of action in MS is not fully understood, but the effects are thought to be mediated through modulating effects on T cells. Also thought to be important is the activation of the nuclear factor erythroid-derived factor 2-related factor 2 pathway, a cellular response pathway induced by stress and affecting the expression of several antioxidant genes.[109]

Clinical trials have shown moderate effect of dimethyl fumarate in patients with RRMS, lowering the annualized relapse rate and the number of lesions on MRI, compared with placebo.[63,64] It also reduced the risk of disability progression compared with placebo in one of the studies,[63] but not the other.[64] Compared with glatiramer acetate, dimethyl fumarate was associated with lower annualized relapse rates and fewer new or enlarging lesions on MRI.[64] Known adverse effects of dimethyl fumarate include flushing and mild to moderate gastrointestinal symptoms.

1.2.6 Natalizumab (I, III, V)

Natalizumab is a humanized monoclonal antibody, given as a monthly intravenous infusion, but more recently also available as a subcutaneous injection administered outside of infusion clinics. It selectively binds to the alpha-4 subunit of the cell adhesion molecule, very late antigen-4, present on the surface of most lymphocytes and monocytes. This prevents the interaction between the very late antigen-4 integrin and its ligand, vascular cell adhesion molecule-1, on brain vascular endothelium. This in turn blocks the transmigration of immune cells across the blood-brain barrier, impeding the formation of new MS lesions.[110]

Clinical trials have shown that natalizumab has a very good effect in patients with RRMS. It has been shown to reduce the risk of sustained progression of disability, the rate of clinical relapses, and the accumulation of lesions on MRI, as compared with placebo.[46] Similar effects were seen for natalizumab as an add-on treatment to ongoing interferon-beta, compared with interferon-beta alone.[47] However, natalizumab does not appear to be an effective treatment for patients with SPMS.[48] Long-term follow-up studies have shown that natalizumab is generally safe and well tolerated.[111] Although, it has also become apparent that treatment with natalizumab increases the risk of developing the severe opportunistic brain infection progressive multifocal leukoencephalopathy.[112]

Progressive Multifocal Leukoencephalopathy

Progressive Multifocal Leukoencephalopathy (PML) is a demyelinating disease of the white matter in the brain, caused by lytic infection of oligodendrocytes with the human polyomavirus John Cunningham Virus (JCV).[112] There is no proven effective treatment for PML and the disease normally leads to death or irreversible neurological disability from progressive cognitive, motor, and visual dysfunction. A positive JCV serology, indicative of a potential latent infection, is present in around 50% of the population and natalizumab therapy likely impose a critical block for the immune system to eradicate an emerging infection in the brain.[112,113] Risk factors for PML include positive anti-JCV serology, long duration of natalizumab treatment, and prior use of immunosuppressants.[113] The risk-benefit of natalizumab in treating MS must be assessed on an individual basis, as the risk of PML in JCV-negative patients is practically non-existing. Retesting of anti-JCV serology is recommended every six months as the seroconversion rate is 1%–2% per year.[114] Due to the risk of PML, treatment with natalizumab is often reserved for MS patients with more aggressive disease or breakthrough activity on any of the first-line DMTs. Apart from natalizumab, also dimethyl fumarate and fingolimod have been associated with an increased, although more modest, risk of PML.

Switching from Natalizumab

Patients with ongoing natalizumab treatment who become JCV positive is an especially difficult patient population that is common in clinical practice. The switching from natalizumab must be managed carefully, as inflammatory activity will quickly return and peak four to seven months after the last dose.[115] Rebound phenomena have also been reported, where the inflammation returns even more aggressively than prior to starting natalizumab,[116–118] and there is no consensus on how these patients should be managed to limit the return of disease activity.[119] Patient populations like these are often not included in randomized controlled trials and have to instead be investigated in observational studies. In Study I (Section 4.1), we compared two different treatment strategies in this specific patient population and found that switching to rituximab had a higher effectiveness and tolerability, compared with switching to fingolimod.

1.2.7 Rituximab (I, III, V)

Rituximab is a chimeric monoclonal antibody targeting the CD20 antigen expressed on B cells, causing rapid and complete depletion of B cells through complement-mediated mechanisms.[120] It is used off-label for MS and is administered as an intravenous infusion, in most cases every six months. The possibility to extend the time between infusions is under investigation. Rituximab is approved for treatment of B-cell lymphoma and leukaemia, systemic vasculitis and rheumatoid arthritis, but has been used off-label to treat several neuroimmunological conditions. It is currently the most common DMT for MS in Sweden, which is unique from an international perspective.[9]

Initial, industry-sponsored clinical trials showed promising results for rituximab in active MS,[93,94,121] but as the patent was nearing expiry, the market authorization holder chose to shift focus away from rituximab. Since then, several clinical trials and observational studies have continued to report positive results for rituximab in active MS, among them Study I, III, and V. Taken together the evidence suggests that rituximab is an effective, safe, and well tolerated treatment for active MS, primarily RRMS. Although, rituximab seems to have some effect also in patients with progressive disease, with an active component. This is discussed further in the literature review in Chapter 2.

1.2.8 Alemtuzumab (IV)

Alemtuzumab, previously used for certain forms of T-cell lymphomas, was the first induction therapy approved for MS. It is a humanized monoclonal antibody targeting CD52, an antigen present on a large proportion of immune cells. Binding to this antigen induces depletion and re-population of B and T lymphocytes, which causes long-lasting changes to adaptive immunity.[122] It is given as an intravenous infusion over five days, with a repeated course of three infusions after one year, although about 25% of patients will require one or more repeated courses later on.[61]

Clinical trials have shown good effect of alemtuzumab in patients with RRMS, reducing the risk of relapses compared with interferon-beta and in one of the studies also reducing the risk of disease progression.[60,61] Infusion-related reactions were recorded in almost all patients in the alemtuzumab groups. Infections, especially cutaneous herpes infections, were more common with alemtuzumab than interferon-beta, and immune thrombocytopenia occurred in 1% of patients in the alemtuzumab groups. Thyroid-associated adverse events were also more common with alemtuzumab, and three patients in the alemtuzumab groups developed thyroid cancer.

Due to the increased risks of thyroid disease and immune-mediated thrombocytopenia, monthly blood testing is recommended until four years after the last dose of alemtuzumab. Post-marketing studies have also found an increased risk of mortality with alemtuzumab treatment, prompting regulatory authorities to restrict the label further.[123] While an induction therapy for MS is an attractive idea, the adverse effects of alemtuzumab limit its widespread use.

In Study IV (Section 4.4), we compared alemtuzumab to AHSCT, another induction-type treatment used in MS, as well as conventional MS DMTs with continued administration.

1.2.9 Hematopoietic Stem Cell Transplantation (IV)

AHSCT is an induction therapy designed to reset the immune system of the patient and achieve long-term suppression of MS inflammatory activity. It is administered in several steps: Haematopoietic Stem Cell (HSC) mobilization, HSC harvesting, ablative conditioning, and HSC transplantation.[124] HSCs are harvested from either the bone marrow or peripheral blood, with the latter first requiring mobilization. Ablative conditioning of the haemato-lymphopoietic system is then performed using high dose chemotherapy, leading to pancytopenia and bone marrow aplasia. Finally, the HSCs are reintroduced to the patient, leading to bone marrow repopulation, recovery of haematopoiesis, and reconstitution of the immune system. Haematopoietic stem cell transplantation is not approved for MS, but is routinely used to treat hematologic malignancies. In the malignancy setting, allogenic transplantation (HSCs from a donor instead of the patient) is more common, which is associated with greater risks than the autologous transplantation (HSCs from the patient themselves) used in MS. In the treatment of MS, AHSCT is mostly saved as a third-line option to be used when one or more conventional therapies have been insufficient.

The evidence for the use of AHSCT in MS is much more limited than for the other MS treatments, and mostly comes from small single arm clinical trials,[98,99,101,102] observational studies,[125–127] or case series.[128,129] One small randomized clinical trial in patients with RRMS and SPMS comparing AHSCT to mitoxantrone, a cytostatic, found a reduced number of lesions on MRI and a reduced annualized relapse rate with AHSCT, but no difference in disability progression.[100] A slightly larger randomized clinical trial among RRMS patients found longer time to disease progression and improved EDSS with AHSCT, compared with conventional DMTs.[103] Together, these studies indicate high effectiveness and reasonable safety for AHSCT in MS, primarily in younger patients with more inflammatory activity.

One of the major concerns with AHSCT is treatment-related mortality, which has limited its more widespread use. Overall treatment-mortality has been reported at 2%, but this also includes the high mortality from the early days of AHSCT (7.3%).[130] In recent years, treatment-related mortality has been shown to be much lower (0.2%).[124] Other adverse effects associated with AHSCT include increased risk of infection, neurological toxicity, reduced fertility, secondary autoimmunity (thyroid disorders most common), and secondary malignancy.[124]

In Study IV (Section 4.4), we compared AHSCT to alemtuzumab, another induction-type treatment used in MS.

1.2.10 Other B-Cell-Depleting Therapies

Due to the initial successful trials for rituximab, B-cell depletion was established as an important target for DMTs in MS. This has led to the development of other anti-CD20 antibody therapies, such as ocrelizumab and ofatumumab. Both are approved for MS and have been shown to be very effective in limiting MS inflammation. However, it is unclear if there is any difference in the effectiveness or safety of these therapies compared with rituximab. Studies comparing ocrelizumab to rituximab are currently ongoing (Section 2.3).

Ocrelizumab

Ocrelizumab is a humanized monoclonal antibody targeting the CD20 antigen, given as an intravenous infusion every six months. Clinical trials have shown very good effect for ocrelizumab, compared with both placebo and interferon-beta, reducing annualized relapse rates, the number of contrast-enhancing lesions on MRI, and the risk of disability progression.[78,80] Unlike most other DMTs, ocrelizumab has also shown some effect in patients with PPMS, reducing both clinical and MRI progression, compared with placebo.[79] Ocrelizumab was reported to be generally well tolerated, but mild infusion-related reactions were more common in the ocrelizumab groups than for placebo on the first infusion, decreasing to levels comparable to placebo for later infusions. However, one woman with high-dose ocrelizumab (2000 mg) developed systemic inflammatory response syndrome of undetermined origin and died.[78]

Ofatumumab

Ofatumumab is a fully human monoclonal antibody targeting the CD20 antigen, given as a subcutaneous injection every month, with more frequent doses during the first month. Clinical trials have shown very good effect for ofatumumab, reducing the number of contrast-enhancing lesions and new or enlarging T2 lesions on MRI, compared with placebo,[88,89] and reducing annualized relapse rates, risk of disability worsening, and lesions on MRI, compared with both interferon-beta and teriflunomide.[80,90] Ofatumumab was generally well tolerated and the number of serious adverse events were similar to the comparison groups, although mild injection-related reaction were common with ofatumumab.

1.2.11 Other Disease-Modifying Therapies

There are several other therapies approved for MS that were not part of any of the studies in this PhD project. These therapies are less commonly used than those mentioned above, mainly because their effectiveness and safety profiles are less desirable or because they are newer versions of more commonly used therapies and have not yet had the time to reach widespread adoption.

Mitoxantrone

Mitoxantrone is a synthetic anthracenedione derivate, given every three months as an intravenous infusion. It was developed for treating acute myeloid leukaemia, but apart from its myelotoxic effects it also has potent anti-inflammatory properties. Although effective, the use of mitoxantrone for MS has steadily decreased due to the risk of serious adverse events, including cardiac decompensation and secondary malignancies, and the increasing availability of other more effective and safer alternatives.[67–70]

Daclizumab (withdrawn)

Daclizumab is a humanized monoclonal antibody targeting CD25 of the interleukin-2 receptor, taken once every month as a subcutaneous injection. It selectively inhibits activated T cells, stimulates regulatory CD56bright natural killer cells, and reduces early T-cell activation and the number of proinflammatory lymphoid tissue inducer cells. Daclizumab was approved for treatment of RRMS based on the results from its clinical trials,[71–73] but was later voluntarily withdrawn by its sponsor in 2018 due to cases of encephalitis that in some instances were fatal.[131]

Teriflunomide

Teriflunomide is a selective immunostatic drug, with anti-inflammatory properties, taken orally once daily. The main therapeutic effects in MS are thought to be mediated by blocking the mitochondrial enzyme dihydroorotate dehydrogenase, an essential enzyme in proliferating lymphocytes. Other effects include changes in cytokine production, expression of cell-surface molecules, and cellular migration. Clinical trials have shown a moderate effect compared with placebo.[55–58] Known adverse effects include upper respiratory and urinary tract infections, gastrointestinal symptoms, and increased liver enzymes.

Cladribine

Cladribine is an active purine nucleoside analogue, taken orally during two weekly sessions every year, for two years. Studies have shown it to be effective in patients with highly active MS, but with a risk of severe lymphopenia and vulnerability to infection.[74–77] An additional safety aspect of cladribine is its interference with DNA synthesis and repair, especially concerning since much of the active MS population is composed of young-adults of child-bearing age.

Pegylated Interferon-Beta

Pegylated interferon-beta is a development of conventional interferon-beta, which is taken as a bi-monthly subcutaneous injection. It has similar effectiveness to regular interferon-beta, but with the benefit of less frequent injections due to the slower release of the drug into the bloodstream.[65,66,132,133] However, the slow release also results in the common adverse effects of interferon (fever, fatigue, headache) lasting up to several days after injection.

Siponimod/Ozanimod/Ponesimod

Siponimod, ozanimod, and ponesimod are oral therapies taken daily, targeting the sphingosine-1-phosphate receptors with similar effects to fingolimod (Section 1.2.4). Siponimod does not bind to sphingosine-1-phosphate receptor 3, reducing the risk of cardiac adverse effects.[81–84] Ozanimod and ponesimod bind selectively to sphingosine-1-phosphate receptor 1, which also reduces the risk of cardiac adverse effects.[85,86,91,92]

Diroximel Fumarate

Diroximel fumarate is an oral therapy taken daily with the same mechanism of action as dimethyl fumarate (Section 1.2.5). It has similar effectiveness to dimethyl fumarate, but with a lower risk of gastrointestinal adverse effects. This is because the metabolization of diroximel fumarate results in less methanol in the small intestine, as compared with dimethyl fumarate.[87]

1.2.12 Other Off-Label Therapies

There is a history of off-label use of several oral and intravenous immunosuppressants for MS, including azathioprine, mychophenolate mofetil, and cyclophosphamide. However, clinical data on effectiveness and safety are limited for these agents in the MS setting.

The main off-label treatments for MS currently used are rituximab (Section 1.2.7) and AHSCT (Section 1.2.9).

1.2.13 Generic Therapies and Biosimilars

As the patents for DMTs expire, more generic preparations, of synthetic drugs, and biosimilars, of biological drugs, become available. These variants of the therapies should have near identical physiological effects to the original preparations, although biosimilars might differ on a molecular level due to less straight-forward production processes. The first generic DMT for MS was a generic version of glatiramer acetate, which was found to have equivalent efficacy, safety, and tolerability to the original, and was approved by the U.S. Food and Drug Administration (FDA) in 2015.[134] In Sweden, use of generics/biosimilars has been limited, although for rituximab, MabThera has started to be replaced by the biosimilars Rixathon, Ritemvia, and Truxima. To date, there are few studies of how these new biosimilars compare to rituximab in MS, but one study has reported equivalent effectiveness and safety for Truxima.[135] Just this year (2022), the patent for fingolimod also expired, resulting in almost all patients switching from Gilenya to the more affordable generic preparations, with intravenous natalizumab soon to follow.

1.3 Multiple Sclerosis and Infection

Patients with MS have been shown to have an increased risk of infection compared with the general non-MS population, regardless of treatment.[136] Newer MS DMTs appear to augment this risk,[51,137–139] while the less potent first-generation injectables do not appear to further increase the risk.[137,138] Herpes virus reactivation is a concern with several of the DMTs, but is more closely linked to fingolimod.[51,140] The most feared infectious adverse event is the life-threatening PML, primarily associated to treatment with natalizumab (Section 1.2.6). Most information about infection risk in MS come from randomized controlled trials, with important limitations to study long-term safety in clinically relevant patient populations (Section 3.2). Large observation studies are needed to further investigate comparative infection risks for different DMTs.

A Canadian cohort study in 2008 of nearly 7000 patients found that exposure to interferon-beta or glatiramer acetate did not change infection risk, while the newer oral DMTs and natalizumab were associated with an increased risk of infection-related physician claims, compared with no treatment.[141] No comparison was made within the group of newer DMTs.

Data on infection risk for off-label rituximab in MS is even scarcer than for the MS-approved DMTs. A descriptive register study of rituximab by our research group found no immediate safety concerns, although patients reported a wide range of infections,.[142] The clinical trials for ocrelizumab reported an increased risk of respiratory tract infections.[79,80] A more recent register-based study from our research group investigated the comparative risk of infection between rituximab, natalizumab, fingolimod, and the older injectable therapies (interferon-beta and glatiramer acetate).[143] Our study confirmed a generally increased infection risk in the MS population, which was modulated by DMT treatment. Among treated patients, interferon-beta and glatiramer acetate carried the lowest risk of infection, while rituximab was associated with the highest risk of hospitalized infection.

1.3.1 COVID-19

Starting in 2019, the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) caused a global pandemic of Coronavirus Disease 2019 (COVID-19) that continued through 2020 and 2021, and forced most of the world to employ widespread preventative measures, including travel limitations, quarantine, and mass testing and vaccination. For individuals with chronic diseases and especially those with treatments affecting the immune system, such as DMTs in MS, there was a lot of uncertainty surrounding the risk of infection and the effectiveness of vaccination. Of special interest to the MS population was the use of anti-CD20 DMTs, mainly rituximab, and their effect on antibody testing, natural immunity, and vaccination. In Sweden, and Stockholm in particular, there was a push to extend the intervals of rituximab treatment to potentially reduce the risk of infection and increase the effectiveness of vaccination. Studies of COVID-19 in MS have reported more severe infection in patients with higher disability, recent corticosteroid treatment, or ongoing treatment with an anti-CD20 agent (rituximab or ocrelizumab).[144–147]

1.4 Multiple Sclerosis and Cancer

Risk of cancer is a long-term concern with modern DMTs, due to their potent effects on the immune system. Several studies have investigated the risk of malignancy in MS populations compared with non-MS populations, while comparisons between different DMTs are rarer.

A study from Norway reported no overall increased risk of cancer in patients with MS, but an increased risk of breast cancer and cancers of the urinary tract.[148] Similarly, a Danish study found no overall increased risk of cancer, and even a lower risk in men with MS, compared with the general population.[149] As in the Norwegian study, there was also an increased risk of breast cancer in women with MS, which persisted after controlling for parity and age at first child delivery. In a larger, French study the overall risk of cancer for participants with MS was similar to that of controls and even less than controls for some cancer types.[150] However, an increased risk of breast cancer could be seen for women with MS treated with immunosuppressive therapy. Interestingly, a Swedish study reported lower overall cancer risk in persons with MS, compared with the general population, except for brain tumours and cancers of the urinary tract.[151] For the increased risk of brain tumours, surveillance bias related to frequent brain imaging could have a role. Lower cancer incidence in MS patients compared with matched controls was also seen in a Canadian study.[152] However, larger tumour sizes at diagnosis suggested diagnosis neglect, possibly contributing to the lower incidence. Collectively, the evidence suggests that MS in itself is not associated with an increased risk of cancer compared with the general population, with the possible exception of certain specific cancer types found in the brain and urinary tract, as well as breast cancer in women with MS.

1.4.1 Disease-Modifying Therapies and Cancer

Few studies compare the risk of cancer between modern MS DMTs in large real-world cohorts. Most existing evidence comes from the respective DMTs clinical trials, or smaller studies and case reports, with evident limitations to compare across therapies.[153] Comparative studies are important to allow patients and physicians to balance the risks and benefits when choosing and prescribing treatment. Study III was the first large, comparative study of cancer risk for MS DMTs, comparing rituximab, fingolimod, and natalizumab (Section 4.3).

Rituximab

In contrast to fingolimod and natalizumab, which are mainly used in MS, rituximab has been used extensively for other indications where the risk of cancer has been studied. Of particular interest are the studies from rheumatoid arthritis, even though rituximab is often combined with methotrexate and used with different dosing regimens than is used for MS. For rituximab in rheumatoid arthritis, a long-term (9.5 years) follow-up of the global clinical trials programme found no increased risk of cancer compared with placebo + methotrexate and no increasing risk of cancer with cumulative exposure to rituximab. It also found no increased risk of cancer for rituximab, when compared with other rheumatoid arthritis populations from observational studies and standardized incidence rates in the general population.[154] In a large observational study, no increased risk of cancer was found for rituximab, when compared with several newer biological rheumatoid arthritis drugs.[155] In the context of MS, one of the randomized controlled trials for ocrelizumab, an anti-CD20 depleting therapy similar to rituximab (Section 1.2.10), reported a higher number of breast cancers in the active arm, compared with placebo.[79] Given the similarity between ocrelizumab and rituximab, a higher incidence of breast cancer could be suspected also for rituximab. However, the incidence rate of breast cancer in the rituximab group of Study III was similar to that of the other therapy groups, both before and after controlling for confounders. It was also lower than the incidence rate reported in the ocrelizumab trial (Section 4.3).

Natalizumab

For natalizumab, case reports have suggested a link to melanoma[156] and an association with primary central nervous system lymphoma,[157] but the evidence is weak. The original clinical trials for natalizumab reported a comparable number of cancers between the active and control groups,[46,47] and the post-marketing surveillance also gives no indication of any increase in malignancy risk.[153] In Study III, natalizumab had the overall lowest cancer rate of the therapy groups, comparable to the general population (Section 4.3), which is in line with the previous evidence.

Fingolimod

Case reports of fingolimod have raised concerns primarily regarding cancer of the skin, but also cancer of the lung, brain, as well as the hematopoietic and lymphatic systems.[153] The initial trial programme for fingolimod reported no difference in overall cancer risk, but a higher number of skin cancers and basal cell carcinomas compared with placebo and interferon-beta.[50–52] Long-term follow-up reported increased risks of both basal cell carcinomas and overall malignancies in the continuous fingolimod group, compared with the interferon-switch group.[158] A later trial reported more malignancies and basal cell carcinomas on fingolimod compared with placebo.[53] Both the FDA label and European Medicines Agency (EMA) product information for fingolimod include lymphoma as a possible adverse effect based on data from pre-marketing clinical trials, and the EMA further includes a warning regarding cutaneous malignancies. Taken together, the results from the clinical trials and their long-term follow-up indicate a higher incidence of cancer on fingolimod compared with both placebo and interferon. In Study III, fingolimod was associated with an increased risk of invasive cancer, compared with both rituximab and the general population (Section 4.3).

