Assessment of mortality rates and comorbidities in patients with multiple sclerosis in Austria and their impact on disability and morphologic brain abnormalities

submitted by

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Declaration

*I hereby declare that this dissertation is my own original work and that I have fully acknowledged by name all of those individuals and organizations that have contributed to the research for this dissertation. Due acknowledgement has been made in the text to all other material used. Throughout this dissertation and in all related publications I followed the guidelines of "**Good Scientific Practice**".*

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Notification

Parts of this doctoral thesis have been published in ***Multiple Sclerosis. (****Mult Scler. 2016 Mar;22(3):340-6. doi: 10.1177/1352458515593405. Epub 2015 Jul 10)*. Parts of these results have already been presented as an oral lecture at the annual meeting of the European Academy of Neurology (EAN) in Istanbul in June 2014. Further results have been presented as an oral lecture at the annual meeting of the European Committee for Treatment and Research in Multiple Sclerosis in September 2016 in London.

The published manuscript was drafted by the doctoral candidate, Alexander Pichler. Therefore, significant parts of the doctoral thesis (the role of brain volume abnormalities) are similar to the published manuscript (with permission of *Multiple Sclerosis*).

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Abbreviations

3T *3-Tesla*

BGV *Basal ganglia volume*

CDMS *Clinically definite multiple sclerosis*

cGMV *Cortical grey matter volume*

CIS *Clinically isolated syndrome*

CNS *Central nervous system*

DMT *Disease modifying therapies*

EDSS *Expanded Disability Status Scale*

FLAIR *Fluid attenuated inversion recovery*

FSL *FMRIB Software Library*

MRI *Magnetic resonance imaging*

MS *Multiple sclerosis*

NBV *Normalized global brain volume*

PBVC *Percentage of brain volume change*

PPMS *Primary progressive MS*

RRMS *Relapsing-remitting MS*

SMRs *Standardised mortality ratios*

SPMS *Secondary progressive MS*

TV *Thalamic volume*

WMV *White matter volume*

Abstract

Background: Multiple sclerosis (MS) is a demyelinating disease of the central nervous system affecting about 2.5 million people worldwide. On a global perspective, MS is associated with a 3-times higher risk of death compared to the general population and life expectancy is about 10 years shorter. The reasons of death appear to be directly related to MS in about 50% of the cases. Other common causes are cardiovascular diseases, cancer and suicide. For Austria, data on the mortality of MS patients and their cause are sparse. Similarly, limited information of the spectrum of comorbidities and the influence of comorbidities, such as arterial hypertension, on the course of MS including morphologic brain abnormalities exists.

Aim: First, actual mortality data and rates for Austria shall be assessed. Second, the type and prevalence of comorbidities in MS and their impact on the course of MS shall be examined. Third, the evolution of brain volume changes over time in relation to the development of the disease and in association with comorbidities shall be evaluated.

Methods: The database of Statistics Austria was used to assess the evolution of the actual mortality rates for Austria on a national and regional basis.

Comorbidities were obtained from a dataset of an initial cohort of 324 patients who were treated ambulatory or stationary at the University hospital of Graz between 2006 and 2012. As MS specific clinical data such as information about the disease duration, disease course etc. were not available in all of these patients, we decided to focus our assessments on a sub-group of 120 individuals who were regularly seen in our outpatient clinic and have consecutive MRI scans ('in house cohort').

Brain volume parameters were obtained using semi-automated software SIENA(X). Imaging parameters included T2-lesion load (T2-LL), normalized brain volume (NBV), cortical grey (cGMV) and white matter volume (WMV) and volumes of the thalami (TV) and basal ganglia (BGV). The percentage of brain volume change (PBVC) over time was assessed using the semi-automated software SIENA.

The evolution of brain volume changes and lesion load was investigated separately with and without inclusion of comorbidities. Additionally, potential sex specific differences and differences regarding the disease course (clinically isolated syndrome, CIS versus clinically definite MS, CDMS) were looked into.

Results: Mortality rates: Between 1970 and 2000, mortality rates in MS (i.e. death due to MS per 100 000 people) decreased in both men and women (1970-79: women 1.43, men 0.91 versus 2000-09: women 0.75, men 0.75). During the last decade, however, a slight increase of mortality rates in women (women 0.79 versus. men 0.62) could be observed. Regional mortality rates showed a slight west to east gradient with lower mortality rates in western regions (Tyrol 0.68 versus Vienna 0.96). In 2014, the mean age at death due to MS was 67.3 years for women and 62.9 years for men.

Assessment of comorbidities: 120 patients with either a CIS; n=63 or with CDMS; n=57 at baseline could be identified. 54 patients had at least one comorbidity. Depression (20%) was the most common comorbidity, followed by arterial hypertension (11.7%) and autoimmune thyreoditis (9.2%). The presence of one or more comorbidities was neither associated with the disease course nor with disability.

Analysis of brain volume changes and lesion load: The mean follow-up period between first and second scan was 43 months. At baseline all brain volume metrics, except cGMV, were significantly lower and the T2-LL was significantly higher in MS compared to CIS. During follow-up, only the PBVC was higher in MS (p=0.008) and this difference was driven by converters from CIS to MS.
When comorbidities are included in this analysis, patients with at least one comorbidity had a significantly lower total brain volume (p=0.001) as well as grey (p=0.06) and white matter volume (0.03) at the baseline MRI scan, whereas the T2-LL was higher (p=0.012). The change of brain volumes and the accrual of lesion load were comparable during the observation.

Conclusion: Mortality rates in Austria were increasing during the last decade, potentially due to a higher awareness of MS in the population. Nevertheless, the mortality rates as well as the age at death are lower in comparison with other western countries, thus indicating that MS might not have been coded as the underlying cause of death in all deceased MS patients in Austria.
It could be shown that comorbidities are present even in the early stages of MS and are associated with lower brain volumes. However, in this study the longitudinal changes of brain volumes and lesion load could not be associated with the presence of comorbidities. Further studies with a longer observation period are needed to evaluate the long term impact of comorbidities on brain volumes.

Zusammenfassung

Einleitung: Die Multiple Sklerose (MS) ist eine demyelinisierende Erkrankung des zentralen Nervensystems, die weltweit ca. 2,5 Millionen Menschen betrifft. Aus globaler Sicht ist das Mortalitätsrisiko von MS-Patienten/innen ungefähr 3mal höher als das der Normalbevölkerung. Die Lebenserwartung ist ca. um 10 Jahre kürzer. In ca. 50% der Fälle wird MS als primäre Todesursache angeführt; weitere häufige Todesursachen bei MS-Patienten/innen sind kardiovaskuläre Erkrankungen, Malignome und Selbstmord. In Österreich existieren nur wenige Daten zur Mortalität bei MS-Patienten/innen. Ähnlich verhält es sich mit Daten zu Begleiterkrankungen, wie beispielsweise Hypertonie, und deren Einfluss auf den Erkrankungsverlauf und Veränderungen der Hirnvolumina.

Ziele: 1.) Erhebung der aktuellen Mortalitätsraten von MS-Patienten/innen in Österreich. 2.) Untersuchung der Anzahl und Art von Komorbiditäten bei MS-Patienten/innen und deren Einfluss auf den Erkrankungsverlauf. 3.) Messung von Hirnvolumsänderungen in der Magnetresonanztomographie (MRT) und deren Beziehung zum Erkrankungsverlauf und dem Vorliegen von Begleiterkrankungen.

Methoden: Über die Datenbank der Statistik Austria wurden Daten zur aktuellen Mortalität auf nationaler und regionaler Ebene bestimmt.

Die Begleiterkrankungen wurden aus einer Kohorte von ursprünglich 324 Patienten/innen mit der Diagnose MS erhoben, welche zwischen 2006–2012 ambulant oder stationär in zumindest einer Abteilung der Medizinischen Universität Graz in Behandlung waren. Da sich im Rahmen der Auswertung herausstellte, dass bei einer Vielzahl dieser Patienten/innen keine MS spezifische Informationen über Erkrankungsdauer oder Erkrankungsverlauf verfügbar waren, wurde beschlossen den Fokus auf eine gut dokumentierte, 120 Patienten/innen umfassende Kohorte der MS Ambulanz der Universitätsklinik für Neurologie Graz zu setzen.

Hirnvolumina wurden mittels der semiautomatischen Software SIENA(X) bestimmt. Die Messung der Hirnvolumina umfasste das normalisierte, globale Hirnvolumen (NBV), das Kortexvolumen (cGMV), das Volumen der weißen Substanz (WMV) und Strukturen der tiefen, grauen Substanz von Thalamus (TV) und den Basalganglien (BGV). Zur Messung der prozentuellen Veränderung des globalen Hirnvolumens (PBVC) über die Zeit wurde die semiautomatische Software SIENA benutzt. Zusätzlich wurde die T2-Läsionslast bestimmt.

Die temporale Veränderung der einzelnen Hirnvolumina und der Läsionslast wurde sowohl mit Einschluss als auch separat ohne Hinzunahme von Begleiterkrankungen untersucht. Zusätzlich wurde auf mögliche geschlechtsspezifische Unterschiede und auf Divergenzen zwischen den verschiedenen Erkrankungsverläufen (klinisch isoliertes Syndrom, CIS versus klinisch gesicherte MS, CDMS) getestet.

Ergebnisse: Mortalitätsraten (bezogen auf 100000 Einwohner): Zwischen 1970 und 2000 ist eine Abnahme der MS-bedingten Mortalität sowohl bei Männern als auch bei Frauen in Österreich zu verzeichnen (1970-79: Gesamt: 1,18; Frauen 1,43; Männer 0,91 versus 2000-09: Gesamt 0,63; Frauen 0,75; Männer 0,75). Innerhalb des vergangenen Jahrzehnts konnte allerdings ein leichter Anstieg vor allem bei Frauen beobachtet werden (Gesamt: 0,82; Frauen 0,79; Männer 0,62). Regional konnte ein leichter West–Ostgradient mit niedrigeren Mortalitätsraten in den westlichen Bundesländern erhoben werden (Tirol 0,68 versus Wien 0,96). 2014 lag das Todesalter bei MS-Patienten/innen im Mittel bei Frauen bei 67,3 Jahren und bei Männern bei 62,9 Jahren.

Komorbiditäten: Es konnten 120 Patienten/innen mit entweder einem CIS, n=63, oder einer CDMS, n=57, identifiziert werden. 54 Patienten/innen hatte zumindest eine Begleiterkrankung. Depression war mit 20% die häufigste Komorbidität, gefolgt von arterieller Hypertonie (11,7%) und Autoimmunthyreoditis (9.2%). Das Vorliegen von einer oder mehreren Begleiterkrankungen hatte weder einen Einfluss auf den Erkrankungsverlauf noch auf die Erkrankungsschwere.

Hirnvolumina und Läsionslast: Zwischen der ersten (baseline) und zweiten (follow-up) Untersuchung lag ein Intervall von durchschnittlich 43 Monaten. Zur baseline zeigten sich alle Hirnvolumsparameter bis auf das cGMV niedriger und die T2-Läsionslast höher bei CDMS als bei CIS. Im zeitlichen Verlauf erwies sich nur die PBVC bei CDMS höher (p=0,008). Dieser Unterschied war in erster Linie durch Patienten/innen ausgelöst, die im Verlauf von CIS zu CDMS konvertierten.

Nach Inklusion der Komorbiditäten in die Analyse konnte gezeigt werden, dass Patienten/innen mit zumindest einer Begleiterkrankung sowohl ein deutlich niedrigeres NBV (p=0,001) als auch ein niedrigeres cGMV (p=0,06) und WMV (0,03) aufwiesen. Auch die T2-Läsionslast war bei Individuen mit Begleiterkrankungen höher (p=0,012). Die Abnahme der Hirnvolumina über die Zeit war unabhängig vom Vorhandensein von Begleiterkrankungen.

Diskussion: Die Mortalitätsraten zeigen in der letzten Dekade einen leichten Anstieg, welcher in erster Linie durch ein höheres Bewusstsein für MS in der Bevölkerung erklärt werden kann. Trotzdem zeigt sich vor allem im Vergleich mit anderen westlichen Ländern, dass die Mortalitätsraten, aber auch die Lebenserwartung in Österreich niedriger sind. Dieser Umstand ist wahrscheinlich dadurch bedingt, dass nicht bei allen verstorbenen Patienten/innen MS als Todesursache angeführt wurde.

Es konnte zusätzlich gezeigt werden, dass Begleiterkrankungen bei MS-Erkrankten bereits in frühen Stadien vorhanden sind und hier bereits einen Einfluss auf Hirnvolumina haben. Longitudinale Veränderungen der Hirnvolumina waren unabhängig vom Vorhandensein von Begleiterkrankung; weitere Untersuchungen mit längeren Untersuchungsintervallen sind daher dringend notwendig.

1. Introduction

Multiple sclerosis (MS) is a chronic demyelinating disease of the central nervous system (CNS) and the leading cause for non-traumatic disability in young adults, affecting more than 2.5 million people worldwide.[3] In 2010 the expected prevalence of MS in Austria was approximately 148 per 100000 inhabitants[4] which is in the range of other central European countries. As this chronic disease begins already in early adulthood and shows a consecutive development of disability as well as a high prevalence, it has a significant socio-economic impact. In that regard, several studies demonstrate that the hospitalisation rate of MS patients is higher compared to the general population.[5,6]

In the last couple of years several research groups could demonstrate that the life expectancy of MS patients is about 10-years shorter[7] and the all cause mortality rate is up to 3.5 times higher compared to the general population.[8,9] The presence of comorbidities such as respiratory tract disorders and cardiovascular diseases are associated with an additional increase of mortality in MS.[9,10] Some authors also report higher mortality rates in female patients.[8]
So far there is only one publication investigating the mortality rates in Austria. In this study, which includes data from 1970 -2001, a continuous reduction of MS-related mortality during the observation period of 32 years of altogether 47% could be observed.[11]

MS itself is thought to be the underlying cause of death in approximately 50% of the patients. Even though MS itself only rarely leads to death for example due a demyelinating plaque within the brainstem,[12] most people die because of complications such as infections related to their immobility in the late stages of the disease, or due to cardiovascular and respiratory tract disorders.[8] Interestingly the reported causes of death in MS patients also differ regionally. However, this might partly be caused by methodological issues given that the assessment and documentation of the cause of death and the associated comorbidities differ between distinct countries.[13] Nevertheless knowledge of the regional mortality rates and causes of death is an emergent necessity as it may indicate the need for consequences in the management of MS patients such as more close monitoring and treatment of comorbidities.

While other European countries like Denmark or Sweden have established MS-registries for the collection and documentation of related information, initiated almost 60 years ago, such comprehensive registries are missing in Austria. While data about mortality and the cause of

death of MS patients are also recorded from different institutions in Austria it is difficult to combine the various databases and data are usually not subject to quality control.