The tumourigenicity of fingolimod has also been studied in the lab setting, where sphingosine-1-phosphate signalling has been implicated in the growth of different forms of tumours, but has also been shown to inhibit metastasis in a mouse melanoma model.[159] Fingolimod was even investigated as a potential anti-tumour drug, with positive effects in some pre-clinical cancer models.[160] Fingolimod may affect tumourigenicity not only through direct effects exerted on cancer cells, but also by impeding tumour surveillance by the immune system. In fact, its potential clinical use as an anti-cancer drug has been questioned based on its immunosuppressive effects, including inhibition of random migration, cytotoxicity, and tumour infiltration of activated T cells in mouse models,[160] as well as inhibition of regulatory T cell proliferation.[161] Thus, the potentially beneficial effects of fingolimod mediated through inhibition of sphingosine-1-phosphate signalling in the tumour may be offset by its suppression of anti-tumour directed immune responses.

In particular, fingolimod's systemic effects on both CD4 and CD8 T cells clearly differs from the consequences of treatment with natalizumab or rituximab, which inhibits the transmigration of immune cells specifically across the blood brain barrier and targets the B cell compartment, respectively.

1.5 Economic Impact and Cost-Effectiveness

Treatment of MS comes at a substantial economic cost, exceeding those of other common neurological conditions, and high also compared with other chronic inflammatory conditions, such as rheumatoid arthritis and psoriasis.[162] In fact, it is the second most costly chronic disease, behind only heart failure.[163] Work capacity declines with advancing disability, resulting in an increase in societal costs.[164] In the US, direct annual healthcare costs for MS average around \$70 000 per patient, with DMT costs for Medicaid almost tripling between 2011 and 2017, to a total of \$1.3 billion.[165,166] This creates a heavy financial burden for both the healthcare system and the person with MS. In Sweden, the mean annual societal cost per patient has been estimated to 244 000 SEK (61% healthcare), 384 000 SEK (45% healthcare), and 888 000 SEK (13% healthcare) for mild, moderate, and severe MS, respectively.[167]

The high costs of MS treatment have put the cost-effectiveness of many DMTs into question and MS patients in the US have reported that out-of-pocket costs are of greater concern than even treatment effectiveness or adverse events.[168] This is corroborated by out-of-pocket costs being associated with decreased adherence to treatment.[169–171] Despite this, neurologists in the US report that they have no available low-cost treatment options to offer their MS patients.[166]

Healthcare systems in wealthy countries are economically strained by the use of MS DMTs, possibly leading to barriers for access to more expensive and effective DMTs. These costs are even more apparent in resource-limited countries, as they might not be able afford highly effective DMTs at all.[172,173] However, the repurposing of drugs approved for other indications may offer cost-effective alternatives. In MS, this primarily concerns the increasing off-label use of rituximab (Section 1.2.7), the most common treatment for MS in Sweden. Compared with MS-approved DMTs, rituximab carries a substantially lower drug price and there is some experience with using rituximab from resource-limited contexts.[174] The price of rituximab was further reduced after 2017, as the patent expired and biosimilars entered the market. The impact of using rituximab instead of MS-approved DMTs on longer-term healthcare costs, including those associated with adverse events, has not previously been assessed. A potential alternative solution to lower the costs of MS treatment is the use of generics/biosimilars of therapies other than rituximab for which patents have expired, however few are currently available (Section 1.2.13).

In a structured PubMed search for studies assessing the cost-effectiveness of MS therapies, only a single study of rituximab was found,[175] in which rituximab was reported to be a cost-effective alternative to natalizumab. However, this was based on a simulation in a small sample rather than real-world data, limiting the interpretability of the results. Most cost-effectiveness research also for other MS DMTs have used Markov-Chain simulation, extrapolating costs from short-term trial data with few participants, which leads to high uncertainty in long-term estimates. The few real-world studies that exist generally suffer from limitations in data availability due to healthcare information being scattered across different regions and/or insurers. These limitations have led to contradictory results and difficulty to determine the relative cost-effectiveness of different treatment alternatives for MS. The lack of cost-effectiveness studies comparing rituximab to MS-approved alternatives was identified as an important issue in the 2022 MS International Federation's recommendations, which were made conditional due to the "very low certainty of the evidence".[176] This was addressed in Study V (Section 4.5), where treatment with rituximab was associated with fewer relapses and considerably lower costs, compared to MS-approved alternatives, highlighting rituximab as an attractive low-cost, highly effective treatment for MS.

The cost-effectiveness aspect of MS treatment is becoming increasingly important as new and often more expensive therapies for MS keep entering the market. Studies investigating the cost-effectiveness of rituximab compared with the MS-approved anti-CD20 therapies ocrelizumab and ofatumumab are needed. This was not addressed in Study V, as few patients receive these newer therapies in Sweden. Further studies are also needed in resource-limited settings where the margins for healthcare costs are much lower.

Chapter 2

Literature Review

A structured literature review of the use of rituximab for the treatment of MS was conducted by searching PubMed[177] with the query:

`"rituximab"[MeSH Terms] AND "multiple sclerosis"[MeSH Terms]`

Review articles were scanned for any studies missed in the PubMed search. All clinical trials of rituximab in MS were included, together with observational studies focused on treatment with intravenous rituximab in adult RRMS patients. Ongoing trials of rituximab in MS were identified at Clinicaltrials.gov.[178] Finally, the currently available evidence for rituximab as a treatment for MS was summarized.

The focus on rituximab was due to its widespread off-label use in Sweden and because the state of the evidence for rituximab in the MS setting is less clear than for the MS-approved therapies.

2.1 Clinical Trials

Bar-Or 2008[121] An open-label, uncontrolled trial of 26 RRMS patients, followed over 72 weeks, evaluating the safety and tolerability of 1000 mg rituximab every six months. Adverse events included mild to moderate infections and infusion-related events, which decreased with later infusions. No serious adverse events were reported. Compared with the year prior to rituximab start, there was a reduction in relapses and fewer contrast-enhancing and T2 lesions were found on MRI from week four through week 72. (Sponsored by Biogen and Genentech)

Hauser 2008[93] HERMES A double-blind, randomized controlled trial of 104 RRMS patients, followed over 48 weeks, evaluating the effectiveness of 2000 mg rituximab divided in two doses two weeks apart, compared with placebo. Patients on rituximab had fewer contrast-enhancing lesions and a lower proportion with relapses throughout the study period. However, more patients in the rituximab group reported infusion-related adverse events, most of which were mild to moderate and decreased for the second infusion. (Sponsored by Biogen and Genentech)

Hawker 2009[94] OLYMPUS A double-blind, randomized controlled trial of 439 PPMS patients, followed over 96/122 week, evaluating the effectiveness and safety of 1000 mg rituximab every six months, compared with placebo. No clear difference in confirmed disease progression or brain volume change was seen between the groups. Patients on rituximab had a smaller increase in T2-lesion volume. In a subgroup analysis of younger patients with more active disease, rituximab was found to delay the time to confirmed disease progression. The proportion of patients reporting serious adverse events were similar between the groups, but those treated with rituximab experienced more serious infections and a greater number of infusion-related adverse events, most of which were mild to moderate and decreased with later infusions. (Sponsored by Biogen and Genentech)

Naismith[179]/Piccio[17] 2010 An open-label, uncontrolled trial of 30 RRMS patients with breakthrough disease on previous therapy, followed over 20/52 weeks (MRI/scores), evaluating the effectiveness of 1500 mg rituximab divided in four infusions over one month. Patients had fewer contrast-enhancing lesions and improved MSFC scores as compared with baseline, and EDSS scores remained stable. Additionally, the study found that B cells were reduced or undetectable and T cells were fewer in the cerebrospinal fluid of the patients after the administration of rituximab, leading the authors to suggest that B cells are critical for the migration of T cells through the blood-brain barrier.

de Flon 2016[180]/2017[181] An open-label, uncontrolled trial of 75 RRMS patients with previous injectable treatment, followed over 24 months, evaluating the effectiveness of 2000 mg rituximab divided in two doses, two weeks apart. Patients had fewer contrast-enhancing lesions, fewer new or enlarged T2 lesions, and reduced levels of neurofilament in the cerebrospinal fluid, compared with the three months run-in period between stopping the injectable treatment and starting rituximab. Additionally, the study found that patients rated their treatment satisfaction higher after switching to rituximab, both at year one and two.

Honce 2019[95] GATEWAYII A double-blind, randomized controlled trial of 55 RRMS patients, followed over three years, evaluating the effectiveness of 2000 mg rituximab divided in two doses two weeks apart as an induction therapy followed by treatment with glatiramer acetate, compared with placebo followed by glatiramer acetate. Patients having received rituximab were less likely to demonstrate evidence of disease activity and had fewer new lesions on MRI. However, the effects of the rituximab induction seemed to fade within the study period.

Cheshmavar 2021[96] An open-label, randomized controlled trial of 84 SPMS patients, followed over 12 months, evaluating the effectiveness and safety of 1000 mg rituximab every six months, compared with glatiramer acetate. No clear differences in EDSS progression, active lesions on MRI, or relapse rate were seen between the groups.

Disanto 2021[182] An open-label, uncontrolled trial of 59 patients (37 RRMS, 22 SPMS), followed over 12 months, evaluating the effectiveness and safety of de-escalating from 1000 mg to 500 mg rituximab, both given every six months. Patients remained stable in their MS on the reduced dose, with no relapses, change in EDSS, or difference in neurofilament levels. An association was seen between greater rituximab load and low levels of immunoglobulin G, with higher risk for infection as a result.

Svenningsson 2022[97] RIFUND-MS A rater-blinded, randomized controlled trial of 200 RRMS patients, followed over 24 months, evaluating the effectiveness and safety of 1000 mg/500 mg rituximab (first dose/subsequent doses) every six months, compared with dimethyl fumarate. Patients in the rituximab group had fewer relapses, but a greater number of mild to moderate infusion-related adverse events. No major safety concerns were reported.

2.2 Observational Studies

Rommer 2016[183] Nationwide (Germany), register study of 56 patients (16 RRMS) treated with rituximab, evaluating safety (mean follow-up 10 months). Mild infusion-related reactions were common and infections were seen in three patients, of which two had to be hospitalized.

Barra 2016[184] Single-centre, retrospective study of 107 patients (54 RRMS) treated with rituximab, evaluating effectiveness and safety (mean follow-up 33 months). Three patients had relapses or lesions on MRI, despite undetectable B cell levels. Mild infusion-related reactions were common and three patients were hospitalized for urinary tract infections.

Salzer 2016[142] Multi-center, retrospective/register study of 822 patients (557 RRMS) treated with rituximab, evaluating effectiveness and safety (mean follow-up 22 months). Annualized relapse rates were low and few patients had contrast-enhancing lesions on MRI. For the patients with RRMS, EDSS remained stable. Mild infusion-related reactions were common and a total of 89 grade-2 adverse events (mostly infections) were recorded in 72 patients. No cases of PML. (I am a co-author)

Spelman 2017[185] Nationwide (Sweden), register, active-comparator study of 1383 RRMS patients (461 rituximab, 922 matched injectables), evaluating effectiveness. Patients on rituximab had a lower relapse rate, better drug survival, and a slight reduction in EDSS, compared with the matched injectables group.

Granqvist 2018[186] Multi-centre, register, active-comparator study of 494 newly diagnosed RRMS patients (120 rituximab, 50 natalizumab, 17 fingolimod, 86 dimethyl fumarate, 215 injectables, 6 other), evaluating effectiveness and drug survival. Treatment with rituximab was associated with fewer relapses and fewer lesions on MRI, compared with injectables and dimethyl fumarate. This effect was also noticeable, but less apparent, when comparing rituximab to natalizumab and fingolimod. Drug survival was better for rituximab compared with all the other groups.

Alcala 2018[187] Multi-centre, retrospective study of 90 patients (31 RRMS) treated with rituximab, evaluating effectiveness and safety. After starting rituximab, patients had reduced annualized relapse rates and during the first year, a small decrease in EDSS was observed. Mild infusion-related reactions were common and three thrombotic events were detected.

Alldredge 2018[188] Single-centre, retrospective study of 64 patients (23 RRMS) treated with rituximab, evaluating effectiveness and safety. Most patients with RRMS were reported to be stable on rituximab. Mild infusion-related reactions were common and one patient chose to stop rituximab treatment due to multiple adverse events.

Scotti 2018[189] Single-centre, retrospective, active-comparator study of 82 patients (43 RRMS) treated with rituximab, evaluating effectiveness and safety. Time to evidence of disease activity was similar between rituximab and a comparator group of 83 natalizumab patients (although with wide confidence intervals). A third of rituximab-treated patients suffered infectious adverse events, of which six required therapy discontinuation.

Yamout 2018[190] Single-centre, retrospective study of 89 patients (59 RRMS) treated with rituximab, evaluating effectiveness and safety. After starting rituximab, patients with RRMS had reduced annualized relapse rates and fewer lesions on MRI, while EDSS remained stable, compared with before treatment start. Mild infusion-related events were common and two patients had serious adverse events requiring surgical intervention, a case of pyoderma gangrenosum vaginalis and an increase in the size of a meningioma. No cases of PML were reported.

Durozard 2019[191] Nationwide (France), retrospective study of 50 RRMS patients treated with rituximab after disease breakthrough on previous DMT, evaluating effectiveness. After switching to rituximab, patients had reduced annualized relapse rates and fewer contrast-enhancing lesions, compared with before the switch.

Alcala 2019[192] Multi-centre, retrospective, active-comparator study of 55 RRMS patients switching to either rituximab (27) or alemtuzumab (28) from fingolimod, evaluating effectiveness and safety (mean follow-up 28.8 months). After switching, patients in both groups showed similar reductions in annualized relapse rates and EDSS. Also, the number of patients with no evidence of disease activity and the occurrence of infusion-related adverse events were similar between rituximab and alemtuzumab groups.

Boremalm 2019[193] Multi-centre, retrospective/register, active-comparator study of 241 RRMS patients (48 rituximab, 105 natalizumab, 55 fingolimod), switching from injectables due to disease breakthrough, evaluating effectiveness and drug survival. Patients on rituximab had a similar risk of relapse as those on natalizumab, and lower than patients receiving fingolimod. Rituximab had better drug survival than both comparators.

Luna 2019[143] Nationwide (Sweden), register, active-comparator study of 6421 RRMS patients (3260 rituximab, 1588 natalizumab, 1535 fingolimod, 2217 injectables), evaluating infection risk. The risk of infection was generally increased for patients with MS, but varied with treatment. After controlling for group differences, the older injectable therapies had the lowest rate of infection, while rituximab had the highest among the newer treatments. However, the use of antiherpetic drugs was lower for rituximab compared with the newer MS-approved alternatives. Importantly, no deadly infections were reported in any of the groups and only one case of PML was recorded in the rituximab group (and one in the fingolimod group), which was consistent with carry-over disease from previous natalizumab treatment. (I am a co-author)

Vollmer 2020[194] Single-centre, retrospective, active-comparator study of 1246 patients (1004 RRMS, 182 rituximab, 451 natalizumab, 271 fingolimod, 342 dimethyl fumarate), evaluating effectiveness. Patients on rituximab had similar disease activity (composite score) and drug survival as patients on natalizumab, but was superior compared with the fingolimod and dimethyl fumarate groups.

Airas 2020[195] Single-centre, retrospective study of 72 patients (31 RRMS) treated with rituximab, evaluating effectiveness and safety. After starting rituximab, the RRMS patients had fewer relapses and contrast-enhancing lesions on MRI. No serious infusion-related reactions were reported, but one patient had to discontinue treatment due to neutropenia.

Bellinvia 2020[196] Single-centre, retrospective study of 69 patients (53 RRMS) treated with rituximab, evaluating effectiveness (mean follow-up 16 months). After starting rituximab, patients had fewer relapses and less activity on MRI, compared with before treatment start.

Mathew 2020[174] Multi-centre, retrospective study of 80 patients (58 RRMS) treated with rituximab, evaluating effectiveness (median follow-up 24 months). After starting rituximab, RRMS patients had no relapses and improved in EDSS, compared with before treatment start. No opportunistic infections or malignancies were reported.

Mazdeh 2020[197] Single-centre, retrospective/prospective study of 70 RRMS patients treated with rituximab, evaluating effectiveness (follow-up 18 months). After starting rituximab, patients had fewer relapses compared with before treatment start, although EDSS remained stable. Mild infusion-related reactions were common for the first infusion.

Zecca 2020[198] Multi-centre, retrospective study of 355 patients (188 RRMS) treated with rituximab, evaluating effectiveness and safety. After starting rituximab, patients with RRMS had reduced annualized relapse rates than before the treatment, and after three years the proportion with EDSS progression remained low. No major safety concerns were reported.

Boremalm 2021[199] Single-centre, retrospective study of 225 RRMS patients treated with rituximab, evaluating effectiveness after dose reduction or therapy discontinuation (mean follow-up 6.5 years). After reducing or ending treatment with rituximab, there were no differences in annualized relapse rates or MRI activity during the study follow-up.

Hou 2021[200] Single-centre, register study of 846 patients (unknown proportion RRMS, 115 rituximab, 204 natalizumab, 267 fingolimod, 260 dimethyl fumarate), evaluating effectiveness (mean follow-up 3.7–7 years). After controlling for group differences, rituximab was associated with fewer relapses compared with natalizumab (rituximab was not compared with fingolimod or dimethyl fumarate).

Perriguey 2022[201] Single-centre, retrospective study of 188 patients (151 RRMS) treated with rituximab, evaluating hypogammaglobulinemia and risk of infection (median follow-up 3.5 years). After starting rituximab, patients had reduced levels of immunoglobulin G, as expected, which correlated with an increased risk of infection. During the study period, 13 severe infections were recorded.

Bribiesca-Contreras 2022[202] Single-centre, retrospective study of 85 patients (73 RRMS) treated with rituximab, evaluating effectiveness (mean follow-up 6.65 years). After starting rituximab, patients had decreased annual relapse rates, lesions on MRI, and EDSS. Only two patients had mild infusion-related reactions and no serious adverse events were reported.

2.3 Ongoing Research

NOR-MS (NCT04121403) An open-label, blinded-endpoint, randomized controlled trial, aiming to test non-inferiority of rituximab compared with cladribine, over eight months. Main outcome is proportion of patients with no new or enlarging lesions on MRI from week 12 to week 96, with secondary outcomes including several efficacy and safety endpoints. The study has a special focus on specific blood and MRI biomarkers for use in personalized MS care and the health-economic consequences of the two therapies. (Norway, estimated primary completion in July 2024)

RIDOSE-MS (NCT03979456) A rater-blinded, randomized controlled trial, aiming to compare two dosing regimens of rituximab, 500 mg twice vs once a year, over four years. Main outcome is proportion of patients with no evidence of disease activity (relapses, MRI lesions, EDSS progression) from year two to year four, with secondary outcomes including several efficacy and safety endpoints. (Sweden, estimated primary completion in June 2025)

OVERLORD-MS (NCT04578639) A double-blind, randomized controlled trial, aiming to test non-inferiority of rituximab compared with ocrelizumab over 30 months. Main outcome is proportion of patients with no new or enlarging lesions on MRI from month six to month 24, with secondary outcomes including several efficacy and safety endpoints. (Norway, estimated primary completion in February 2025)

DanNORMS (NCT04688788) A double-blind, randomized controlled trial, aiming to test non-inferiority of rituximab compared with ocrelizumab over 24 to 36 (extension) months. Main outcome is proportion of patients with no new or enlarging lesions on MRI from month six to month 24, with secondary outcomes including several efficacy and safety endpoints. (Denmark, estimated primary completion in April 2025)

2.4 Summary

The results from the first clinical trials of rituximab in MS appeared in 2008/2009, sponsored by Biogen and Genentech.[93,94,121] These studies demonstrated that rituximab was well tolerated and effective in limiting inflammation in patients with active MS, although results for progressive MS were less positive. Despite these promising reports, the market authorization holder chose to shift their focus away from rituximab to the newer anti-CD20 antibody ocrelizumab. In the years that followed, several smaller trials, most of which were open-label and uncontrolled, continued to report positive results for rituximab in active MS,[17,179–181] while one trial which focused entirely on patients with SPMS found no benefit of rituximab.[96] Other trials focused on specific aspects of treatment. Rituximab as an induction therapy was found to have initial positive effects that seemed to fade over time when no new infusion was given,[95] and de-escalation of rituximab from 1000 mg to 500 mg biannually did not result in return of disease, and is likely preferable from a safety perspective.[182] It would take around a decade since the initial trials were reported until there was another large randomized controlled trial for rituximab in MS, RIFUND-MS. This trial was at the initiative of academia, without any industry sponsors, and showed, as expected, that rituximab is an effective and safe treatment for patients with active MS.[97]

Apart from the clinical trials, many observational studies have also been published based on different populations and registers. Many of these have been small and without an active comparator,[174,183,187,188,190,191,195–197,202] but a few have had large cohorts[142,184,198,199,201] and/or an active-comparator design,[143,185,186,189,192–194,200] including Study I, III, and V of this PhD project.

Taken together the evidence suggests that rituximab is an effective, safe, and well tolerated treatment for active MS, primarily for RRMS, although with some apparent effect also in patients with progressive disease, as long as there is an active inflammatory component. The most common adverse events are mild infusion-related reactions that become less likely with additional infusions, but there is also an increased risk of infections, including severe infections requiring hospitalization. Compared with other MS DMTs, rituximab is more effective than injectables, dimethyl fumarate and fingolimod, and have similar or slightly greater effectiveness than natalizumab. The effect of rituximab is mostly constrained to the prevention of relapses and MRI activity, while EDSS is largely unaffected, at least in the short- to medium-term.

Chapter 3

Methods

Epidemiology started as a field of science in the 19th century, focused on studying infectious diseases and epidemics. Since then, the field has evolved to also include non-communicable diseases, with the central principles and methods of epidemiology formalized in the later part of the 20th century. Because of the evolution of the field, there are many definitions of what epidemiology is. Although there is some consensus today, no canonical definition exists.[203] The Oxford Reference *A Dictionary of Epidemiology*[204] defines epidemiology as:

> The study of the occurrence and distribution of health-related events, states, and processes in specified populations, including the study of the determinants influencing such processes, and the application of this knowledge to control relevant health problems.

In other words, epidemiological research investigates diseases and other health events in different populations, including what causes them and what can be done to prevent or treat them. To achieve this, good study design (Section 3.1), high-quality data (Sections 3.2 and 3.3), and sound statistical methods (Sections 3.4–3.13) are needed.

3.1 Study Design

A clinical study can be designed in many different ways depending on the context, available resources, and the research question of interest. Broadly, clinical study designs can be divided into *experimental* and *observational*. Experimental clinical studies are those involving an intervention controlled by the investigators, where the aim is to assess the efficacy, safety, or other effects of this intervention. In observational studies, there is no intervention by the investigators on the participants of the study, instead one or more groups of participants are observed with the aim to describe or identify differences between the groups.

Experimental clinical studies, also called clinical trials, are said to be *controlled* if the group of interest is compared with a reference group and *randomized* if the allocation to the groups is done through randomization. Because of its ability to remove confounding bias through randomization, the *randomized controlled trial* is the gold standard in clinical research on interventions. However, randomized controlled trials are often unfeasible due to limited resources, ethical concerns, or other practical restrictions. In these cases, we must instead rely on observational designs (Section 3.2). Common types of observational study designs include *case-series*, *cross-sectional*, *case-control*, and *cohort* studies.

Case-Series A simple design where investigators follow a small group of participants, take detailed records of each participants clinical experience, and aggregate these using descriptive statistics.

Cross-Sectional Also known as a prevalence study, it investigates a population at a specified time point, often with the aim to find the prevalence of a disease or other conditions.

Case-Control Two groups of participants having experienced different outcomes are identified and data on their previous exposures collected. These data are then used to differentiate the two groups with the goal to identify possible causes of the outcome. Case-control studies are less resource-intensive than cohort studies, especially for rare outcomes, but the often-retrospective nature of the collected data increases the risks of bias. Results are often reported as odds ratios, but if incidence density sampling is used (Section 3.12) other types of statistics are also available.

Cohort One or more groups of interest are identified and followed over time to identify differences in conditions or events between the groups. In contrast to case-control studies, a cohort design follows the participants from the time of inclusion, identifying any events of interest from that point onwards. Different types of statistics can be reported.

Study I, III, IV, and V were all cohort studies with prospectively collected register data, comparing groups of patients starting different MS DMTs, while Study II was a descriptive study assessing the data of the Swedish MS Register.

Recently, there has been an increasing focus on observational clinical studies using *real-world data*, prospectively collected in the clinical setting.

3.2 Real-World Data and Evidence

Real-world data and real-world evidence are terms that have become widely used in recent years, and although there is no universally accepted definition, it generally refers to data and evidence generated from sources other than traditional clinical trials.[205] These alternative sources are often aggregates of data collected in clinical practice, such as electronic health records, healthcare and demographic registers, and claims and billing databases.

The same characteristics that make the traditional randomized controlled trials the gold standard in medical research (Section 3.1), can also make them less generalizable, as the studied populations often differ from those encountered in clinical practice. This is especially true for niche, but clinically important patient groups, such as MS patients with aggressive disease that have to switch from natalizumab due to the risk of PML, as in Study II (Section 4.2). Furthermore, randomized controlled trials rarely include data on interactions with concomitant therapy or disease. The high cost of clinical trials also limits their size and follow-up time, making them less suitable for studying rare or long-term outcomes, such as cancer as in Study III (Section 4.3). Sometimes it is not even possible or ethically defensible to randomize patients to receive a specific exposure (e.g. smoking or environmental hazards such as asbestos), which makes observational real-world data the only option for studying these.

Because of the limitations of randomized controlled trials, together with methodological advancements in the design and analysis of observational studies, real-world evidence has an increasingly important role in medical research. However, it is important to acknowledge that real-world studies come with their own difficulties concerning data quality, the risk of confounding, and other methodological challenges. Real-world evidence is never better than the data it is based on. Thus, it is important to assess the quality of this data (Study II) and to have insight into how it was collected and processed.

3.3 Data Sources

Good-quality data are a prerequisite for good-quality epidemiological studies. Fortunately, Sweden has an excellent repertoire of health and demographic registers available for researchers. The main health registers (the Patient Register, the Prescribed Drug Register, and the Cancer Register, among others) are governed by the *National Board of Health and Welfare* (Socialstyrelsen), while the demographic registers are held by *Statistics Sweden* (Statistikmyndigheten SCB) and additional registers on reimbursements by the *Swedish Social Insurance Agency* (Försäkringskassan). In addition to the national registers with mandatory participation, there are several "quality registers" (kvalitetsregister) for specific diseases or topics maintained by individual organizations, such as the Swedish MS Register by *Svenska Neuroregister*.

Every register is by itself a valuable source of information, but the true power of the registers comes from linking them together through the Swedish personal identification number (personnummer), a unique identifier of every resident in Sweden. Combining data in this way allows for broader research questions and more robust confounder control. These registers have been instrumental in all the studies of this PhD project and represent an enormous effort by register holders and individual government and healthcare workers to gather, structure, and make the data available for research.

3.3.1 The Swedish Multiple Sclerosis Register

The Swedish MS Register is a publicly funded nationwide register collecting prospective data on MS patients in Sweden.[206] It was introduced in 2000 and has since become an integral part of the clinical documentation at Sweden's neurological clinics and a core part of MS research, providing data for over 180 scientific reports to date.[9] Data in the MS Register are recorded by physicians and nurses and include MS diagnosis, onset, and type, as well as therapies, visits, clinical scales, relapses, MRI reports, and lab tests.

Although participation is voluntary, coverage is estimated to around 80% of the Swedish MS population. In random sampling of the patients in the register at around 15 different neurological clinics, no erroneous diagnoses of MS were found compared with clinical charts.[9,206,207] In Study II, we found that data were of generally good quality, especially for therapies, but with some issues of missingness for infusions, MRIs, and relapses (Section 4.2). A later (2021) validation of the MS Register in one region of Sweden found no erroneous diagnosis of MS, but a slightly lower coverage (71%) than the estimated national average.[208]

3.3.2 The Patient Register

Inpatient visits to public hospitals have been recorded in the Patient Register since 1964.[209] Registration became mandatory in 1984 and included all in-patient care by 1987. From 2001 the register also covers outpatient visits to a physician, including day surgeries and psychiatric care, from both public and private healthcare providers. Primary care data is not available in the register, but there are efforts to also include this. Diagnoses in the patient register are recorded as International Classification of Diseases (ICD) codes, since 1997 ICD-10.

The register is updated every month with continuous quality control. Data are generally of good quality and underreporting is low for recent years, but some types of information have had to improve over time (e.g. time points for emergency visits, involuntary psychiatric care, and forensic psychiatric care). An external review in 2011 found that the validity of the register was high for most, but not all diagnoses (positive predictive value generally 85%–95%).[210] The authors speculated that the proportion of valid diagnoses were likely higher for patients with severe disease or related complications, compared with those with milder disease and no complications. They also identified "diagnostic drift" due to financial incentives to register certain diagnoses to increase reimbursement as a risk to the validity of the data, although quantification of this issue is difficult.

3.3.3 The Prescribed Drug Register

The National Prescribed Drug Register was established in 2005 and contains data on all prescription drugs collected at pharmacies in Sweden, as well as for dispensed medical devices and medical consumables within the pharmaceutical benefits scheme.[211,212] However, pharmacological treatment in inpatient care or nursing homes, as well as over-the-counter medications and vaccines, are not included. Drugs in the prescribed drug register can be identified through their Anatomical Therapeutic Chemical Classification System (ATC) codes. The register is updated every month with continuous quality control and as the processes are largely automated and tied to the pharmacies' reimbursements, there is virtually no missing data.

3.3.4 The Cancer Register

The National Cancer Register was founded in 1958 and collects data on every newly detected cancer diagnosed in clinical, morphological, or laboratory examinations, as well as those diagnosed at autopsy.[213] The register is constructed as six regional registers, tied to the oncology centres in each region. Cancers can be identified through different coding schemes, including ICD-7, ICD-9, ICD-O/2, ICD-O/3, and WHO/HS/CANC/24.1. Stage is available from 2004, coded according to FIGO for gynaecological tumours and TNM for the rest (not recorded for cancers in the brain or cranial nerves, lymphoma, or leukaemia).

The register is updated annually with continuous quality control. An external validation for the year 1998, found that the overall completeness was high with low levels of underreporting, although the missing data seemed to vary with patient characteristics which could pose a problem for specific research questions.[214] Furthermore, since the cancer register does not accept reports from death certificates, underreporting is highly dependent on the lethality of the cancer, meaning that, for example, breast cancer cases are mostly complete while pancreatic and lung cancers will be missing to a greater extent.[215]

3.3.5 Demographic Registers

Statistics Sweden (Statistikmyndigheten SCB) holds data on the entire Swedish population, including births, deaths, emigration, immigration, age, sex, country of birth, and citizenship, among others.[216] Data have high completeness with close to 100% of births and deaths, 95% of immigrations, and 91% of emigrations, within 30 days of the event and higher proportions over time.[217] Further data on education, income, occupation, and reimbursements is available in Statistics Sweden's Longitudinal Integrated Database for Health Insurance and Labour Market Studies (LISA) database. Reimbursements include sick leave, disability pension, unemployment benefits, and social welfare payments. Data have overall high quality, with 95% completeness for occupation and 98% for education, and 85% estimated accuracy for education.[218]

3.4 Population, Sample, and Variables

Epidemiological studies generally infer information about a *population* by measuring *variables* in a *sample* from that same population. A population is a set of entities that can be existing and finite (every person with MS) or hypothetical and potentially infinite (every person that will ever have MS). A sample is a subset of a population, chosen to represent the entire population in analyses, as gathering information about the whole population might not be feasible or even possible.

The variables measured are statistically modelled as *random variables* quantifying the observations of conceptual random processes, and can be continuous (any value in an interval) or discrete (distinct values). The probability of observing a specific value for a random variable is described by its *probability distribution*. A probability distribution is a mathematical model, defined by its *probability density function* (continuous) or *probability mass function* (discrete), describing the probability of observing a value in a specified interval (continuous) or set (discrete) of possible values. Probability distributions are generally specified by one or more *parameters*, affecting their central tendency (mean) and variability (standard deviation/variance).

One of the better-known probability distributions is the *Normal Distribution* ($\mathcal{N}$), which is defined by a probability density function (f) with two parameters, corresponding to the distribution's mean (μ) and variance (σ^2).

$$\mathcal{N}(\mu, \sigma^2) \qquad f(x) = \frac{1}{\sigma\sqrt{2\pi}} e^{-\frac{1}{2}\left(\frac{x-\mu}{\sigma}\right)^2} \tag{3.1}$$

Other common probability distributions include the *Binomial Distribution* for modelling the number of successes in a fixed number of independent trials and the *Poisson Distribution* for the number of occurrences of an event in a fixed interval of time or space.

Given a representative sample of a population of interest, we can observe values of random variables in the sample to *estimate* the parameters for the corresponding random variables in the population.

3.5 Estimation

To estimate the *population parameters* of the probability distribution for a random variable from a sample, we find the values of the corresponding *sample statistics* that best support the observed data.[a]

For example, we might be interested in describing the height of all people living in Sweden. The height (H) can be modelled as a random variable with an assumed normal distribution $H \sim \mathcal{N}(\mu_H, \sigma_H^2)$, whose values we can observe by measuring the heights in a sample of people from Sweden (Table 3.1). Based on the observations in the sample, we want to estimate the population parameters μ_H and σ_H^2, describing the populations mean and variance, with the sample statistics θ_{h0} and θ_{h1}.

[a]A population parameter is a characteristic of a population, while a sample statistic is a characteristic of a sample.

Table 3.1: Example data showing observed values of the modelled random variables height and weight from a sample ($n = 10$) of the population of people living in Sweden

i	h_i (cm)	w_i (cm)
1	177	77
2	160	89
3	174	109
4	144	48
5	181	117
6	147	71
7	170	105
8	168	66
9	158	58
10	213	131

$$\begin{aligned} \theta_{h0} &\approx \mu_H \\ \theta_{h1} &\approx \sigma_H^2 \end{aligned} \tag{3.2}$$

In this simple example, the values of θ_{h0} and θ_{h1} that best support the observed data ($n = 10$) are the sample mean and unbiased sample variance.

$$\begin{aligned} \hat{\theta}_{h0} &= \frac{\sum_{i=1}^{n} h_i}{n} = 169.2 \\ \hat{\theta}_{h1} &= \frac{1}{n-1} \sum_{i=1}^{n} (h_i - \theta_{h0})^2 = 386.8 \end{aligned} \tag{3.3}$$

Our estimate ("best guess") for the random variable height ($\hat{h}$) in the population of Swedish people, given the observed values in the sample, is then a normal distribution with mean 169.2 and variance 386.8.

$$\hat{h} \sim \mathcal{N}(\hat{\theta}_{h0} = 169.2, \hat{\theta}_{h1} = 386.8) \tag{3.4}$$

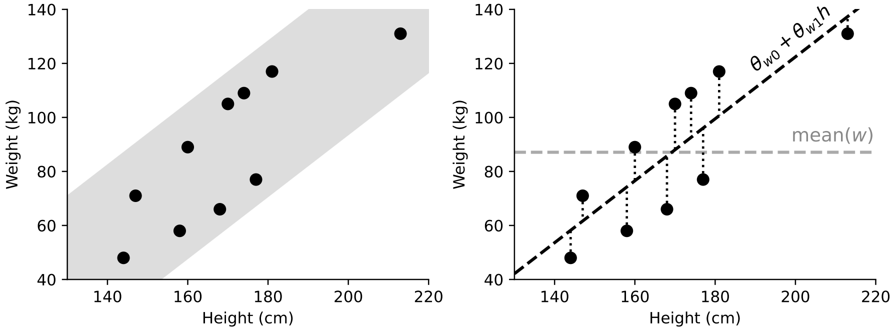


Figure 3.1: Scatter plot of weight and height, indicating that these variables are associated as taller heights correspond to heavier weights (left). The same scatter plot with displayed residuals and the regression line $\theta_{w0} + \theta_{w1}h$, and the unconditional sample mean shown as reference (right)

Assessing a single variable works well for descriptive statistics, but we are often more interested in investigating *associations* between variables. If we extend our example by also collecting information on weight in our sample (Table 3.1), we can estimate the association between weight and height. Expressed differently, we can find the best estimates of the population parameters for the weight distribution *conditional* on height. This is called the *conditional probability distribution* of weight given height, in contrast to the *marginal probability distribution* of weight independent of height (or any other variables). For the conditional probability distribution of weight, we could for example assume a linear increase or decrease in the mean value of the weight distribution by height, while assuming no association between height and the variance of weight.

$$\begin{aligned} \theta_{w0} + \theta_{w1}h &\approx \mu_W \\ \theta_{w2} &\approx \sigma_W^2 \end{aligned} \tag{3.5}$$

We can check the plausibility of this assumption by inspecting the scatter plot of height and weight to see if they appear to vary dependently (Figure 3.1, left).

One intuitive way to find the values of θ_{w0} and θ_{w1} that best support the observed data in this example is by minimizing the sum of squared residuals in what is called Ordinary

Least Squares regression. Put differently, we want to minimize the sum of the squared differences between the observed values of weight in our sample (w) and the *regression line* $\theta_{w0} + \theta_{w1}h$ (Figure 3.1, right).

$$\hat{\theta}_{w0}, \hat{\theta}_{w1} = \underset{\theta_{w0},\theta_{w1}}{\arg\min} \sum_{i=1}^{n} (w_i - (\theta_{w0} + \theta_{w1}h_i))^2 = -107, 1.15 \tag{3.6}$$

Where w_i and h_i are the individual observations of weight and height for participant i. The actual minimization is solved using matrix algebra, which is outside the scope of this text. As the variance of the weight distribution is not dependent on height, the best fit for the sample statistic θ_{w2} is the unbiased variance of the sample, as in the previous example. Our estimate for the random variable weight ($\hat{w}$) in the population of Swedish people conditional on height, given the observed values in the sample, is a normal distribution with mean varying with height and a variance of 756.

$$\begin{aligned} \hat{w}(h) &\sim \mathcal{N}(\hat{\theta}_{w0} + \hat{\theta}_{w1}h, \hat{\theta}_{w2}) \\ &= \mathcal{N}(-107 + 1.15h, 756) \end{aligned} \tag{3.7}$$

This can be expressed differently as the equation:

$$\hat{w}(h) = -107 + 1.15h + \epsilon_{\hat{w}} \qquad \epsilon_{\hat{w}} \sim \mathcal{N}(0, 756) \tag{3.8}$$

Where $\epsilon_{\hat{w}}$ denotes the normally distributed error around the expected value of the weight. Written in this form, it is easier to understand the interpretation of the estimated parameters and we can see that a 1 cm increase in height corresponds to an average increase of 1.15 kg ($\hat{\theta}_{w1}$) in weight.

$$\hat{w}(1) - \hat{w}(0) = -107 + 1.15 \times 1 + \epsilon_{\hat{w}} - (-107 + 1.15 \times 0 + \epsilon_{\hat{w}}) = 1.15 \tag{3.9}$$

In the more general case, the population parameters $\mathbf{\Theta} = \{\Theta_1, \Theta_2, \dots, \Theta_k\}$ of a probability distribution $\mathcal{D}$ for a random variable X can depend on any number of other random variables $\mathbf{Z} = \{Z_1, Z_2, \dots, Z_l\}$, in any combination of terms $f_k(\cdot)$, including polynomials and interaction terms. The estimation of the population parameters by finding the values of the sample statistics that best support the observed data is then often done with *maximum likelihood estimation* (Section 3.7).

$$X \sim \mathcal{D}(\mathbf{\Theta}) \qquad \Theta_1 = f_1(\mathbf{Z}), \Theta_2 = f_2(\mathbf{Z}), \dots, \Theta_k = f_k(\mathbf{Z}) \tag{3.10}$$

Apart from the *point estimate* of a sample statistic, it is also important to assess "how likely" this estimate is given the data. By correctly managing uncertainty, we can place the estimate in an interval of values within which we can be reasonably certain that the value of the population parameter lies (Section 3.8).

In cases where the goal is to estimate associations between variables, as in the example with weight and height above, *regression analysis* is a common method of choice.

3.6 Regression Analysis

In regression analysis,[b] the associations between a *dependent* random variable and one or more *independent* random variables are estimated. More generally, both the dependent and the independent random variables can have any probability distribution with any number of parameters. However, in practice, a limited number of typical regression models are used for the situations most common in epidemiological research. Frequently used regression models include the *generalized linear models* and the *Cox proportional hazards model*.

[b] The term regression comes from the early use of this statistical method in biology, where the *regression toward the mean* phenomenon was observed

3.6.1 Generalized Linear Models

In the generalized linear model, a dependent random variable Y is assumed to have a probability distribution $\mathcal{D}$ with the expected value (mean) defined by the independent random variables $\mathbf{X}$ through a *link function* $g(\cdot)$. The choices of probability distribution and link function depends on the type of dependent variable and the research question. Some of the combinations of distribution and link function are so common that they have their own names.

Linear Regression

Distribution: Normal
Link: Identity

$$\begin{aligned} Y &\sim \mathcal{N}(\mu, \sigma^2) & \mu &= \text{mean of Y} \\ g(\mu) &= \mathbf{X\Theta} & g(X) &= X \\ \mu &= \Theta_0 + \Theta_1 X_1 + \Theta_2 X_2 + \cdots + \Theta_k X_k \end{aligned} \tag{3.11}$$

Linear regression models the dependent variable as continuous and the parameters can be interpreted as the average difference in the dependent variable between levels of the independent variables.

$$\begin{aligned} \mu_{X_1=1} - \mu_{X_1=0} &= \Theta_0 + \Theta_1 \times 1 + \cdots + \Theta_k X_k - (\Theta_0 + \Theta_1 \times 0 + \cdots + \Theta_k X_k) \\ \mu_{X_1=1} - \mu_{X_1=0} &= \Theta_1 \end{aligned} \tag{3.12}$$

Logistic Regression

Distribution: Binomial ($n = 1$, i.e. one trial)
Link: Logit

$$\begin{aligned}
Y &\sim \mathcal{B}(1, p) && p = \text{probability of Y} \\
g(p) &= \mathbf{X\Theta} && g(x) = \ln\left(\frac{x}{1-x}\right) \\
\ln\left(\frac{p}{1-p}\right) &= \Theta_0 + \Theta_1 X_1 + \Theta_2 X_2 + \cdots + \Theta_k X_k && \frac{p}{1-p} = \text{Odds} \\
\text{Odds} &= \exp(\Theta_0 + \Theta_1 X_1 + \Theta_2 X_2 + \cdots + \Theta_k X_k)
\end{aligned} \tag{3.13}$$

Logistic regression models the dependent variable as binary and the exponentiated parameters can be interpreted as Odds Ratios (ORs) of the dependent variable between levels of the independent variables.

$$\begin{aligned}
\text{OR}_{\frac{X=1}{X=0}} &= \frac{\text{Odds}_{X=1}}{\text{Odds}_{X=0}} = \frac{\exp(\Theta_0 + \Theta_1 \times 1 + \cdots + \Theta_k X_k)}{\exp(\Theta_0 + \Theta_1 \times 0 + \cdots + \Theta_k X_k)} \\
&= \frac{e^{\Theta_0} e^{\Theta_1} \cdots e^{\Theta_k X_k}}{e^{\Theta_0} e^{\Theta_2} \cdots e^{\Theta_k X_k}} = e^{\Theta_1}
\end{aligned} \tag{3.14}$$

Multinomial logistic regression is a generalization of logistic regression for discrete dependent variables with more than two possible values. Logistic regression was used in Study I to model having lesions on MRI and in Study II to identify predictors of having missing data in the MS Register. Multinomial logistic regression was used in Study III and V to model the probability of receiving a specific treatment in the estimation of the inverse probability of treatment weights (Section 3.12.2).