For long the role of comorbidities in MS has been neglected. However, in the last couple of years increasing interest came from reports which could show an association of comorbidities with the course of MS, e.g. that the coexistence of cardio-vascular diseases was associated with more rapid disease progression.[14] Other studies could demonstrate a correlation between the presence of comorbidities and the morphological changes observed on magnetic resonance imaging (MRI) of the brain such as higher rates of brain volume loss and a higher lesion load in MS patients with than without cardiovascular comorbidities.[15] This is an important finding considering that several investigations could demonstrate that pronounced brain volume loss, which occurs already in early stages of MS and higher lesion load are associated with higher disability and a more rapid decline of cognitive functions.[16–19] However, long term data on the impact of comorbidities on brain volume loss and lesion load are still missing.

MRI nowadays is an established diagnostic tool for MS and it is also used to monitor the disease over time. It has some predictive value for disability progression, even though a prognosis about the disease course on an individual basis is impossible. Nevertheless, it could be demonstrated that the accrual of lesion load is associated with the grade of disability which develops over a period of 20 years of follow-up.[20] Even more promising results could be obtained by measuring the brain volume changes in MS patients. Several studies could demonstrate that the loss of global brain volume as well as of compartmental brain volumes such as gray matter volume, are associated with the progression of disability and of cognitive impairment.[16–19] However, most of these studies focused on a single phase of the disease or only looked for changes in single brain regions. Comprehensive studies with a longitudinal assessment comparing different measures of global, compartmental and regional brain atrophy are rare.

These aspects served to define three distinct objectives for this doctoral thesis. These objectives were

1) to provide actual mortality rates of MS and to identify the causes of death of MS patients in Austria using the database of Statistics Austria

2) to identify the frequency of comorbidities in MS patients in Austria and

3) to investigate the evolution of brain volume loss and lesion load and their association with the presence of comorbidities

Given the lack of systematic registries in Austria, the collection and assessment of data regarding mortality and comorbidities in order to answer the two initial research questions posed a major challenge in this thesis. We had speculated to use the local hospital patient database as source of information. However, this was complicated by the fact that MS patients are mostly treated in outpatient departments and by practising neurologists or family physicians who do not participate in shared and accessible documentation systems. We therefore decided to also defined a third objective, i.e. to investigate the impact of comorbidities on the evolution of brain volume loss and lesion load in a subset of MS patients who are regularly seen in the outpatient clinic of the department of Neurology of the Medical University of Graz.

For a better understanding of this complex and heterogeneous disease the following chapter gives a short introduction to general aspects of MS, associated causes of death, the interaction with potential comorbidities and an overview of morphologic brain abnormalities.

2. Background

2.1 Epidemiology

More than 2.5 million people worldwide are affected with MS. The rates of MS vary regionally with a trend of a decreasing prevalence from north to south. Austria has a prevalence of 148 per 100000 inhabitants[4], which is in line with other central European countries.[21] Significantly higher rates are reported for example for Scotland with approximately 187 patients per 100000 inhabitants.[22] This regional differences in the proportion of affected patients is likely explained by a genetic predisposition and/or environmental factors as potential triggers for MS.[23]

2.2 Aetiology

During the last decades intensive research has been carried out to find an etiologic factor for the development of MS, but until now the exact cause still remains unknown. It is assumed that multiple factors including age, genetics, viral infections and several environmental/ nutritional factors contribute to the development of the disease.[24]

The importance of genetic factors has been documented in several ways. Twin studies showed a significantly higher concordance for MS of 25% in monozygotic twins while in dizygotic twins a concordance rate of only 5% could be demonstrated.[25] Other investigations showed that first grade relatives of MS patients have a relative risk of 5% for the development of MS, which is about 20-50 times higher compared to the general population.[26]

Also viral infections as for example the Epstein-Barr virus (EBV) are in discussion as a potential trigger for MS. A post-mortem analysis of MS brain tissue showed that EBV might be reactivated in MS-lesions and that B-Cells express specific markers indicating a latent infection with the virus.[27,28] In contrast, other investigations could not find an association between EBV and MS.[29,30]

Other potential causes which are still under investigation are the influence of sun light exposure, environmental pollution, cigarette smoking and several toxins.[31,32] In the last couple of years several studies could demonstrate that lower Vitamin D levels are associated with a higher risk of MS and an increased disease activity.[33,34]

2.3 Histopathology

The typical sclerotic plaques which represent the end stage of the acute inflammatory process is a result of inflammation, demyelination, remyelination and in the end axonal and neuronal degeneration.[24] Predilection spots for these plaques are the periventricular white matter, the brain stem, the juxta-cortical region, the cerebellum, and the white matter of the cervical cord.[35] Nevertheless, due to post mortem studies and the implementation of advanced MRI techniques such as double inversion recovery (DIR) it could be demonstrated that MS is not a sole disease of the cerebral white matter but that inflammatory processes also affect grey matter structures even in the early stages of the disease.[36] These findings are of direct clinical relevance because they correlate with disability and disease progression in relapsing remitting MS as well as in progressive MS.[37,38]

The key players during the acute inflammatory process are auto-reactive lymphocytes which migrate across the blood-brain barrier due to failure in the cell regulation.[24] Histopathologic studies of MS plaques revealed that especially in the early stages of the plaque evolution CD8+ lymphocytes and macrophages are the major cell population.[39] But also B-cells drive disease activity due to several mechanisms such as antigen presentation, cytokine and antibody production.[40]

In the late stages of the disease active inflammatory processes decrease, while neurodegenerative processes become more prominent.[41]

2.4 Clinical characteristics of multiple sclerosis

2.4.1 Sex ratio

The sex ratio is clearly to the disadvantage of female patients with a female to male ratio of 2-3:1. This sex ratio increased between 1930 and 1990 (from 2.35 to 2.73) especially in patients with a relapsing remitting disease course and also shows a latitudinal gradient with a decreasing ratio from North to South.[42]

Interestingly though, in primary progressive MS (PPMS) the sex ratio is equal.[43]

2.4.2 Signs and symptoms

About two thirds of all patients suffer their first clinical attack between the age of 20 to 40 years with a peak at the age of 30.[44]

The first episode of a demylinating event is termed clinically isolated syndrome (CIS) if (the later described) criteria for definitive MS are not yet fulfilled,. Per definition neurological deficits must persist at least for 24 hours to be considered as an attack.[45] About two thirds of all patients with a history of a CIS will have further attacks and convert to relapsing-remitting MS (RRMS).[46]

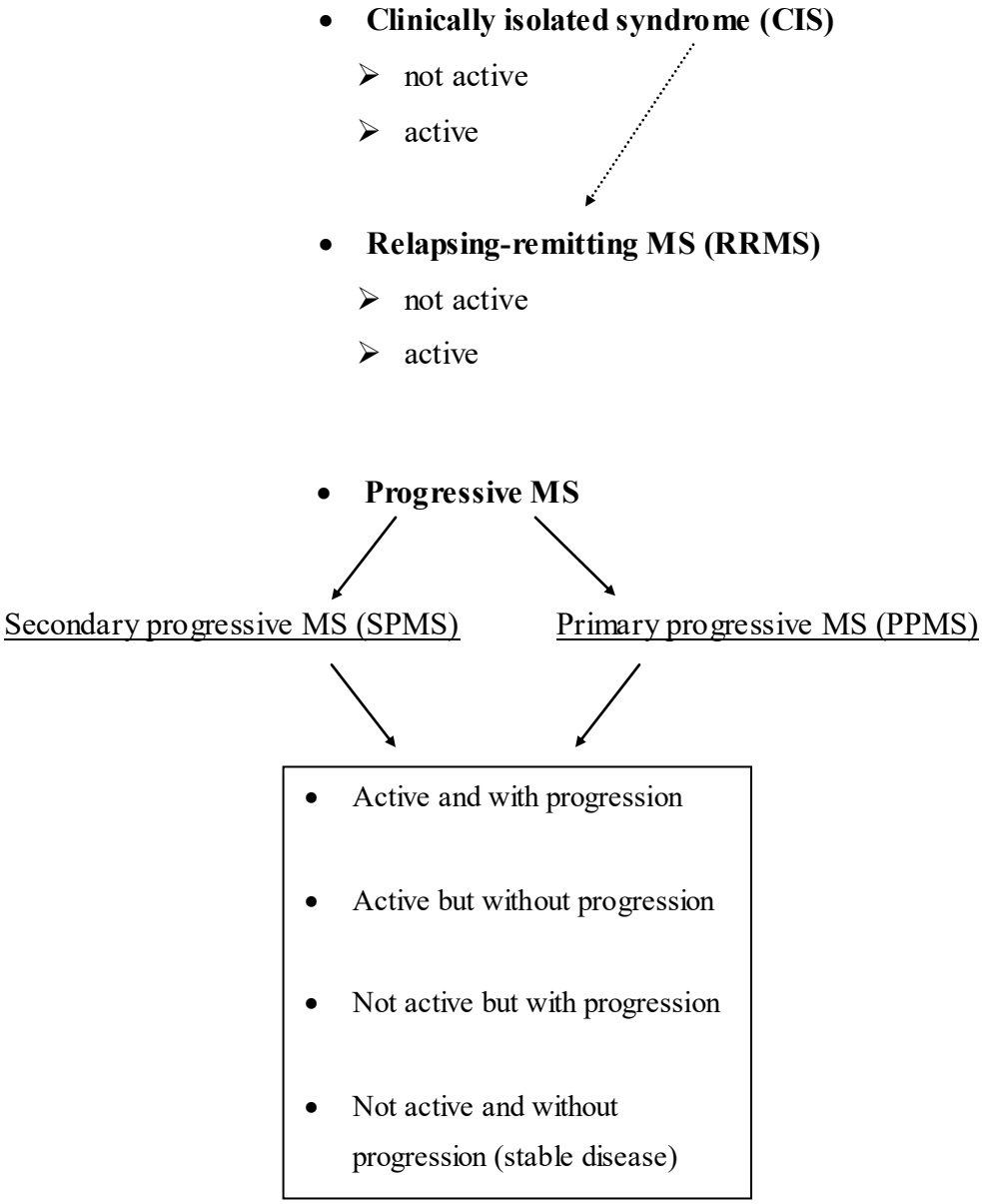
The clinical manifestation of multiple sclerosis depends on the location of the plaque in the CNS. Typically, the first episode of more than two thirds of all patients occurs in a mono symptomatic manner[43], e.g. with either sensory loss, visual blurring, motor function deficits, ataxia, bladder-, bowel- or sexual dysfunction[47] (see table 1). Thus, none of the listed symptoms are specific for MS but they are nevertheless rather characteristic. A more specific symptom for MS is the Lhermitte's sign, which is characterised by electrical sensations beginning in the neck and radiating into the limbs when the head of the patients is bending forward. This phenomenon is suggestive of an involvement of the cervical cord or the caudal medulla oblongata.[24]

Table 1: Typical symptoms of MS at disease onset

Initial symptomes (%)	(Poser, et al. 982)[48] N= 1571	(Confavreux et al., 2000)[49] N= 1844	(Myhr et al., 2001) N= 220
Pyramidal	43	52	38
Sensory	43	-	44
Brainstem/ Cerebellum	24	9	66
Optic neurits	33	18	31
Bladder/Bowel	9	-	2
Cognitive dysfunction	4	-	-

2.4.3 Disease course

MS has different disease courses based on the number of clinical relapses, time to disease progression and development of new lesions on MRI. According to the revised definition of MS types of Lublin et al.[50] MS is categorized in:



- Activity is referred to clinical relapses and/or MRI changes (gadolinium-enhancing lesions, new/enlarging T2 lesion)
- Progression should be assessed by clinical evaluation at least once yearly

The most common disease course at the beginning of MS is the RRMS form in about 85% of all patients.[47,49] RRMS is characterised by repeated occurrence of focal-neurological deficits called relapses with a full or at least partial recovery between the attacks. A relapse in turn is defined as a persistent neurological deficit for more than 24h-hours, which is not triggered by an infection or other external causes.

Studies of untreated cohorts could show that about 10 years after disease onset approximately 40% of RRMS patients develop a SPMS, which is defined as a continuous progression of disability with sporadic relapses especially in the early phase of this stage. After 25 years the number of patients converting to SPMS increases up to 85%.[47,49]

About 10% of MS patients show a constant worsening of disability right from the disease onset without any relapses or phases of remission, which is termed as PPMS.

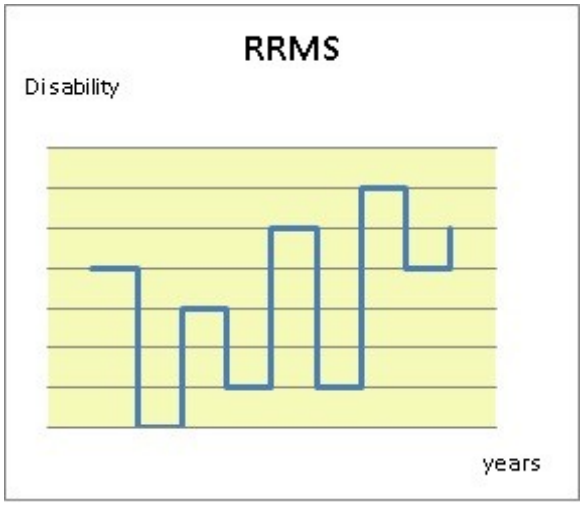


Figure 1: Relapsing remitting disease course over time

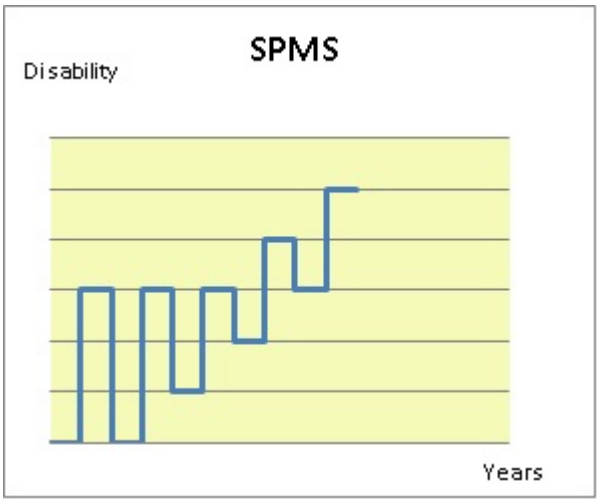


Figure 2: Secondary progressive disease course over time

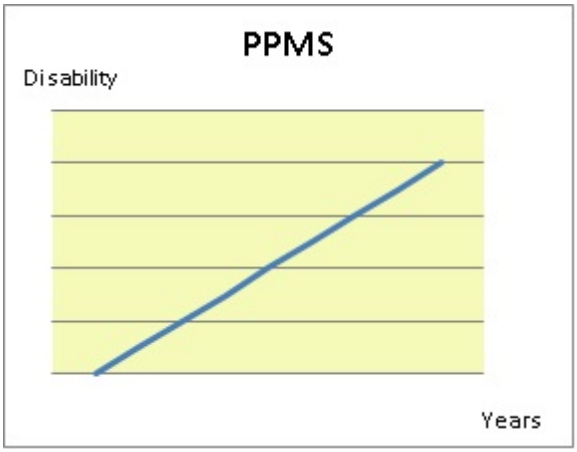


Figure 3: Primary progressive disease course over time

2.4.4 Rating neurological impairment in multiple sclerosis

The level of disability is commonly rated according to the Expanded Disability Status Scale (EDSS), which was firstly introduced in 1983.[1] The EDSS is an ordinal scale which ranges from 0 to 10 (see table 2). A score of 0 indicated the absence of any neurological deficits while a score of 10 is equivalent to death due to MS. Up to a score of 4 the EDSS is mainly rated on the basis of 8 functional scores. Individuals with a score higher than 4 are rated according to their walking distance (see table 2). Even though the EDSS is sometimes criticized because it does not include all clinical information, e.g. ignores more subtle cognitive dysfunction, it is still the most common rating instrument in clinical studies and is therefore also used in this thesis.

Table 2: The Expanded disability status score (EDSS) adapted from Kurtzke JF.[1]

Score	Description
0.0	Normal neurological exam (all grades 0 in Functional Systems [FS]; cerebral grade 1 acceptable).
1.0	No disability, minimal signs in one FS (ie, grade 1, excluding cerebral grade 1)
1.5	No disability, minimal signs in more than one FS (more than one grade 1 excluding cerebral grade 1).
2.0	Minimal disability in one FS (one FS grade 2, others 0 or 1).
2.5	Minimal disability in two FS (two FS grade 2, others 0 or 1).
3.0	Moderate disability in one FS (one FS grade 3, others 0 or 1) or mild disability in three or four FS (three/four FS grade 2, others 0 or 1) though fully ambulatory.
3.5	Fully ambulatory but with moderate disability in one FS (one grade 3) and one or two FS grade 2; or two FS grade 3; or five FS grade 2 (others 0 or 1).
4.0	Fully ambulatory without aid, self-sufficient, up and about some twelve hours a day despite relatively severe disability consisting of one FS grade 4 (others 0 or 1), or combinations of lesser grades exceeding limits of previous steps. Able to walk without aid or rest for some 500 meters.
4.5	Fully ambulatory without aid, up and about much of the day, able to work a full day, may otherwise have some limitation of full activity or require minimal assistance, characterised by relatively severe disability, usually consisting of one FS grade 4 (others grade 0 or 1), or combinations of lesser grades exceeding limits of previous steps. Able to walk without aid or rest for some 300 meters.
5.0	Ambulatory without aid or rest for some 200 meters; disability severe enough to impair full daily activities (e.g. to work a full day without special provisions). (Usual FS equivalents are one grade 5 alone, others 0 or 1; or combinations of lesser grades usually exceeding specifications for step 4.0).
5.5	Ambulatory without aid or rest for about 100 meters; disability severe enough to preclude full daily activities. (Usual FS equivalents are one grade 5 alone, others 0 or 1; or combinations of lesser grades usually exceeding specifications for step 4.0).
6.0	Intermittent or unilateral constant assistance (cane, crutch, or brace) required to walk about 100 meters with or without resting. (Usual FS equivalents are combinations with more than two FS grade 3+).
6.5	Constant bilateral assistance (cane, crutch, or brace) required to walk about 20 meters without resting. (Usual FS equivalents are combinations with more than two FS grade 3+).
7.0	Unable to walk beyond 5 meters even with aid, essentially restricted to wheelchair; wheels self in standard wheelchair and transfers alone; up and about in wheelchair some 12 hours a day. (Usual FS equivalents are combinations with more than one FS grade 4+; very rarely, pyramidal grade 5 alone).
7.5	Unable to take more than a few steps; restricted to wheelchair; may need aid in transfer; wheels self but cannot carry on in standard wheelchair a full day; may require motorised wheelchair.

	(Usual FS equivalents are combinations with more than one FS grade 4+).
8.0	Essentially restricted to bed or chair or perambulated in wheelchair; but may be out of bed itself much of the day; retains many self-care functions; generally has effective use of arms. (Usual FS equivalents are combinations, generally grade 4+ in several systems).
8.5	Essentially restricted to bed much of the day; has some effective use of the arm(s); retains some self-care functions. (Usual FS equivalents are combinations, generally grade 4+ in several systems).
9.0	Helpless bed patient; can communicate and eat. (Usual FS equivalents are combinations, mostly grade 4+ in several systems).
9.5	Totally helpless bed patient; unable to communicate effectively or eat/swallow. (Usual FS equivalents are combinations, almost all grade 4+).
10	Death due to MS.
Functional system score FSS (FS: pyramidal, cerebellar, brainstem, sensory, spincter, visual, mental) are scored from 0-5(6): 0=normal; 5(6)=severe disability	

Source: http://www.edmus.org/en/proj/ms_edss.html

2.5 Diagnosis of multiple sclerosis

The key features of the correct diagnosis of MS are the presence of suggestive symptoms with dissemination in space and time and the absence of evidence for any other disorder. This means that attacks characteristic of MS occur at least one month up to several years apart in different anatomical regions. For example, a patient who presented with an isolated optic neuritis and 1 year later with a sensory deficit in the arm would be diagnosed as clinically definite multiple sclerosis (CDMS) if no better explanation for these events could be found.[51]

With the advent of MRI with its high sensitivity and specificity[52] the correct diagnosis of MS need not rely so much on repeated relapses but can be made very often already with one MRI after the first clinical attack if the patient fulfils specific criteria,[2] which are explained in table 3.

The use of other diagnostic methods like lumbar puncture to look for oligoclonal bands in the cerbro-spinal fluid is still used, in particular to strengthen the immune-mediated origin of the disease and for differential diagnostic considerations. Nevertheless, CSF findings are not mandatory in current diagnostic criteria.[2]

Table 3: Diagnostic criteria of MS according to the McDonald criteria 2010[2]

Clinical (Attacks)	Lesions	Additional Criteria
2 or more	Objective clinical evidence of 2 or more lesions or objective clinical evidence of 1 lesion with reasonable historical evidence of a prior attack	None. Clinical evidence alone will suffice; additional evidence desirable but must be consistent with MS
2 or more	Objective clinical evidence of 1 lesion	Dissemination in space, demonstrated by • ≥ 1T2 lesion in at least two MS typical CNS regions (periventricular, juxtacortical, infratentorial, spinal cord); OR • Await further clinical attack implicating a different CNS site
1	Objective clinical evidence of 2 or more lesions	Dissemination in time, demonstrated by • Simultaneous asymptomatic contrast-enhancing and non-enhancing lesions at any time; OR • A new T2 and/or contrast-enhancing lesions(s) on follow-up MRI, irrespective of its timing; OR • Await a second clinical attack
1	Objective clinical evidence of 1 lesion	Dissemination in space, demonstrated by • ≥1T2 lesion in at least two MS typical CNS regions (periventricular, juxtacortical, infratentorial, spinal cord); OR • Await further clinical attack implicating a different CNS site **AND** Dissemination in time, demonstrated by Simultaneous asymptomatic contrast-enhancing and non-enhancing lesions at any time; OR • A new T2 and/or contrast-enhancing lesions(s) on follow-up MIR, irrespective of its timing; OR • Await a second clinical attack

2.6 Magnetic resonance imaging in multiple sclerosis

During the last two decades MRI has become an important tool not only for diagnostic purposes but also as an instrument to monitor disease activity, which can occur even without any clinical signs. Therefore it became also an important tool for clinical decision making especially considering the increasing number of available immunomodulatory treatments.[53]

On conventional MRI of the brain MS-lesions are best visualized on T2-sequences. Since the relaxation time of a lesion on T2 is longer than that of the remaining white matter, lesions appear hyperintense. As the CSF is also bright on T2 periventricular lesions are more difficult to identify. Therefore special techniques like the fluid attenuated inversion recovery (FLAIR) sequence have been developed which suppress the signal from CSF and thereby allow a better of visibility of plaques at the edge of the ventricles.[54]

MS lesions are typically well circumscribed with a round to ovoid shape and a size of a few millimetres up to one centimetre.[55] But even lesions with a size of several centimetres also termed as "tumefactive" lesions can occur.[56]

Active MS lesions show gadolinium enhancement on T1-weighted sequences as a consequence of their impaired blood-brain barrier[57] (see figure 5). Gadolinium enhancement belongs to the first signs of a newly developing lesion in the CNS and may occur with or without clinical symptoms.[58] Contrast enhancement can persist for 4-6 weeks. If the patient is treated with steroids in case of an acute relapse, this interval is significantly shorter.[59]

Between 10 and 20% of all MS-plaques are hypointense on T1-sequences indicating persistent demyelination and axonal loss. In the literature these hypointense lesions are termed ’black holes’.[60]

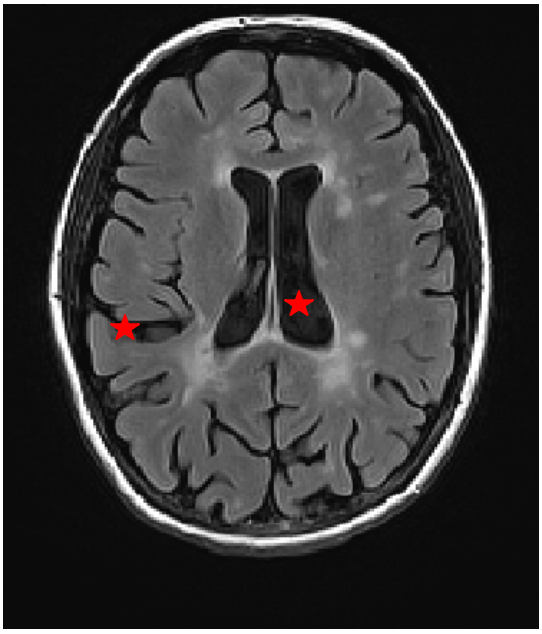


Figure 4: Typical hyperintense partly confluent lesions adjacent to the ventricles on this FLAIR sequence in a 25 year old female patient with an active RRMS. Notice the widening of ventricular and subarachnoid spaces indicating brain volume loss (red star)

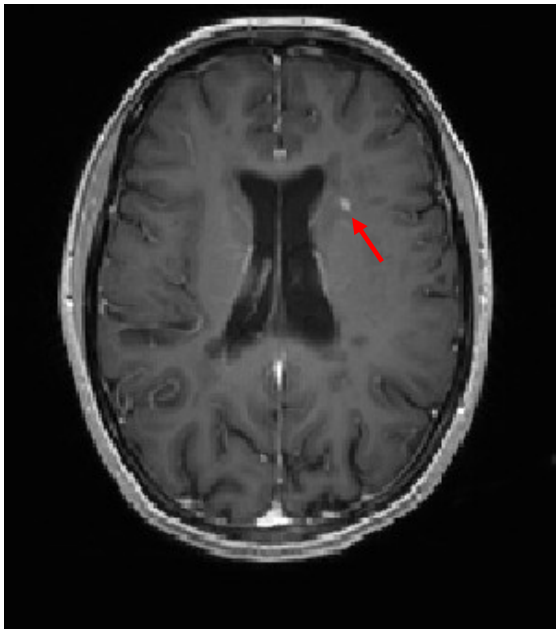


Figure 5: The same patient showing a gadolinium enhancing lesion on a T1-sequence (red arrow), representing active inflammation

2.7 Treatment options in multiple sclerosis

Since to date MS is not curable the therapeutic options of MS are divided into treatment of acute relapses and long-term immunomodulatory regimens.

The treatment of acute relapses with steroids (Methylprednisolone) has been 'state of the art' for decades with the aim to fasten recovery and to prevent persistent neurological deficits Typically patients receive a steroid pulse of 1000 mg Methylprednisolone over a period of 3-5 days.[61] With severe relapses especially when not responding to steroids or if steroids are contraindicated plasmapheresis can be used alternatively.[62]

Disease modifying therapies (DMT) are approved since the early nineties (Interferon beta) with the goal to reduce the frequency of relapses, to slow down the disease progression,[63] and to reduce the evolution of new lesions and brain atrophy on MRI.[64]

A DMT is indicated in patients with RRMS, but also in patients with a CIS, who have a high risk of conversion to clinically definite MS.[65]
Interferon beta and Copaxone, which are administered either subcutaneously or intramuscularly, have been well established agents which good data for safety and efficacy for

more than 20 years.[66]
Newer agents for the treatment for RRMS in an orally pharmaceutical form are Dimethylfumarat and Teriflunomide.[67,68]

If disease activity still progresses under the basic therapy regimes, three potential escalating therapy options exist.
Natalizumab, a monoclonal antibody is administered once monthly and shows a reduction rate of relapses of 68% and progression of disability in 42% but can rarely cause progressive multifocal leukencephalopathy, a severe infection which can lead to death or persisting neurological deficits.[69]
Fingolimod, administered orally, is the second approved agent for escalation therapy.[70]
Alemtuzumab a humanized monoclonal antibody that targets CD52 was also approved by local authorities recently for the use in patients with a very active disease course of MS.[71]

Compared to RRMS the therapy options for SPMS are sparse. Interferon beta or Mitoxantrone, an immunosupressive agent, can be prescribed to reduce neurological disability in SPMS patients with additional relapses.[72,73]

Thus far no proven treatment has been licensed for PPMS.