Poisson Regression

Distribution: Poisson
Link: Natural logarithm

$$
\begin{aligned}
Y &\sim \mathcal{P}(\lambda) & \lambda &= \text{expected count of Y} \\
g(\lambda) &= \mathbf{X\Theta} & g(X) &= \ln(X) \\
\ln(\lambda) &= \Theta_0 + \Theta_1 X_1 + \Theta_2 X_2 + \cdots + \Theta_k X_k
\end{aligned}
\tag{3.15}
$$

Poisson regression models the dependent variable as a count, but can be extended to model Y as a rate of events over some exposure (often time) with an *offset* term, ln(exposure).

$$
\begin{aligned}
Y \sim \mathcal{P}(\text{rate}) \qquad \text{rate} &= \frac{\lambda}{\text{exposure}} \\
\ln\left(\frac{\lambda}{\text{exposure}}\right) &= \Theta_0 + \Theta_1 X_1 + \Theta_2 X_2 + \cdots + \Theta_k X_k \\
\ln(\lambda) &= \ln(\text{exposure}) + \Theta_0 + \Theta_1 X_1 + \Theta_2 X_2 + \cdots + \Theta_k X_k
\end{aligned}
\tag{3.16}
$$

When modelling a rate, the exponentiated parameters can be interpreted as Incidence Rate Ratios (IRRs) of the dependent variable between levels of the independent variables.

$$
\begin{aligned}
\text{IRR}_{\frac{X=1}{X=0}} &= \frac{\text{IR}_{X=1}}{\text{IR}_{X=0}} = \frac{\exp(\Theta_0 + \Theta_1 \times 1 + \cdots + \Theta_k X_k)}{\exp(\Theta_0 + \Theta_1 \times 0 + \cdots + \Theta_k X_k)} \\
&= \frac{e^{\Theta_0} e^{\Theta_1} \ldots e^{\Theta_k X_k}}{e^{\Theta_0} e^{\Theta_2} \ldots e^{\Theta_k X_k}} = e^{\Theta_1}
\end{aligned}
\tag{3.17}
$$

3.6.2 Cox Proportional Hazards Model

The Cox proportional hazards model differs from the generalized linear model in that it models survival, specifically the hazard function (h), over time (t).

$$
\mathrm{h}(t) = \mathrm{h}_0(t)\exp(\mathbf{X\Theta}) \qquad \mathrm{h}_0(t) \text{ is the baseline hazard at time } t \tag{3.18}
$$

The hazard function is an event rate at a specified time, conditional on survival until that same timepoint, and can be thought of as the “instantaneous probability” of observing an event at this time.

$$h(t) = \lim_{\Delta t \to 0} \frac{\Pr(t \leq T < t + \Delta t \mid T \geq t)}{\Delta t} \tag{3.19}$$

The exponentiated parameters of a Cox model can be interpreted as Hazard Ratios (HRs) of the dependent variable between levels of the independent variables, given that the proportional hazards assumption is valid. If it is not, the parameters instead represent the average HRs over the observed time.

$$\begin{aligned}
\mathrm{HR}_{\frac{X=1}{X=0}} &= \{\text{proportional hazards}\} = \mathrm{HR}_{\frac{X=1}{X=0}}(t) = \frac{\mathrm{h}_{X=1}(t)}{\mathrm{h}_{X=0}(t)} \\
&= \frac{\mathrm{h}_0(t)\exp(\Theta_1 \times 1 + \cdots + \Theta_k X_k)}{\mathrm{h}_0(t)\exp(\Theta_1 \times 0 + \cdots + \Theta_k X_k)} \\
&= \frac{\mathrm{h}_0(t)e^{\Theta_1}e^{\Theta_2 X_2}\cdots e^{\Theta_k X_k}}{\mathrm{h}_0(t)e^{\Theta_2 X_2}\cdots e^{\Theta_k X_k}} = e^{\Theta_1}
\end{aligned} \tag{3.20}$$

Cox regression was used in Study I, III, IV, and V to model time to first event for relapses, cancers, and drug discontinuation.

3.7 Likelihood

Likelihood describes how well any particular statistical model is supported by the observed values in a sample, with a higher likelihood indicating better support. It is commonly used in regression analysis to estimate the sample statistics that best support the observed data through *maximum likelihood estimation*. Importantly, the likelihood is not to be confused for a probability, as explained by Fisher (emphasis is mine):[219]

> I stress this because in spite of the emphasis that I have always laid upon the difference between probability and likelihood there is still a tendency to treat likelihood as though it were a sort of probability. The first result is thus that there are two different measures of rational belief appropriate to different cases. **Knowing the population we can express our incomplete knowledge of, or expectation of, the sample in terms of probability; knowing the sample we can express our incomplete knowledge of the**

population in terms of likelihood. We can state the relative likelihood that an unknown correlation is +0.6, but not the probability that it lies in the range .595–.605.

For a discrete random variable X with a probability distribution defined by a parameter θ, the likelihood function $\mathcal{L}$ of a model with parameter value $\hat{\theta}$, given the observed value x, is the probability of that value. Stated differently, the likelihood of a model is the probability to observe the data at hand, if that particular model were true.

$$\mathcal{L}(\hat{\theta} \mid x) = \Pr(X = x \mid \theta = \hat{\theta}) = f_X(x \mid \hat{\theta}) \tag{3.21}$$

Where f_X is the probability mass function of the probability distribution for X. For an observed value x from a continuous random variable X, f_X is instead the probability density function.

$$\mathcal{L}(\hat{\theta} \mid x) = f_X(x \mid \hat{\theta}) \tag{3.22}$$

The *likelihood function* expresses the likelihood for all possible values of the parameter θ given a sample $x \sim X$.

$$\mathcal{L}(\theta \mid x) = f_X(x \mid \theta) \tag{3.23}$$

More generally, our sample is a collection of n observations $\mathbf{x} = (x_1, x_2, \ldots x_n)$ from a random variable X with a probability distribution expressed through k parameters $\boldsymbol{\theta} = (\theta_1, \theta_2, \ldots \theta_k)$ and a density function $f(\mathbf{x} \mid \boldsymbol{\theta})$.

$$\mathcal{L}(\boldsymbol{\theta} \mid \mathbf{x}) = f(\mathbf{x} \mid \boldsymbol{\theta}) \tag{3.24}$$

Given that X is an independent and identically distributed random variable, the density function $f(\mathbf{x} \mid \boldsymbol{\theta})$ is the product of univariate density functions.

$$f(\mathbf{x} \mid \boldsymbol{\theta}) = \prod_{i=1}^{n} f^{\mathsf{univar}}(x_i \mid \boldsymbol{\theta}) \tag{3.25}$$

Thus, the total sample likelihood is a product of the likelihood of all individual observations. The value of any one likelihood is by itself meaningless and it is first when we compare two or more likelihoods for different values of θ that they become informative (i.e. the model with higher likelihood would be preferred over the model with lower likelihood). A simple way to compare likelihoods is with the *likelihood ratio*.

$$\text{Likelihood Ratio} = \frac{\mathcal{L}(\boldsymbol{\theta}_0 \mid \mathbf{x})}{\mathcal{L}(\boldsymbol{\theta}_1 \mid \mathbf{x})} \tag{3.26}$$

3.7.1 Log-Likelihood

In practice, it is often more convenient to work with the natural logarithm of the likelihood function $\ell(\boldsymbol{\theta} \mid \mathbf{x})$, called the *log-likelihood*.

$$\ell(\boldsymbol{\theta} \mid \mathbf{x}) = \ln \mathcal{L}(\boldsymbol{\theta} \mid \mathbf{x}) \tag{3.27}$$

The log-likelihood is commonly used in *maximum likelihood estimation* in regression models. This is possible because the maximum of $\ell(\boldsymbol{\theta} \mid \mathbf{x})$ and $\mathcal{L}(\boldsymbol{\theta} \mid \mathbf{x})$ occur at the same $\boldsymbol{\theta}$, as the logarithm is a monotonic function.

The log-likelihood transforms the product of univariate density functions (3.25) into a sum.

$$\ell(\boldsymbol{\theta} \mid \mathbf{x}) = \ln \mathcal{L}(\boldsymbol{\theta} \mid \mathbf{x}) = \ln f(\mathbf{x} \mid \boldsymbol{\theta}) = \ln \prod_{i=1}^{n} f^{\mathsf{univar}}(x_i \mid \boldsymbol{\theta}) = \sum_{i=1}^{n} \ln f^{\mathsf{univar}}(x_i \mid \boldsymbol{\theta}) \tag{3.28}$$

And the likelihood ratio (3.26) into a difference.

$$\ln \frac{\mathcal{L}(\boldsymbol{\theta}_0 \mid \mathbf{x})}{\mathcal{L}(\boldsymbol{\theta}_1 \mid \mathbf{x})} = \ln \mathcal{L}(\boldsymbol{\theta}_0 \mid \mathbf{x}) - \ln \mathcal{L}(\boldsymbol{\theta}_1 \mid \mathbf{x}) = \ell(\boldsymbol{\theta}_0 \mid \mathbf{x}) - \ell(\boldsymbol{\theta}_1 \mid \mathbf{x}) \tag{3.29}$$

3.7.2 Maximum Likelihood Estimation

Maximum likelihood estimation can be used to estimate the sample statistics that are the most likely given the observed data, and it is widely used to estimate the coefficients in regression analysis (Section 3.6). The goal is to find the *maximum likelihood estimate* $\hat{\boldsymbol{\theta}}$ of a sample statistic $\boldsymbol{\theta}$ that maximizes the likelihood function $\mathcal{L}(\boldsymbol{\theta} \mid \mathbf{x})$ for the observed values $\mathbf{x}$:

$$\hat{\boldsymbol{\theta}} = \arg\max_{\boldsymbol{\theta}} \mathcal{L}(\boldsymbol{\theta} \mid \mathbf{x}) \tag{3.30}$$

Figure 3.2 illustrates maximum likelihood estimation for a sample of $n = 10$ observed values of the random variable X from an assumed normal distribution with standard deviation 1 and mean θ. To find the maximum likelihood estimate $\hat{\theta}$ we observe how the likelihood (in this case the log-likelihood) behaves for different values of θ. We find that among $\theta = (-4, -2, 0, 2, 4)$, the value $\hat{\theta} = 0$ resulted in the highest log-likelihood (-23.26) and is our maximum likelihood estimate. Of course, when estimating maximum likelihood in "the real world", for example when fitting a regression model, more sophisticated maximization algorithms are used.

3.8 Uncertainty and Standard Error

When inferring characteristics about a population from a sample, there will always be a measure of uncertainty for every estimated sample statistic. This uncertainty can be quantified by the *standard error* (SE), which is the standard deviation of the *sampling distribution* for the statistic. When reporting results, the standard error is often used to create a *p-value* or a *confidence interval*.

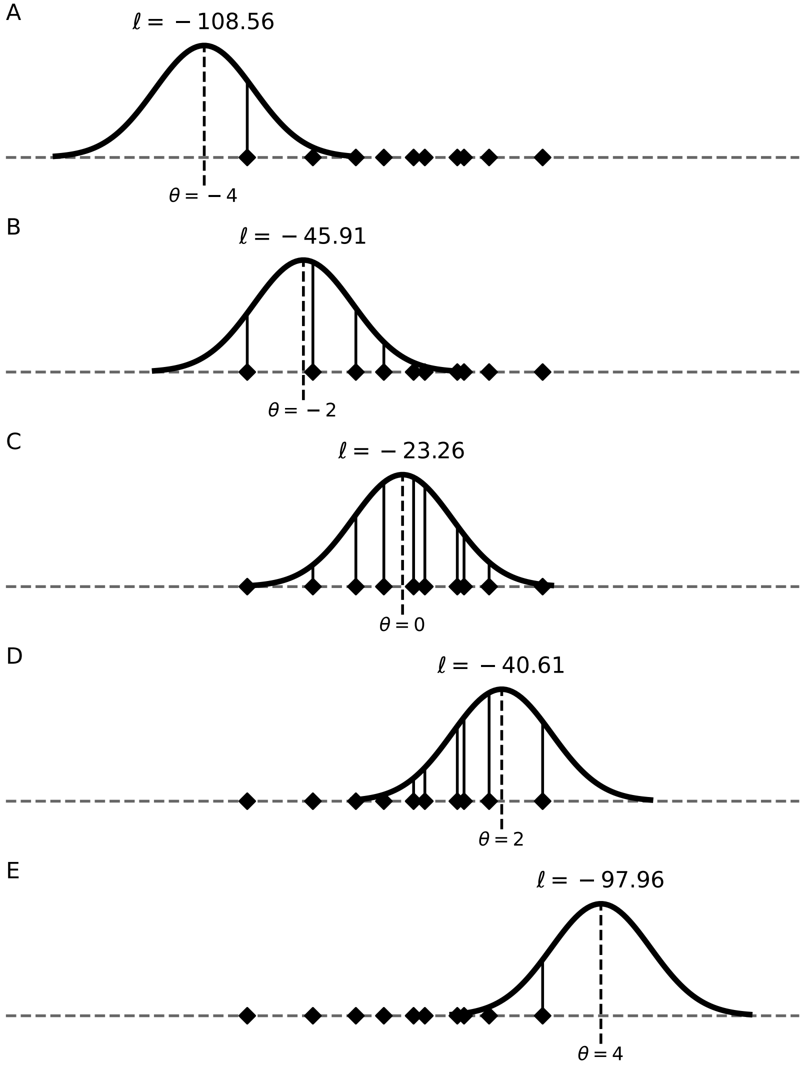


Figure 3.2: Maximum likelihood estimation for a random sample of $n = 10$ observations from an assumed normal distribution $\mathcal{N}(\theta, 1)$. Among $\theta = (-4, -2, 0, 2, 4)$, the value $\hat{\theta} = 0$ (C) gave the highest log-likelihood (ℓ) and is our maximum likelihood estimate.

3.8.1 Estimating the Standard Error

The standard error is itself a random variable that depends on the size of the sample and the variability of the measurement. How the standard error is estimated depends on the chosen test statistic and includes summary statistics, maximum likelihood, and bootstrapping. For maximum-likelihood estimation (Section 3.7.2), common in regression analysis (Section 3.6), the Hessian (matrix form of the second derivative) of the likelihood is used to estimate the standard error.

3.8.2 Robust Standard Error

Generally, in regression analysis, the variance of the dependent variable is assumed to be unaffected by the independent variables. If this is not the case, there is *heteroskedasticity* and we need to use heteroskedasticity-robust standard errors when fitting the regression model. An extension of heteroskedasticity-robust standard errors are the cluster-robust standard errors, used when observations can be divided into groups (clusters) within which the observations are not strictly independent. This can for example happen if the same patient contributes multiple observations.

3.8.3 P-Value

The p-value is used in *null-hypothesis testing* to quantify the probability of getting a result at least as extreme as the one observed, assuming that the *null hypothesis* is correct. The smaller the p-value, the less support for the null hypothesis. A result rejecting the null hypothesis by reaching a p-value below a *significance level* (α) is often called "statistically significant". A commonly chosen significance level is 5% ($\alpha = 0.05$), for which we would reject the null hypothesis if the p-value is below 0.05. For an estimated coefficient in a regression model ($\hat{\theta}$), p-values are often estimated based on the *t statistic* (t).

$$
\begin{aligned}
t_{\hat{\theta}} &= \frac{\hat{\theta}}{\mathrm{SE}(\hat{\theta})} \\
p &= 2F_{\mathcal{T}(\nu)}(t_{\hat{\theta}}) \qquad \text{(two sided)}
\end{aligned}
\tag{3.31}
$$

Where $F_{\mathcal{T}(\nu)}$ is the *cumulative distribution function* of the *t-distribution* ($\mathcal{T}$) with $\nu = n-k$ degrees of freedom, when estimating k coefficients in a sample of size n.

3.8.4 Confidence Interval

The confidence interval is used to quantify the uncertainty of an estimate of an unknown parameter at a specific *confidence level* $(1-\alpha)$, often 95% with $\alpha = 0.05$. The confidence level denotes the probability that the true value of the parameter lies inside any one confidence interval, in the series of confidence intervals that we would get if the study was repeated with different samples. In other words, the true value of the parameter would lie in the 95% confidence interval for 95% of the repeated studies (Figure 3.3). For an estimated coefficient in a regression model ($\hat{\theta}$), the 95% confidence interval can be estimated from the standard error, given that the sampling distribution of the coefficient is asymptotically normal with increasing sample size, due to the *central limit theorem*.

$$\begin{aligned} \mathrm{CI}_{\mathrm{lower}} &= \hat{\theta} + Q_{\mathcal{N}}\left(\frac{\alpha}{2} = 0.025\right)\mathrm{SE}(\hat{\theta}) = \hat{\theta} - 1.96\mathrm{SE}(\hat{\theta}) \\ \mathrm{CI}_{\mathrm{upper}} &= \hat{\theta} + Q_{\mathcal{N}}\left(1 - \frac{\alpha}{2} = 0.975\right)\mathrm{SE}(\hat{\theta}) = \hat{\theta} + 1.96\mathrm{SE}(\hat{\theta}) \end{aligned} \tag{3.32}$$

Where $Q_{\mathcal{N}}$ is the *quantile function* (inverse cumulative distribution function) of the standard normal distribution. This is equivalent to shifting and rescaling the standard normal distribution into a normal distribution around the coefficient with the standard error as the standard deviation (i.e. the estimated sampling distribution of the coefficient), and using this distribution to find the values of the confidence interval at the desired confidence level.

$$\begin{aligned} \mathcal{N}(\mu = 0, \sigma^2 = 1) &\rightarrow \mathcal{N}(\mu = \hat{\theta}, \sigma^2 = \mathrm{SE}(\hat{\theta})^2) \\ \mathrm{CI} &= \left[Q_{\mathcal{N}(\hat{\theta}, \mathrm{SE}(\hat{\theta})^2)}(\alpha/2), Q_{\mathcal{N}(\hat{\theta}, \mathrm{SE}(\hat{\theta})^2)}(1 - \alpha/2)\right] \end{aligned} \tag{3.33}$$

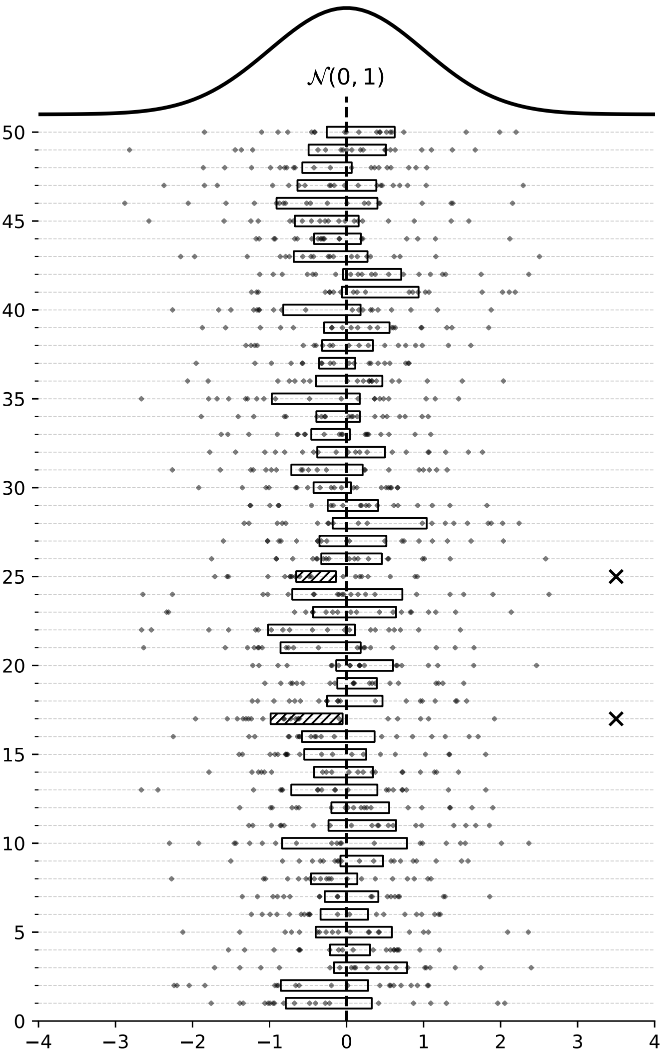


Figure 3.3: Confidence intervals with 95% confidence level for the individual means of $k = 50$ samples of $n = 20$ observations taken from a normal distribution $\mathcal{N}(0, 1)$. We expect that the confidence intervals contain the true value of the mean ($\mu = 0$) 95% of the time. Two of the intervals do not contain the true value $\frac{50-2}{50} = 0.96 = 96\%$

3.8.5 P-Values vs Confidence Intervals

According to the American Statistical Association, the p-value is often misused and misinterpreted.[220] There is a tendency to disregard results that are just above the significance threshold without considering the larger context of evidence and to interpret the p-value as the probability that the null hypothesis is true. These concerns have sparked a heated debate around the p-value's place in inferential statistics and it has been suggested that the p-value should be abandoned in favour of the confidence interval (or other measures, such as likelihood ratios or Bayes factors).

3.8.6 Bootstrapping

Bootstrapping is a resampling method using random sampling with replacement to non-parametrically estimate the sampling distribution of sample statistics. It is used when the assumptions of the desired parametric model are uncertain or when the formula for estimating the standard error is prohibitively complicated. One way to construct a confidence interval, non-parametrically, for a bootstrapped sample statistic $\hat{\theta}$, at confidence level $1 - \alpha$, is by using the quantiles of the estimated sampling distribution $Q_{\hat{\theta}}(\cdot)$ corresponding to the confidence level.

$$\begin{aligned} \mathrm{CI} &= \left[Q_{\hat{\theta}}\left(\frac{\alpha}{2}\right), Q_{\hat{\theta}}\left(1 - \frac{\alpha}{2}\right)\right] \\ \mathrm{CI} &= [Q_{\hat{\theta}}(0.025), Q_{\hat{\theta}}(0.975)] \quad \text{for a 95\% confidence interval} \end{aligned} \tag{3.34}$$

3.9 Causal Inference

When studying health-related events in epidemiology, we generally measure *statistical associations*. However, in many cases, we are interested in a *causal interpretation* of our findings, for which associations alone are not enough. Much effort has gone into defining a true *causal effect* and in the 1970's Donald Rubin developed a formal definition using *potential outcomes and counterfactuals*. In the 1980's, James Robins developed statistical methods for estimating causal effects in longitudinal studies and in the 1990's Judea Pearl introduced the Directed Acyclic Graph (DAG) as a tool for epidemiology. To understand why this is important, we first need to understand the terms *association* and *causation*, and the difference between them.