2.8 Mortality in multiple sclerosis

2.8.1 Divergence in studies on life expectancy

Life expectancy is a frequently asked question of most newly diagnosed MS-patients to their treating physician. Data on this important issue suggest that the life expectancy in MS patients is about 6 to 14 years shorter compared to the general population.[7,8,74] However, several investigations show diverging results when considering the interval between disease onset and death, cause of death and sex specific mortality.[13]

For example, the time from disease onset until death varies regionally between 24 years in Scotland to 45 years in New Zealand.[75] These differences can only partly be explained by different numbers of patients and follow-up intervals.[13] Conflicting results also exist regarding the trend of mortality over time. While a big Danish study showed that the standardised mortality ratios (SMRs), ie the ratio of observed deaths in a study group to the expected deaths in the general population, has about 50% decreased in MS patients during the

last decades,[7] an observation in Canada did not show any longitudinal changes of the SMRs.[74] A recently published meta-analysis also suggested that the excess of mortality in MS did not change during the last 50 years.[76]

For Austria so far there is only on study that investigated the mortality in MS patients between 1970 and 2001 and showed a decline of mortality by 47% within this period. The mean age of death in this study was 57 years[11] and was lower compared to other studies ranging from 58.4 to 76.7 years at the time of death.[74,77]

Diverging results exist also regarding sex specific differences of mortality. Several studies could demonstrate that females have longer survival rates compared to males[74,78,79], while others could not identify any differences[80] or even found a higher mortality risk in female patients.[11]

It is also important to consider that much of the published data refers to patients, who did not benefit from the initiation of DMTs during the early nineties. Newer studies showed that patients treated with DMTs might have an advantage of survival compared to untreated individuals. In a trial consisting of 372 individuals with consecutive follow-up data of 21 years, which originally investigated the safety and efficacy of Interferon beta 1a, the patients who had been randomized to the treatment arm showed a significant reduction of all cause mortality (hazard rate 46%) compared to the placebo group.[66] Thus the influence of DMTs on mortality needs further evaluation. Interestingly, death has never been used as an outcome measure in therapeutic trials.[13]

2.8.2 Causes of death in MS patients

Despite being a chronic disabling disease, MS is only in rare cases a direct cause of death, as for example in cases of brainstem involvement. In fact, most individuals die because of MS related complications, such as pneumonia or sepsis, in particular if the patients are bedridden in the late stage of the disease, which correlates with EDSS scores of 8 to 9 prior death. In these cases MS should be recorded as the underlying cause of death on the death certificate.[13]

The percentage of patients with MS as the primary cause of death according to the death certificate fluctuates among different studies ranging from 47% in a Canadian cohort of patients[81] up to 75% in France.[78] This variation among different countries is related to methodological issues regarding the assessment of the cause of death in MS patients. The sources used to determine the cause of death are various among different studies, even though

most of the investigations of mortality use national registries. Furthermore, the assessment of the cause of death can be biased, as for example in cases where the clinical examination is made by doctors who have limited knowledge of the clinical history of the patient, or in patients with additional medical issues.[13]

Other common causes of death in MS patients are cardiovascular disease, cancer, respiratory disease and suicide. In most studies cardiovascular diseases account as primary cause of death for as many as 10 to 20% of the patients.[81,82] A Finish cohort even showed a rate of 26%.[83] The number of cancer related deaths is comparable among different studies and amounts to approximately 10%.[7,8,80,82] Higher rates of death due to cancer are reported in Canada and Finland only.[81,83] Suicide is considered to be higher in the MS cohort compared to the general population. Interestingly though, there is a wide range in the incidence of suicide among different geographical regions which varies from 2.5% in Norway[79] to 28.6% in Canada.[81] However, these observations are probably also influenced by methodological issues, as for example Finland with an observed suicide rate of 5% among MS patients[83] also has a very high suicide rate in the general population, Very often suicides are reported as 'accidents' to avoid a social stigma and therefore the real rate of suicides might even be higher.[13]

So far, no data about causes of death of MS patients exist in Austria. Potential reasons are discussed later (see below Chapter 2.9).

2.9 The role of comorbidities in multiple sclerosis

A comorbidity is defined as the total burden of illness other than the specific disease of interest.[84]

Comorbidities are very common in the general population and are typically increasing with age. Frequent conditions are cardiovascular diseases, arterial hypertension, hyperlipidemia, or psychiatric disorders such as depression. The role of such comorbidities in other neurological disorders like epilepsy or Alzheimer disease is already of great interest, as for example patients with epilepsy tend to have additional psychiatric disorders and therefore need special health care services more often than the general population.[85]

In the last couple of years the role of comorbidities also became a focus of interest in MS, e.g. several studies could demonstrate that patients with MS suffer more often from psychiatric

disorders than the general population. But also other conditions like chronic lung diseases tend to be more common in individuals with MS.[14]

Depression belongs to the most commonly observed comorbidities in MS patients with a life time incidence of up to 50 %, which is about 3-times higher compared to the general population.[86] However, this might not be directly disease related, but rather associated with a poorer quality of life and with more pronounced disability. In a reciprocal context data suggest that the adherence to disease modifying therapy is reduced in patients with depression.[87]

Also the presence of vascular comorbidities, such as ischemic heart disease or stroke, might be slightly higher in MS patients compared to the general population. This accumulation is potentially related to limited physical activity due to disability and higher rates of smoking and overweight within the MS population.[88] Smoking itself is associated with a higher likelihood of a more rapid disease progression in MS.[89]

The presence of vascular comorbidities such as arterial hypertension, hyperlipidemia or diabetes in MS patients lead also to a more rapid progression of disability, especially the ability to walk independently is affected.[90]

Besides the impact of comorbidities, there are of course socio-economic challenges for individual patients. In view of an annual hospitalisation rate of MS patients of up to 25.8%, which is significantly higher compared to the general population, the burden for the social community becomes evident.[91] In this context it is interesting that in a Canadian cohort of more than 5.700 MS patients the majority of hospital stays (91%) was non MS-related. The three most common reasons for hospitalisation were digestive, genitourinary and circulatory system conditions. However, compared to a control cohort of more than 28.000, MS patients stayed significantly longer in the hospital than patients from the general population (8.3 versus 7.4 days) and were also significantly younger at the time of admission (48.6 versus 54.9 years).[5]

It is also known that the presence of comorbidities in MS patients increases their mortality risk. However, this increase is not specific for MS, but also observed in the general population.[10] For Austria data regarding the prevalence and the impact of comorbidities in MS are still lacking.

2.10 Morphologic brain abnormalities in multiple sclerosis

The third cornerstone of this thesis focuses on morphologic brain abnormalities regarding lesion load and brain volume changes in MS patients using MRI and the potential correlation of those parameters with comorbidities, disability and disease progression.

The identification of factors associated with disease progression and accumulation of disability has been an important field of research for several years in MS, because the prediction of the disease course solely based on clinical variables is insufficient.
Therefore, the use of conventional MRI is not only reserved for diagnostic considerations, but is also used to identify prognostic factors for prediction of the further development of MS, even though a single parameter for an individual prognosis of the disease is still missing.

Several studies could show that the lesion load, which is measured on T2-weighted sequences, is associated with later disability.[20,92] Therefore the measurement of the lesion load is used in clinical trials as one outcome parameter to monitor treatment efficacy.[93]

Even more promising results could be obtained by analysing brain volume changes in patients with MS as they reflect neurodegenerative processes and axonal loss. It could be demonstrated that measuring atrophy is better associated with disability and disease course than lesion load alone.[94,95] It was even suggested that the rate of brain atrophy may predict later disability, especially when assessed early on.[16,19]

Nevertheless, when and to what extent the brain starts to shrink due to MS is still not fully clear. In part, this is due to the fact that investigations of this issue often focused only on single phases of the disease. It is also not fully clear if certain compartments or regions of the brain undergo preferential volume loss. Due to continuous advancements of semi- and fully-automated software, the measurement of brain volume changes with a separate evaluation of grey and white matter volumes and of specific brain structures has now become much easier and more reliable.[96]

Different studies could demonstrate that the volume loss of the brain starts already in the earliest stages of the disease. Spanish investigators for example could demonstrate that the global volume loss measured as percentage of brain volume change (PBVC) occurred within the first year after CIS.[16] In this study brain volume loss was also significantly associated with conversion from CIS to definite MS. Similar results could also be shown in several other studies.[17–19]

In addition to the global loss of brain volume, several studies focused on brain volume changes of specific compartments like the grey and white matter, or on specific structures like the thalami or basal ganglia. It could be shown that especially the loss of grey matter is associated with disability and cognitive impairment. For example, Fillipi et al. could demonstrate that grey matter loss in a cohort of 73 patients obtained from MRI at baseline and after 12 months was a strong predictor for pronounced disability and cognitive decline after an observation period of 13 years.[94] Zividanov et al. provided similar results investigating 180 patients over 5 years. Patients who had a significant disease progression (n=90) showed a higher loss of the cortical grey matter and thalamic volume compared to individuals with a stable disease course.[97]
Not surprisingly, brain atrophy is used as an important outcome parameter in controlled clinical trials to measure treatment efficacy.[98] In contrast, the use of brain volumetric measurements in clinical routine is still under investigation

Important limitations of volume measurements also come from the complex interplay of different disease mechanisms and the effects of therapy. In patients with active inflammatory processes oedema can lead to an increase of brain volume despite damage to the brain parenchyma. Consecutive treatment with steroids in case of an acute relapse or the initiation of a DMT can reduce inflammation and oedema and, although being beneficial, can thus induce pronounced volume loss. In the literature this effect is known by the term pseudoatrophy.[99]

So far, studies on the role of comorbidities on brain volume changes and the relation to disability are sparse. This is remarkable considering that for example a higher level of Hb1AC, a marker of glycated hemogloblin indicating diabetes mellitus, was associated with higher rates of global brain volume loss in individuals without any specific neurological disease.[100] In a recent cross-sectional study the presence of arterial hypertension and heart disease were associated with a lower grey matter and cortical volumes.[15]. Also smoking could be identified as a risk factor for pronounced loss of brain volume and higher lesion load in MS patients.[101] However, data regarding the longitudinal evolution of morphologic brain abnormalities in MS patients in the context of accompanying diseases is still missing.

3. Aim

Based on current understanding as outlined in the introduction this thesis encompasses 3 major objectives that meet current challenges in MS research:

The first objective of this thesis is to gather data about the actual mortality rates of MS patients in Austria. The difficulty of this task consists in the lack of systematic demographic MS registries in Austria, so that it is crucial to point out the importance of such a comprehensive access based on the model of the Nordic countries.

The second objective mainly consists of the evaluation of the prevalence and effect of comorbidities on the disease course of MS, a field in which very little research has been done in Austria so far.

The investigation of morphologic brain abnormalities in patients in the early stages of MS and the potential correlation of those parameters with comorbidities, disability and disease course finally makes up the last objective of the thesis.

Despite their heterogeneity all three objectives meet current and pressing challenges in MS research and merit a joint discussion that might allow insights which could not be gained from a separate analysis.

4. Methods

4.1 Assessment of the mortality rate due to MS

Mortality data were derived from the database of 'Statistic Austria'. This partially free accessible database contains information about the reason of death for all kinds of diseases, annual hospitalisation rates, a cancer registry, population based data etc..

The total number of death due to MS was available from 1970 until 2014. The MS mortality statistics are based primarily on the death certificate indicating MS as the underlying cause of death.

To maintain a better comparability with existing mortality data from the group of Ekstern et al.[11] the average mortality rate was calculated based on 10-year-periods, for example from 1970 until 1979 and 1980 until 1989 and so on.

The number of deaths was normalized according to age and is referred by cases per 100000 inhabitants. Separate sex specific and regional analyses of the mortality rates were also performed.

The MS specific mortality rates were then compared with mortality rates of the general population. For this thesis it was not possible to calculate standardised mortality rates (SMR) because no information about the age composition of MS patients in Austria was available.

Age at time of death was recorded for MS patients (if accessible) and compared to the general population using life expectancy tables of the Statistics Austria.

4.2 Assessment of comorbidities in MS

For the investigation of comorbidities we decided to use a regional approach in respect of the above mentioned lack of systematic registries. We chose this approach for this project also to evaluate how accessible those data are with respect to local regulations including data protection and ethical considerations. We therefore collaborated with the Institute for Medical statistics of the University of Graz. We looked for patients with the diagnosis of MS (ICD 10 Code G35.0), respectively CIS, who had been in contact with the facilities of the Medical University of Graz. All these contacts are recorded in the OPEN MEDOCS system. Based on these contacts we collected the recorded reasons for the admission and the recorded comorbidities.

In a second step we aimed to link these data to those patients, who are treated and observed in our MS outpatient clinic, because these patients have a fully documented clinical history including age at disease onset, disability grade, current therapies, MRI data etc..
In the next step we tested if the assessed comorbidities are associated with clinical and morphological parameters.

4.3 Assessment of morphologic brain abnormalities

For the analysis of morphologic brain abnormalities, which represents the third aim of this thesis, we retrospectively looked for MS patients who had at least two separate MRI scans between 2006 and 2012.. To ensure the quality of our imaging results only patients who had an identical scanning protocol on the same 3-Tesla (3T) MRI scanner were included in the study.

Further inclusion criteria were:

- a baseline diagnosis of a CIS suggestive of MS[45] (n=63) or of CDMS fulfilling the McDonald diagnostic criteria 2005[102] (RRMS=53; SPMS=4);
- a detailed neurological examination including the Expanded Disability Status Scale (EDSS)[1] at baseline and follow-up MRI;
- a fully documented clinical history, including the use of DMT;
- an interval of at least 18 months between the baseline and follow-up MRI;
- a minimum interval of eight weeks between the administration of steroids and the MRI to avoid potential volume shifting effects,

These inclusion and exclusion criteria resulted in a final cohort of 120 patients.

Clinical variables including the age at baseline, age at disease onset, gender, disease duration, annualized relapse rate and the number of patients receiving a disease modifying therapy were recorded.

The main aim of this analysis was to assess how the distinct morphologic brain parameters consisting of brain volume variables and lesion load are associated with clinical variables like the disease severity and if there is a difference between the distinct stages of MS (CIS/RRMS). We also tested if these morphologic brain abnormalities have a predictive value for the conversion from CIS to definite MS.

Hence, conversion was defined as either a second clinical relapse or radiological fulfilment of the criteria for dissemination of time and space according to the McDonald diagnostic criteria 2005.[102]

In a second step the assessed comorbidities were added in the analysis to evaluate their impact on the distinct brain volume parameters and lesion load.

This study was approved by the ethics committee of the Medical University of Graz.

4.4 Magnetic resonance imaging (MRI) protocol

All MRI examinations were performed with a single 3T machine (Siemens Tim Trio, Siemens Healthcare, Erlangen, Germany) using a phased-array head coil with 12 receiver elements and a consistent imaging protocol as previously described.[103] In brief, structural imaging included a fluid-attenuated inversion recovery (FLAIR) sequence (repetition time [TR]/echo time [TE]/inversion time [TI] = 9,000 ms/69 ms/2,500 ms, in plane resolution 0.9 × 0.9 mm, slice thickness 3 mm, acquisition time 4 minutes 22 seconds) and a T1-weighted 3-dimensional magnetization-prepared rapid gradient echo (MPRAGE) sequence with 1 mm isotropic resolution (TR/TE/TI/flip angle = 1,900 ms/2.19 ms/900 ms/9°, acquisition time 6 minutes 1 second).

4.5 Brain volume and T2-lesion load measurement

Estimates for global brain volume (NBV), for cortical grey matter volume (cGMV) and white matter volume (WMV) normalized for subject head size were assessed using SIENAX.[104]

> 'SIENAX starts by extracting brain and skull images from the single whole-head input data.[105] The brain image is then affine-registered to MNI152 space[106,107] (using the skull image to determine the registration scaling); this is primarily in order to obtain the volumetric scaling factor, to be used as a normalisation for head size. Next, tissue-

type segmentation with partial volume estimation is carried out[108] in order to calculate total volume of brain tissue (including separate estimates of volumes of grey matter, white matter, peripheral grey matter and ventricular CSF).'

Because of its central role for different cortical and subcortical connections[109], and potential association with disease progression in MS[110], the volume of the thalami (TV), and volumes of basal ganglia (BGV) structures including putamen, caudate nucleus, globus pallidus were also determined using FIRST as part of FSL.[111] Additionally, the percentage of brain volume change (PBVC) was assessed using SIENA as part of FSL.[104]

'SIENA starts by extracting brain and skull images from the two-timepoint whole-head input data.[105] The two brain images are then aligned to each other[106,107] (using the skull images to constrain the registration scaling); both brain images are resampled into the space halfway between the two. Next, tissue-type segmentation is carried out[108] in order to find brain/non-brain edge points, and then perpendicular edge displacement (between the two time points) is estimated at these edge points. Finally, the mean edge displacement is converted into a (global) estimate of percentage brain volume change between the two time points.'

T2-lesion load was measured, as described earlier, using the semi-automated software DispImage.[112] For that purpose, masks defining the lesions on FLAIR images were created and the total lesion load was calculated by multiplying the area of all masks by the slice thickness.[112]

For further analyses, all MRI measures were annualized to account for individual differences in the observation period.

4.6 Statistical analysis

Mortality data were obtained using the public access to Statistics Austria (www.statistik.at). The data were grouped, if applicable, in 5 year periods from 1970 till 2014. In addition general and sex specific mortality rates were calculated per 100000 inhabitants based on the population number during the respective periods.

All data was tested for distribution using histograms and the Kolmogorov-Smirnov test. In case of non-parametric distribution of data, the Mann-Whitney U test and the Kruskal Wallis test were used. In other cases, the 2-tailed student-t-test and ANOVA were applied.

Correlations between clinical variables, comorbidities and conventional MRI-parameters were assessed according to the Pearson and Spearman correlation coefficients. Fisher's exact test was used to analyse contingency tables. To assess the contribution of PBVC in predicting conversion from CIS to MS and to evaluate the effect of PBVC on the time of conversion to MS and on clinical disability, we also created quartiles of PBVC with individuals in the first quartile and fourth quartile having the highest and lowest amount of annual brain volume decline, respectively. Comparisons were performed between cohorts with CIS or MS at baseline and the CIS subgroups remaining CIS or converting to MS.

5. Results

5.1 Mortality rates in Austria between 1970 and 2014

Mortality rates of the general population in Austria between 1970 and 2014 are shown in table 4. Overall, the mortality rates in Austria are constantly declining since 1970. The same trend could be observed for mortality rates of MS patients normalized for age, which were constantly decreasing from 1970 until 2000. However, from 2000 to 2014 the numbers of deceased patients increased again (see table 5). In total, 3724 deaths (1270 men and 2454 women) with MS as the underlying cause were recorded from 1970 to 2014.

In the sex specific analysis, a similar trend could be observed. Within all periods except the last decade women had a higher mortality rate compared to men.

As information on the age composition of the MS population in Austria is not available, it proved impossible to calculate standardised mortality ratios (SMR).
However, to generate a better impression on how mortality rates might differ between the general population and the MS patients, the proportion of deaths between both groups was calculated for the period of 2010–2014 based on the expected number of 148 MS patients per 100000 people in Austria and the available mortality rate of the general population, which is shown in table 4. This ratio was 0.0054 in the MS cohort and 0.003 in the general population and therefore almost double in the MS cohort.
Age specific mortality rates were available for 2014 and are shown in table 6.

The average ratio male to female during all periods was 1:1.92. The ratio was highest in the period from 1970 to 1979 with 1: 2.71. During the subsequent periods it decreased to 1:1.67 for the period between 2000 and 2009. After that, the ratio slightly increased to 1:1.86 for the period from 2010–2014.

Table 4: Mortality rates of the general population in Austria between 1970 and 2014 per 100000 people

Period	Overall	Male	Female
1970-1979	858	1116	682
1980-1989	716	949	559
1990-1999	577	768	445
2000-2009	455	592	352
2010-2014	395	506	307

Table 5: Overall and sex specific mortality rates of MS patients in Austria between 1970 and 2014 per 100000 people

Period	Overall	Male	Female
1970-1979	1.18	0.91	1.43
1980-1989	0.78	0.61	0.92
1990-1999	0.53	0.45	0.64
2000-2009	0.63	0.75	0.75
2010-2014	0.82	0.62	0.79

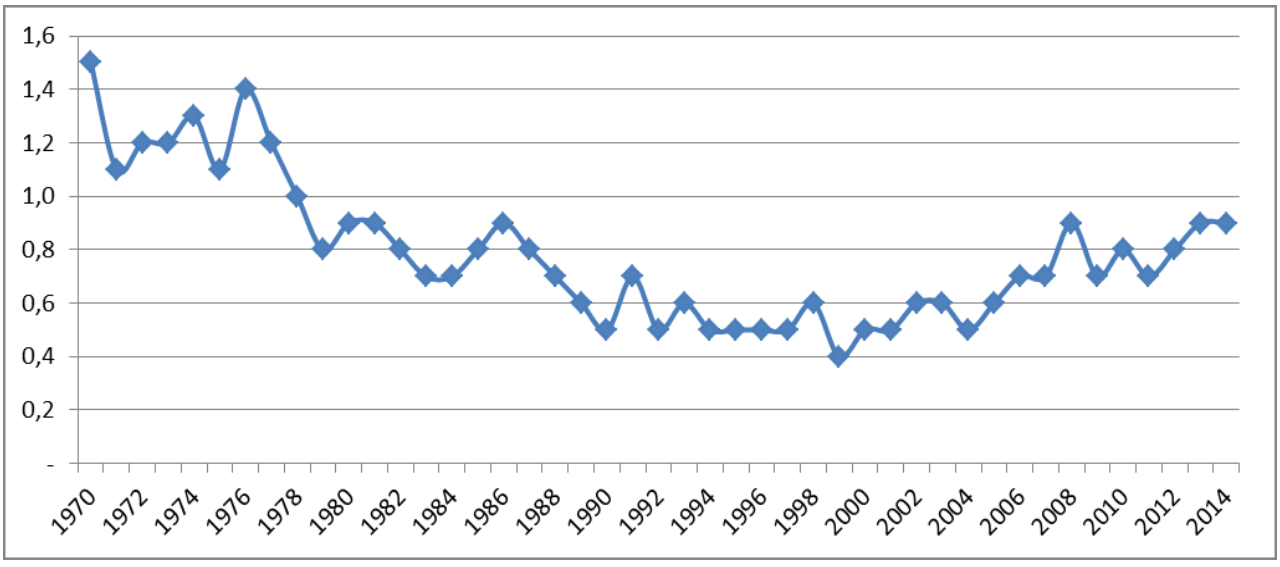


Figure 6: Overall mortality rate of MS in Austria between 1970 and 2014 per 100000

Table 6: Age specific mortality rates of the general population and MS patients in Austria per 100000 in 2014

Age	Mortality rate of the general population	Mortality rate of MS patients
30 to 49	69.5	1.15
50 to 69	468.5	3.01
70 to 89	2168.4	3.23
90 and older	9723.2	4.35

For the analysis of the regional MS mortality rates data were available from 1970 to 2014. In all regions a decrease of MS related mortality until the late nineties could be observed. After the turn of the millennium, mortality rates increased in all regions, thus mirroring a national trend.

It could also be observed that the mortality rates differ between the distinct regions throughout the observation period of 32 years. A slight west to east gradient with higher mortality rates in the eastern regions could be demonstrated (see table 7 and figure 8).

Table 7: Regional mortality rates in Austria between 1970 and 2014

Period	Burgenland	Carinthia	Lower Austria	Upper Austria	Salzburg	Styria	Tyrol	Vorarlberg	Vienna
1970-1979	0.84	1.27	1	1.15	0.86	1.07	0.77	0.79	1.61
1980-1989	0.62	0.96	0.84	0.80	0.66	0.87	0.60	1	0.63
1990-1999	0.35	0.37	0.61	0.73	0.49	0.56	0.49	0.52	0.45
2000-2009	0.70	0.46	0.65	0.60	0.64	0.69	0.53	0.58	0.69
2010-2014	0.94	0.82	0.76	0.72	0.76	0.78	0.68	0.86	0.96

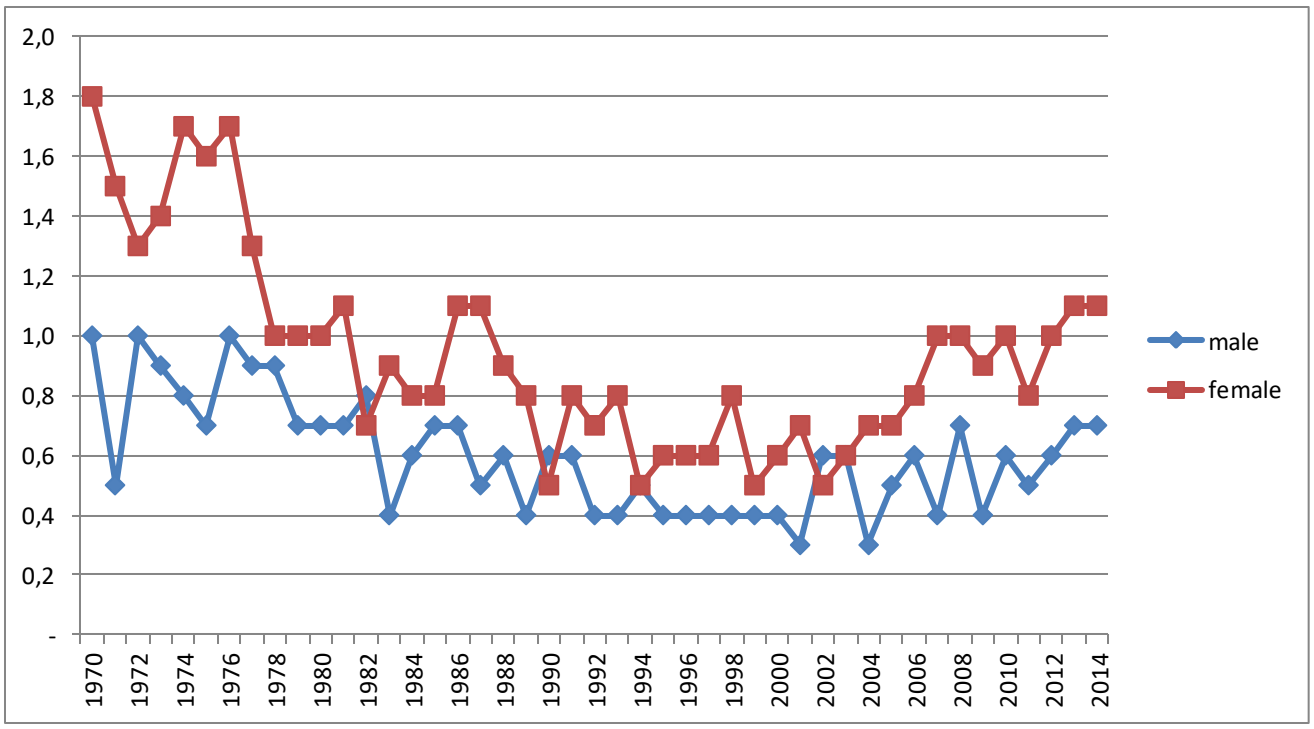


Figure 7: Sex specific mortality rate in Austria between 1970 and 2014 per 100000

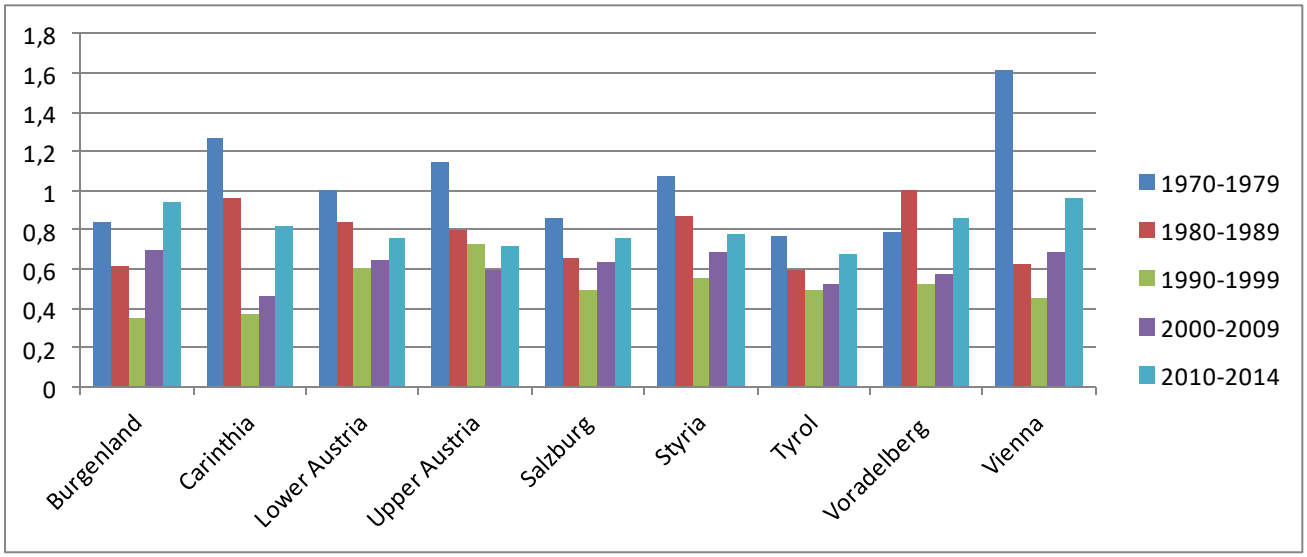


Figure 8: Regional mortality rates in Austria between 1970 and 2014

5.1.1 Life expectancy of the general population and of MS patients

The life expectancy of the general population in Austria has been rising almost linearly since 1950 for both sexes (see figure 9).

For MS patients, the average age at death was accessible for 2014 with a mean age of 62.9 years for men and 67.3 years for women.

To correlate these results with individuals of the general population, life expectancy tables of Statistics Austria were used. Individuals born around 1950 had a life expectancy at the time of birth of 61 years for men and 66 years for women. However, if people reached the age of 60 in the year 2000 their expected remaining life expectancy was still 19.9 years for men and 24.1 years for women. In 2010, the life expectancy of the general population is now estimated at 77.9 years for men and 82.2 years for women. According to this data, life expectancy when suffering from MS is about 18 years shorter in male patients and 17 years shorter in female patients compared to the general population.