3.9.1 Independence and Association

Two random variables can be either *independent* or *associated*. X and Y are independent ($Y \perp\!\!\!\perp X$) if the probability distribution of one variable is the same between all levels of the other, and associated if the probability distribution of one variable is different between at least some levels of the other.

$$Y \perp\!\!\!\perp X \iff \Pr(Y = 1|X = 0) = \Pr(Y = 1|X = 1) = \Pr(Y = 1) \tag{3.35}$$

Here X and Y are said to be *marginally independent* (3.35), in contrast to *conditionally independent* given variables $\mathbf{Z}$ (3.36).

$$Y \perp\!\!\!\perp X \mid \mathbf{Z} \iff \Pr(Y = 1|X = 0, \mathbf{Z}) = \Pr(Y = 1|X = 1, \mathbf{Z}) = \Pr(Y = 1 \mid \mathbf{Z}) \tag{3.36}$$

Association between variables is a prerequisite for, but does not imply causation.

3.9.2 Causation

Several models exist for defining causality, but here we will focus on the potential outcomes model, where two hypothetical scenarios are compared: the outcome had the subject been exposed (Y_1) and the outcome had the subject not been exposed (Y_0), everything else being equal. Y_0 and Y_1 are the *potential outcomes*, and if they differ the exposure is said to have a causal effect on the outcome. Realistically, we can only ever observe one of the potential outcomes, the *factual outcome*, while the *counterfactual outcome* remains unknown. This makes it impossible to assess individual causal effects, while population causal effects can be estimated. An exposure is said to have a population causal effect on Y if:

$$\Pr(Y_0 = 1) \neq \Pr(Y_1 = 1) \tag{3.37}$$

And no causal effect if:

$$\Pr(Y_0 = 1) = \Pr(Y_1 = 1) \tag{3.38}$$

Just as we can have conditional association, we can also have *conditional causality*.

$$\Pr(Y_0 = 1 \mid \mathbf{Z}) \neq \Pr(Y_1 = 1 \mid \mathbf{Z}) \tag{3.39}$$

When exposure and outcome are not binary, the causal effect can be defined in terms of the *expected value* of Y when an exposure X changes from $X = 0$ to $X = x$.

$$\mathrm{E}(Y_0 = 1 \mid \mathbf{Z}) \neq \mathrm{E}(Y_x = 1 \mid \mathbf{Z}) \tag{3.40}$$

A statistical association is equal to a causal effect if there are *no systematic differences* between the exposed and unexposed. This is true if the distributions of the potential outcomes Y_0 and Y_1 are the same for those with exposure $X = 0$ and $X = 1$, respectively, as for every subject. In other words, the potential outcomes are independent of the exposure. This is called *exchangeability* between the exposed and unexposed and holds true if:

$$(Y_0, Y_1) \perp\!\!\!\perp X \iff \begin{cases} \Pr(Y_0 = 1 | X = 0) = \Pr(Y_0 = 1) \\ \Pr(Y_1 = 1 | X = 1) = \Pr(Y_1 = 1) \end{cases} \tag{3.41}$$

Exchangeability can never be tested given the data, but can be strived for by limiting systematic differences between the exposed and unexposed groups in the study design (e.g. in a randomized controlled trial) and/or in the analysis by controlling for potential confounders through conditioning on variables or reweighting of the sample (Section 3.12).

It can be difficult to think in terms of above equations when assessing how well we are achieving exchangeability in our studies. For discussing issues around exchangeability, such as different types of bias (Section 3.11), it is easier to use a graphical representation of causality, the DAG.

3.10 Directed Acyclic Graphs (DAG)

The DAG is a mathematical concept originating from graph theory and computer science, brought over to epidemiology to visualize causal structures. A DAG is defined as a finite directed graph with no directed cycles. DAGs can model many different types of data and they are frequently used in epidemiology to simplify the interpretation and communication of complicated topics in causal inference, as well as for covariate selection in observational studies. The basic building blocks of a DAG are the *nodes* (sometimes called vertices) connected by directed *edges*. Figure 3.4 shows an example DAG.

In epidemiology, DAGs are used to represent the underlying causal structures of a data-generating process and they are formally connected to the potential outcomes and counterfactuals causal framework through non-parametric structural equations. In this context, every node represents an event occurring at a definite time and every edge represent a possible causal relationship from the *early time node* to the *late time node* of that edge. This means that time always flows in the direction of the edges, ensuring acyclicness (i.e. no causal loops). Assumptions in the DAG are encoded by the edges:

An edge from X to Y means that X *may* cause Y and Y *does not cause* X

No edge from X to Y means that X *does not cause* Y

Any connection of edges between two nodes makes a path, regardless of the direction of the edges and any intermediate nodes. The edges along a path can form different patterns at the nodes (Figure 3.4):

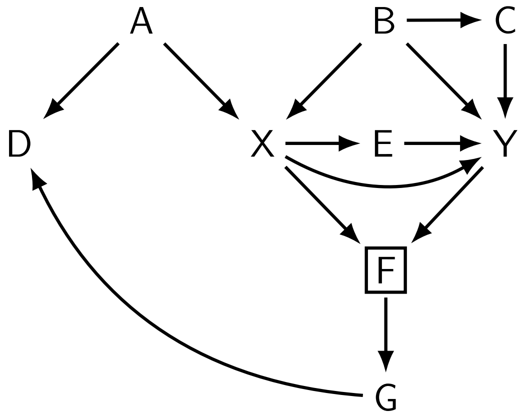


Figure 3.4: Example of a directed acyclic graph, where the letters are nodes and the arrows the directed edges. A square around a node is often used to denote that a variable has been conditioned on (Section 3.12)

Chain The entry edge and exit edge have the *same direction* (→ E →)

Fork The entry edge and exit edge have *opposite directions, away from the node* (← B →)

Inverted Fork/Collider The entry edge and exit edge have *opposite directions, towards the node* (→ F ←)

A path between two nodes is said to be *closed* if it has any *unconditioned* colliders or any *conditioned* chains or forks, and open otherwise. A node (representing a random variable) can be conditioned on in many different ways, e.g. by stratification or as a covariate in regression modelling (Section 3.12). Any open path between two nodes implies a *statistical association* between those nodes and any open path between two nodes *following the direction of the edges* implies *causality* in that direction. As we are often only interested in the causal association between nodes, the goal becomes to manipulate the DAG, through study design and conditioning, so that only the causal path we are interested in remains open. If the desired path is the only open path, it follows that the association we measure in our data is a causal one. In reality, however, there will always be concerns about residual confounding or other types of bias affecting the association.

3.11 Bias/Systematic Error

Bias are systematic errors in the analyses that cause the estimated sample statistics to systematically differ from the population parameters we are interested in. It is important to differentiate bias from precision and generalizability, as these are often conflated. Precision has to do with the uncertainty of our estimation of the sample statistic, given the variability and size of the data (Section 3.8). It is possible to be have good precision even if the statistic is biased (and the other way around), which would mean that we could accurately estimate the statistic given our model, but that the value we get is systematically different from the population value we are looking for. Generalizability on the other hand has nothing to do with a study's *internal validity*, but instead considers if the results found for one study population are applicable to another, often called a study's *external validity* (e.g. if the results from a study of adults can reasonably be applied to children).

In epidemiology, bias is generally categorized into the three types: *confounding*, *collider stratification bias* (selection bias), and *information bias*. To understand these biases it is useful to visualize the causal structure using a DAG (Section 3.10).

3.11.1 Confounding

Confounding occurs when there are common causes for the exposure (X) and the outcome (Y), leading to an open non-causal path and a potential non-causal association between X and Y (Figure 3.5).

We can limit confounding by closing all non-causal open paths in the DAG. In the example in figure 3.5, we would do this by conditioning on the variable C in the DAG to the left and either or both C1 and C2 in the DAG to the right (Figure 3.6).

Confounding by Indication is confounding that occurs when the outcome is influenced by an indication for the exposure. E.g. severe disease might prompt aggressive

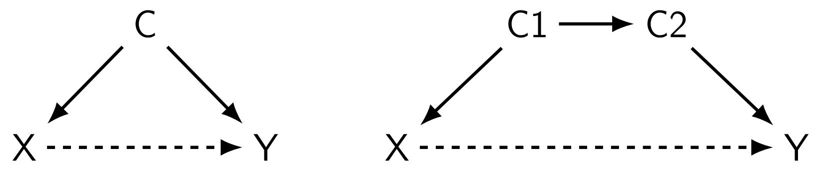


Figure 3.5: Two examples of confounding of the association between X and Y, as C, C1, and C2 are common causes of X and Y. The dashed lines illustrate the causal associations we are interested in

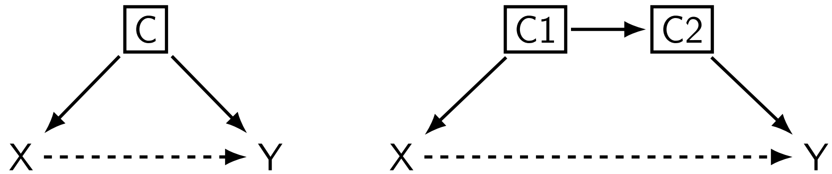


Figure 3.6: Conditioning on the variable C in the DAG to the left and either or both C1 and C2 in the DAG to the right removes the confounding. The dashed lines illustrate the causal associations we are interested in

treatment and at the same time increase the risk of death, leading to a confounded association suggesting that aggressive treatment might increase the risk of death.

Residual Confounding refers to any remaining confounding in the model, often from unmeasured variables (Figure 3.7), but also due to imperfect measurement and potential model misspecification for the variables included. All studies will have a degree of residual confounding unless there is randomization of the exposure, as in a randomized controlled trial.

3.11.2 Collider Stratification Bias

Collider stratification bias (sometimes called selection bias) occurs when there are common descendants of the exposure (X) and the outcome (Y) that have been conditioned on, leading to an open non-causal path and a potential non-causal association between X and Y (Figure 3.8).

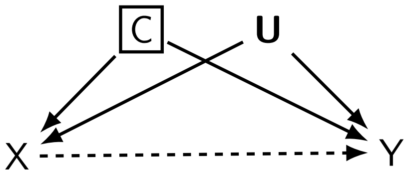


Figure 3.7: Example of residual confounding of the association between X and Y, as U is a common cause of both X and Y. Since U is unmeasured it cannot be conditioned on. The dashed line illustrates the causal association we are interested in

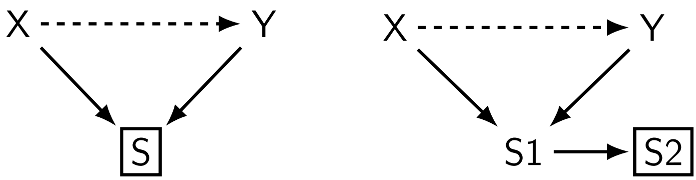


Figure 3.8: Two examples of collider stratification bias of the association between X and Y, as S and S2 are common descendants of X and Y and has been conditioned on. Notice that S2 does not have to be a direct descendent of X and Y to cause bias. The dashed lines illustrate the causal associations we are interested in

Collider stratification bias is difficult to correct for if it has been introduced as a consequence of the study design. Sometimes it is possible to close the open path by conditioning on another variable in a chain or a fork along that path. It is important to take care not to introduce this type of bias in an analysis by conditioning on variables that are colliders in the DAG.

Example

Collider stratification bias can feel unintuitive, so it can help to look at an example. Imagine a hospital that only treats people with gall stones or kidney stones, no other health conditions. If we want to study the association between gall stones and kidney stones by recruiting participants from this hospital, we will be introducing collider stratification bias. This is because we have conditioned on (in this case selected) the hospital where the patients are treated. Applied to figure 3.8 (left), the probability of having a gall stone would be X, the probability of having a kidney stone Y, and being in

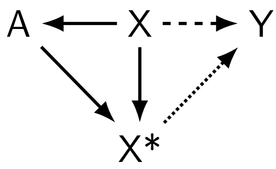


Figure 3.9: A DAG illustrating information bias, where X is the true value and X* is the misclassified measured value. The dashed lines illustrate the causal associations we are interested in and the dotted line is the association we are actually measuring

the hospital the conditioned-on S. Any one patient in that hospital is there for only two possible reasons. That means if a patient is not at the hospital because of gall stones, the probability of them having kidney stones is much higher than in the general population, and vice versa. The association between gall stones and kidney stones will therefore be biased and risk being underestimated with this study design.

3.11.3 Information Bias

Information bias (also called misclassification bias) refers to the bias that can occur when there are systematic differences in the measurement error or misclassification of a variable. When this happens, we are in reality measuring the association between the misclassified variable X* and Y, rather than the association we are interested in between X and Y (Figure 3.9).

Example

An intuitive example occurs when the value being measured affects the measuring itself, such as a scale not being able to measure values above a certain value. If the scale then shows the maximum value (e.g. 120 kg) for each measurement that in truth is heavier, we have misclassification bias. Applied to figure 3.9, the true weight would be X, the misclassified measured weight would be X*, and A would be the scale failing.

3.12 Controlling for Variables

By controlling (adjusting) for variables in analyses, we can limit confounding and get closer to exchangeability between the assessed groups. This can be done by either *conditioning* on variables or *reweighting* of the study sample. Conditioning on variables can be done through stratification or by including them in a regression model of the exposure and the outcome of interest. This is equivalent to closing open paths in a DAG and gives us a *conditional effect* of the exposure on the outcome. An alternative approach is to use weighting techniques to transform the study sample into a pseudo-sample for which no associations exist between the exposure and the potential confounders included in the model. This can be done through standardization, matching, or inverse probability weighting, and gives us the *marginal effect* of the exposure on the outcome. Marginal effects are also what is estimated in randomized controlled trials, as long as no variables were additionally controlled for in the analysis.

Stratification The most intuitive and straight-forward way to condition on variables, but limited in the number of covariates that can be conditioned on. The sample is divided into groups and analysed separately, based on the covariate patterns of the variables conditioned on. For example, separate analyses for males and females would condition on sex.

Regression Modelling In regression analyses (Section 3.6), any variable included in the model in addition to the exposure is conditioned on.

Standardization With standardization, group-specific rates are reweighted to be comparable between exposure levels. For example, treatment effects within age-groups can be reweighted to find the marginal treatment effect independent of age in a population with a specific age distribution. Standardization can be direct or indirect.

Matching With matching, participants are selected into the study based on similarity in some covariates, between either exposure or outcome levels. For example, for every participant with exposure A, a matched cohort study could select a fixed number of participants with the same sex, but exposure B. Such a selection guarantees similar distributions of covariates between the exposure levels, but risks excluding a large portion of the available sample population. To avoid introducing bias, *incidence density sampling* (risk-set sampling) with replacement should be used, meaning that the sampling to the matched group is done from the population at risk for the exposure/outcome at the time of the matching (e.g. at the time of exposure) and that participants in this population can be repeatedly chosen as a match.

Inverse Probability Weighting With inverse probability weighting, the study sample is reweighted into a pseudo-sample in which marginal treatment effects can be estimated. See below (Section 3.12.2).

Marginal and conditional effects are equal for collapsible statistics (e.g. means and risk differences) if the variables conditioned on have the same distributions between all levels of exposure or outcome and there is no effect-modification by variables with different distributions between the sample and pseudo-sample, but may differ for non-collapsible statistics (e.g. ORs and HRs) even under these conditions. When they differ, the interpretation of conditional and marginal effects is not the same, as stated by Austin in his paper on propensity score methods:[221]

> A conditional treatment effect is the average effect of treatment on the individual. A marginal treatment effect is the average effect of treatment on the population

As a simplified example, if a treatment has a reported *conditional* OR of 0.5 for a negative outcome, compared with no treatment, a physician can assume that giving this treatment to a patient will lower that patient's odds of the outcome by 50%, given that the reported effect was conditioned on all the information the physician knows about the patient. However, if a treatment instead has a *marginal* OR of 0.5, the physician can assume that if they give this treatment to all their patients, it will lower the entire patient population's odds of the outcome by 50%. In both cases, the study population for which the OR was estimated, needs to be generalizable to the physician's patient (conditional) or patient population (marginal).

In our studies, we have mainly focused on regression modelling with and without inverse probability of treatment weighting.

3.12.1 Propensity Score

The propensity score is the probability of observing the factual exposure for each individual, conditional on other variables. It is commonly estimated in a regression model (Section 3.6) with the exposure as the dependent variable. The propensity score can then be used as a matching criterion, as a variable in a regression model, or in *inverse probability weighting* to estimate marginal effects by controlling for the variables included in the model used to estimate the score.

3.12.2 Inverse Probability Weighting

Inverse probability weighting allows for controlling of potential confounding variables by analysing a weighted pseudo-population, giving marginal estimates of sample statistics. If the exposure is a treatment, this method is referred to as *inverse probability of treatment weighting*. Inverse probability weighting is performed by first calculating the propensity score, i.e. probability of having a specific level of the exposure $X = x$ given the potential confounders $\mathbf{Z}$.

$$\mathrm{ps} = \Pr(X = x \mid \mathbf{Z}) \tag{3.42}$$

The inverse of the propensity score is then assigned as the weight for each subject i.

$$W_i = \frac{1}{\mathrm{ps}} = \frac{1}{\Pr(X_i = x_i \mid \mathbf{Z}_i)} \tag{3.43}$$

Where X_i and $\mathbf{Z}_i$ are the outcomes of those variables for observation i.

To avoid extreme values, the weights can be stabilized by normalizing the conditional probability of having a specific exposure level by the marginal probability for that same exposure level.

$$W_i^s = \frac{\Pr(X_i = x_i)}{\Pr(X_i = x_i \mid \mathbf{Z}_i)} \tag{3.44}$$

To further avoid extreme values, the weights can also be truncated to a quantile of their distribution (e.g. 99th percentile) with the quantile function for the distribution of stabilized weights Q_{W^s}.

$$W_i^t = \begin{cases} W_i^s & \text{if } W_i^s \leq Q_{W^s}(0.99) \\ Q_{W^s}(0.99) & \text{if } W_i^s > Q_{W^s}(0.99) \end{cases} \tag{3.45}$$

After estimation, and possibly stabilization and truncation, the weights can be applied to the study sample to create a pseudo-population that is balanced for potential confounders between levels of exposure.

3.12.3 Assessing Balance in Covariates

To assess the balance in the covariates, we can compare their distributions between levels of exposure. This is usually summarized as the mean and standard deviation (continuous variables) or number and proportion (discrete variables) in the Table 1 of a study. However, it can be difficult to know how big of a difference is "too big". To get a measure that is comparable between covariates of different sizes and scales we can use the *standardized mean difference* to compare two exposure levels to each other. The standardized mean difference is constructed by taking the difference between the means of the variable in the two groups ($\bar{x}, \bar{y}$) and dividing it by the combined standard deviations (s_x, s_y).

$$\text{SMD} = \frac{\bar{x} - \bar{y}}{\sqrt{\frac{s_x^2 + s_y^2}{2}}} \tag{3.46}$$

A standardized mean difference between exposure levels of less than 0.2 is generally considered well-balanced.[222]

3.13 Missing Data

Missing data is a ubiquitous problem in research and occurs when the outcome of the variables of interest is not recorded for all participants in the sample. Even if there is only a low amount of missingness in several variables, many study participants will have at least one missing observation. Depending on the mechanism of missingness and how it is dealt with in the analyses, the missing data can reduce precision and generalizability, as well as introduce bias. Missingness generating mechanisms are typically divided into three types:

Missing Completely at Random (MCAR) occurs when the causes of the missing data are unrelated to the data itself, i.e. the probability of missing is equal for all participants. In this case, the issue is only the loss of information and the missingness will not bias the results, but MCAR is often unrealistic in real-world data.

Missing at Random (MAR) occurs when the causes of the missing data depend on the observed data. This type of missingness might bias analyses, but can be addressed as the data are MCAR within groups of the observed data (i.e. conditional on some variables). This scenario is more realistic than MCAR and generally what is assumed by modern methods for dealing with missing data.

Missing Not at Random (MNAR) occurs when the causes of the missing data are unknown, not available in the data, or when missingness is determined by the missing values themselves. This type of missing data might bias analyses and can only be handled by gathering more information about the causes of the missingness.

Regardless of the type of generating mechanism, missingness has to be dealt with in some way before data can be analysed. Several techniques exist, although many of them with significant limitations:

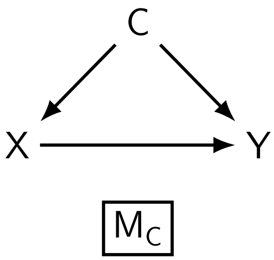
Complete-Case Analysis In a complete-case analysis, all observations with missing data on the variables of interest are discarded. In the case of MCAR, this does not introduce bias, but will lower precision as the sample size is reduced (Figure 3.10a). However, in cases of MAR or MNAR, the complete case analysis might limit generalizability by changing the distribution of the outcome (Figures 3.10b and 3.10d) or introduce collider stratification bias (Figures 3.10c and 3.10e). The complete-case analysis might also make it difficult to compare crude and adjusted estimates, or two estimates adjusted for different covariates, as they will be based on different samples. Despite its limitations, the complete-case analysis is still a common way to handle missing data.

Missing Category To deal with missingness in categorical variables, missing data can be defined as its own category. This allows for the use of all observations in the analysis, but as it combines different unknown values into a single heterogeneous category it will be difficult to interpret the coefficient for this category and controlling for a variable with a missing category will not correctly control for any confounding it introduces. Furthermore, estimates might still suffer from selection bias.

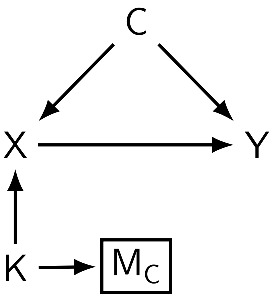
Carry-Forward Imputation In carry forward imputation, missing observations in a time-series are imputed using the previously observed value. This method is commonly used for the outcomes in randomized-controlled trials for the participants that are lost to follow-up. However, it ignores changes over time and, in reality, just compares the outcomes for different participants at different time points. Since data is imputed, a loss in variability will result in artificially small standard errors.

Simple-Mean Imputation In simple-mean imputation, missing observations are imputed using the sample mean. This will weaken any associations in the data, as the imputed values are not associated with anything, and will not correctly control for any confounding. Furthermore, estimates might still suffer from selection bias. Since data is imputed, a loss in variability will result in artificially small standard errors.