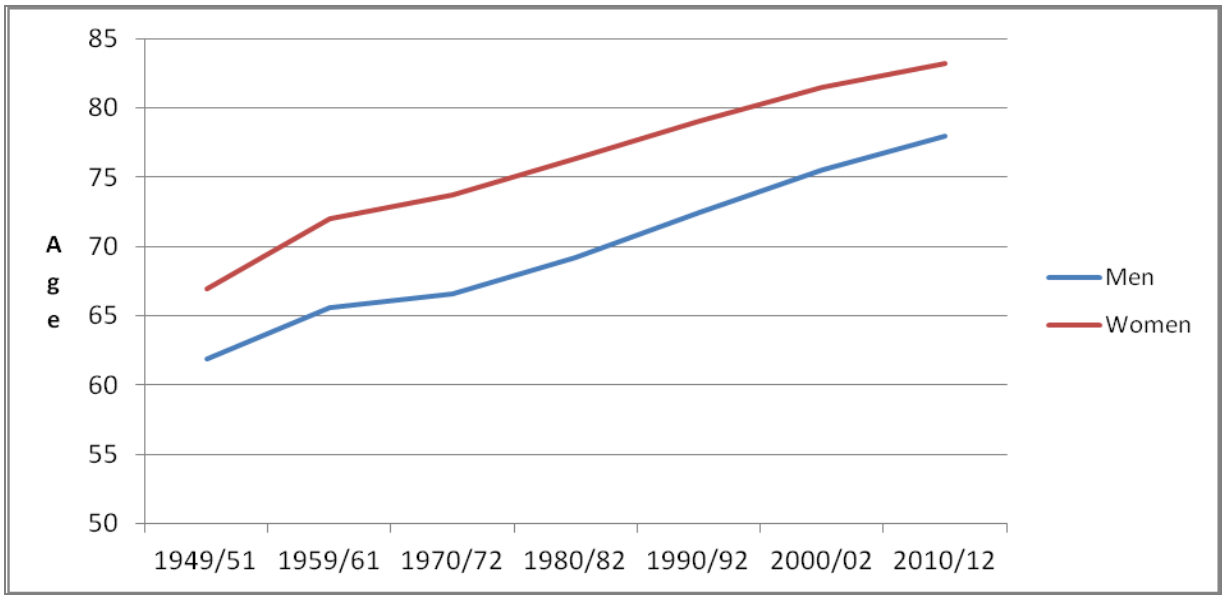


Figure 9: Life expectancy of the general population in Austria between 1950 - 2012

5.2 Comorbidities in patients with multiple sclerosis

Comorbidities were assessed using a dataset from the Institute for Medical Informatics, Statistics and Documentation of the University of Graz. Between 2006 and 2012, 811 hospital contacts – accounting for 324 individuals with a diagnosis of MS – to the several facilities of the Medical University of Graz including the departments of internal medicine, surgery, gynaecology, dermatology, neurology, ophthalmology, psychiatry and dermatology could be identified. 120 MS patients were regularly seen in the outpatient clinic of the Department of Neurology, Medical University of Graz (i.e. 'in house cohort'). Given that 47 patients formed part of the overall cohort of 324 patients as well as the 'in house cohort', these patients have been subtracted from the 324 patients-cohort, thus limiting this group to a total of 277 patients ('total cohort'). The 'in house cohort' (n= 120) and the 'total cohort' (n= 277) were analysed separately as described on the next pages.

The demographic variables of the 'total cohort', as far as accessible, and the number of comorbidities of this total cohort are shown in table 8.
Depression was the most frequently recorded condition, followed by arterial hypertension and hyperlipidemia. Malignancies have only been reported in 1.8% of patients.

Table 8: Age at time of assessment and number of comorbidities in the 'total cohort'

	Total n=277	**Female n=188 (69.3%)**	**Male n=89 (32.8%)**	**p-value**
	N (%)	**N (%)**	**N (%)**	
Age at time of assessment	40.4 ± 13.7	40.2 ± 13.9	40.0 ± 13.3	0.831
Arterial hypertension	25 (9.0)	14 (7.4)	11 (12.4)	0.186
Hyperlipidemia	25 (9.0)	17 (9.0)	8 (9.0)	0.988
Diabetes mellitus	5 (1.8)	2 (1.1)	3 (3.4)	0.332
Epilepsy	5 (1.8)	5 (2.7)	0	0.180
Autoimmune thyreoditis	15 (5.4)	13 (6.9)	0	0.155
Malignancies	5 (1.8)	3 (1.6)	2 (2.2)	0.516
Depression	36 (13)	26 (13.8)	10 (11.2)	0.347

Given that very few patients that form part of the 'total cohort' were regularly seen in our MS outpatient clinic, we do not dispose of more detailed clinical data, such as information about the age at onset of MS, the disease course, or information about actual MS specific treatments of these patients. In that regard, any evaluation of a potential association of comorbidities with the disease course would be - at best - highly speculative and more likely in vain. Therefore, we focused our attention on the 'in house cohort' of 120 people. Clinical data for this 'in house cohort' are particularly detailed and complete and seemed thus very promising for further investigations on the role of comorbidities and as a point of comparison to check whether the number of the above listed comorbidities (table 8) is representative. The same 'in house cohort' was also used to analyse brain morphologic abnormalities and their potential correlation with disease severity and comorbidities (see chapter 5.3.4).

Of the 120 patients of the 'in house cohort' 63 were classified as CIS and 57 had a CDMS at baseline. While the mean age at baseline and the mean follow-up time were comparable between CIS and MS patients, the latter were younger at disease onset. The female to male ratio was about 1.9:1, which can be considered as typical for MS. No sex specific differences could be demonstrated for all demographic variables. During follow-up, 33 CIS patients (52.4%) converted to MS after a mean period of 26 months. Twenty of these converters had a second attack, while 13 individuals were diagnosed as MS on radiological terms according to the McDonald diagnostic criteria 2005.[102] As expected, MS patients had a higher EDSS at baseline and at follow-up compared to those with CIS. Four of the 49 patients with relapsing-remitting MS at baseline developed secondary progressive MS.

The baseline demographic data of the 'in house cohort' are listed in table 9.

Table 9: Clinical and demographic characteristics of the investigated 'in house cohort'

	All patients n=120	CIS n=63	MS n=57	p-value
Sex female (%)	78 (65%)	41 (65.1)	37 (64.9)	n.s.[c]
Age at baseline, years	33.7 ± 9.3	32.6 ± 8.4	35.0 ± 10.2	n.s.[b]
Age at onset, years	29.0 ± 9.1	31.7 ± 8.5	26.0 ± 8.8	<0.001[b]
Disease duration baseline, months	56.0 (0-320.0)	2.0 (1.0-7.0)	89.0 (43.0-151.0)	<0.001[a]
Interval baseline – follow-up, months	42.8 (18-79.0)	42.0 (18.0-79.0)	43.0 (30.5-53.5)	n.s.[a]
EDSS baseline[d]	1.7 (0.0-7.5)	1.5 (0.0-2.0)	2.0 (1.0-3.5)	n.s.[a]
EDSS follow-up[e]	1.5 (0.0-8.0)	1.2 (0.0-2.0)	2.5 (1.0-3.5)	0.029[a]
Annualized relapse rate in RRMS	0.29 ± 0.33	n.a.	0.29 ± 0.33	
Disease modifying therapy n (%)				
Baseline	46 (38.3)	13 (20.6)	33 (57.9)	<0.001[c]
Follow-up	80 (66.7)	39 (61.9)	41 (71.9)	n.s.[c]

CIS= clinically isolated syndrome; MS= multiple sclerosis

EDSS= Expanded Disability Status Scale n.a.= not applicable; n.s.= not significant

a= Mann-Whitney-U-test; b= Student-T-test; c= x^2 contingency test; d= EDSS baseline to follow-up in CIS; e= EDSS baseline to follow-up in MS

Data are presented as either mean ± standard deviation in case of normal distribution of data or median (interquartile range IQR) in case of non-parametric distribution

The percentage of comorbidities of the 'in house cohort' was quite comparable with that of the 'total cohort'. Depression was also the most common condition but with a higher frequency of 20%. Arterial hypertension and hyperlipidemia were also commonly recorded comorbidities in the 'in house cohort' and showed quite similar rates compared to the 'total cohort'.

In total, about half of all patients (n=54) had at least one comorbidity. 9 individuals had more than1, 3 had more than 2, and one patient had 4 comorbidities.
There was no significant difference between female and male patients regarding all comorbidities (p=0.251). A separate analysis of distinct comorbidities showed that only thyroid disease was more common in female patients (female n=11 versus male n=0, p=0.008). All other comorbidities were equally distributed between both genders (see table 10).

Table 10: Number of the assessed comorbidities for the 'in house cohort'

	Total n=120 N (%)	**Female n=77 (64.2%)** N (%)	**Male n=43 (35.8%)** N (%)	**p-value**
Arterial hypertension	14 (11.7)	7 (9.0)	7 (16)	0.241
Hyperlipidemia	9 (7.5)	5 (6.4)	4 (9.3)	0.389
Lung Disease	3 (2.5)	2 (2.6)	1 (0.02)	0.280
Epilepsy	2 (1.7)	2 (2.6)	0	0.541
Autoimmune thyreoditis	11 (9.2)	11 (14.2)	0	0.008
Other autoimmune disorders	3 (2.5)	3 (3.8)	0	0.551
Malignancies	1 (0.8)	1 (1.3)	0	1.0
Depression	24 (20)	19 (24.6)	5 (11.6)	0.080

Not surprisingly, patients with one or more comorbidities were older at disease onset (mean age 27.1 versus 32.2 years p=0.017). Having a comorbidity was neither associated with the last known EDSS nor the annualized relapse rate during the observation period.

25% of all patients had a positive smoking status. Smoking was neither associated with disability nor with the disease course. There was also no association between smoking and other cardiovascular risk factors such as arterial hypertension and hyperlipidemia. Unfortunately, the number of pack years was not available. There was no significant sex difference regarding the smoking status.

The results regarding the correlation of comorbidities with morphologic brain abnormalities will be presented in the next chapter.

5.3 Morphologic brain abnormalities and their correlation with comorbidities

5.3.1 Brain atrophy markers and lesion load

Baseline volumes of global, compartmental and regional brain fractions as well as the T2-lesion load of the 'in-house cohort' at baseline are shown in table 11. The NBV, cGMV, WMV, TV and BGV were all higher and the T2-lesion load was lower in CIS compared to MS.

During follow-up, compartmental as well as regional atrophy metrics changed to a similar extent in CIS and MS. Only PBVC was significantly lower in CIS than in MS patients (p=0.008) (see table 12).

In a subgroup analysis, patients converting from CIS to MS had a higher PBVC (p=0.029) during follow-up than those who remained CIS. The annualized decline of NBV, cGMV, TV and BGV was more pronounced for converters without reaching statistical significance (see table 12 and figure 10).

Table 11: Baseline MRI-parameters of the 'in house cohort' dependent on their disease course

	CIS n=63	MS n=57	p-value
NBV (cm^3)	1571.12 ± 72.87	1523.51 ± 116.46	0.019[b]
cGMV (cm^3)	653.80 ± 49.83	635.39 ± 60.50	n.s.[b]
WMV (cm^3)	776.51 ± 49.71	743.86 ± 93.95	0.017[b]
TV (cm^3)	10.83 ± 1.37	9.80 ± 1.57	0.001[b]
BGV (cm^3)	12.21 ± 1.47	11.37 ± 1.85	0.008[b]
T2 –LL (cm^3)	1.41 (0.52-3.34)	4.09 (0.67-12.91)	0.003[a]

CIS= clinically isolated syndrome; MS= multiple sclerosis

NBV= normalized brain volume; cGMV= cortical grey matter volume; WMV= white matter volume; T2-LL= T2 lesion load; TV= thalamic volume; BGV= basal ganglia volume

a= Mann-Whitney-U-test

b= Student-T-test

Data are presented either as mean ± standard deviation in case of normal distribution of data or median (interquartile range IQR) in case of non-parametric distribution

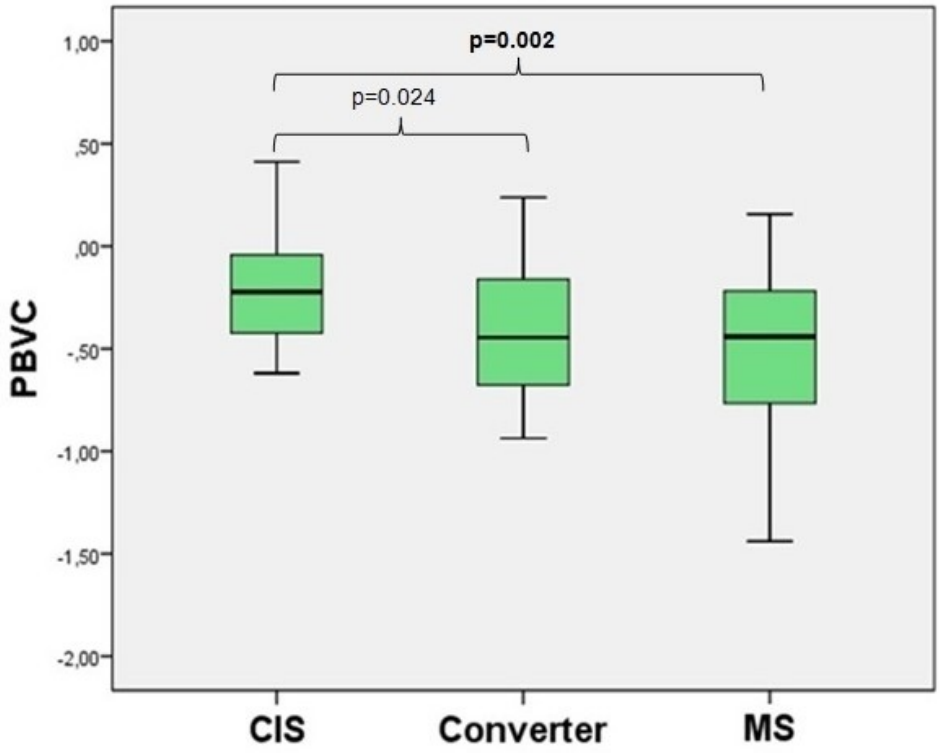


Figure 10: Percentage of brain volume change (PBVC) during follow-up between patients with a clinically isolated syndrome (CIS), converters to multiple sclerosis (MS) and patients with MS from the study onset.
p-values have been corrected for multiple comparison.