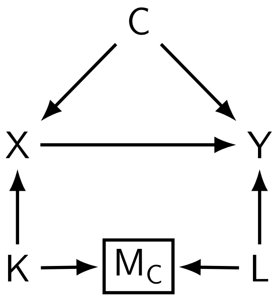
Regression-Mean Imputation Regression-mean imputation extends simple-mean imputation by estimating the mean used to impute missing values conditional on some covariates. This is better than the simple method and can remove collider-stratification bias if the conditional model is specified correctly, however it still



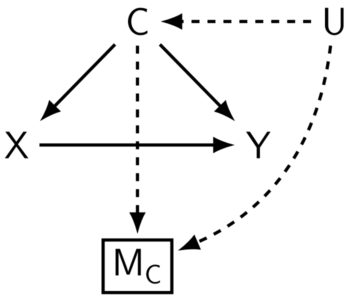
(a) MCAR (no bias)



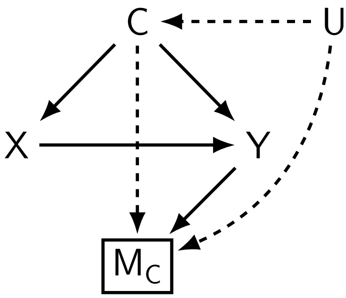
(b) MAR without bias



(c) MAR with bias



(d) MNAR without bias



(e) MNAR with bias

Figure 3.10: Directed acyclic graphs of missingness mechanisms in the context of a complete case analysis (only the participants with complete data are included). In (d) and (e), only one of the dashed paths from C to M_C are necessary, either the direct path or a path through the unmeasured variables U. MCAR=Missing Completely at Random, MAR=Missing at Random, MNAR=Missing Not at Random

suffers from the same loss in variability as the previous two imputation methods, which will result in artificially small standard errors.

None of the above methods should generally be used to deal with missing data in epidemiological studies, as they have severe limitations, can introduce further bias, and result in too small standard errors (in the case of the imputation methods). However, all is not lost, as there is a technique that has gained increased recognition as an adequate way of dealing with missingness: *multiple imputation*.

3.13.1 Multiple Imputation

Multiple imputation is an imputation technique where missing data are imputed several times to account for the inherent uncertainty of the imputations themselves, thus avoiding the artificially small standard errors produced by the imputation methods mentioned above.[223]

The general idea is that several new datasets are created from the original incomplete data by imputing the missing values from their estimated probability distributions, leading to slightly different imputed values in every imputed dataset. The imputed datasets can then be analysed separately and the results pooled according to *Rubin's rules*.[224] Figure 3.11 shows a schematic of a multiple imputation workflow.

Multiple imputation using the Fully Conditional Specification, and specifically the special case of Multiple Imputation by Chained Equations (MICE), has become a popular method for imputing missing values.[225] With MICE, the variables with missing values are imputed one by one using a chain of regression models, considering all other variables used in later analyses.

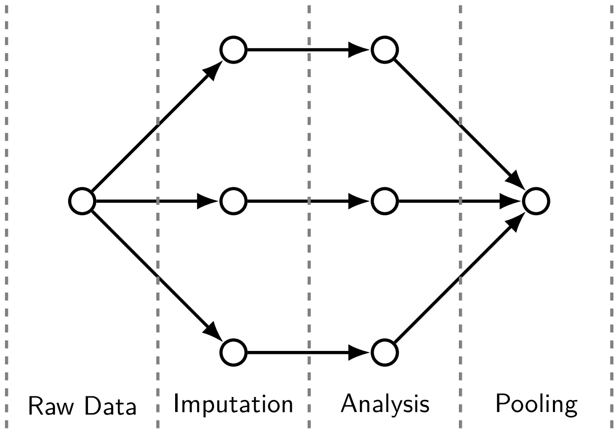


Figure 3.11: Schematic of Multiple Imputation

Chapter 4

Results

The collective aim of this PhD project, with its five studies, was to investigate different risk-benefit aspects of DMTs for MS, with a special focus on rituximab.

In **Study I** we assessed the effectiveness of rituximab and fingolimod in patients having to switch from treatment with natalizumab due to JCV positivity and the risk of PML. Following this we took the chance to assess the data quality of the Swedish MS Register in **Study II**, as part of the larger COMBAT-MS project. In **Study III** we investigated the risk of cancer, an important safety aspect of DMTs due to the role of the immune system in cancer surveillance. **Study IV** focused on assessing the safety of the induction therapies AHSCT and alemtuzumab, compared with continuously administered MS DMTs. Finally, in **Study V**, we assessed the cost-effectiveness of rituximab compared with MS-approved alternatives, in an effort to inform the decisions of clinicians and policymakers.

4.1 Study I: Therapy Switch

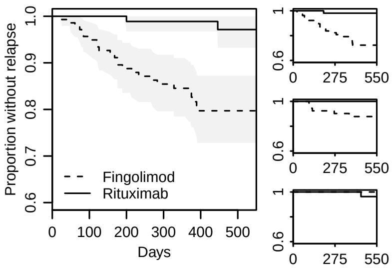
Study I focused on patients treated with natalizumab that due to becoming seropositive for JCV had to switch therapy because of the risk of PML (Section 1.2.6). At the time of the study, rituximab had started becoming a more popular treatment option for MS in Sweden, competing with conventional, MS-approved therapies. However, there were still major differences in the use between regions in Sweden, and there was no consensus on how and for which patients rituximab should be used.

Previous research had shown that disease activity returns after stopping treatment with natalizumab, peaking between four and seven months after the last dose.[115,226] There had also been reports about rebound phenomena, where disease activity returns more severely than before the start of therapy.[116–118] Limiting the washout period to less than three months had been shown to improve the outcome and starting fingolimod after natalizumab cessation had been found to be moderately effective.[227–229] No data were available supporting the use of rituximab in this patient group.

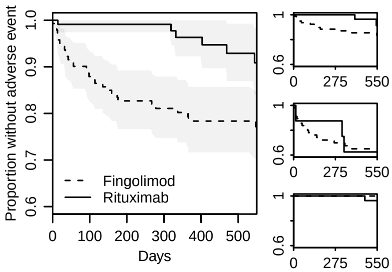
We collected data on all RRMS patients switching from natalizumab to either fingolimod or rituximab due to JCV positivity, at three large MS centres in Sweden: Karolinska (Stockholm), Sahlgrenska (Gothenburg), and Norrland's (Umeå) University Hospitals. Patients were identified through the Swedish MS Register (Section 3.3.1) and data corroborated with clinical chart review. Outcomes were contrast-enhancing and T2 lesions on MRI, clinical relapses, adverse events, and drug survival, within 1.5 years of rituximab or fingolimod start.

In 256 patients, 114 on rituximab and 142 on fingolimod, less disease activity was observed in the rituximab group within the first 1.5 years from therapy switch: 1% vs 16% had contrast-enhancing lesions and 2% vs 18% experienced a clinical relapse. Controlled for possible confounders, the OR of getting a contrast-enhancing lesion was 0.05 (95% Confidence Interval (CI) 0.00–0.22) and the HR of getting a first relapse was 0.09 (95% CI 0.02–0.40), comparing rituximab to fingolimod. The rituximab group also reported fewer adverse events and significantly better drug survival, compared with fingolimod. Figure 4.1 shows survival plots for time to first relapse, adverse event, and therapy discontinuation, as well as the cumulative incidence for different causes of therapy discontinuation.

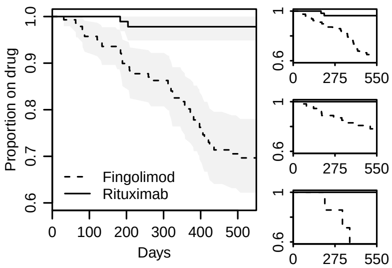
In summary, for RRMS patients switching from natalizumab due to JCV positivity, rituximab appears to be more effective at preventing relapses and contrast-enhancing



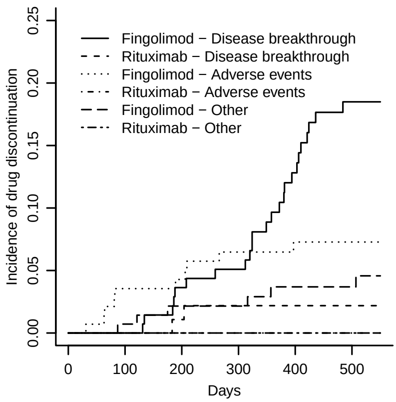
(a) Relapses



(b) Adverse Events



(c) Drug Discontinuation



(d) Reasons for Discontinuation

Figure 4.1: Outcomes Study I — Survival curves within 1.5 years of treatment start for time to first relapse (a), adverse event (b), and drug discontinuation (c), as well as cumulative incidence curves of reasons for discontinuing treatment with rituximab or fingolimod (d). The curves “Rituximab - Adverse events” and “Rituximab - Other” are both zero. Main cohort displayed in each large plot and individual centres in smaller plots: Stockholm (top), Gothenburg (middle), and Umeå (bottom)

lesions and better tolerated, making it the superior choice over fingolimod in this patient population. These findings are also interesting in relation to treatment of highly active MS in general.

4.2 Study II: Register Validation

The Swedish MS Register is used extensively for research and already at the time of this study it had provided data for over 100 scientific reports, a number that has since grown to 180 (Section 3.3.1).[9,206] Having used the Swedish MS Register in Study I and planning for it to be a core part in future projects linked to other registers, we were fortunate to have an opportunity to validate its data within the bounds of the COMBAT-MS project. This project began with a clinical chart review to update the data in the MS Register for the study cohort.

More than 3000 patients were included in the COMBAT-MS clinical chart review, updating the MS Register. This allowed us to compare the data before and after the update to draw conclusions about the quality of the data for the included patients, as well as for the register in general.

Data collection from clinical charts into the MS Register was conducted by personnel at Sweden's University Clinics. Lists of patients and instructions for standardized data collection were distributed, and clinics were instructed to add or correct any missing or erroneous data in the register for patient information, disease characteristics, therapies, EDSS and other scores, clinical relapses, and MRI. We identified observations that had changed, been removed, or added in the MS Register after the update. Clinics were reimbursed per completed patient review to assure high compliance.

A total of 3012 patients were included in the update. Few observations had been removed (below 3%) or changed (below 7%), except for MRI where 34% of observations had modified data. Observations added to the register ranged from a 5% increase for therapies to 71% for MRIs. Recorded relapses increased by 35%, however most had occurred before treatment started. Before the update, in the first three years after therapy start, relapses had a sensitivity of 78% and a specificity above 99%. The same pattern was

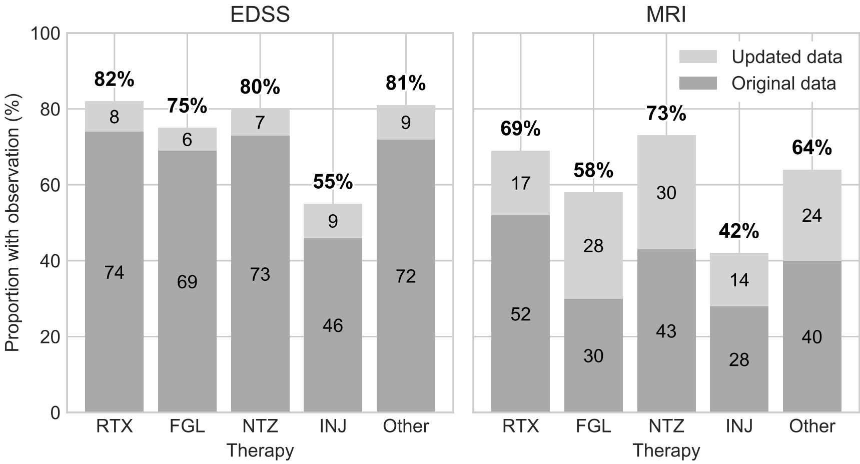


Figure 4.2: Outcomes Study II — Bar plot of proportion of therapy starts with an observation of EDSS [−180 days, +30 days] (left) and MRI [−90 days, +30 days] (right), important for confounder control. EDSS=Expanded Disability Status Scale, MRI=Magnetic Resonance Imaging, RTX=Rituximab, FGL=Fingolimod, NTZ=Natalizumab, INJ=Injectables

seen for the relative increase of contrast-enhancing lesions on MRI, although with a sensitivity of 51%. Proportion of therapy episodes with observations of EDSS and MRI around treatment start, an important timepoint for confounder control, increased after the update, especially for MRI (Figure 4.2). However, missingness due to lack of original data remained, with the highest proportion in the injectables group.

In summary, data on treatment and EDSS was reasonably complete, while MRIs were often missing or incomplete, but could to some extent be recovered from the medical records. Missingness varied by clinic, and compared with newer DMTs, the proportion of missing was higher for the older injectable therapies. The better data quality for newer therapies is likely, at least in part, due to the Immunomodulation and Multiple Sclerosis Epidemiology (IMSE) studies, initiated with the launch of natalizumab and working closely with the Swedish MS Register to improve the register data for each new treatment becoming available for MS.

4.3 Study III: Therapies and Cancer

After investigating rituximab effectiveness in Study I and assessing the quality of our available data in Study II, we now turned our attention to important safety aspects of DMTs. For most immunomodulatory treatments, the major concerns are increased risks of infection and cancer, as well as in some cases secondary autoimmune disorders (see Study IV, Section 4.4). In this study we focused on the risk of cancer for the three most commonly used DMTs in Sweden at the time, while risk of infection was assessed in a parallel study by our research group.[143]

Despite their increasingly widespread use and the growing evidence to support superior effectiveness of modern DMTs compared with older injectable therapies, relatively few studies have addressed comparative safety aspects, such as cancer. Given the potency of these therapies to affect the immune system, such studies are much needed to establish the risk-benefit profiles necessary for treatment decisions in the clinic (Section 1.4).[230,231] As clinical trials are limited in their ability to capture rare safety outcomes, due to generally small sample sizes, short follow-up, and issues with real-world generalizability, population-based register studies are ideal for investigating these outcomes.

Data from the Swedish MS Register was linked to the Cancer Register and other national healthcare and census registers (Section 3.3). Therapy episodes of fingolimod, natalizumab, and rituximab were included and matched to a general-population reference cohort. We used an ever-treated approach where therapy episodes were followed even after the patient had stopped receiving that specific treatment. Cancers were identified in the national Cancer Register and limited to the first ever diagnosis of each assessed cancer type. Multiple imputation by chained equations (Section 3.13.1) was used to deal with missing data and inverse probability of treatment weighting (Section 3.12.2) was used to control for potential confounding.

In 7477 therapy starts, we identified 78 invasive cancers. After controlling for possible confounding, we found an increased risk of invasive cancer for fingolimod, compared with both rituximab, HR 1.68 (95% CI 1.00–2.84), and the general population HR 1.53 (95% CI 0.98–2.38) (Figure 4.3). It was not possible to assess if any specific type

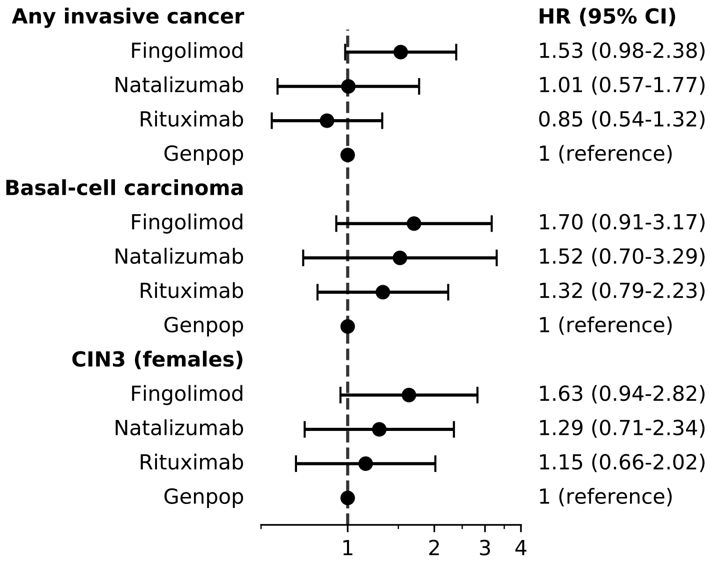


Figure 4.3: Outcomes Study III — Forest plot of adjusted hazard ratios with 95% confidence intervals, with the general population as the reference and time since therapy start as the time scale. HR=Hazard Ratio, CI=Confidence Interval, CIN3=Cervical Intraepithelial Neoplasia grade 3

of cancer was driving this difference. No increased risk was found for rituximab or natalizumab compared with the general population.

Although no previous data were available on cancer risk for rituximab treatment in MS, our findings were in line with previous studies of rituximab in rheumatoid arthritis.[154,155] Interestingly, for ocrelizumab, another CD20 antibody that was recently approved for MS (Section 1.2.10), a higher incidence of breast cancer was reported in one of its trials, which was not the case for rituximab in our study. The clinical trials and long-term follow-up of fingolimod have yielded inconclusive results related to cancer risk,[50–53,158,232] but the product information from both the FDA and EMA include lymphoma as a possible adverse event, and the EMA further warns about cutaneous cancers. Our results support a possible increased risk of cancer with fingolimod. For natalizumab, our results replicate those of its trials and post-marketing surveillance.[46,47,153]

4.4 Study IV: AHSCT and Alemtuzumab

After concentrating mostly on rituximab in the previous studies, we now switched focus to another treatment for MS not formally approved by regulatory authorities, AHSCT. As an induction therapy, AHSCT, and similarly alemtuzumab, has a special appeal in that it is the closest we have come to "cure" MS. Alemtuzumab was the first induction therapy to be approved for RRMS, but AHSCT has been used off-label for MS since 1995 (Sections 1.2.8 and 1.2.9).

Both AHSCT and alemtuzumab cause long-lasting changes to adaptive immunity and the hope is that an initial round of treatment can keep the MS-induced inflammation at bay for many years without needing additional therapy. The choice between AHSCT and alemtuzumab has mostly been influenced by the local experience and availability of the treatment at the treatment centres. Widespread use of these therapies has been limited due to fears of long-term adverse effects and in Sweden AHSCT and alemtuzumab are mostly used in patients with aggressive RRMS and breakthrough disease on conventional therapy. The aim of this study was to assess and compare safety outcomes after treatment with AHSCT and alemtuzumab, and contrast these to non-induction MS therapies.

We used data from the Swedish MS Register linked to national healthcare and demographic registers (Section 3.3) to identify patients having received AHSCT or alemtuzumab, and matched these to a cohort of patients receiving non-induction therapies. Primary outcomes were death, thyroid disease, non-thyroid autoimmune disease, and infection.

Among 139 patients treated with AHSCT and 132 patients treated with alemtuzumab, we observed one death (Incidence Rate (IR) per 1000 person-years 1.7, [95% CI 0.0–9.6]) in the AHSCT group and four deaths (IR 8.6, 95% CI 2.3–22.0) in the alemtuzumab group. In the non-induction reference group, the mortality rate was 0.7 (95% CI 0.3–1.3). The incidence of thyroid disease was the highest in the alemtuzumab group, IR 109 (95% CI 75–154) vs 34 (95% CI 18–56) for AHSCT, and 5.3 (95% CI 3.9–7.1) for the non-induction therapies (Figure 4.4). Non-thyroid autoimmunity was rare in all groups. The incidence of infection was higher in the AHSCT group, IR 275 (95% CI 213–350) vs IR 56 (95% CI 34–87) for alemtuzumab, and 52 (95% CI 47–58) for

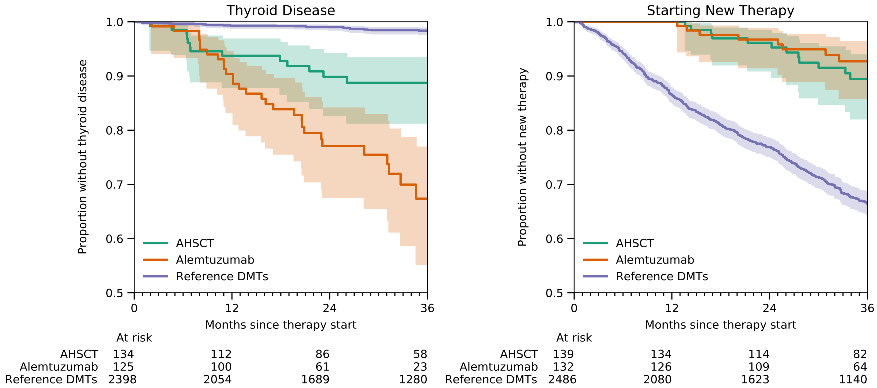


Figure 4.4: Outcomes Study IV — Survival curves of proportion of patients without thyroid disease (left) and not starting a new therapy (right). AHSCT=Autologous Hematopoietic Stem Cell Transplantation, DMT=Disease-Modifying Therapy

the non-induction therapies. The rate of infection was highest just after the AHSCT procedure and levelled off six months after treatment start (Figure 4.5).

One of the four deaths after alemtuzumab was directly linked to the treatment (cytomegalovirus reactivation), while the only death in the AHSCT group was a suicide. The mortality rate for AHSCT was lower in our study than in most previous reports,[124] which could be a consequence of the patient population, as AHSCT was previously more often used in older patients with more disability, but could also be due to a shift towards less aggressive ablative conditioning. An increased risk of infection is a known adverse effect to AHSCT treatment, but this risk seems to quickly diminish a few months after therapy (Figure 4.5). The incidence of thyroid disease with alemtuzumab was higher in our study than previously reported in clinical trials, but in line with later observational data.[59–61,233] The rate of starting a new therapy was significantly lower with both AHSCT and alemtuzumab, compared with the non-induction therapies, indicating that they in fact do work as induction treatments (Figure 4.4).