Table 12: Annualized change of brain atrophy parameters and T2 lesion load in CIS and MS and in the subgroups of CIS patients remaining CIS or converting to MS

	All CIS n=63	MS n=57	P	Remaining CIS n=30	Converter to MS n=33	p
NBV (cm^3)	-6.13 ± 10.86	-6.46 ± 14.40	n.s.[b]	-5.98 ± 8.77	-6.26 ± 12.60	n.s.[b]
cGMV (cm^3)	-3.81 ± 8.37	-3.45 ± 11.97	n.s.[b]	-2.80 ± 9.17	-4.72 ± 7.58	n.s.[b]
WMV (cm^3)	-2.08 (-4.34-2.19)	-1.22 (-4.84-2.05)	n.s.[a]	-2.86 (-5.5-2.61)	-1.23 (-4.12-1.07)	n.s.[a]
PBVC %	-0.36 ± 0.45	-0.52 ± 0.35	**0.008**[b]	-0.23 ± 0.41	-0.47 ± 0.47	**0.029**[b]
TV (cm^3)	-0.071 ± 0.109	-0.056 ± 0.152	n.s.[b]	-0.052 ± 0.067	-0.087 ± 0.135	n.s.[b]
BGV (cm^3)	-0.084 ± 0.166	-0.084 ± 0.143	n.s.[b]	-0.059 ± 0.108	-0.106 ± 0.203	n.s.[b]
T2 –LL (cm^3)	0.01 (-0.15-0.12)	-0.01 (-0.41-0.13)	n.s.[a]	-0.01 (-0.12-0.10)	0.03 (-0.15-0.15)	n.s.[a]

CIS= clinically isolated syndrome; MS= multiple sclerosis
NBV= normalized brain volume; cGMV= cortical grey matter volume; WMV= white matter volume; PBVC= percentage of brain volume change
T2-LL= T2 lesion load
TV= thalamic volume
BGV= basal ganglia volume
a= Mann-Whitney-U-test
b= Student-T-test

Data are presented as either mean ± standard deviation in case of normal distribution of data or median (interquartile range IQR) in case of non-parametric distribution

5.3.2 Correlation of MRI metrics

In the entire CIS-cohort, PBVC correlated with the decline of WMV (r=0.714; p=<0.001), cGMV (r=0.484; p<0.001) and THV (r=0.251; p=0.049). Similar findings were observed in patients with MS, where PBVC also correlated with the decline of WMV (r=0.441; p=0.001), GMV (r=0.356; p=0.007) and THV (r=0.301; p=0.028). There were also significant correlations between PBVC and T2-LL at baseline (r= -0.408; p= 0.002) and at follow-up (r= -0.356; p= 0.009).

In CIS converters, PBVC correlated with the change of WMV (r=0.668; p<0.0001), cGMV (r=0.525; p=0.002) and THV (r=0.420; p=0.015).

5.3.3 Clinical associations

There was no association between the decline of global, compartmental and regional brain volume parameters with disability neither for the entire CIS cohort nor for MS patients. Only in the combined analysis of CIS and MS, the decline of the PBVC weakly correlated with higher EDSS values at baseline (r= -0.219; p=0.016) and at follow-up (r= -0.201; p=0.028) and also with a higher annualized relapse rate (r= -0.208; p=0.023).

5.3.4 Associations of comorbidities with brain volume changes and lesion load

Comparisons of the baseline MRI parameters between individuals without or with at least one comorbidity are shown in table 13. Patients with at least one comorbidity had a significantly lower total brain volume as well as grey and white matter volume at their baseline MRI scan than those without any comorbidity. Also the subcortical grey matter volumes of the thalami and basal ganglia were significantly lower in patients with comorbidities. Patients with at least one comorbidity also showed a significantly higher lesion load at the baseline MRI.

Table 13: Baseline MRI parameters depending on the presence of no or at least one comorbidity

	No comorbidity n=66	One or more comorbidities n=54	p-value
NBV (cm^3)	1573.99 ± 82.19	1523.51 ± 116.46	0.001[b]
cGMV (cm^3)	657.63 ± 56.87	635.39 ± 60.50	0.06[b]
WMV (cm^3)	773.91 ± 44.05	743.86 ± 93.95	0.038[b]
TV (cm^3)	10.79 ± 1.52	9.82 ± 1.43	0.001[b]
BGV (cm^3)	12.14 ± 1.57	11.41± 1.78	0.02[b]
T2 –LL (cm^3)	1.23 (0.45-4.05)	2.08 (0.90-11.36)	0.012[a]

CIS= clinically isolated syndrome; MS= multiple sclerosis
NBV= normalized brain volume; cGMV= cortical grey matter volume; WMV= white matter volume; T2-LL= T2 lesion load; TV= thalamic volume; BGV= basal ganglia volume
a= Mann-Whitney-U-test
b= Student-T-test

Data are presented either as mean ± standard deviation in case of normal distribution of data or median (interquartile range IQR) in case of non-parametric distribution

During follow-up MRI variables changed to a similar extent in both groups.

In the separate analysis of each assessed comorbidity, patients with depression showed a lower volume of the thalami and basal ganglia at baseline and higher lesion load at baseline (median 1.48cm^3 versus 3.62 cm^3, p=0.015), as well as at follow-up (1.47cm^3 versus 3.63cm^3, p=0.024). The annualized loss of brain volume and the accrual of lesion load during the observation period was similar compared to patients without depression.

Arterial hypertension was associated with a lower total brain volume at baseline and lower cortical grey matter volume at baseline and follow-up. The accrual of lesion load during the observation period was higher compared to patients without high blood pressure (-0.16cm^3 versus 0.4cm^3, p=0.046).

Arterial hypertension and depression were not related with each other given that only 4 patients with depression also had a high blood pressure.

In this cohort the smoking status was not associated with any of the assessed MRI variables.

There was no association of the sum of comorbidities with brain volume changes and lesion load.

5.3.5 Analysis of extremes of brain volume change

To explore if the magnitude of PBVC could serve to identify patients with a more severe disease also on an individual basis, we generated quartiles of PBVC. Each quartile contained 30 patients. The number and percentage of individuals grouped in the distinct quartiles according to their phenotype is given in table 14. As can be seen, only one of the 30 patients remaining CIS during follow-up was grouped in the quartile with the highest brain volume loss, but otherwise phenotypes were rather evenly distributed over all quartiles.

Table 14: Quartiles of the PBVC in relation to the clinical phenotype

Quartiles PBVC	**Q1**	**Q2**	**Q3**	**Q4**
	-2.10% to -0.64%	**-0.63% to -0.41%**	**-0.40% to -0.18%**	**-0.18% to 0.78%**
Remaining CIS n=30	1	8	9	12
	3.3%	26.6%	30.0%	40.0%
Converter to MS n=33	10	8	5	10
	30.3%	24.2%	15.1%	30.3%
MS n=57	19	14	16	8
	33.3%	24.5%	28.0%	14.0%

Q= quartile

PBVC= percentage of brain volume change

CIS= clinically isolated syndrome

MS= multiple sclerosis

Table 15 shows the clinical characteristics according to the quartiles of PBVC. Patients grouped in the highest PBVC quartile, ranging from -2.10% to -0.64%, showed a trend towards a higher annualized relapse rate compared to patients in the fourth quartile representing those patients with the lowest PBVC (-0.18 to 0.78%) (0.36 versus 0.24 p=0.039). A trend could be observed for higher EDSS values in the first and second quartiles (representing patients with the highest PBVC) compared to the lower quartiles (p=0.01). The time interval between CIS and conversion to MS was comparable across the quartiles.

Table 15: Demographic and clinical variables at baseline and follow-up in relation to quartiles of PBVC

	Q1 -2.10% to -0.64%	Q2 -0.63% to -0.41%	Q3 -0.40% to -0.18%	Q4 -0.18% to 0.78%	p-value
Sex female (%)	20 (66.7)	22 (73.3)	20 (66.7)	16(53.3)	n.s.[c]
Age at baseline, years	33.6 ± 11.3	34.4 ± 9.5	32.6 ± 8.8	34.5 ± 7.7	n.s.[b]
Age at onset, years	27.2 ± 9.1	29.4 ± 8.6	27.7 ± 10.0	31.6 ± 8.3	n.s.[b]
Disease duration baseline, months	28.5 (2.75-112.7)	21.0 (2.0-96.0)	13.5 (2.0-95.2)	6.5 (1.0-34.0)	n.s.[a]
Interval baseline – follow-up MRI, months	35.5 (24.7-50.7)	44.0 (26.7-64.2)	43.0 (35.0-50.0)	42.5 (31.5-54.0)	n.s.[a]
EDSS baseline	1.7 (1.0-3.1)	2.0 (0.7-3.1)	1.2 (0.0-2.0)	1.2 (0.7-2.0)	n.s.[a]
EDSS follow-up	2.0 (1.5-3.5)	2.0 (1.0-3.6)	0.0 (0.0-2.1)	1.5 (1.0-2.0)	**0.01**[a]
Annualized relapse rate	0.3 (0.0-0.67)	0.0 (0.0-0.48)	0.0 (0.0-0.43)	0.0 (0.0-0.37)	n.s[a]
Disease modifying therapy n (%)					
Baseline	14 (46.7)	11 (36.7)	11 (36.7)	11 (33.3)	n.s.[c]
Follow-up	23 (76.7)	23 (76.7)	17 (56.7)	17 (56.7)	n.s.[c]

Q= Quartile
EDSS= Expanded Disability Status Scale
a= Kruskal-Wallis-test
b= ANOVA
c= x^2 contingency test

Data are presented as either mean ± standard deviation in case of normal distribution of data or median (interquartile range IQR) in case of non-parametric distribution

6. Discussion

This thesis deals with the development of mortality rates of MS patients in Austria. Furthermore, it explores how comorbidities of MS patients can be assessed despite the absence of systematic registries.
As an additional objective the temporal evolution of brain volume changes in a sub-cohort of patients with early MS and the potential associations with comorbidities is investigated.

6.1 Mortality of multiple sclerosis in Austria

So far data about mortality and reasons of death of MS patients in Austria are rare. Up to now only one publication investigated the trend of mortality from 1970 - 2001 and showed a general decline of death rates.[11] For this thesis the data collected from the database of Statistics Austria was reviewed and updated until 2014.

It could be demonstrated that from 1970 the overall mortality rate was decreasing until the turn of the millennium. In contrast, from 2000 onwards the mortality rate was increasing again, especially in female MS patients. The prediction of an increase in mortality for the period of 2010 until 2020 ranging from 1.13 to 1.32 per 100000 by Ekstern et al.[11] could be confirmed – however, so far it is lower than expected.
Similar trends could be observed in a Canadian cohort from 1975 till 2009.[113] Regardless, the overall mortality rate per 100000 as well as the sex specific mortality rate in this Canadian cohort was higher (overall 1.33, male 0.99, female 1.45) compared to the Austrian MS population (overall 0.82, male 0.62, female 0.79). The trend towards a higher mortality rate for the female MS population in Austria could also be demonstrated in other investigations.[79,114] The difference in the mortality rates between female and male patients in the Austrian cohort was highest between 1970 until 1979 (1.43 versus 0.91). For the period between 2000 and 2009 no difference could be observed (0.75 each) and for the last five years the mortality rates increased in the female group (from 0.75 to 0.79) while decreasing in males (from 0.75 to 0.62).

This sex specific difference of mortality is potentially caused by a higher and still increasing prevalence of MS in females. Given that this prevalence is probably also more widely known among physicians MS is probably also more often reported as the cause of death on the death

certificates of female patients.[115] Nevertheless, other studies exist that found no difference of sex specific death rates or even higher rates in male patients.[80] So far there is no clear explanation for this divergence of results. They might partly be explained by a higher prevalence of distinct comorbidities, for example of cardiovascular diseases, in male MS patients in some studies[116]; but even this factor may differ between distinct regions.[117] On the other hand there are reports, which suggest that the prevalence of cardiovascular comorbidities are slightly higher in male patients, but that the effect of these cardiovascular risk factors on mortality are higher in women.[118]

In comparison to the general population the crude mortality ratio was about double in the MS cohort in this investigation. However, this result can only be interpreted as a trend, because the calculation of standardised rates was not possible due to the lack of information on the age composition of the MS population in Austria.

An analysis of the regional trend of mortality in Austria shows that a slight east-west gradient with higher mortality rates in the eastern regions of Austria, which has already been observed by Ekstern et al., still exists.[11] For example, during the period of 2010–2014 the mortality rate was 0.96 per 100000 in Vienna and 0.68 per 10000 in Tyrol. Possible explanations might include a higher awareness of MS in more urban areas and therefore a better codification on death certificates.[11]

In 2014 the average age at death from MS in Austria was 62.9 years for men and 67.3 years for women. Compared with the data from Ekstern et al., an increase of life expectancy of 6.2 years for men and 7.7 years for women since 2001 could be observed.[11] Life expectancy tables of Statistics Austria show that, in general, the life expectancy of individuals born around 1950 was at time of birth about 61 years for men and 66 years for women. However, if people reached the age of 60 in the year 2000 the expected remaining time of life was still 19.9 years for men and 24.1 years for women.

Even though the life expectancy for MS patients has been increasing constantly since 1970 the life expectancy of MS patients compared to the general population in the year 2014 according to the assessed data was about 17 years lower for male patients and about 18 years for females.

Compared to other investigations the age at death in Austria seems to be significantly lower. In a Canadian cohort investigating 6917 MS patients the median age at death was 74.3 years

in men and 78.6 years for women. The life expectancy in this cohort was about 6 years less compared to the general population.[74]

In the Danish MS-registry, which encompasses about 10000 individuals with MS, a survival analysis could demonstrate that life expectancy is about 10 years shorter in individuals with MS compared to the age matched general population.[7]

The surprisingly low age at death in Austria compared to the above listed investigations might be partly explained by methodological issues. In the database of 'Statistics Austria' the reason for death is recorded according to the death certificates, which contain the primary cause of death as voiced by the responsible physician. It can be expected that, especially in older individuals who suffer from additional diseases, MS might not always be coded as the underlying cause of death.
This hypothesis is based on several other investigations which could demonstrate that MS is recorded in only about 50-60 % of MS patients as the primary cause of death.[7,8,79,80] Other common causes of death indicated in MS patients are cardiovascular disease, cancer, respiratory diseases and suicide.[7,8,79,80] Unfortunately, it was not possible to assess how many patients with MS in Austria die due to the above listed diseases. Thus, the low age at death in Austria may primarily reflect those individuals with a more progressive disease course, suffering from severe disability. This assumption gets support from a French investigation where it was shown that progressive MS was associated with a significantly reduced probability of being alive 25 years after disease onset compared to RRMS (73% versus 91%).[114]

It can be speculated that patients suffering from a more pronounced disease course also did not benefit from disease modifying therapies, which came on the market during the early nineties. Long term data, for example on Interferon beta, could show that patients treated in the early stages of the disease had a reduced hazard rate of dying by 46.8%.[66]

Unfortunately, the assessment of the length of survival for the Austrian MS patients could not be obtained given that clinical data such as age at disease onset are not available in the database of 'Statistic Austria'. However, general studies show that the survival rate is significantly shorter in males compared to female patients. In a Danish cohort, which encompasses more the 9800 individuals with MS, the mean survival rate in men was 28 years compared to women with 33 years.[7] Together, these findings once again highlight the emerging and pressing need for comprehensive and systemic registries based on the models of

Denmark, France or Norway, where all patients with MS are recorded and have been recorded, at least in part, since 1945.