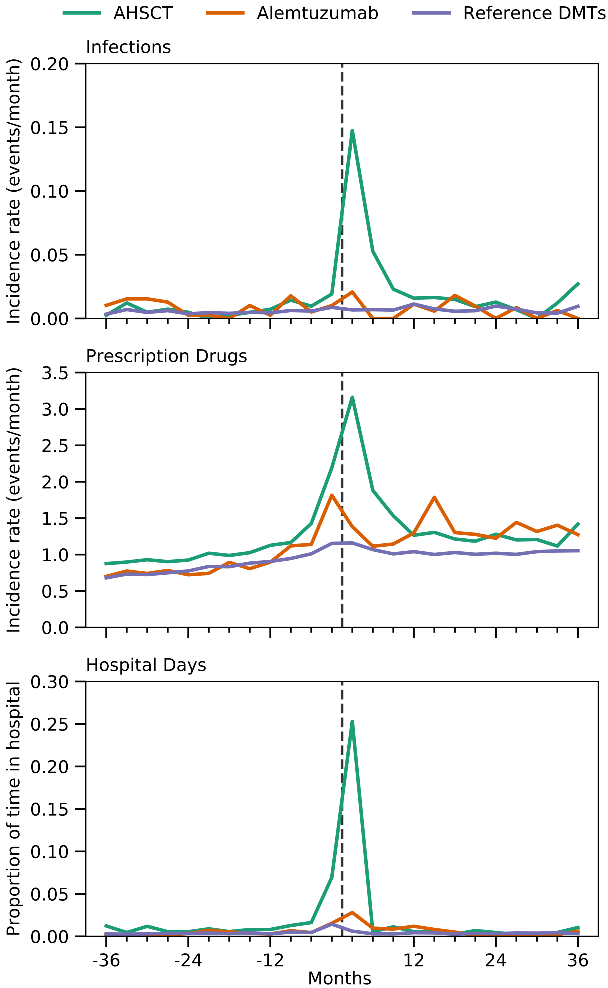


Figure 4.5: Outcomes Study IV — Plots of incidence rates (events per month) for diagnosed infections (top) and prescription of drugs (middle), as well as the proportion of time spent in inpatient care (bottom). Three-month intervals, three years before and after therapy start (dashed line). AHSCT=Autologous Hematopoietic Stem Cell Transplantation, DMT=Disease-Modifying Therapy

4.5 Study V: Therapy Cost-Effectiveness

Having studied the effectiveness and safety of rituximab, we finally wanted to investigate its cost-effectiveness to help guide clinicians and policymakers as rituximab continues to grow in popularity as a treatment for MS. At this point, rituximab had become the most commonly used MS DMT in Sweden, in spite of the lack of cost-effectiveness data comparing it to MS-approved alternatives.

Treatment for MS is associated with high costs, compared with both other neurological disorders and inflammatory conditions (Section 1.5).[162,165] This puts a heavy financial burden on both the individual and society, and have raised questions about the cost-effectiveness of MS DMTs.[234] The high costs of MS treatment strain healthcare systems in wealthy countries, while resource-limited countries might not be able to afford modern MS treatment at all. One way to lower costs and provide treatment for all who need it is to repurpose drugs approved for other indications, making rituximab a prime candidate in the MS setting.

We included all patients in the Swedish MS Register treated with rituximab, natalizumab, fingolimod, or dimethyl fumarate, linked to the national healthcare and demographic registers (Section 3.3). We used a new-user, active-comparator design with an ever-treated approach to follow-up. Costs included MS therapies, non-MS prescription drugs, specialized outpatient visits, and inpatient care. The effectiveness was measured as the accumulated number of clinical relapses. Missing data was handled using multiple imputation (Section 3.13.1), baseline characteristics were balanced using inverse probability of treatment weighting (Section 3.12.2), and confidence intervals were estimated with bootstrapping (Section 3.8.6).

In a total of 5924 included therapy starts, adjusted mean healthcare costs over five years were for rituximab €27 313 (95% CI 26315–28470), natalizumab €92 946 (89054–97393), fingolimod €84 187 (82306–86011), and dimethyl fumarate €61 808 (59139–65018). The main cost driver for all groups was the index therapy, although lower for rituximab (Figure 4.6). Adjusted number of relapses per patient were for rituximab 0.10 (95% CI 0.08–0.12), natalizumab 0.22 (0.19–0.26), fingolimod 0.31 (0.28–0.36), and dimethyl fumarate 0.31 (0.26–0.37). Figure 4.7 shows the mean cumulative number of relapses over time and Figure 4.8 shows the cost-effectiveness plane.

Rituximab was superior to the other therapies, with both lower costs and fewer relapses. Mean cost savings per patient over five years were €34 000–€66 000 and mean number of prevented relapses 0.12–0.21 (Figure 4.8). This translates into total cost savings of €307 000 000 and 1063 prevented relapses, over five years, for a cohort of MS patients similar to those in our study, if they were all assigned rituximab compared with if they were all assigned MS-approved therapies. The cost savings and lower number of relapses with rituximab were possible without any increase in other individual healthcare costs, such as prescription drugs, specialized outpatient visits, or inpatient care.

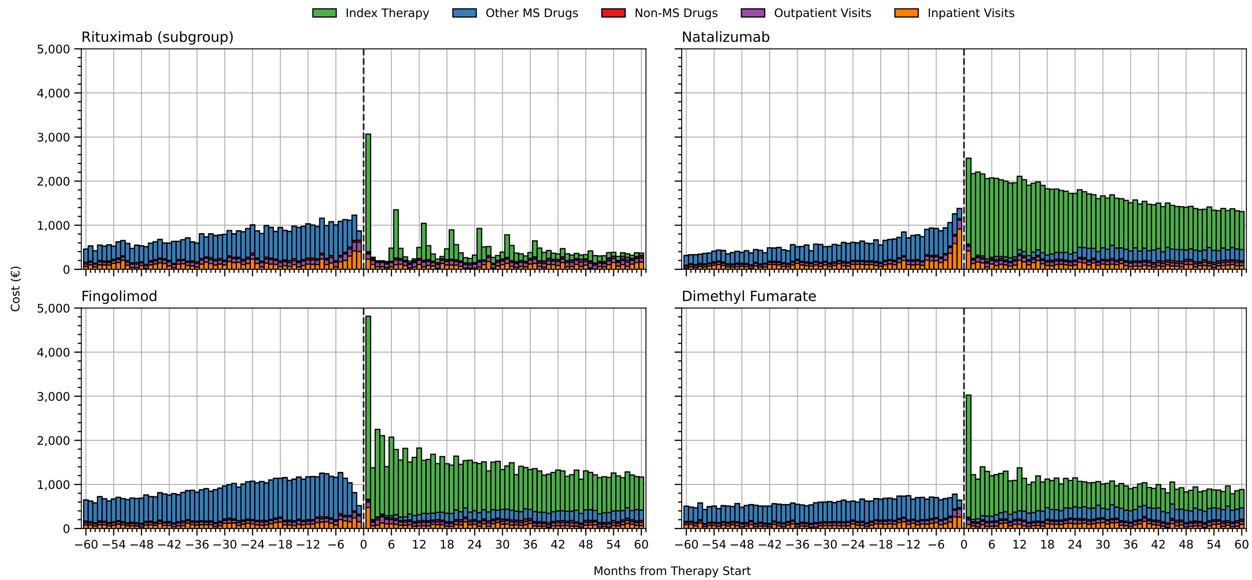


Figure 4.6: Outcomes Study V — Stacked bar charts of mean monthly healthcare costs in Euro (€) per therapy episode, before and after therapy start (dashed line), stratified by therapy. MS=Multiple Sclerosis

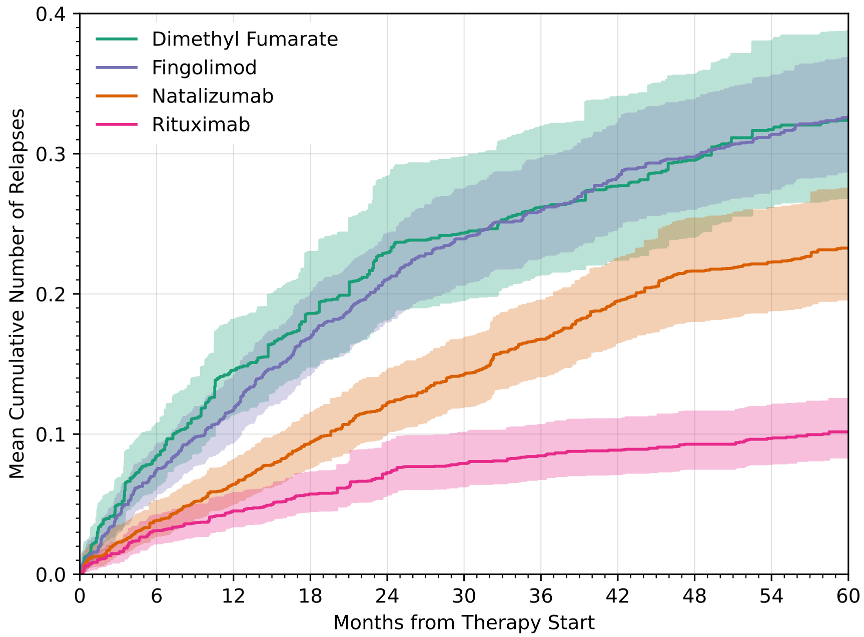


Figure 4.7: Outcomes Study V — Plot of adjusted mean cumulative number of relapses up to five years after therapy start, stratified by therapy. Shaded regions depict 95% pointwise confidence intervals

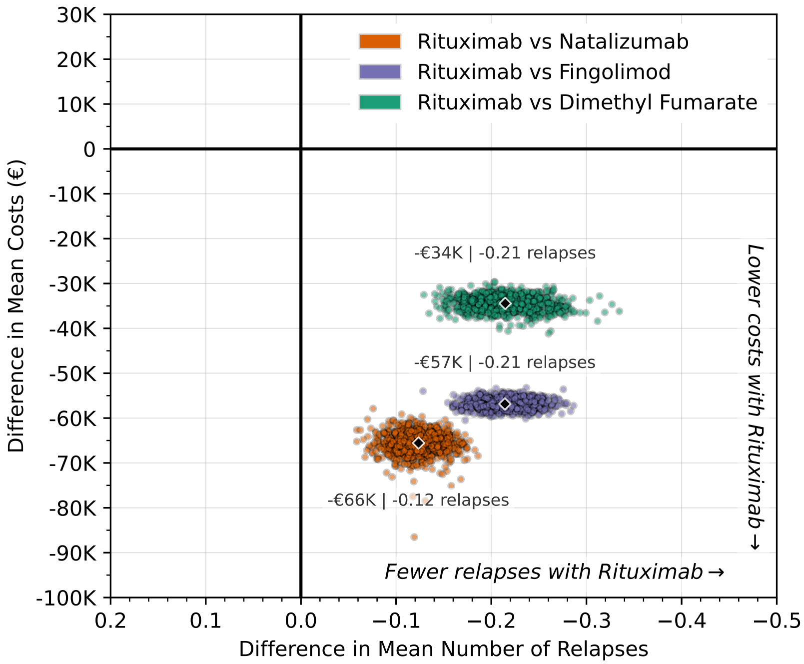


Figure 4.8: Outcomes Study V — Cost-effectiveness plane with clouds of point estimates for the adjusted mean differences in costs in Euro (€) and number of accumulated relapses, per therapy episode, five years after therapy start, stratified by therapy. Each coloured marker depicts the estimate from a single bootstrap sample and the aggregated median estimates are shown as black diamonds and text. Rituximab as the reference

Chapter 5

Discussion

When I began my research in 2015, rituximab was gaining in popularity as an alternative to the MS-approved DMTs in Sweden. However, despite its increased use for MS, there was no clear consensus about how rituximab should be dosed or which types of patients should receive it. This led to great variation in the use of rituximab between different treatment centres and regions in Sweden. Increasing clinical experience suggested that rituximab was a good, sometimes even better, alternative to established therapies, but there was a lack of formal studies backing this notion. The main evidence, at that time, came from two clinical trials, one in RRMS[93] and the other for patients with PPMS[94] (Section 1.2.7). Both trials reported promising results for rituximab over placebo in patients with active disease. However, as rituximab was nearing its commercial end-of-life due to an expiring patent, the market authorization holder chose to shift focus to ocrelizumab, another anti-CD20 antibody targeting the same antigen as rituximab. Ocrelizumab was eventually approved for both RRMS and PPMS in 2017 (FDA) and 2018 (EMA). That left several years during which anti-CD20 treatment for MS was thought to be effective based on the studies for rituximab, but no formally approved anti-CD20 therapy was available.

In Sweden, physicians are trusted with “free prescribing rights” (fri förskrivningsrätt), which means that a licensed physician can prescribe a treatment outside that treatment’s indication as long as the decision is based on “science and proven experience”. This right is central to healthcare in Sweden and allows for medical treatment in situations where it would not be possible otherwise, such as in the treatment of children (use of therapies approved only for adults) and in end-of-life care. This right is also the reason why Swedish neurologists can use rituximab to treat MS, despite the fact that no formal indication has been provided by the pharmaceutical industry.

After the initial positive results from the trials and given the long experience of using rituximab in the treatment of rheumatoid arthritis, Swedish neurologists started using rituximab for MS patients where approved alternatives had failed or were unsuitable. With more use came increased clinical experience and soon the use of rituximab for MS had spread to many parts of Sweden. Although, regional differences remained and there was an ongoing discussion among neurologists about rituximab’s legitimacy as a treatment for MS. It was in this context we set out to assess rituximab’s effectiveness in Study I.

The results from Study I (Section 4.1) verified what neurologists had already observed in the clinic: that rituximab is an effective treatment for MS also in real-world populations. We found it to be even more effective than the MS-approved alternative, fingolimod. Not everyone was convinced, however, and there was critique from the pharmaceutical industry’s trade organization in Sweden, Läkemedelsindustriföreningen (LIF), concerning the validity of the Swedish physicians’ “free prescribing rights”. In 2016, LIF sent a request to the Swedish government to investigate how the “free prescribing right” should be interpreted in relation to the laws governing the approval of medical treatments.[235,236] LIF argued that it is not sustainable with systematic and widespread use of therapies outside their indication. However, the verdict of the Swedish government’s medical organizations (*Socialstyrelsen*, *Läkemedelsverket*, *Tandvårds- och läkemedelsförmånsverket*, and *Inspektionen för vård och omsorg*), with the support from the Swedish physicians’ professional organization (*Svenska Läkaresällskapet*) and trade union (*Sveriges läkarförbund*), was that the “free prescribing right” is a vital, core part of the Swedish medical system that should be protected.[237,238] The parties involved were in agreement that this right must be used responsibly. Therefore, if off-label treatments are used, they need to be followed up to identify possible adverse outcomes.

Other researchers raised concerns about possible negative effects of rituximab in MS, as demonstrated by the letter to the editor of the Annals of Neurology by Marignier et al. concerning our Study I. It highlighted valid concerns regarding the risk of PML and the timing of rituximab initiation after stopping treatment with natalizumab due to JCV positivity. Marignier et al. argued that more research was needed before we could draw any conclusions on the risk-benefit and timing of rituximab (and fingolimod) treatment, especially in the group of patients previously treated with natalizumab, where the risk of PML is much greater. At the time, there were no studies specifically addressing these important issues. However, the experience from rituximab use in the treatment of rheumatoid arthritis indicated that the PML risk with rituximab was in line with other immunomodulatory therapies and lower than with natalizumab.[239] As we also shared these concerns about possible unknown adverse effects of rituximab treatment in MS, we set out to investigate the two most common fears related to immunomodulatory treatment: infections, including PML, and cancer.

The infection study,[143] carried out by colleagues in the research group, directly addressed the concerns of PML with rituximab raised by Marignier et al. and echoed by others. This study found that there was a generally increased risk of infections for persons with MS compared with the general population and that this risk differed depending on the treatment. The lowest rate of infection was seen for the older injectable therapies, interferon-beta and glatiramer acetate, while among the newer treatments, rituximab had the highest rate of hospitalized infections. However, among the 8600 included therapy episodes (of which 1535 were with fingolimod and 3260 were with rituximab) only two occurrences of PML were recorded, one with fingolimod and one with rituximab. In both cases, the patient had recently switched from natalizumab, consistent with a carry-over infection diagnosed when natalizumab concentrations declined enough for an immune reconstitution syndrome to occur. So, although there seems to be an increase in the risk of infection with rituximab use, no increased risk of PML was found.

Concerns about the risk of cancer were addressed in Study III (Section 4.3), where we found no increased risk for rituximab and a potential increase for fingolimod. This is especially interesting given the results from one of the ocrelizumab trials (Section 1.2.10), where a greater number of breast cancers were reported among patients with ocrelizumab, compared with placebo (IR 26.1 vs 0 per 10 000 person-years).[79] As we did not find an increased risk of breast cancer for rituximab in our study, the higher risk with ocrelizumab could be due to the high dose of ocrelizumab administered, relative to what is commonly used for rituximab. However, it could also be a chance finding.

Study I, III, and the infection study[143] contributed to the increasing use of rituximab in Sweden. These studies were only possible because Swedish neurologists saw the potential of rituximab for helping their patients and started using it off-label in small scale, based on the evidence available at the time. This generated the data needed to perform our observational studies, cementing rituximab's role as an important treatment option for persons with MS. With rituximab now well-established in Swedish MS care and also gaining interest internationally, there is an opportunity to raise further global awareness about this treatment option. Apart from being an effective treatment for MS, rituximab is additionally unique in that it is less expensive than most other MS DMTs.

We quantified these differences in costs in Study V (Section 4.5), which assessed the cost-effectiveness of rituximab compared with MS-approved DMTs. In the literature review for this study, we only found a single cost-effectiveness evaluation of rituximab in MS. It found rituximab to be a cost-effective alternative to natalizumab. However, this study was based on a simulation in a small sample (n=120) rather than real-world data, limiting the interpretability of the results. In fact, most cost-effectiveness studies also for other DMTs in MS use Markov-Chain simulation to extrapolate costs from short-term trial data with few participants, leading to high uncertainty in long-term estimates.

The few real-world cost-effectiveness studies that exist for MS DMTs suffer from limitations in data availability, due to scattered healthcare information across regions and/or insurers. These limitations have led to contradictory results and difficulty to determine the relative cost-effectiveness of different MS therapies. The lack of cost-effectiveness studies specifically comparing rituximab to MS-approved alternatives was identified as an important issue in the 2022 MS International Federation's recommendations for rituximab, where the recommendations had to be made conditional due to the "very low certainty of the evidence".[176]

Although as of yet unpublished and unreviewed, our findings in Study V showed that treatment with rituximab in MS led to fewer relapses and lower costs over a five-year period, compared with MS-approved DMTs. This made rituximab the most cost-effective alternative among the MS therapies most used in Sweden. Cost-effectiveness is important in all contexts with finite resources, not the least in healthcare, but is of greatest consequence in resource-limited settings or countries where much of the healthcare costs are deferred to the individual. This can make newer, more effective treatments for MS prohibitively expensive and therefore not available for many persons with MS.[173] In these settings, rituximab could provide an affordable and easily available alternative, with equal or better relapse prevention compared with the more expensive alternatives. This also applies in wealthier countries with tax-funded universal healthcare given that resources are finite, which can lead to barriers for accessing more effective and expensive DMTs.[172]

Taken together, the results from Study I, III, and V, viewed in the context of the currently available research (Chapter 2), show that rituximab is an effective, safe, and cost-efficient treatment option for patients with RRMS. The primary remaining barrier for wider adoption had been the lack of formal randomized controlled trials, which was changed when the RIFUND-MS trial[97] recently reported its findings (Section 2.1), and additional trials are underway (Section 2.3).

The growing international interest for rituximab in the MS setting was demonstrated by the collaboration between our research group, researchers from Kaiser Permanente in California, and the US non-profit institute Patient-Centered Outcomes Research Institute (PCORI), initiated in 2016. PCORI sponsored the COMBAT-MS project that allowed for the validation of the Swedish MS Register in Study II, as well as extensive prospective data collection further improving the quality of the register as a source of data for epidemiological research. The COMBAT-MS project has generated several publications (Study II, III, and IV, among others[143,240–247]) and is currently preparing its final reports.

5.1 The Swedish MS Register

The COMBAT-MS project was a great opportunity for us to validate the main data source for MS research in Sweden, resulting in Study II. There was a growing awareness that long-term, post-marketing studies using real-world data are needed to complement more limited clinical trials, especially for monitoring off-label treatments, such as rituximab and AHSCT. Large clinical registers such as the Swedish MS Register have key roles in aggregating and making data available to researchers. However, the validity of any study depends on the data it is based on and can be limited by issues of misclassification, missingness, and incomplete coverage. In Study II, we mainly focused on the accuracy and completeness of the register and it was regrettable that we were unable to also investigate the coverage compared with the National Patient Register. The register holders themselves report a coverage of 80%, but it is not entirely clear what this proportion is based on and it does not seem to have changed between 2015 and 2022.[9,206] Interestingly, a study in one region of Sweden found a local coverage of 71%, lower than the estimated national coverage.[208] Unfortunately, when Study II was conducted we had not yet received the data for our first register linkage, including the data from the Patient Register, and we had to make do with what we could assess from the MS Register alone. Assuming that the estimated national coverage of 80% holds true, the MS Register covers almost the entire Swedish MS population and bias due to selection should be low. However, it is still likely that there are regional differences in the use of the register as a clinical documentation tool, possibly also changing over time. This might not only affect the coverage of the data, but also the quality, which is important for the generalizability of the results from Study II, as it focused on MS patients at Sweden's University Hospitals. However, since the majority of Swedish MS patients are followed at one of these centres, this aspect is most important if the register is used to study a specific region in Sweden, rather than the Swedish MS population as a whole. Further validation of the coverage and the regional quality of the MS Register is needed.

5.1.1 Quality Assurance and Ease of Use

Apart from external validation of the Swedish MS Register, there are also several steps the register holder can, and in some cases have, taken to improve the quality of the data in the register. It is important that checks are in place for when users of the register enter data, so that erroneous information can be identified and prevented. More of these checks have been added to the register over the years, but from the data that the register delivers, it is obvious that this was not always the case. For this reason, it is important that quality-assurance testing is also performed on the existing data. This could take the form of mostly automated checks to identify unreasonable values (e.g. dates) and then contacting the responsible clinics to address any issues found.

Apart from avoiding errors in the data, it is important that the register is easy and quick to use for physicians, nurses, and patients, to remove any potential barriers for entering information. For patients, this has been partly implemented with a section of the register where patients themselves can fill in forms resulting in different clinical scales. These forms can be filled in at home, before a visit to the clinic, or while in the waiting room. For physicians and nurses, it would reduce the administrative workload to have relevant data automatically transferred from the clinical chart system to the MS Register, without having to manually add the information in both, as is the case today.

Better automatic quality assurance and frictionless data entry would likely increase data accuracy and completeness significantly. This would be especially beneficial for data on EDSS, relapses, and MRI, which are regularly recorded as part of the clinical routine, but are still often missing in the register (Section 4.2).

5.1.2 The Reuse of Data

One aspect of register-based research that I have not seen discussed much in the context of MS is the reuse of data that happens when several independent studies draw their samples from the same register data. The results of these studies are then often presented as individual pieces of evidence, building the bigger picture of what we know about MS and the effects of treatment with DMTs. However, this does not consider that these studies are, in many cases, reporting different aspects of the same patient population. If this would have been done within the same study, questions around the issue of multiple testing would surely arise. There is still a great need for larger observational studies like those included in this PhD project, but they cannot all be based on the Swedish MS population. Sweden is of course not alone in having high-quality registers, but there is a need to spread the knowledge and infrastructure that makes Sweden a prominent producer of epidemiological research to other countries.