6.2 Comorbidities of MS and their clinical consequences

As already shown, comorbidities are associated with mortality in MS patients. But also the role of comorbidities on the diseases course and morphologic brain abnormalities are in the focus of interest in several research networks. For Austria, so far there are no data about the potential impact of comorbidities on MS patients. Therefore, one aim of this thesis was to analyse if and how such data could be accessed in Austria. We decided to start with a local approach using the local patient database MEDOCS. We used this method because the MEDOCS system is used in all public hospitals in Styria and contains medical information from different departments since 2000. We also intended to assess data obtained by general practitioners and private hospitals, even though we were fully aware that these data might not be readily accessible. For this purpose, we contacted local health insurances. Unfortunately however, these institutions were not able to provide data about comorbidities obtained outside public hospitals, mainly because no systemic coding of diseases exists outside clinical institutions and thus data cannot be assessed by the regional branches of the national health insurances. We therefore focused on MS patients who were treated in at least one of the distinct institutions of Medical University hospital of Graz within a period of 6 years and who had a documented MS specific clinical history.

In a cohort consisting of 120 patients, depression (20%) was the most common comorbidity. This percentage is lower compared to a meta-analysis including 118 studies that investigated psychiatric disorders in MS. In this article the mean prevalence of depression was reported with 23.7% in MS patients. Nevertheless, there was a range from 3.8% to 68.4%, which was mainly caused by different approaches of assessment of depression.[119] Studies using only administrative data found rates of about 12%.[120] In view of other studies, which observed much higher rates of depression by using structured interviews and standardised rating scales,[121] it can be assumed that the assessed frequency of depression in our study might be underestimated.

The prevalence of depression in MS is about three times higher compared to the general population and also higher compared to other chronic diseases.[122] To date there is no clear explanation for the high rates of psychiatric disorders, in particular of depression in MS. A

complex interplay of neuropathological changes with several psychological factors such as perceived helplessness, uncertainty and perceptions of disability, has been suggested.[123] However, depression in MS is associated with a lower quality of life, higher rates of fatigue reduced adherence to therapy[124] and in some studies also with higher levels of disability.[125] Even though the latter could not be shown in our study, which might partly be related to the overall low level of disability in our cohort, it highlights the necessity of an early diagnosis, because depression is considered as a treatable condition.[126]

Cardiovascular comorbidities including arterial hypertension and hyperlipidemia were other common diseases of our cohort. Arterial hypertension could be observed in 11.7%, which is a lower rate compared to a current meta-analysis investigating the prevalence of cardiovascular diseases in MS patients. In this paper an average rate of 18.7% could be observed.[88] In the same paper a prevalence of 10.7% for hyperlipidemia could be observed, which is higher compared to our patients with 7.5% of affected patients.

Not surprisingly, patients with arterial hypertension were significantly older at disease onset and at baseline. No association with disability or disease course could be shown, which is first and foremost related to the short observation interval. Other authors could demonstrate that vascular comorbidities are associated with an shorter interval and a higher risk for an impaired walking ability defined as a proportion of patients needing unilateral walking assistance.[90] Even though the pathogenetic mechanism for the progression of disability and in MS patients with cardiovascular disease are not well understood, there are some hypotheses which suggest that cardiovascular diseases induce peripheral inflammation leading to the activation of the inflammatory cascade. The elevated activation of cellular and humoral components may increase demyelination and neurodegeneration.[127] Besides the effect at the pathophysiological level, there are some additive effects of cardiovascular comorbidities. Diabetes mellitus, for example, can cause polyneuropathy leading to pronounced gait impairment.[90]
Due to the group size it was not possible to give a reliable statement on whether cardiovascular comorbidities are more frequent in MS patients. Based on the literature, cardiovascular diseases seem to be more frequent in MS. Even though MS itself may not cause cardiovascular diseases it can be assumed that, due to the progression of disability in particular in the later stages of the disease, individuals are less physically active and therefore are more prone to cardiovascular diseases such as arterial hypertension or hyperlipidemia.[127]

Autoimmune thyroid disease was the third common comorbidity in this thesis, affecting 9.2% of all patients and was observed only in women. This finding is in line with other investigations showing a prevalence of thyroid disease of 9.5% in MS patients with a female predominance.[128] A similar result could also be shown in an Austrian study in which a frequency of thyroid disease of 8.7% in female MS patients was observed. This finding was comparable with the frequency of 9.2% in a non-MS control group.[129]

There was only one case of cancer (cervix carcinoma) in the observed cohort, even though this finding is not representative due to the relatively short observation period of 6 years.

We are aware that this analysis of comorbidities has several limitations. First, compared to other investigations the observed cohort is relatively small. Even though we were able to identify 324 patients with MS in a first step, roughly only half of these patients have been seen in our outpatient clinic on a regular basis and can thus provide useable data on the disease course. Second, the number of comorbidities might be underestimated given that we could only use a dataset of the University hospital of Graz. Furthermore, it can be assumed that not all additional medical issues in MS patients, as for example arterial hypertension, need to be treated in a tertiary care hospital. Thus, it can be expected that even if individuals with MS visit other institutions of the Medical University of Graz, for example the department of surgery in case of a broken leg, additional medical conditions are not given an ICD code. Third, regarding the assessment there are also some limitations, because the diagnosis is limited to self-reports of patients and to external diagnosis, for example by the general practitioner of the patient's choice. Systematic testing with special depression scores was not available and therefore it was also not possible to sub-classify depression into mild, moderate or severe depression as performed in prospective studies.[130] Fourth, the investigated cohort represents first and foremost patients in the early phase of MS. Thus, we do not have information on the effect and the frequency of comorbidities in patients with more progressive forms of the disease. Fifth, due to the small cohort size and the lack of an age matched control group it was not possible to make a statement about the prevalence of the observed comorbidities compared to the general population.

Despite these limitations the number of most of the reported comorbidities in this cohort was comparable respectively slightly lower to other investigations[88,119,131,132] and could be tested in a small, but very well documented cohort of patients. Our findings highlight the importance of recognizing, monitoring and treating the distinct comorbidities appropriately on an

individual level, especially considering that to date the full consequences of comorbidities in patients with MS over the long-term are unknown.

6.3 Morphologic brain abnormalities and correlations with comorbidities

Measurement of lesion load and of brain volume changes has become a widely accepted method to investigate brain pathology in MS. Lesion load is an established marker in clinical trials to monitor treatment efficacy. During the last couple of years brain volume is increasingly considered as an additional important outcome variable in treatment trials.[98,133] This leads some to advocate atrophy measures for routine clinical application in view of increasingly robust and more easily applicable measurement techniques, although the appropriate setting in the clinical routine outside well controlled clinical trials is not yet fully clear. As mentioned above there is an increasing awareness of comorbidities in patients with multiple sclerosis but little is known about potential associations of comorbidities with morphologic brain abnormalities.

In this longitudinal 3-Tesla MRI-study, we thus investigated the evolution of various markers of brain volume changes that were derived from routine clinical follow-up of patients in a tertiary care hospital.

The early stages of MS in this context are of special interest because treatment decisions are less informed by the long-term cause and current disease modifying treatments are known to be most efficacious.

We found that over a mean follow-up period of 43 months, CIS patients had a significantly lower PBVC than patients with MS. However, this was only due to the fact that the patients who remained CIS had a lower PBVC than those who converted to MS or were MS. In contrast there was no difference between converters from CIS to MS and patients who were MS at the study entry.

These findings are in line with earlier work on this issue. In one study investigating 176 CIS patients at baseline and one year after disease onset, those individuals who experienced a second attack (n=76) had a significantly higher PBVC compared to the remaining CIS cohort (-0.65% versus +0.06% $p<0.001$) after a mean follow-up period of 53 months.[16] Another finding in that study was a shorter interval to a second attack in the quintile of patients with the highest PBVC.[16]

To see if the change in PBVC may also allow to predict conversion from CIS to MS, we created quartiles of PBVC, but could not detect significant associations. This illustrates the limitation of such metrics on an individual basis. It cannot be excluded, however, that the overall low rate of PBVC in our CIS cohort during the observational period compared to other investigations may have contributed to this negative finding.[16,134,135]

It is noteworthy that significant differences between CIS and MS and especially between CIS patients converting or not-converting to MS could only be observed for PBVC. The annualized decline of the compartmental and regional brain volumes during follow-up was similar between CIS and MS or at least followed a trend similar to the PBVC between converters and non-converters, except for the WMV. However, these differences did not reach statistical significance. This suggests PBVC as the most robust metric and little gain in sensitivity for disease progression by adding further compartmental or regional volumetric analyses. When comparing our findings to those of earlier studies on longitudinal compartmental and regional volume changes,[16,95,97] the relatively low overall disease progression in our cohort – both in clinical and morphologic terms – which certainly mitigated possible differences has to be considered. Some associations could be detected between the magnitude of PBVC and the patients' clinical evolution over time on a group level. Individuals grouped in the highest two quartiles of PBVC showed more pronounced disability at baseline than those in the lower quartiles and the difference in median EDSS became significant with follow-up. A more pronounced decline of PBVC was also related to a higher annualized relapse rate. These observations are in line with several other reports that show a positive correlation between brain volume changes and clinical disability.[94,95,136]

In the analysis of the association of comorbidities with lesion load and brain volume abnormalities, having at least one comorbidity was associated with a lower total brain volume as well as with lower grey and white matter volumes at baseline. This result was triggered primarily by arterial hypertension. This observation is in line with recent work from Kappus et al.. Their study assessed 326 patients with RRMS, 163 patients with progressive MS and 61 with CIS. Arterial Hypertension and heart disease in this study were associated with a lower total and cortical grey matter volume in MS patients.[15]

This observation is not very surprising given that the relationship between cardiovascular risk factors and accelerated brain atrophy is a common finding even in patients without a specific cerebro-vascular disease.[100] A recently published study investigating 663 individuals without MS but with cardiovascular disease could demonstrate that higher blood pressure levels were

associated with the progression of subcortical brain volume loss at follow-up. Declining blood pressure levels during the observation period were associated with less progression of atrophy.[137] So far the pathophysiological processes leading to the pronounced brain volume loss in individuals with cardiovascular risk factors are not fully understood. One potential reason might be a decreased arterial supply due to stiffness of the arterial walls with disturbance of the cerebral autoregulation, which in the following leads to hypoperfusion of brain parenchmya resulting in increased atrophy.[137]

Diabetes mellitus is another highly endemic disease in western countries and is considered to increase oxidative stress levels and to alter inflammatory processes in the brain which may promote loss of brain volume.[138]

To date it is not possible to give a reliable statement on whether cardiovascular diseases have a direct impact on the inflammatory and neurodegenerative processes in MS. In the study of Kappus et al. it has been postulated that the pronounced atrophy can be interpreted as an additional effect of cardiovascular diseases due to the mechanism described above.[15]

Depression could be identified as another comorbidity associated with brain morphologic abnormalities in this thesis. MS patients with depression had a significantly higher lesion load at baseline and at follow-up and a lower thalamic and basal ganglia volume at baseline regardless of the disease course. This finding is in line with other investigations showing an association of white matter lesions[139] and the atrophy of deep grey matter structures with depression beyond physical disability.[140]

The smoking status in this study was neither associated with brain volumes nor lesion load, which deviated from other investigations. Zividanov et al. could demonstrate that higher lesion loads and pronounced brain atrophy is strongly associated with smoking.[101] This is not unexpected given that several ingredients of cigarettes are known as potential triggers for inflammation in other autoimmune diseases[141] due to the alteration of several cytokines and pro inflammatory factors like C-reactive protein or interleukin 6.[142] The negative finding in the investigated cohort might partly be explained by the lack of the exact number of pack years, which was not possible to obtain retrospectively. Other investigations have shown that higher numbers of pack years were associated with higher disability over a mean follow-up period of about 3 years, but data about lesion load and brain volume changes were not assessed in that study.[143]

The decline of brain volume during the observation period was not associated with comorbidities, which might be related to the relatively short observation period of about 42 months.

For the interpretation of the results of this study, a few limitations relating to the study design and setting have to be taken into account. First, our study cohort represents MS outpatients who are seen on a regular basis and undergo consecutive MRI scans. Data were collected as part of the clinical routine and may therefore be somewhat more variable in quality than those from stringently controlled trials. Second, the assessment of comorbidities was done retrospectively and was dependent on the correct coding in our medical record system MEDOCS. Most of the comorbidities were self-reported and therefore the real number might be higher, especially in cases of depression; this was not tested specifically. Third, as mentioned above, this cohort represents first and foremost individuals in the early stages of MS and it was not possible to assess the role of comorbidities in more progressive forms of MS. Due to the higher grade of disability and the lack of therapeutic options in progressive stages, patients with very advanced MS are rarely seen in our outpatient clinic.

Irrespective of the aforementioned limitations, this work bears undoubtedly direct relation to patient management and illustrates the difficulties of incorporating volumetric measurements and comorbidities into clinical decision-making.

6.4 Summary and future perspectives

Mortality rates in Austria have been slightly increasing during the last decade, which is related first and foremost to the higher awareness of MS in the population. Nevertheless, the reported mortality rates and the age at death of MS patients in Austria are lower compared to other regions. This is probably caused by methodological issues given that MS as the underlying cause of death is mostly reported in patients with more progressive forms of MS. Other potential causes of death, such as cardiovascular disease or cancer, were not accessible. The only way to assess these data more thoroughly in the future is to implement systematic registries comparable to those in North America or Northern European countries.

In 2006 an MS-registry has been established in Austria and currently encompasses about 2100 patients. This number corresponds to approximately one sixth of all MS patients in Austria, where a number of about 12500 cases regarding a prevalence rate of 148 per 100000 can be

expected. This registry was created to monitor the safety and efficacy of specific MS treatments, which became mandatory according to the social insurance companies after the approval of Natalizumab. Thus, at the moment the registry contains primarily patients with an active disease course requiring a DMT. Nevertheless, for the future it is important to report all patients with MS regardless of their disease course and whether they are monitored in clinical institutions or in medical practices in order to get a full overview of comorbidities and causes of death. It is not enough to refer to data from abroad, because – as mentioned above – the prevalence of specific comorbidities significantly varies between distinct regions. This might be explained by locally different social status, different exposition to risk factors like smoking, which is especially high in Austria, and access and quality of the local health care institutions. As such, registries are not only of statistical interest, but also offer the opportunity to gather important information about the quality of care for MS patients.

The impact of comorbidities on the disease course and their potential correlation with brain volume parameters is poorly investigated. Thus, the relation of comorbidities with clinical and MRI variables were also explored in this thesis. While no association could be demonstrated with the (overall low) disability and disease course, it could be demonstrated that the presence of cardiovascular risk factors such as arterial hypertension is associated with a lower whole brain and cortical grey matter volume regardless of the disease course. A lower thalamic and basal ganglia volume was associated with the presence of depression.

These results underline the necessity of an early diagnosis and aggressive therapy of comorbidities hopefully leading to a better long-term prognosis. Given that some treatments are associated with higher risks, for example in case of cardiac diseases, our results also emphasize the necessity to carefully and adequately assess comorbidities - particularly in view of the increasing therapeutic options.

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