5.2 Hematopoietic Stem Cell Transplantation

Study IV (Section 1.2.9) was a slight departure from our focus on rituximab, to instead investigate AHSCT. This was motivated by the fact that AHSCT, similarly to rituximab, is not approved for use in MS. As a result, there are few studies assessing key aspects of this treatment. Specifically, the safety of AHSCT has been a concern and a limitation for its more widespread adoption. As induction-type treatments, AHSCT together with the MS-approved alemtuzumab, hold a special appeal in that they are as close as we currently can get to "cure" MS. It is also interesting to note that rituximab might in the future be considered an induction therapy as well, depending on how long it is possible to stretch the intervals between infusions (Section 5.4.6).

The primary safety concern with AHSCT is treatment-related mortality, which was as high as 7% in the early days of AHSCT, but has since decreased to more tolerable levels (0.2%).[124] In Study IV, there was only a single death in the AHSCT group of 139 patients, a suicide more than six years after AHSCT treatment. The mean follow-up for the AHSCT group was 4.2 years and given that most severe treatment-related adverse events occur close to the administration of the treatment, our study supports that the contemporary treatment-related mortality of AHSCT is low. This is likely explained by the use of less aggressive ablative conditioning regimens and better routines for preventing infection, compared to when AHSCT was introduced.

Another Swedish observational study, published after Study IV, also compared AHSCT to alemtuzumab using the Swedish MS Register, but chose to focus on slightly different outcomes.[248] This study found that patients treated with AHSCT were more likely to remain disease free, but had more adverse events in the first 100 days, after which adverse events were more common for alemtuzumab.

A case-series from the United Kingdom in 2021 included 120 patients treated with AHSCT, of which more than half had progressive forms of MS.[249] This study found no new relapses in 87% of patients and no new MRI lesions in 85% of patients, at four years after treatment start. However, they also reported three transplantation-related deaths within 100 days (2.5%), all caused by fluid overload followed by cardiac or respiratory failure. The higher mortality compared with Study IV is likely due to these patients being older and with more MS-related disability (mean age 50 years and EDSS 6.5 at baseline), as well as possible differences in the conditioning regimens.

Taken together the current evidence for AHSCT in MS suggests that AHSCT provides long-term remission of MS inflammation in most patients, with higher effectiveness in patients with active, non-progressive disease. The treatment-related mortality has been significantly reduced since the early days of AHSCT and is now in line with other DMTs for MS, although mortality seems to increase for older patients with higher levels of disability. AHSCT is a suitable treatment choice for younger patients with aggressive, active MS and without severe disabilities, where conventional therapies are insufficient. This also is in line with the Swedish MS Society's (*Svenska MS-Sällskapet*) recommendations.[250]

5.3 Methodological Considerations

There were several specific methodological considerations of interest for the studies in this PhD project, relating to data quality and missingness, confounders and other types of bias, sampling and follow-up, as well as therapy specific details.

5.3.1 Data Quality and Missing Data

One of the main strengths of the studies included in this thesis is the use of data from linked health-care and demographic registers (Section 3.3). This allowed us to study outcomes and control for baseline characteristics that would not be possible with only a single data source. However, there were still aspects of data quality that need to be addressed. For the Swedish MS Register, this mostly relates to the coverage of the register and the completeness of the data (Section 5.1). In Study I, data were complemented through clinical chart review, while missingness in Study III and V was addressed through multiple imputation for the baseline characteristics. Importantly, there were never any missing data for exposure or outcome measures. For the national registers, the main data quality issues were the lack of general-practice outpatient care in the Patient Register (Section 3.3.2) and the risk of missing data on lethal cancers in the cancer register (Section 3.3.4). The lack of general practice data is likely to have resulted in the underestimation of some diagnosis-based outcomes. However, this is mostly a concern for milder adverse events, as more serious illness shows up in the available data for inpatient or specialized outpatient care. The missing data on lethal cancers would have been a bigger issue if we had assessed specific cancers with especially poor outcome, such as pancreatic cancer or lung cancer. However, Study III was not powered enough for this type of analysis.

5.3.2 Covariate Selection

Due to the breadth of variables available through the register linkages in Study III and V, it was possible to include many different covariates in analyses to control for potential confounding. It was especially important to control for variables relating to disease activity at baseline, such as previous therapies, relapses, MRI lesions, EDSS and other scores, disease duration, and type of MS. We also controlled for demographic factors, such as age, sex, place of birth, and education level, as well as the general health of the patient with variables for antidepressants, antidiabetics, antipsychotics, arrhythmia, major acute cardiovascular events, serious infections, cancer, hospitalizations, sick leave, and disability pension. In Study III, we also included previous immunosuppressive or glucocorticoid use. In Study I and IV, we used the subset of these variables that were available in the MS Register.

5.3.3 Residual Confounding

Residual confounding is a methodological challenge in all non-randomized studies (Section 3.11.1), including those in this thesis. Baseline differences were noted between the therapy groups in all the comparative studies (I, III, IV, and V).

In **Study I**, patients treated with rituximab had shorter washout time and longer time on the previous natalizumab treatment. However, the results remained stable after controlling for these differences, as well as in a sensitivity analysis restricted to patients with less than three months of washout time. There were also differences between treatment centres. Patients from Stockholm were younger than those from Gothenburg, suggesting that the threshold for switching JCV-positive natalizumab patients to other therapies was lower in Stockholm. There was also a difference in the follow-up time, where patients on rituximab were on average followed for a shorter time, compared with those with fingolimod, likely due to rituximab being part of the treatment repertoire for MS a shorter time than fingolimod. This difference was managed through the multivariable Cox proportional hazards models.

In **Study III**, differences in baseline characteristics were managed through inverse probability of treatment weighting. However, we had no information on diet, alcohol use, workplace exposure to carcinogens, obesity, sunlight exposure, or smoking, all well-known risk factors for cancer. Although these variables are not likely to influence the choice of therapy, they cannot be excluded as potential confounders. Several general health markers and demographic variables were included as proxies, to limit this potential confounding.

In **Study IV**, there were only minor baseline differences between the two main groups, and together with the size of the effects and the clear temporal relationship with therapy start, it is unlikely that the observed effects could be caused by confounding alone.

In **Study V**, patients with natalizumab had fewer previous therapies, more active disease, and were younger. However, a wide range of demographic and clinical factors were controlled for in the analyses through inverse probability of treatment weighting and there were overall small differences in healthcare resource use and costs, other than for the index therapy. It is therefore unlikely that residual confounding could have substantially altered the main conclusions.

5.3.4 Therapy Episodes and Follow-Up

Two of the more difficult methodological aspects of our studies to communicate to reviewers and fellow researchers were the choice of therapy episodes as the unit of observation and using an ever-treated approach to follow-up.

For all the comparative studies in this thesis (I, III, IV, and V), we sampled *therapy episodes* from the MS Register, rather than patients. In Study I and IV, these end up being the same thing, as every patient could at most have a single eligible therapy episode, due to how the inclusion criteria were specified. However, in Study III and V, a single patient could contribute with several therapy episodes, at different times in their life. In fact, this is the case for most patients not starting with rituximab as their first therapy, as many of them need to switch therapy later on due to breakthrough disease, tolerability issues, or JCV positivity in the case of natalizumab. Focusing on therapy episodes, rather than following individual patients, might not be immediately intuitive, but it has the advantage of allowing us to use all of our data and not limit the study population to only those with first or second line of therapy. This is important for precision, generalizability, and avoiding selection bias, but raises other challenges that need to be handled in the analysis. The main issue is that we are now comparing more heterogenous groups at different stages of treatment and disease. We addressed this by controlling for key baseline characteristics, such as number of previous therapies and disease duration.

In Study III, IV, and V, we used an ever-treated approach to follow-up, in contrast to on-treatment, meaning that we followed therapy episodes also after the patient had stopped receiving the therapy or even started a different one. This is similar to the intention-to-treat approach that is recommended in randomized controlled trials, in that it avoids differential loss to follow-up that could bias the results (Section 3.11.2). It also more closely resembles the real-world clinical situation where an MS patient and their physician have to decide on a DMT. They need to consider aspects of treatment often missed in randomized controlled trials and on-treatment analyses, such as long-term effects of treatment or the increased risk of rebound disease after ending certain therapies. The trade-off with the ever-treated approach is that we risk diluting any associations in the data that are the strongest while still receiving a specific treatment, e.g. herpes virus reactivation while being actively treated with fingolimod. However, since short-term, on-treatment effects are better assessed in clinical trials, we chose to lean into the strengths of our chosen study design.

As a consequence of both studying therapy episodes and using the ever-treated approach to follow-up, the observations in our studies were no longer completely independent, as they were clustered among individual patients. This risks artificially decreasing the standard error, unless we take it into account in the analyses (3.8), by using cluster-robust standard errors (Study III) or non-parametric estimation of the standard error, e.g. through bootstrapping (Study V). This approach also affects the interpretation of our results, changing it from "what happens while a patient is receiving therapy X" to "what happens if a patient starts therapy X", which is often the more clinically relevant question.

5.3.5 Rituximab Dose and Interval

Since rituximab is not formally approved for MS, there is no label or product information specifying the official dose and interval length to be used. Initially, the treatment regimen for rheumatoid arthritis was adopted, but with the increasing use of rituximab in Sweden, a consensus started to form about the dosing used in the MS setting. The majority of MS patients treated with rituximab in Sweden now receive an infusion of 500 mg every six months, which is a lower dose than what is commonly used internationally. This lower dose is seemingly not affecting the effectiveness of rituximab, but might contribute to the relatively low rates of adverse events in our studies.

Looking back through the data in the Swedish MS Register, it is clear that several different treatment regimens for rituximab have existed, mostly differing in the use of higher doses when initiating treatment. However, some patients received regular doses of 1000 mg. Since the COVID-19 pandemic, it has become more common to spread out infusions with longer intervals of one to two years. There is a risk that patients with a lower dose or longer intervals between infusions would be undertreated and thereby have an increased risk of breakthrough disease, but since outcomes relating to insufficient effectiveness are so rare for rituximab in our studies, this risk is likely small. Until recently, the differences in infusion interval length have been relatively small, but this might change now that prolonged intervals between infusions are being used more widely, something future studies will have to consider.

5.3.6 Surveillance Bias

The increasingly long infusion intervals for rituximab, as well as the differences between type and frequency of administration for the other MS DMTs, increase the risk for *surveillance bias*, a type of missingness. Patients that more frequently visit the clinic for infusions or treatment follow-up are more likely to report also less serious adverse effects or relapses, while those with only rare contact with healthcare might forget to bring these up at their next visit. However, this potential bias should be limited by the national guidelines for follow-up and assessment of MS patients, which state that patients should see a neurologist and have an MRI scan at least once a year. Patients are also more regularly followed up by nurses and are asked to fill in assessment forms from home. Even so, it is still possible that a patient on natalizumab that goes in for infusions every month will be more inclined to report healthcare events, compared with a patient receiving rituximab infusions every six to 24 months.

5.3.7 Immortal Time Bias

There is a risk of immortal time bias in the way we sample therapy episodes in the MS Register, due to the fact that some patients will have first received treatment and then been added to the register at some later point. These patients would be guaranteed to have been available/alive in the time between therapy start and inclusion in the register. Patients who die or are lost to follow-up in some other way during this time would never be registered. We manage this by excluding therapy episodes started long before the patient was added to the MS Register (more than 90 days).

5.3.8 Cost-Effectiveness Outcomes

Cost-effectiveness analyses commonly use Quality-Adjusted Life-Years (QALYs) as the main effectiveness measure, reflecting both the quality and quantity of life. Incremental costs per QALY additionally allow for comparisons of cost-effectiveness results across different diseases, but are less relevant when comparing treatment options within a specific disease. In Study V, we chose to not use QALYs based on disability progression measured with EDSS, as the available data for this measure was insufficient. In addition, EDSS is a relatively insensitive measure over the medium term, despite observed differences in relapses and lesions on MRI.

Study V was restricted to healthcare-related costs, not including societal costs, such as productivity loss. These are important disease-associated costs, but are also difficult to generalize across different social welfare systems, making the results less generalizable.

5.4 Future Research

Our understanding of MS, and consequently what treatments are available, has come a long way in a short time, but there is much left to discover. We still have an incomplete understanding of exactly what causes MS and why some individuals are at a greater risk than others. The progress so far has led to dramatic improvements in the lives of persons with MS, but there is still a long way to go until MS is the manageable condition we hope for. Several key aspects need to be investigated over the coming years.

5.4.1 Progressive Worsening

As MS DMTs have become increasingly more effective at limiting acute inflammatory activity and relapses, it has become apparent that even in the absence of new lesions, many patients report progressive worsening. This mainly concerns diffuse symptoms relating to cognition, memory, or fatigue. Previously, progressive disease was thought to only be present in SPMS and PPMS, as neurological disability in RRMS was attributed to relapse activity. There are currently no effective treatment options that target this progression outside the inflammatory relapses, making it an important target for future research.

5.4.2 Reparative Treatment

A type of treatment that has not yet seen much progress in MS is reparative therapy, with the ability to reverse some or all of the neurological damage and disability that can accumulate over the lifetime of an MS patient. The potentials for neuroreparative therapies are not limited to MS and would likely revolutionize neurorehabilitation across many health conditions, as patients suffering stroke, traumatic damage, or just age-related degeneration would benefit greatly.

5.4.3 Curative Treatment and Preventative Measures

The long-term goals in MS care are to find curative treatments and preventative measures. As our understanding of MS evolves, we move closer to these goals. Of particular interest are the recent reports that EBV infection is closely linked to the development of MS. As EBV forms a latent infection by hiding in B-cell populations, research into the effects of B-cell-depleting therapies on the virus' ability to remain hidden could potentially lead to new treatment approaches. However, since latent infection with EBV is so prevalent in most populations, reinfection would likely be a problem. A more robust approach would be to develop a vaccine that could protect against primary EBV infection and thus potentially also MS. It is possible that the advancements in vaccine development and production during the COVID-19 pandemic could speed up this process.

5.4.4 Novel Disease Markers

Neurofilaments are part of the neuronal cytoskeleton and can be used as a measure of axonal damage to assess ongoing harm in the central nervous system. Initially, neurofilaments were only measured intrathecally (in cerebrospinal fluid from the spinal canal), but it has recently become possible to also measure them in peripheral blood. In the clinic, neurofilament can be a complement to MRI for assessing ongoing inflammatory activity or progressive worsening. When reliable peripheral measurements become more widely available, it could possibly be used by patients themselves to monitor for relapses and disease progression, similar to blood-sugar monitoring in persons with diabetes. In this way, patients and physicians would have an early-warning system for breakthrough disease or conversion to SPMS. Apart from the clinical benefits, neurofilament also has great potential as an outcome measure in studies of MS therapies, especially if it could be measured frequently and easily in peripheral blood. This would allow for a much finer timescale and better precision to assess the start of relapses. It would also make it possible to find sub-clinical disease that might otherwise be missed.

Brain atrophy is another measure with the potential to benefit both clinical care and serve as an outcome measure in research. As the technology used for MRI advances, we are better able to map and follow changes in the brain and spinal cord for persons with MS. Brain atrophy is a marker that is thought to be more closely linked to progressive worsening than the inflammatory lesions, and precise and frequently measured observations of brain atrophy would be a valuable outcome measure for future studies of DMTs targeting progressive MS.

5.4.5 Personalized Medicine

As not all individuals react the same way to the therapies they receive, it would be of great value to identify factors that can predict for which patients a specific treatment would be especially beneficial or harmful. This would allow patients and physicians to make more well-informed decisions when choosing a DMT. It would also give the patient a better estimation of their short- and long-term prognosis. Identifying variables that could be used in this way requires large datasets of patients with detailed information on many different variables. Although we are not there yet, this might be possible in the future if we find a way to better integrate the MS Register with the clinical chart system and collaborate with other countries to collect data in a standardized way.

5.4.6 Rituximab Infusion Intervals

Growing clinical experience suggests that the time between rituximab infusions can be prolonged from the now standard six months, without risking the return of inflammatory activity and possibly with fewer adverse effects (including easier timing for effective vaccinations). During the COVID-19 pandemic, rituximab infusions had to be given more sparsely due to administrative and social-distancing concerns. The clinical experience from this was that it is possible to prolong the time between rituximab infusions from six months to one to two years, and possibly even longer, without return of disease activity in most patients. One trial evaluating rituximab once per year vs every six months is currently ongoing (Section 2.3).

5.4.7 Rituximab vs Ocrelizumab vs Ofatumumab

Studies are needed to compare rituximab to the MS-approved B-cell-depleting therapies ocrelizumab and ofatumumab. Apart from assessing the relative effectiveness and safety, it is also important to evaluate the comparative cost-effectiveness. Two currently ongoing trials are comparing rituximab with ocrelizumab (Section 2.3). There is also a great opportunity for observational, register-based studies to clarify any potential differences among the B-cell-depleting therapies for MS. Unfortunately for Swedish researchers, rituximab is already so established for MS in Sweden that few patients receive the newer MS-approved anti-CD20 therapies, leading to a lack of data for these comparisons.

5.4.8 Rituximab Biosimilars

There are limited data on the equivalency of rituximab biosimilars to the original, MabThera. Further studies are needed to evaluate the effectiveness and safety of these new biosimilars to ensure that they provide the same effective protection, with no additional safety concerns. Of specific interest are the formation and clinical relevance of anti-drug antibodies.

5.5 Ethical Considerations

There are several ethical considerations in epidemiological research, and medical research in general. Four important topics are sensitive information, informed consent, effective comparisons, and open science and data.

The studies included in this thesis all had ethical approval:

Study I 2009/2107-31/2, 2013/445-31
Study II 2017/32-31/4
Study III/IV 2017/700-31/4
Study V 2021-02384

5.5.1 Sensitive Information

Epidemiological studies generally require a lot of sensitive information on large groups of participants to allow unbiased estimation of associations. However, these data also have the potential to cause personal, professional, and financial harm to the participants, if leaked or stolen. Security breaches of healthcare information are increasing and a US study identified 2149 breaches of health data between 2010 and 2017, comprising a total of 176 million records.[251]

The potential risks associated with research data have to be weighed against the benefits of the research in question. Study participants themselves can benefit from the research through the advancement of healthcare for their specific condition, and many studies also benefit society as a whole through a healthier population with less work loss and overall reduced costs. Specifically, treatment with rituximab and AHSCT would be unethical to *not* follow-up on, as these treatments are given outside of their respective indications.

We have taken several measures to minimize the potential risks associated with our study data. We have ethical permission for all studies and we follow the EU's General Data Protection Regulation (GDPR). Data are stored on physically protected, encrypted servers that are only accessed by authorized researchers. All register linkage data have been masked, with the personal identification number removed, and only aggregated data are finally reported.

5.5.2 Informed Consent

The scope of patient information and consent varies between types of studies and data sources. In studies directly affecting the individual (e.g. clinical trials), it is mandatory to collect informed consent from all participants. For epidemiological studies using registers, it is not as straightforward. Registers such as the MS Register allow participants to opt out of studies, although their data are still collected for clinical use. The national healthcare and demographic registers gather data on all citizens, with no option to not participate. This intrudes on the individual's privacy and autonomy, as data will be collected even without their consent. However, this has to again be contrasted with the benefits that research can provide. Asking for consent can also be an intrusion in life of the potential participants. It would, for example, not be feasible for all observational studies using a specific register to ask for consent from all individuals included in that register, as these individuals would then be bombarded with requests.

5.5.3 Effective Comparisons

Many of the randomized controlled trials for MS DMTs thus far have used a placebo control group, although this is starting to change. However, it can no longer be considered ethically defensible to use placebo or first-generation injectable therapies as comparisons for new treatments. The most obvious reason for this is that we now have effective treatments for MS, which should not be withheld from patients because they are participating in a study. Apart from causing unnecessary harm to patients, comparisons to placebo are also no longer especially informative. All therapies currently available for MS are more effective than placebo. What physicians and patients need to know is the *relative* effectiveness and safety of treatments. Of course, there is a risk to the pharmaceutical company developing a new drug to compare it to the most effective competitor, as there is a higher probability that the new treatment will not be as effective. However, this cannot be a reason for withholding proper treatment from the patients that need them.

5.5.4 Open Science and Data

Recently there has been a push for more reproducible research and open data. This means that all the material used in a study, including code, documentation, and data, should be made available to the scientific community. Code and documentation are often easily shared and, in many fields, this is also true for data. In clinical research, however, sharing data would mean sharing sensitive information about individual persons, although masked, which would be a breach of privacy and, with current legislation, illegal.

Chapter 6

Conclusions

Through the five studies in this PhD project, we validated the data in the Swedish MS Register, investigated the safety of AHSCT compared with alemtuzumab, and assessed the effectiveness, safety, and cost-effectiveness of rituximab compared with fingolimod, natalizumab, and dimethyl fumarate. In the context of the larger body of research, we conclude the following.

- The data in the Swedish MS Register is of generally good quality, especially for data relating to therapies, making the register a good resource for pharmacoepidemiologic studies. However, there are some issues with missing data, primarily for rituximab infusions, MRIs, and relapses. There are several actions that could be taken to further improve the accuracy and completeness of the data in the register.

- AHSCT is an effective and safe induction treatment for MS. Treatment-related mortality is similar that of other DMTs, for younger patients without severe disability. However, the risk of infection is increased around the time of treatment and there is a risk of autoimmune thyroid disease. This necessitates proper prophylactic care and monitoring.

- Rituximab is a safe and well tolerated treatment for MS, with no increased risk of cancer, PML, or death. However, rituximab increases the risk of both mild and severe infections (shown outside the studies of this thesis), which is important to consider in any risk-benefit assessment. Consideration is also needed for vaccination and vaccine timing, as was made clear during the COVID-19 pandemic.
- Rituximab is an effective treatment for reducing inflammatory disease activity in MS, leading to fewer clinical relapses, fewer contrast-enhancing lesions on MRI, and better drug survival.
- Rituximab is a cost-effective treatment for MS, with both higher effectiveness and lower healthcare, costs compared with MS-approved alternatives. Importantly, the lower drug costs were not offset by any increase in other healthcare costs.

6.1 Recommendations

- AHSCT should be considered for younger patients with aggressive, active MS, no severe disabilities, and where alternatives are insufficient.
- Rituximab should be considered as an alternative first-line therapy for most MS patients with an active non-progressive disease.
- Rituximab should be provided to MS patients in low-resource settings, where more expensive MS-approved alternatives are unavailable.

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